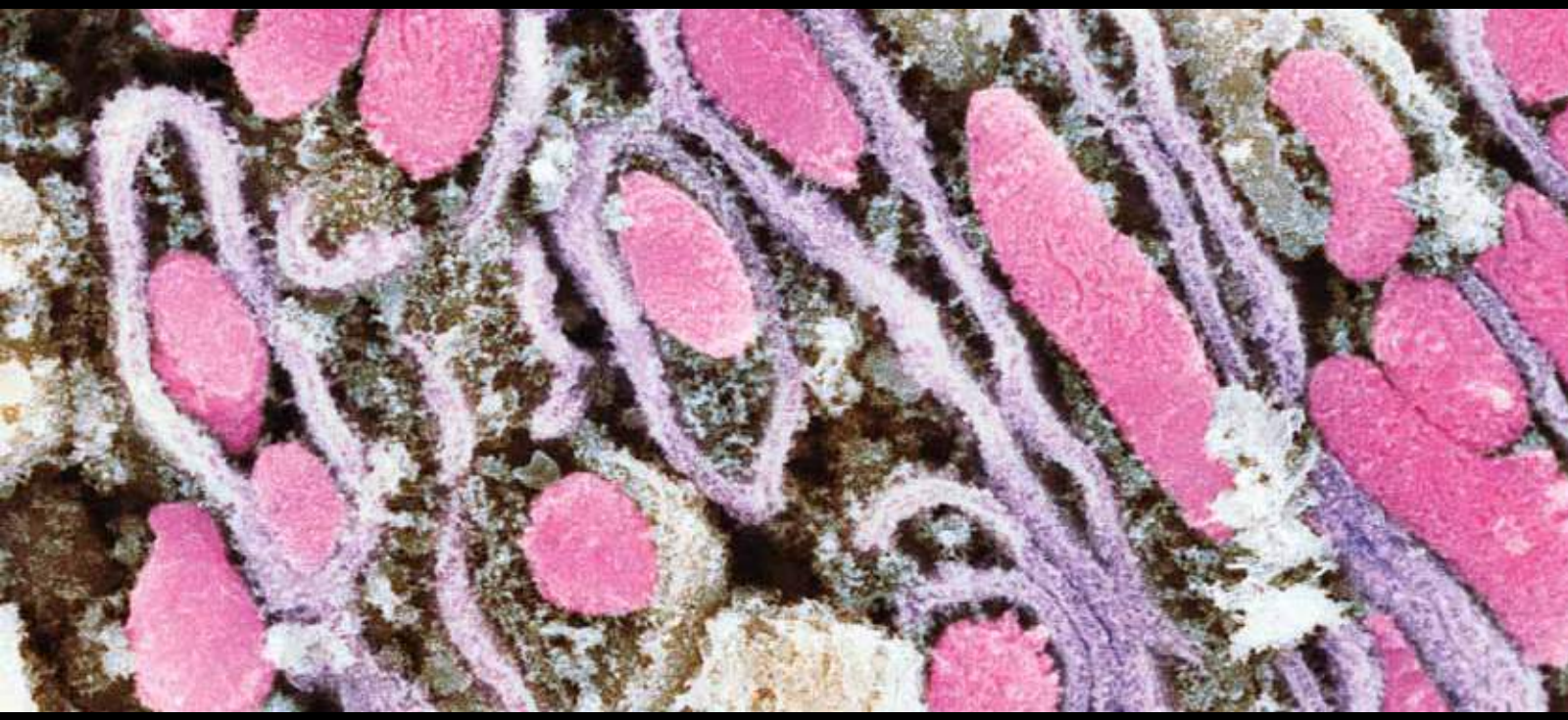
International Journal of Nephrology

Pediatric Nephrology

Guest Editors: Michel Fischbach, Patrick Niaudet, Franz Schaefer, and Lesly Rees



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Editorial Board

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Editorial
Pediatric Nephrology

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Pediatric nephrology covers a large field of medical developments, including genetics, care strategies, and renal preservation. Most of the pediatric kidney diseases are congenital, some of them familial with a precise heredity. Growing, the children will be adult patients emphasizing the importance of better knowledge by the nephrologist of this kidney disease, to optimize transition from pediatric to adult care. Therefore, this special issue is of major interest for "all." It was only possible to do this "issue" with the partnership of many "Friends."

Several Topics Are "Invited Reviews Resulting from Recognized Expert Teams. The "*Hemolytic uremic syndrome: new developments in pathogenesis and treatment*" by P. Niaudet and O. Boyer can be divided into two forms, typical and atypical HUS. Postdiarrhea HUS, that is, typical D+ (Shiga-toxin enterohemorrhagic Escherichia coli (EHEC) or Shigella), generally of good prognosis is the most common form of HUS in children accounting for 90 percent of all cases. Atypical HUS (aHUS) is a heterogeneous disorder which is responsible for only 10 percent of cases in children. Thrombotic microangiopathy may affect other organ systems, including the central nervous system, with poorer prognosis. In the past 15 years, mutations in complement regulators of the alternative pathway have been identified in almost 60% of cases, leading to excessive complement activation. Eculizumab, a monoclonal antibody binding to C5, has been used in patients with aHUS on native kidney or recurrence of aHUS after transplantation with very encouraging results.

The "*The history of cystinosis: lessons for clinical management*" by P. Goodyer is marked by a few sudden leaps forward in our understanding rather than by a sustained research effort fuelled by the larger research community. Major conceptual breakthroughs include (a) its discovery in 1903, (b) recognition of the renal Fanconi syndrome, (c) realization that tissue accumulation of cystine reflects a defective channel in the lysosomal membrane, (d) translation of this discovery to trials of cysteamine, (e) discovery of the *CTNS* gene, and (f) report of successful stem cell therapy in the cystinotic mouse.

The "*Primary hyperoxalurias*" as marked by P. Cochat et al. are inborn errors in the metabolism of glyoxylate and oxalate. Early initiation of conservative treatment, including high fluid intake, inhibitors of calcium oxalate crystallization, and pyridoxine in responsive cases, can help to maintain renal function in compliant subjects. In end-stage renal disease patients, the best outcomes have been achieved with combined liver-kidney transplantation which corrects the enzyme defect.

In the paper by J. A. Sayer et al., the "nephronophthisis" is recognized as a ciliopathy. NPHP is a recessive monogenic disorder [19], meaning that two mutations (homozygous or compound heterozygous) in a single gene are sufficient to cause disease. Thirteen genes have been identified in affected families with NPHP, and these genes currently allow 30% of cases with NPHP to be "solved" in terms of a molecular diagnosis. NPH is a leading genetic cause of established renal failure (ERF) in children and young adults.

The "*Renal mitochondrial cytopathies*" are a group of rare diseases that are characterized by frequent multisystemic involvement and extreme variability of phenotype, including renal involvement. Most frequently patients present a tubular defect that is consistent with complete De Toni-Debré-Fanconi syndrome in most severe forms. More rarely, patients present with chronic tubulointerstitial nephritis, cystic renal diseases, or primary glomerular involvement.

The "*Molecular and genetic basis of inherited nephrotic syndrome*" describes the novel concept of the glomerular filtration barrier, highly dynamic slit diaphragm proteins. The "*Nephrotic syndrome in children: from bench to treatment*" allows a review of the clinical management of NS in children. Most of the cases are steroid sensitive, but fifty percents of the latter recur frequently and necessitate a prevention of relapses by nonsteroid drugs. But, their long-term prognosis is overall good. On the contrary, steroid-resistant nephrotic syndrome (SRINS) leads often to end-stage renal failure.

The "*Recurrent focal segmental glomerulosclerosis: a discrete clinical entity*" describes that some FSGSs are caused by mutations podocyte slit diaphragm genes, that is, hereditary FSGS. Thereby, it is increasingly clear that the steroid-resistant form of FSGS that recurs in the renal allografts (R-FSGSs) constitutes a distinct clinical entity.

The "*Primary molecular disorders and secondary biological adaptations in Bartter syndrome*" describes the Bartter syndrome as a hereditary disorder that has been characterized by the association of hypokalemia, alkalosis, and the hypertrophy of the juxtaglomerular complex with secondary hyperaldosteronism and normal blood pressure. The genetic causes of Bartter syndrome primarily affect molecular structures directly involved in the sodium reabsorption at the level of the Henle loop.

The "*Acute renal replacement therapy in pediatrics*" gives out the current knowledge on the dialysis modalities: when to apply and, how to apply.

Chronic dialysis remains a life event for most of the children on end-stage renal disease despite the growing place of preemptive kidney transplantation. "*Peritoneal dialysis tailored to pediatric needs*" and "*Optimal hemodialysis prescription: do children need more than a urea dialysis dose?*" offer a review on the necessarily optimized dialysis strategies for children based on collaborative clinical experiences. The concept of the "adapted automatic peritoneal dialysis" is described, as well as the place for a "convective volume" to add to the limited urea dialysis dose. "Daily" hemodiafiltration appears as an optimal package to promote statural catch up growth.

Several Topics Reflect Original Experiences. This special issue covers several original experiences: "*Once-daily tacrolimus extended-release formulation: 1 year after conversion in stable pediatric kidney transplant recipients,*" "*The hyponatremic hypertensive syndrome in a preterm infant: a case of severe hyponatremia with neurological sequels,*" "*Depressive symptomatology in children and adolescents with chronic renal insufficiency undergoing chronic dialysis,*" "*(Pro)renin receptor in kidney development and disease,*" and "*Urinary angiotensinogen as a biomarker of nephropathy in childhood.*"

Together, the lecturers should improve their "pediatric nephrology" knowledge, from bench to bedside.

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Review Article

Recurrent Focal Segmental Glomerulosclerosis: A Discrete Clinical Entity

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Focal segmental glomerulosclerosis refers to a set of particular histopathologic lesions in which steroid-resistant podocyte injury leads to patchy adhesions between the glomerular tuft and Bowman's capsule, followed by progressive glomerulosclerosis and proteinuric renal failure. Because of the nonspecific nature of this lesion, it has been difficult to classify the various forms of primary nephrotic syndrome in children. However, with the recognition of hereditary FSGS caused by mutations podocyte slit diaphragm genes, it is increasingly clear that the steroid-resistant form of FSGS that recurs in the renal allografts (R-FSGS) constitutes a distinct clinical entity. Capitalizing on recent studies in which patients have been screened for slit diaphragm gene mutations, this review focuses on the natural history and pathogenesis of R-FSGS.

1. R-FSGS in the Context of Steroid-Resistant Nephrotic Syndrome

A recent population-based study in the Gironde region of France reported an incidence of about 2.3 pediatric cases of idiopathic nephrotic syndrome for every 100,000 children <15 years of age [1]. The majority (91%) of these children exhibited a classical steroid-responsive relapsing disease, in which there may be podocyte foot process effacement noted on renal biopsy but no progressive renal insufficiency. Estimates of steroid-responsiveness are somewhat lower (80%) in referral populations or cohorts that include adults [2]. Among those who fail to respond to daily steroid therapy for 4 weeks, renal biopsies usually reveal a progressive lesion in which early podocyte detachment from the glomerular basement membrane is associated with segmental hyalinosis of the glomerular capillary tuft and, eventually, fibrotic adhesions to Bowman's capsule. It is the patchy distribution of these lesions within the glomerulus and the initial sparing of some glomeruli that warrant the pathologic descriptor, focal segmental glomerulosclerosis (FSGS).

In New York City, about two thirds of steroid-resistant nephrotic syndrome (SRNS) patients display FSGS on renal biopsy [3]. In the setting of SRNS, these lesions are associated with high risk of progressive renal failure [4]. Collectively, these children comprise nearly 15% of the dialysis and renal transplant population in North America. However, the FSGS lesion is not pathognomonic for a specific clinical entity but rather reflects any process that leads to patchy irreversible podocyte injury. In addition to the many disorders that cause "secondary" glomerulosclerosis, children with steroid-sensitive nephrotic syndrome (SSNS) may show mild FSGS lesions at presentation or later when biopsied for development of steroid resistance [5]. Yet, if steroid responsiveness is documented at any time, patients rarely have progressive renal insufficiency [6–11]. Conversely, some children with SRNS display only minimal histopathologic changes on initial biopsy, yet eventually develop end-stage renal disease. Thus, it has been difficult to dissect out the discrete clinical syndromes in children who present with idiopathic nephrotic syndrome in childhood.

Among the SRNS patients who develop FSGS lesions and end-stage renal disease, about one third are now known to harbor mutations of genes encoding components of the podocyte slit diaphragm or adaptor proteins which link this structure to the podocyte cytoskeleton. These patients are generally unresponsive to immunosuppressive therapy but do very well after renal transplantation. Among children

without pathogenic mutations of slit diaphragm genes, 30–50% exhibit recurrence of proteinuria and then gradually develop *de novo* FSGS lesions in the renal allograft. This phenomenon, first reported by Rich [12] and then Hoyer et al. [13], is generally taken as *prima facie* evidence for a circulating "FSGS factor" in the allograft recipient and serves as the signature for a distinct form of SRNS. Capitalizing on recent studies which screen out the most common gene mutations, this review focuses on the natural history and pathogenesis of steroid-resistant recurrent FSGS (R-FSGS).

2. Clinical Characteristics of Recurrent FSGS (R-FSGS)

Recurrence of FSGS in a subset of renal allografts has been extensively documented. However, until recently, it has been perplexing why some cases of primary FSGS recur while others do not. With broader screening for the recessive forms of genetic FSGS, the natural history of R-FSGS is now emerging with greater clarity. At one point, it was suggested that R-FSGS patients might differ from children with steroid-responsive nephrotic syndrome only in that the former carry a heterozygous mutation of a slit diaphragm gene. However, this does not seem to be the case [14]. In general, R-FSGS children have no identifiable mutations of slit diaphragm genes including nonpathogenic variants such as the R229Q polymorphism of the podocin gene [15, 16].

In 2010, Canaud reviewed 77 cases of idiopathic steroid-resistant nephrotic syndrome with FSGS who received a renal allograft [17]; the 42 patients who exhibited recurrent proteinuric disease had no demonstrable slit diaphragm gene mutations. Age at the time of presentation (10.4 ± 10 years) and delay before end-stage renal disease (4.8 ± 2.3 years) was similar to the group that showed no recurrence of proteinuria. At initial presentation, all were steroid resistant but 10/77 showed only minimal changes on renal biopsy. In the others, the FSGS lesion was subclassified according to 2004 "Columbia" criteria [18]; about half had a non-specific (NOS) lesion, 22.2% had the cellular variant, 12.9% showed collapsing lesions, and 7.8% had perihilar, and 7.8% had "tip lesions." Thus, there is no distinctive pathologic subtype that distinguishes R-FSGS from forms of FSGS that do not recur after transplantation. Furthermore, as FSGS lesions gradually appeared in the allograft, less than 10% showed the same Columbia pattern as in the initial biopsy. In an earlier study of 19 R-FSGS patients, greater fidelity of the pathologic subtype before and after transplantation was reported [19], but it seems most likely that the glomerular histopathology reflects variability in the host response to podocyte injury or to modifications induced by treatment rather than a recognizable marker of the underlying etiology.

Clinical features of R-FSGS in the allograft roughly recapitulate the initial presentation. However, recurrence affords an opportunity to dissect the features of the earliest stage in detail. Heavy proteinuria may develop within hours of transplantation; this occurs in the absence of any FSGS lesions. Although proteinuria may rarely be delayed for several months (1/42), the majority in Canaud's study exhibited proteinuria within 48 hours (32/42) or within the first three weeks (9/42) [17]. About 90% of R-FSGS patients exhibit glomerulosclerosis when they first come to medical attention [17], but it is clear that FSGS lesions appear gradually in the allograft. Canaud identified FSGS in one patient by day 15 but documented the lesion in 28% of biopsies at 3 months and 38% by 12 months after transplant [17]. Appearance of FSGS lesions might have been more rapid in the absence of plasmapheresis and high-dose calcineurin inhibitors, but it seems that irreversible podocyte damage takes time and occurs well after the disruption of podocyte slit diaphragms that marks rapid onset of proteinuria.

3. The Putative Circulating Podocyte-Toxic Factor in R-FSGS

There is little doubt that patients with R-FSGS have acquired a circulating factor (or factors) that rapidly affects podocyte biology. Electron microscopy shows podocyte effacement at the time of recurrent proteinuria in the allograft and if patient plasma is applied to human podocytes *in vitro*, the cellular cytoskeleton is deranged within 6 hours [3, 20]. Sharma et al. argued that podocyte dysfunction in R-FSGS could be due to lack of a normal circulating factor, since replacement of FSGS plasma with normal plasma allows podocyte cytoskeleton recovery *in vitro* [21]. However, R-FSGS is transiently responsive to plasmapheresis [22]; this effect can be seen even when albumin (rather than fresh plasma) is used as the replacement fluid (Figure 1). Furthermore, Lagrue et al. reported the case of a woman, who had previously given birth to a normal child, and then developed SRNS with FSGS lesions [23]. In her two subsequent pregnancies, heavy proteinuria was evident in each newborn but resolved within 2-3 weeks [23]. This interesting observation shows that the FSGS factor can cross the placenta and suggests that podocytes can recover once exposure to the factor is ended. On the other hand, it also suggests that the circulating factor can persist for many days in the infant circulation or that podocytes need some time to recover.

For many years, it has been assumed that the putative circulating factor is a cytokine derived from T-lymphocytes. This hypothesis was proposed by Shaloub in 1986, when he encountered a man with relapsing nephrotic syndrome and leukemia, involving a malignancy of natural killer-like T-lymphocytes bearing a chromosome 10 translocation; proteinuria resolved following successful chemotherapy of the malignancy [24]. Supporting this idea, Le Berre et al. reported that Buffalo/Mna mice develop a spontaneous form of R-FSGS which is ameliorated by infusion of $CD4^+CD25^+$ $FoxP3^+$ lymphocytes or by LF15-0195, a drug that increases the level of these cells in the circulation [25]. Similarly, Bao et al. found that R-FSGS, induced in mice by an antipodocyte antibody, is linked to a decay activating factor-dependent T-cell response [26]. However, recent reports have documented an effect of the CD20 B-cell antibody, rituximab, on relapsing steroid-dependent nephrotic syndrome [27–29], and Bagga described an effect of rituximab on steroid-resistant FSGS [30]. Thus, the time-honored assumption that human R-FSGS is a direct consequence of T- lymphocyte dysfunction must be reconsidered.

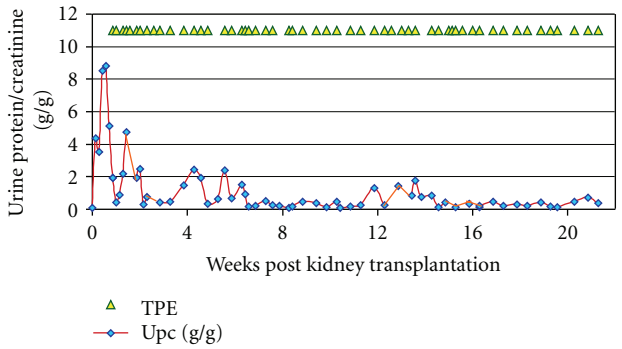

FIGURE 1: Immediate recurrence of FSGS (rFSGS) following deceased donor kidney transplantation. A 15-year-old girl with steroid-resistant FSGS had bilateral nephrectomy prior to transplantation. Proteinuria recurred within the first 12 hours but was controlled with intensive plasma exchange therapy (1.5 plasma volumes with albumin replacement). Efforts to wean plasmapheresis lead to a rise in urine protein/creatinine ratio (g/g) on several occasions. Each triangle represents a plasma exchange.

Several groups have tried to identify circulating podocyte-toxic factors in patients with nephrotic syndrome. Savin and Sharma reported a 30–50 kDa factor in FSGS serum which appears to alter the permeability of isolated rat glomeruli to albumin, after exposure to 2% patient serum for 10 minutes [31, 32]. In this assay, the investigators measured the increase in glomerular volume that accompanies switch of culture medium from 5 g/L to 1 g/L albumin; unfortunately, it is difficult to know whether this assay reflects a change in permeability of the slit diaphragm or the capillary wall. In other reports, hemopexin, cardiotropin-like cytokine1, and soluble urokinase receptor have been proposed as potential candidates, but direct evidence for their involvement in R-FSGS is lacking to date.

Most recently, Wei et al. reported that plasma levels of the soluble urokinase plasminogen activating receptor (suPAR) are elevated above an apparent threshold level (>3 ng/mL) in about two thirds of steroid-resistant FSGS patients and that suPAR induces proteinuria 24 hours after infusion into mice [33]. In elegant experiments with various suPAR mouse mutants, they found that this involves binding to and activation of β3integrin at the podocyte surface. In previous studies, they showed that β3integrin activation promotes cell mobility via small GTPases (Cdc42 and Rac1) that affect the cytoskeleton and showed that constitutive activation of β3integrin causes proteinuria [33]. These observations strongly implicate suPAR-induced β3integrin activation as a central mechanism causing proteinuria in FSGS. It is puzzling, however, why one third of their FSGS cohort had suPAR levels within the normal range (1–3 ng/mL). Conceivably, these patients have another circulating factor that stimulates local uPAR production by the podocyte or they may have some other, as yet unknown, circulating FSGS factor. Another paradox is that patients with chondrosarcoma produce elevated plasma levels of suPAR but uniformly do not develop proteinuria [34]. Wei et al. postulated that the suPAR released by chondrosarcoma cells may be functionally different due to alternative protein processing [33]. While some perplexing questions remain, activation of podocyte β3integrin by circulating suPAR may be central to the pathogenesis of R-FSGS.

In 2009, Leroy et al. reported the case of a 12-year-old boy with R-FSGS in whom infusion of anti-TNF-alpha antibody induced rapid but transient complete remission. Remission was transient, but with each relapse, proteinuria resolved after infusion of etanercept, a synthetic fusion protein that blocks TNF alpha interaction with its receptor [35, 36]. In a preliminary report from the FONT study group, two of nine children with R-FSGS exhibited complete remission of proteinuria [35]. In an interesting case report, Assadi described a pregnant woman with HELLP syndrome (hemolysis, thrombocytopenia, elevated liver enzymes) and elevated circulating levels of TNF-alpha, whose newborn baby had nephrotic syndrome that resolved postnatally [37]; although the baby received hydrocortisone, the authors argue that transient proteinuria was most likely the result of transplacental TNF-alpha, since levels of the cytokine fell in parallel with resolution of proteinuria. TNF-alpha is expressed by lymphocytes and monocytes; podocytes express TNF-alpha R2 receptors and respond to cytokine stimulation by producing TNF-alpha themselves [38]. These observations suggest that in some cases of R-FSGS, TNF-alpha may constitute another circulating factor driving podocyte injury and raises some interesting questions about its relationship to the suPAR/β3integrin pathway.

4. Pathogenesis of Proteinuria and FSGS Lesions in the Affected Allograft

The effect of R-FSGS serum on the allograft glomerular filtration barrier is rapid and it is not uncommon to identify proteinuria in the immediate postoperative period [17]. Similarly, R-FSGS plasma disturbs the podocyte cytoskeleton within hours *in vitro* [39, 40] (Figure 2). These actin-based fibers are crucial for support of podocyte foot processes and, in biopsies performed shortly after transplantation, foot process effacement is seen by electron micrography. Furthermore, the actin cytoskeleton is linked indirectly via scaffold proteins to the slit diaphragm complex and, *in vitro*, R-FSGS plasma disperses nephrin from the slit diaphragms [20, 39, 41]. Interestingly, however, recurrence of proteinuria is sometimes delayed for weeks [17]. It is unclear whether late onset of recurrent proteinuria in the allograft represents a less aggressive form of the disease or whether primary progression of proteinuria was slower in such patients.

As noted above, there is often considerable delay between the onset of proteinuria and the gradual appearance of FSGS histopathologic lesions in the allograft. Current evidence supports the view that patchy irreversible injury to the glomerulus is preceded by podocyte detachment from the GBM [42–45]. FSGS plasma has been shown to disperse nonmuscle myosin from actin stress fibers *in vitro* [41]. Conceivably, loss of this contractile element in the podocyte cytoskeleton contributes to detachment by compromising cell contractility during pulsatile blood flow through the glomerular capillary. Another possibility is that podocyte

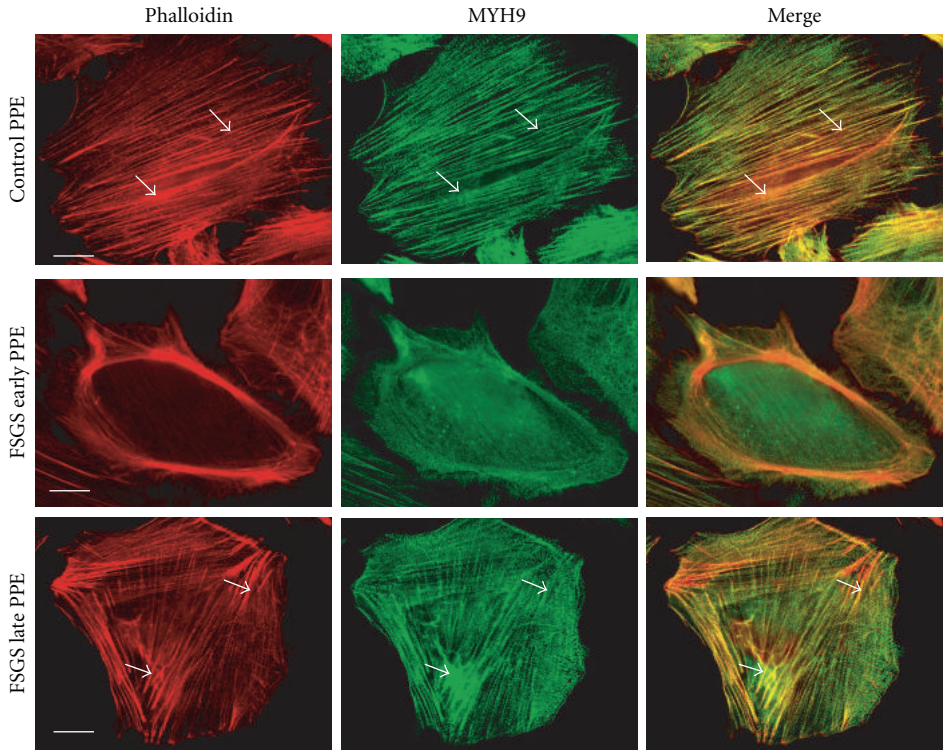

Figure 2: Plasmapheresis effluent from a patient with a recurrent FSGS disrupts the cytoskeleton of human podocytes in culture. Immortalized human podocytes (gift from Dr. Saleem) were incubated for 6 hours with 10% plasmapheresis effluent from a control patient undergoing plasmapheresis for a nonrenal disease (upper panel); PPE from a patient with recurrent FSGS collected at the start of procedure (middle panels, "early PPE"); end of plasmapheresis (lower panels, "late PPE"). The "early PPE," but not the late PPE FSGS sample disrupts polymerized actin (phalliodin staining, red) and nonmuscle mysosin II (staining with anti-MYH9 antibody-green); arrowheads: actin stress fibers; scale bar 5 mc.

detachment is disruption of focal adhesion complex proteins at the podocyte's basolateral surface. Chen reported that podocyte detachment and podocyturia is associated with decreased integrin expression in FSGS patients [16]. Babyeva has demonstrated rapid loss of podocyte focal adhesion complexes following exposure to R-FSGS plasma *in vitro* [2].

Although the severity of podocyte injury and detachment from the glomerular basement membrane may be driven by the same circulating factor that rapidly causes proteinuria in the allograft, it has been difficult to understand why histopathologic lesions appear only after months or years. Some insight into this paradox has recently come from two converging lines of investigation that assign an important role to the host response to podocyte injury in determining the long-term outcome. In 2005, Dijkman performed detailed analysis of a patient with R-FSGS, in whom Bowman's space and the glomerular tuft were "invaded" by parietal epithelial cells [46]. Several groups have provided strong support for the hypothesis that glomerular epithelial cells seen in the "proliferative" form of FSGS are derived from Bowman's capsule rather than arising through transdifferentiation of mature podocytes as had been proposed earlier [47–49]. The second set of important observations has come from Romagnani's group who have shown that mature kidneys retain a subset of renal progenitor cells in a putative stem cell niche at the urinary pole of Bowman's capsule. Their observations provide indirect evidence that these progenitor cells replace damaged podocytes throughout life. Taken together these two sets of observations raise the interesting possibility that FSGS lesions represent a disturbance of the normal process of podocyte replacement—occurring either when the system is overwhelmed by the magnitude of podocyte detachment or dysregulated by the circulating factor itself. In this view, proteinuria may reflect that immediate effect of the FSGS factor on podocytes, but the gradual appearance of glomerular lesions reflects insufficiency of the normal podocyte replacement process.

5. Treatment of R-FSGS

Although R-FSGS may be driven by a disorder of the immune system, the prospect of achieving sustained remission with immunosuppressive agents alone is limited. (a) Most R-FSGS patients were treated unsuccessfully with immunosuppressive agents (usually prednisone and calcineurin inhibitors ± cyclophosphamide for the primary disease in their native kidneys). (b) FSGS recurs in the allograft despite standard transplant immunosuppression. However, numerous groups have reported a rapid effect of plasmapheresis on proteinuria

in some patients with R-FSGS [50]. When plasmapheresis is initiated shortly after disease recurrence, proteinuria improves substantially or resolves in up to three quarters of children; typically this involves three or four 1.5 x plasma volume exchanges per week for several weeks [51].

Unfortunately, many children who show excellent initial response to plasmapheresis recur as the frequency of treatments is weaned. In these children, a variety of sustained immunosuppressive regimens have been tried. Salomon reported rapid and sustained remission of R-FSGS in 30% of children treated with higher doses of calcineurin inhibitors (trough levels of 250–300 ng/mL for 3 weeks), proposing that this might overcome the effect of hyperlipidemia on down-regulation of LDL receptors that mediate cellular uptake of the drug [52]. Remission was even higher (70%) among children who also received intensive plasmapheresis [52]. In a pilot study of adults with R-FSGS, Canaud used a combination of high dose (2 mg/kg by intravenous infusion for 2 weeks, plus oral cyclosporine to achieve 2-hour levels of 1200–1400 ng/mL, thereafter) and protracted plasmapheresis (1.5-plasma volume exchanges against albumin X3/week for three weeks with slowly decreasing frequency over nine months). With this protocol, sustained remission was achieved in nine of ten patients [22]. Dall'Amico et al. reported sustained remission in 7 of 11 patients treated with plasmapheresis and oral cyclophosphamide 2 mg/kg/day for 2-3 months [53]. Interestingly, among 30 reported cases of R-FSGS, about 50% have been reported to undergo sustained complete urinary remission following administration of one or two doses of rituximab 375 mg/m^2 [54–56]. It is unclear whether the infusion of anti-CD20 antibody eliminates production of the circulating FSGS factor by B-cells, whether the depletion of B-cells indirectly alters T-cell function, or whether rituximab acts directly on podocytes [57].

6. Conclusion

About 30–50% of children with steroid-resistant nephrotic syndrome develop rapid recurrent proteinuria and thereafter develop slowly progressive FSGS in their allografts. Recurrent FSGS defines a distinct clinical entity involving a putative circulating factor that rapidly disrupts the podocyte slit diaphragm and then leads to irreversible podocyte injury. Until proven otherwise, it should be presumed that the pathogenesis of R-FSGS is distinct from steroid-sensitive idiopathic nephrotic syndrome and that heterozygous mutations of slit diaphragm genes have little impact on clinical features of the disease.

Acknowledgments

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Review Article

The History of Cystinosis: Lessons for Clinical Management

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Cystinosis is a rare disorder, and, accordingly, progress on the understanding and treatment of this disease has been relatively slow. Although cystinosis was identified over 100 years ago, the history of cystinosis is marked by a few sudden leaps forward in our understanding rather than by a sustained research effort fuelled by the larger research community. Major conceptual breakthroughs include (a) its discovery in 1903, (b) recognition of the renal Fanconi syndrome, (c) realization that tissue accumulation of cystine reflects a defective channel in the lysosomal membrane, (d) translation of this discovery to trials of cysteamine, (e) discovery of the *CTNS* gene, and (f) report of successful stem cell therapy in the cystinotic mouse. This paper focuses on the importance management lessons from these milestones and the potential new therapeutic strategies which may be looming in the near future.

1. Cystine Accumulation in Cystinosis

Cystinosis as an autosomal recessive disorder caused by mutations of the *CTNS* gene on chromosome 17, which encodes a ubiquitous cystine-specific transporter (cystinosin) in the lysosomal membrane (Figure 1). Since cystinosin facilitates efflux from the lysosome, homozygous *CTNS* mutations result in massive intralysosomal accumulation of cystine in crystals which apparently disrupt the organelle, leading to apoptotic cell death and progressive organ dysfunction. Over 90 mutations of the *CTNS* coding and splice regions have been reported. About half of the cystinosis alleles in the Western populations are caused by a 57.2 kb deletion which extends from the tenth *CTNS* exon through the adjacent SHPK and first two exons of the *TRPV1* (capsaicin receptor) gene ( ). This deletion is thought to have arisen in Northern Germany in about 500 AD.

An unsavoury character from Zurich, Emil Aberhalden's scientific career was described by Otto Westphal as "a fraud from beginning to end." Nevertheless, Aberhalden seems to have been the first to identify cystinosis. In 1903, he described a child with severe growth failure in infancy who had cystine crystals in the liver and spleen at autopsy [1]. Tissue accumulation of cystine remains the diagnostic hallmark of cystinosis. Elevation of cystine can be quantified directly from chorionic villus samples at week 9-10 of gestation or can be measured in fetal cells from amniotic fluid prior to 20 weeks of gestation (Jackson Prenatal Dx 2005). At birth, placental cystine (2–5 umoles/g protein) is well above normal (0.1–0.2 umoles/g protein). Although corneal crystals may be seen with a slit lamp by about 1 year of age, these deposits represent coalescence of extracellular cystine left by apoptotic cells and may not be present at birth. As infants develop growth failure at 4–6 months of age, diagnostic elevation of cystine levels can be measured in circulating leukocytes. However, the absolute level of leukocyte cystine varies appreciably from laboratory to laboratory, and lymphocyte cystine (5X normal) is lower than that in polymorphonuclear leukocytes. Since results are expressed as micromoles of cystine per mg cellular protein in the sample, variability in the reported leukocyte cystine level depends, in large part, on how the white cells are isolated at the point of care, rather than from variation induced by the method of cystine measurement (cystine binding assay, automated amino acid analyzer, or HPLC). To minimize variation, the University of California at San Diego has published a protocol for leukocyte isolation (http://biochemgen.ucsd.edu/cystinosis/). This protocol emphasizes prompt leukocyte separation by mixing blood with an equal volume of acid-citrate-dextran solution, allowing the red blood cells to settle by gravity for one hour. Red cells remaining in the upper layer are then

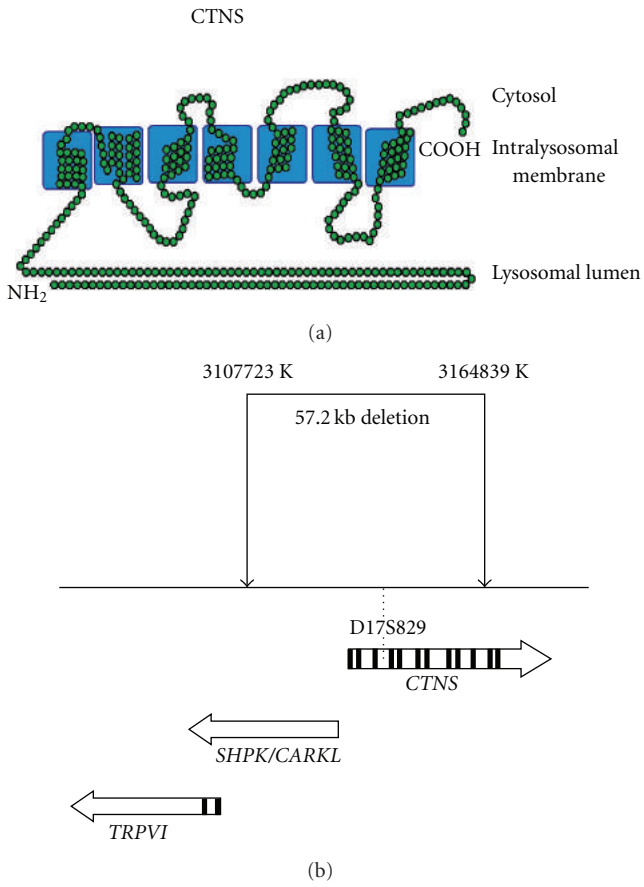

Figure 1: (a) Depiction of *CTNS* protein spanning the lysosomal membrane, (b) the common 57.2 kb deletion extending upstream from *CTNS* exon 10 through the first two exons of *TRPV1*.

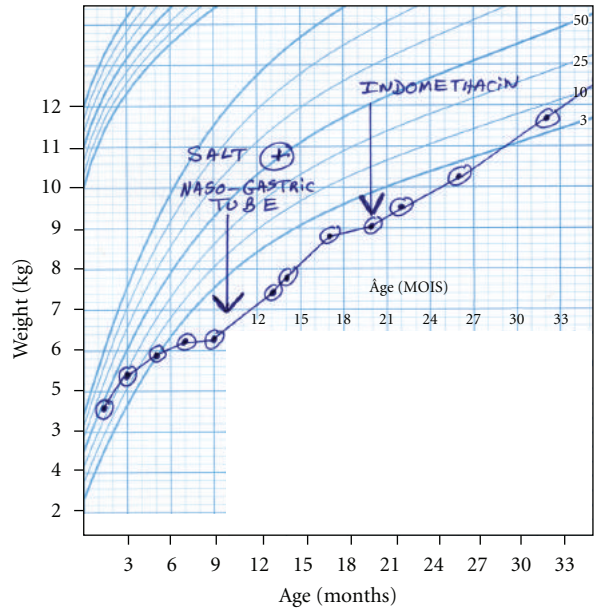

Figure 2: Growth curve for a cystinotic child. (1) Growth velocity fall off at 4–6 months of age improves with salt supplements and increased fluid volume delivered by a nasogastric tube. (2) Slowing of growth velocity at 17–19 months of age is improved by introduction of indomethacin to reduce electrolyte losses by 25–30%.

lysed; the sample is acidified and frozen for shipping to a central laboratory for protein and cystine determinations. Using this method, leukocyte cystine is nearly undetectable in normal subjects (0–0.12 umoles (half-cystine/gram cell protein) whereas heterozygotes have about 4 times this value, and cystinotic levels are about fifty times normal, usually ranging from 1–10 umoles half-cystine/g protein.

2. Cystinosis and the Renal Fanconi's Syndrome

In 1924, Lignac expanded on the observations of Aberhalden to point out that children with cystinosis often present with profound rickets [2]. In 1931, Fanconi was the first to perceive that cystinosis is associated with urinary wasting of substances that are normally reabsorbed during their passage through the renal tubules [3]. The full picture of this tubulopathy was expanded by DeToni, who explained the rickets by documenting urinary phosphate wasting [4] and Debre et al. who noted excretion of organic acids [5]. Fanconi's further contribution to the subject came in 1936, when he recognized the similarities between these cases, referred to the disease as nephrotic-glycosuric dwarfism with hypophosphatemic rickets and suggested that the organic acids found in the urine might be amino acids [6]. In 1947, Dent characterized the urinary amino acid and protein profile of cystinotic children and proved that this was due to defective absorption [7].

Unlike most other tissues which deal with the ubiquitous turnover of cellular proteins, the renal proximal tubule must also contend with the enormous daily load of low-molecular-weight proteins contained in glomerular filtrate. These proteins undergo endocytotic uptake as they pass through the proximal tubular lumen and are delivered to lysosomes where they are degraded into their constituent amino acids. Interestingly, newborns may appear normal at birth and exhibit only mild amino aciduria. However, between 4 and 6 months of age proximal tubular dysfunction emerges, and infants fall off their growth curves (Figure 2). By a year of age, apoptotic cell death causes progressive atrophy of the proximal tubule, leading to the "swan neck" deformity described by Clay et al. [8].

With the recognition of the renal Fanconi's syndrome, clinicians were able to extend the lives of cystinotic children by replacing the crucial constituents lost in urine. These therapeutic strategies have been developed over many years and have been reviewed by others [9], but several points are worthy of note as follows.

(1) Hypophosphatemic rickets can be largely reversed by divided daily oral supplements of sodium phosphate. However, since phosphate salts are cathartic, the dose should be gradually increased to 50–100 mg/kg/day phosphate over several weeks so that peak serum phosphate levels (at 1 hour) are eventually brought into the low-normal range. Furthermore, since oral

phosphate acts as a calcium binder and stimulates parathyroid hormone release, the final oral phosphate dose should be matched by enough calcitriol (10–50 ug/kg/day) to ensure adequate uptake of dietary calcium to maintain normal levels of intact parathyroid hormone.

(2) Repair of chronic volume contraction is important for growth. In small infants, this nearly always requires placement of a nasogastric tube and, eventually, a gastrostomy tube. Estimates of urine volume and natriuresis in timed urine collections can then be used to guide fluid intake and salt supplementation and normalize plasma renin level.

(3) In small infants, indomethacin (1.5–3 mg/kg/day) may reduce urine volume and electrolyte losses by about one-third. However, it should be accompanied by omeprazole or another modern proton pump inhibitor. Emma has suggested that it may be prudent to discontinue indomethacin as the child approaches school age and has reported that introduction of an ACE inhibitor at this time may prolong renal function [10].

(4) Proximal renal tubular acidosis and urinary potassium losses may require large oral supplements of bicarbonate and potassium that may cause vomiting. Correction is best achieved by a mix of sodium bicarbonate, potassium citrate, and potassium chloride.

Although the early years are dominated by the impact of the renal Fanconi's syndrome, untreated children develop a number of nonrenal manifestations. Hair color is usually blond or much lighter than that in the parents. However, it is unclear whether this represents a genetically linked trait or a disturbance of pigmentation, since many examples of black hair have been reported. Corneal crystal deposition becomes increasingly evident during the second year of life. While some children need little therapy, photophobia is common, and most patients begin to use sunglasses in early childhood. Since tear production may be diminished, lubricant eye drops are helpful. If blepharospasm begins to interfere with daily life, children should be treated with topical cysteamine eye drops. Although corneal crystals do not seem to disturb visual acuity, severe untreated corneal involvement may lead to band keratophy which can obscure vision. Progressive dysfunction of the thyroid gland may become evident within the first decade, and elevation of serum TSH levels should prompt oral thyroxine replacement.

3. Cystinosis and Progressive Renal Failure

In 1952, Bickel and Smellie pointed out that cystinotic children develop inexorable loss of glomerular filtration despite treatment of the renal Fanconi's syndrome [11]. In untreated cystinotics, multinucleated podocytes are seen in glomeruli [12, 13], and tubular proteinuria is overshadowed by progressive focal segmental glomerulosclerosis associated with nephrotic-range proteinuria and progressive renal insufficiency [14]. By 1974, this prognosis had not changed much, leading Royer to remark in his textbook of Pediatric Nephrology that children with cystinosis usually survive until the age of about 10–12 years, when they succumb to end-stage renal failure [15].

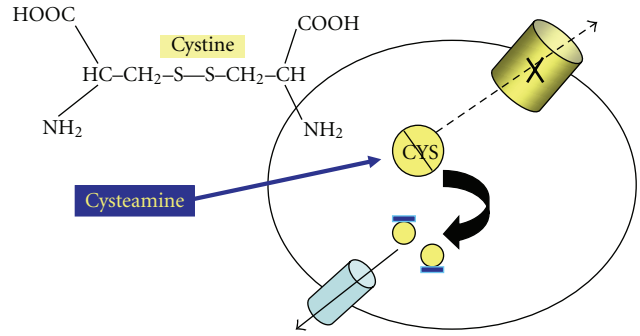

Figure 3: Cystine-depleting effect of cysteamine. Cystine forms mixed sulfides with intralysosomal cystine that are able to efflux into the cytoplasm via alternative lysosomal channels.

It was about this time, however, that advances in pediatric renal transplantation allowed the first cystinotic children to leap past this roadblock. In 1970, Mahoney et al. reported excellent renal allograft survival in four cystinotic children and pointed out that, as expected, there was no evidence of recurrent cystinosis in the allograft [16]. As cystinotics develop end-stage renal disease, they may have heavy proteinuria and high urine volumes. However, the incidence of graft thrombosis is not different than that in the general population, and children are accustomed to drinking large volumes prior to transplantation [14]. Thus, it is not entirely evident that they need pretransplant nephrectomise, and this decision should be approached carefully.

4. Cystinosis and Cysteamine

In the late 1960s, investigators at the US National Institutes of Health discovered that the accumulation of cystine in patient tissues was due to a defect in efflux of free cystine from lysosomes isolated from cystinotic fibroblasts or leukocytes [17–22]. Patrick and Lake demonstrated ascertained by electron microscopy that the intracellular crystals in cystinotic tissues were surrounded by lysosomal membranes [23]. Reasoning that progressive tissue injury might be ameliorated if cystine could be mobilized from the lysosomes; Schneider reported in 1976 that the sulfhydryl reagent, cysteamine, could react with intralysosomal cystine, permitting efflux of resulting mixed disulfides via alternative channels in the lysosomal membrane (Figure 3) [24]. Gahl went on to show that protracted oral therapy with cysteamine-depleted organ cystine [25] and organized a North American trial of cysteamine that demonstrated slowing of progressive renal failure, particularly in those children who began therapy before the age of two years and were adherent to the prescribed dose (1.3 gm/m^2, divided qid) [26]. In a retrospective review of 100 adults with cystinosis, Gahl reported that late complications of cystinosis (diabetes mellitus,

muscle wasting, hypothyroidism, pulmonary dysfunction, and death) decreased with time on cysteamine therapy [27].

Despite the apparent benefits of long-term cysteamine therapy, there is no question that strict adherence to the medication is difficult. For that reason, leukocyte cystine should be monitored on a regular basis. In early childhood, when most patients receive cysteamine via nasogastric tubes or under parental supervision, leukocyte cystine falls abruptly to about 15% of baseline values which is close to the heterozygous range and usually below 0.5 umoles half-cystine/g protein (Figure 4). However, following a variable "honeymoon period," mean leukocyte cystine tends to drift gradually upwards, and the number of frankly non-compliant patients increases significantly in adolescence (Goodyer, unpublished). In 2010, Dohil et al. reported their experience comparing enteric-coated cysteamine (25 mg/kg) with the standard cysteamine bitartrate (47 mg/kg) [28]. They demonstrated equivalent mean serum cysteamine levels and noted no significant clinical deterioration while taking the enteric-coated preparation twice a day for 1-2 years [28]. A phase II clinical trial of enteric-coated cysteamine (Raptor Pharmaceutical) is now nearing completion, and it is hoped that adherence will be improved by the twice-a-day dosing schedule.

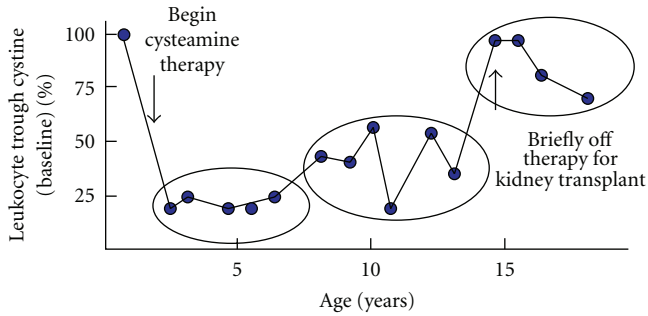

Figure 4: Leukocyte monitoring of leukocyte cystine levels in 50 Canadian children with nephropathic cystinosis. Introduction of oral cysteamine therapy (1.3 g/m^2/day in four divided doses) in early childhood achieves a rapid reduction of trough 6–8 hours after a single dose. Following a variable "honeymoon period," mean leukocyte cystine gradually drifts upwards as compliance decreases. Cystine levels return to baseline when stopped briefly for transplant or when teenagers discontinue therapy.

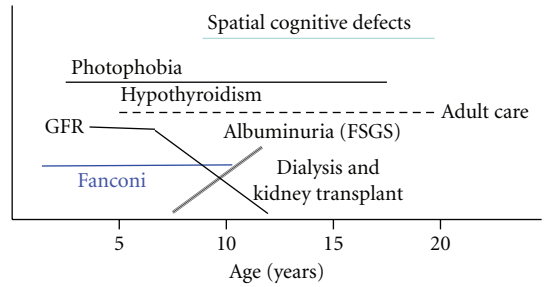

Figure 5: Natural history of untreated cystinosis.

5. Adult Complications of Cystinosis

With the advent of renal transplantation and cysteamine therapy, the natural history of cystinosis has been shifted (Figure 5). Nesterova and Gahl have reviewed the late complications of cystinosis, including myopathy, encephalopathy, diabetes mellitus, male infertility, retinal degeneration, hypothyroidism and coronary artery calcifications [29]. In general, the severity of these complications correlates with the number of years without cysteamine treatment. However, cysteamine therapy does not prevent the need for eventual renal transplantation and cannot eliminate the many debilitating complications that arise in adulthood. In 1988, Sonies et al. reported the case of a 20-year-old male with distal muscle atrophy and progressive loss of the ability to sit without support, but sensation and nerve conduction were intact. In a review of 101 posttransplant patients, about half had evidence of significant myopathy, particularly debilitating was the loss of pharyngeal musculature causing dysphagia and oromotor dysfunction interfering with speech [30]. Myopathy contributes to light exercise intolerance and pulmonary dysfunction as well.

Trauner et al. has recently described the subtle spatial perception deficit in children with cystinosis [31]. Young cystinotic children might have subtle learning disabilities that could be overcome with learning interventions that diminish reliance on spatial perception skills. After the age of twenty, untreated cystinotic adults may develop cortical dysfunction with progressive confusion, tremor, and seizures [32]. Broyer et al. reported that only 5% of patients have evidence of encephalopathy at age 23, but this increases rapidly to 45% by age 27 [33]. Interestingly, Broyer et al. found that late introduction of cysteamine seemed to improve some manifestations of the encephalopathy [33]. Mueller described a cystinotic woman who developed some memory loss with mild cortical atrophy at age of 29 evident by magnetic resonance imaging. With introduction of cysteamine, cerebral function and cortical atrophy were stable, but distal myopathy continued to progress. If this case is representative, it may be difficult to avert muscle wasting in adult cystinosis. Although growth hormone has been used to accelerate somatic growth in cystinotic children, its effects on muscle wasting in the adult have not been studied.

Experience with cystinosis in adulthood offers several management lessons. Firstly, that cysteamine therapy should be started as early as possible and continued throughout life; renal transplantation does not diminish the need for continued therapy, and even late introduction of cysteamine may provide clinical benefits. Secondly, adherence to cysteamine therapy is especially difficult during teenage years and strategies to improve compliance and facilitate the transition to adult care systems are crucial. Thirdly, the many forms of organ dysfunction that arise during adulthood warrant specialized cystinosis-specific outpatient care units, since management usually requires expertise well beyond the skills of the nephrologist who may preside over care at the time renal transplantation is needed.

6. Cystinosis and Stem Cell Therapy

In 2009, Syres et al. reported successful hematopoietic stem cell therapy of cystinosis in mice [34]. Cells from a wild-type mouse infused into *CTNS* (−/−) recipients were able to home to damaged tissues, reducing tissue cystine levels by 90% and normalizing organ function. This remarkable observation raises this immediate question of whether a similar strategy might be feasible in humans. One scenario would involve standard bone marrow transplantation, in which bone marrow stem cells from a healthy donor are infused into the cystinotic patient who has undergone marrow-ablative chemotherapy. Alternatively, the patient's own stem cells might be corrected by transfection with the wild-type *CTNS* gene *in vitro* and reinfused. Although there remain a number of practical problems to solve before human trials are considered, work in the mutant mouse model has opened a new hope for the treatment of cystinosis.

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Review Article

Primary Molecular Disorders and Secondary Biological Adaptations in Bartter Syndrome

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Bartter syndrome is a hereditary disorder that has been characterized by the association of hypokalemia, alkalosis, and the hypertrophy of the juxtaglomerular complex with secondary hyperaldosteronism and normal blood pressure. By contrast, the genetic causes of Bartter syndrome primarily affect molecular structures directly involved in the sodium reabsorption at the level of the Henle loop. The ensuing urinary sodium wasting and chronic sodium depletion are responsible for the contraction of the extracellular volume, the activation of the renin-aldosterone axis, the secretion of prostaglandins, and the biological adaptations of downstream tubular segments, meaning the distal convoluted tubule and the collecting duct. These secondary biological adaptations lead to hypokalemia and alkalosis, illustrating a close integration of the solutes regulation in the tubular structures.

1. Introduction

Bartter syndrome is a hereditary disorder that has been characterized by the association of hypokalemia, alkalosis and the hypertrophy of the juxtaglomerular complex with secondary hyperaldosteronism and normal blood pressure [1]. By contrast, the genetic causes of Bartter syndrome primarily affect molecular structures directly involved in the sodium reabsorption at the level of the Henle loop (see the functional segmentation of sodium reabsorption in Figure 1 and [2, 3]. The ensuing urinary sodium wasting and chronic sodium depletion are responsible for the contraction of the extracellular volume, the activation of the renin-aldosterone axis, the secretion of prostaglandins, and the biological adaptations of downstream tubular segments, meaning the distal convoluted tubule and the collecting duct. These secondary biological adaptations lead to hypokalemia and alkalosis, illustrating a close integration of the solutes regulation in the tubular structures.

2. Primary Molecular Defects and Direct Consequences

Genome alterations leading to the Bartter syndrome affect the genes encoding different molecules involved in the sodium reabsorption at the level of the large ascending limb of the Henle loop (Figure 2). Sodium-Potassium-Chloride Cotransporter (NKCC2, gene *SLC12A1*) is responsible for Bartter type I, the inwardly rectified potassium channel (ROMK or rectifying outer medullary potassium channel, gene *KCNJ1*), for Bartter type II, the chloride channel Kb (ClCKb, gene *CLCNKB;*) for Bartter type III, the barttin (gene *BSND)* for Bartter type IV [3]. A complete deletion of *CLCNKB* gene with additional alterations of the *ClCK-A* gene leads to a severe form of Bartter syndrome similar to Bartter syndrome type IV [4, 5]. A biological phenotype of Bartter syndrome has also been reported in different damages of molecular structures that directly or indirectly affect sodium reabsorption in the Henle loop: the calcium-sensing

receptor (*CaSR*, activating mutation L125P) that mainly leads to hypocalcemia and hypercalciuria, sometimes referred as type V Bartter syndrome [6], the Chloride channel-5 (ClC5) [7], and the cystinosin [8]. Gitelman syndrome, due to the alteration of the *SLC12A3* gene that encodes the Sodium chloride cotransporter in the distal convolution, is also marked by sodium wasting, hyperreninism, hyperaldosteronisme,and hypochloremic alkalosis but differs from Bartter syndrome by hypomagnesemia and hypocalciuria [3]. The basolateral potassium channel (*KCNJ10)* is the cause of the EAST syndrome (Epilepsy, Ataxia, Sensorineural deafness, and Tubulopathy) that also displays a phenotype closer from Gitelman syndrome than from Bartter syndrome [9]. The chronic use of furosemide that blocks the NKCC2 leads to an acquired biological phenotype of Bartter syndrome [10]. Nephrotoxic agents (aminoglycosides, amphotericin B, and heavy metal intoxication) have also been reported to be sometime associated with a phenotype of Bartter syndrome [11–13]. Extrarenal loss of sodium chloride by the gastrointestinal tract (congenital chloride diarrhea) or by the skin (cystic fibrosis) may also display a biological phenotype of Bartter syndrome but these patients that have a normal renal tubular function differed from true Bartter syndrome by a very low excretion fraction of sodium [14, 15].

2.1. Urinary Sodium Wasting. Assuming a glomerular filtration rate of 100 mL/min and a serum sodium concentration of 140 mmol/L, about 20 mol/day of sodium are filtered everyday, equivalent to the amount of 1.2 kg of salt. Under physiological conditions, renal tubules are capable of reabsorbing 99 to 100% of filtered sodium and water. The primary consequence of a defect in the Henle loop is a failure to reabsorb 30% of the filtered sodium. The massive amount of sodium that is delivered downstream to the end of the Henle loop exceeds the possibility of compensation by the distal convoluted tubule and the collecting duct and leads to urinary sodium wasting [3].

2.2. Urinary Concentration and Dilution Defect. A second consequence of the defect of Henle loop is the abolition of the corticopapillary osmolar gradient that prevents the ability to concentrate the final urine over the plasma osmolality. In addition, patients display a partial impairment in diluting urine far below the plasma osmolality while half of the capacity of diluting urine is due to the anhydrous reabsorption of sodium chloride in the Henle loop, and the second half is due to the anhydrous reabsorption of sodium chloride in the distal convoluted tubule [16].

2.3. Hypercalciuria. Urinary calcium wasting is due to the abolition of the transtubular potential, the subsequent inability to passively drive divalent cations through the intercellular space in the Henle loop (see Figure 2) and the patent failure of downstream adaptation. The chronic loss of calcium is frequently responsible for an increased bone resorption and a decrease of bone mineralization that is proportional to the level of urine calcium [17]. Calcium supplementation may prevent these bone alterations.

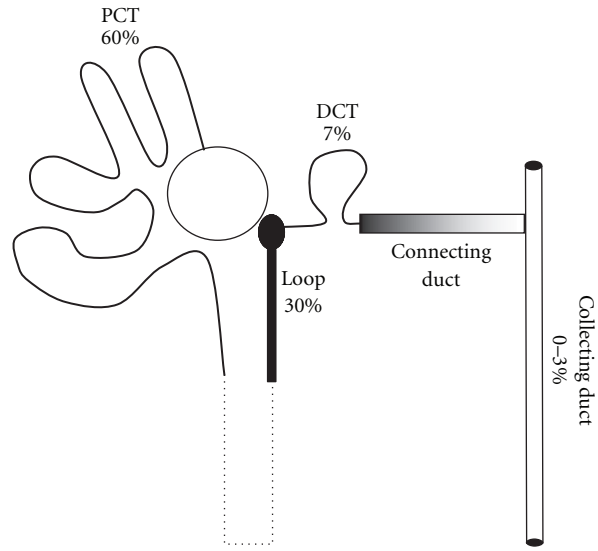

Figure 1: *Segmentation of sodium reabsorption*. Sodium reabsorption in tubular epithelial cells proceeds along a general two-step mechanism that includes a/an active extrusion of intracellular sodium ions by the basolateral sodium pump that is common to all tubular segments, b/ a passive apical entry of sodium dissipating the electrochemical gradient generated by the sodium pump via an exchanger or a cotransporter or a sodium channel that is specific to each tubular segment. Briefly, 175 L/1.73 m^2 of plasma roughly containing 20 moles of sodium chloride are filtered every day by the glomeruli, and 99 to 100% of this amount is reabsorbed in the convoluted and straight tubules (60%), the thick ascending limb of the Henle loop (30%), the distal convoluted tubule (7%), the connecting and the collecting duct (0 to 3%, controlled by aldosterone and angiotensin-2). The thin descending and ascending limbs of the Henle loop do not display any capacities to reabsorb sodium. Proximal tubular failure leads to the Fanconi syndrome where sodium wasting is associated with numerous solute wasting (potassium, bicarbonates, calcium, phosphates, glucose, aminoacids). Failure of the large ascending limb of the Henle loop is responsible for the Bartter syndrome, of the distal convoluted tubule for the Gitelman syndrome, and of the collecting duct for type 1 Pseudohypoaldosteronism [3].

3. Secondary Biological Adaptations

3.1. Hyperreninism. The extracellular volume is a sodium salt solution that is closely maintained to a steady-state osmolality of 300 mosmol/Kg. Chronic sodium depletion subsequently leads to a contraction of the extracellular volume. Hypotension, failure to thrive, and at the biological level, either an increased concentration of the total plasma protides or a high hematocrit are the common signs of extracellular volume contraction [19, 20]. Chronic hypovolemia results in a stimulation of the renin axis and enhances the production of angiotensin-2 that stimulates the sodium reabsorption in the principal cell of the collecting duct (Figure 3) [21].

3.2. Hyperaldosteronism. Angiotensin-2 directly increases the secretion of aldosterone by the *granulosa* of the adrenal glands [22]. Aldosterone adds to the stimulation of the

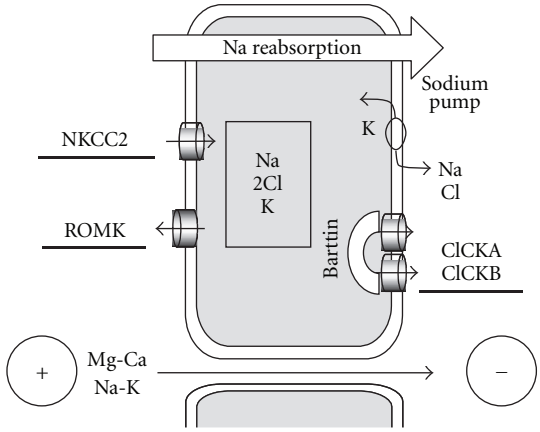

Figure 2: *Structures involved in sodium reabsorption in the Henle loop.* The large part of the Henle loop is mainly dedicated to sodium reabsorption. The system is vectorized and energized by the sodium pump at the basolateral face of the cell. KCNJ10 (not drawn on the figure) is supposed to recycle potassium at the basolateral face of the cell allowing fueling of the sodium pump in extracellular potassium. At the apical face, the sodium is cotransported with 1 potassium and 2 chlorides by the NKCC2 cotransporter. The four sites of the NKCC2 have to be occupied to generate an electroneutral transport. As the urinary fluid is 30-fold more concentrated in sodium than in potassium, potassium ions are recycled in the urine fluid at the luminal face of the cell through the ROMK (rectified outer medullary potassium) channel, in order to provide enough potassium for a continuous activity of the NKCC2. The 2 chlorides are reabsorbed at the basolateral face of the cell by the chloride channels CLCKA and B. Those channels need to be addressed and clustered at the basolateral membrane by the barttin. The main regulator of sodium reabsorption in the Henle loop is the vasopressin (also referred as antidiuretic hormone). The asymmetry of charges in the lumen (excess of potassium) and in the interstitium (excess of chloride) generates a potential according to the 3^{rd} principle of thermodynamics (Nernst formula). The paracellular transport of cations (calcium and magnesium as well as sodium and potassium in some extent) through the intercellular space is allowed by special claudins (claudines16 and 19, also referred as paracellins) and dissipates the transepithelial potential of the Henle loop [2, 3].

sodium reabsorption in the collecting duct and also likely stimulates those in the distal convoluted tubule [23]. Hyperaldosteronism may be dampened, relatively to a high renin stimulation, by hypokalemia and potassium depletion that directly interfere with aldosterone secretion in the *granulosa* [22].

3.3. Hypokalemia. As in any case of hyperaldosteronism, the exacerbation of the sodium reabsorption is closely associated to an exacerbation of the potassium secretion due to the stimulation of the crossed transport activity of sodium and potassium by the sodium pump. Consistently, hyperaldosteronism and urinary potassium wasting improve when sodium supplementation is increased [24]. Conversely, hypokalaemia and potassium depletion may improve with angioconvertase inhibitors, likely through a decrease of production of angiotensin-2 and the deactivation of the collecting duct [25]. Accordingly, patients treated with enalapril had a fall in blood pressure and a decrease in glomerular filtration rate [25].

3.4. Alkalosis. The mechanism of alkalosis might be explained by an effect of aldosterone on the expression of the proton ATPase located in the α-intercalated cell but, α-intercalated cell does not express the mineralocorticoid receptor [26, 27]. A second possibility is that the potassium depletion might be responsible for a *de novo* expression of the gastric proton-potassium ATPase (sensitive to omeprazole) at the luminal face of the principal cell in order to reabsorb a part of the secreted potassium. For each potassium ion that is internalized, 1 hydrogen is extruded in the urine by the proton potassium ATPase, generating alkalosis [18, 28]. This adaptation that has been fully demonstrated in rat models of potassium depletion might also apply to humans.

3.5. Complications of Potassium Depletion. Hypokalemic periodic paralysis and rhabdomyolysis has been reported for hypokalaemia below 2 mmol/L and fully resolved following the reload of the system with potassium chloride [29, 30]. Prolonged QT interval has been evidenced with hypokalemia below 2 mmol/L, and subsequent aborted sudden cardiac death may occur [31, 32]. Potassium depletion also induces coronary microvascular and myocardial defects during exercise in patients with Bartter syndrome [33].

3.6. Polyuria. The mechanism of polyuria in Bartter syndrome has to be considered differently in the antenatal period and after birth. In the antenatal period, the final urine delivered by a normal fetus is hypoosmolar suggesting that the water reabsorption in the collecting duct is not yet matured before birth while the anhydrous reabsorption of sodium chloride is fully functional in the Henle loop [34, 35]. In the amniotic fluid, the fetal urine is balanced with the fetal plasma that circulates in the fetal membranes (amnion and chorion) [34]. In physiological conditions, the difference of the osmotic pressure between the amniotic fluid and the fetal plasma strongly favors a water outflow from the amniotic fluid into the capillaries of the fetal membranes [36]. Indeed, 5 types of aquaporins (AQP-1, -3, -4, -8, -9) are strongly expressed in the amnion and the chorion [37]. In fetus with Bartter syndrome, the amniotic fluid is fed with the fetal urine that is released at the end of the proximal convoluted tubule and not modified in the Henle loop. In these conditions, the fetal urinary osmolality is equal to those of the fetal plasma, likely preventing any water transfer from the amniotic fluid into the fetal plasma and leading to a polyhydramnios. The concentration of solutes remains balanced in the amniotic fluid while the total protein concentration (the solid phase) is diluted [38]. After birth, the polyuria is supposed to be generated by the addition of the inability of the kidneys to concentrate the final urine over the plasma osmolality and the increase of excreted osmoles due to the urinary sodium and potassium wasting.

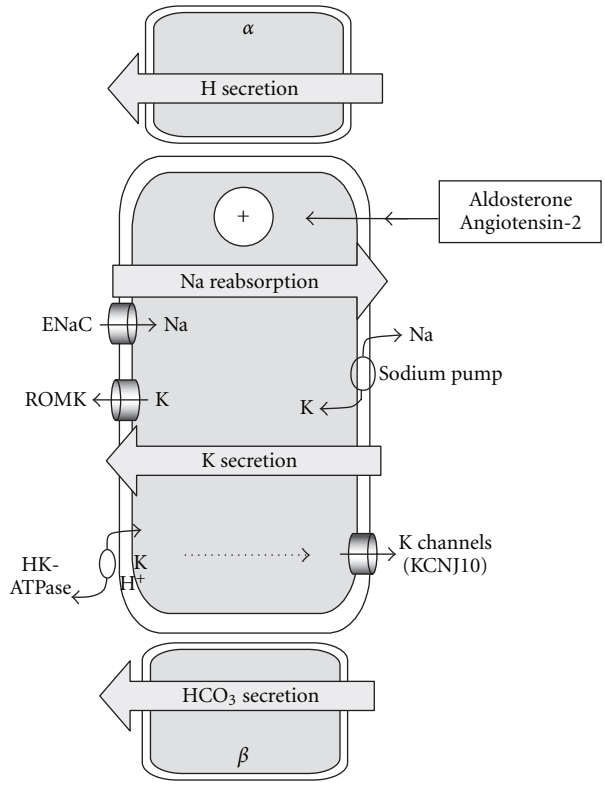

Figure 3: *Activation of the principal cell of the collecting duct by aldosterone and angiotensin-2*. The collecting duct is a composite structure made of 3 types of cells: the principal cell that assumes sodium and water reabsorption and potassium secretion, the α-intercalated cell dedicated to hydrogen secretion, and the β-intercalated cell dedicated to bicarbonate secretion. In the principal cell, the reabsorption of sodium is vectorized through the epithelial sodium channel at the apical side of the principal cell and the sodium pump at the basolateral side. Potassium ions are cross-transported against sodium ions by the sodium pump and secreted in the lumen by the ROMK channel that is the same ROMK channel expressed in the Henle loop. Chronic sodium depletion and the subsequent extracellular volume contraction lead to an activation of the renin-angiotensin2-aldosterone axis that leads to the enhancement of sodium reabsorption and an exacerbated cross-secretion of potassium by the principal cell. The subsequent potassium depletion leads to hypokalemia and likely induces the expression of the gastric proton-potassium pump (inhibited by omeprazole) at the apical face of the principal cell leading to an excessive secretion of hydrogen and a subsequent alkalosis [18].

3.7. Hyperprostaglandinuria. Hyperprostaglandinuria is not specific of Bartter syndrome and has been either reported in sodium depletion without Bartter syndrome [39, 40] or in water deprivation [41–43]. Tissular secretion of PGE_2 is stimulated by the vasopressin [44] either secondary to hypovolemia due to a sodium depletion or hypernatremia. In the kidney, cyclooxygenase-1 (COX1) is highly expressed in the collecting duct, and cyclooxygenase-2 (COX2) is expressed predominantly in the renal medullary interstitial cells, in the cortical thick ascending limb and in the cells associated with the macula densa. In addition, prostaglandin E synthase (PGES1) is highly expressed in the collecting duct and also detected in the thick ascending limb and the macula densa [45].

4. Specificities according to the Molecular Defect.

4.1. SLC12A1 (NKCC2). Mutations of *SLC12A1* gene have been associated with the most typical form of antenatal Bartter syndrome featuring early onset of polyhydramnios, neonatal polyuria, and dehydration requiring massive amount of water and sodium supplementation in the first days of life, detectable nephrocalcinosis on renal ultrasound within the first month, and high plasma level of renin and secondary hypokalemic alkalosis following the first days of life [46, 47]. A delayed onset in life has also been exceptionally reported in cases with a residual function of the NKCC2 cotransporter [48].

4.2. ROMK (KCNJ1). Mutations in the *KCNJ1* gene display the same clinical phenotype as described in the NKCC2 section, but transient hyperkalemia and acidosis are observed during the first month of life [46, 47, 49]. Kalemia secondarily decreases along the course of the disease, but hypokalemia frequently failed to develop in these patients [47, 49]. A delayed onset in life has also been exceptionally reported in cases with a residual function of the ROMK channel [50].

4.3. ClCK-b (CLCNKB). Mutations in the *CLCNKB* gene give the most variable clinical phenotypes from early polyhydramnios and severe neonatal polyuria [46, 47] to classic

Bartter syndrome with a delayed diagnosis in childhood and failure to thrive [51] and to the phenotype of Gitelman syndrome without polyuria [52]. Hypercalciuria may lack in some patients with *CLCNKB* mutations [46].

4.4. Barttin (BSND). Barttin mutations are marked by deafness [53]. Most of the patients have a severe tubular disease, but a few adults have been recognized on deafness with a mild renal phenotype [54]. Hypercalciuria may also lack in a set of patients with *BNSD* mutations [46].

5. Treatment

Undelayed management of neonates with fully symptomatic antenatal Bartter syndrome is a necessity. Prenatal diagnosis relies on the occurrence of polyhydramnios without any classical cause of polyhydramnios (mainly maternal diabetes and fetal gastrointestinal tract malformation). The analysis of sodium, potassium, and chloride concentration relatively to those of total protein and alphafetoprotein in the amniotic fluid (the Bartter Index) is a reliable tool to confirm the diagnosis when familial history is lacking [38].

5.1. Sodium Chloride and Water. According to the physiopathology of the disease, the primary treatment of Bartter syndrome, at least in the neonatal period and in childhood, is the supplementation in sodium chloride. Data are scarce but some reports show that the amounts of sodium chloride needed to balance the extracellular volume, and prevent dehydration and hypovolemia to limit the stimulation of the renin axis, may reach 50 mmol/Kg/day in neonates [46, 55, 56]. Polyuria may occur immediately after birth prior to any sodium supplementation and requires water inputs that have sometimes to be rapidly increased up to 500 mL/Kg/day within the first days of life [55, 56]. Secondarily, continuous supplementation with water and sodium chloride either through a nasogastric tube or a gastrostomy may be necessary to improve growth in weight and length during the first months or years of life [57, 58]. A free access to sodium chloride and water when children gain food autonomy is as much as important that a free access to water for the children that are affected with a nephrogenic diabetes insipidus. Intravenous infusions of sodium salt solutions are necessary to prevent life-threatening dehydration in case of vomiting or diarrhea.

5.2. Potassium Chloride. Sodium chloride supplementation should be the best way to preserve children from hypokalemia and potassium depletion through the control of the extracellular volume and the renin-aldosterone axis. [59] Nevertheless, in some patients, the amounts of sodium chloride that would balance the extracellular volume and the renin system are so elevated that they are not acceptable by the patient. A potassium-rich diet and potassium chloride supplementation are subsequently needed.

5.3. Indomethacin. Indometacin has been used either prior to birth to prevent the recurrence of a polyhydramnios or after birth to limit the polyuria [60, 61]. In the fetus with a Bartter syndrome, the amount of urine that is delivered at the end of the proximal convoluted tubule is directly proportional to the glomerular filtration rate. Therefore, the effect of indomethacin in preventing the recurrence of polyhydramnios following a drain is likely due to its action on glomerular vasculature, the reduction of the fetal glomerular filtration rate, and the subsequent reduction of fetal urine output [62]. After birth, prostaglandins are paradoxically potent inhibitors of the sodium reabsorption in the collecting duct through their specific receptor EP_1 and EP_2 [45, 63]. In addition, inhibition of prostaglandin synthesis during fasting in healthy man increases renal sodium absorption through a possible direct regulation of the epithelial sodium channels [64]. In the same time, prostaglandins are likely to be responsible for a decrease of the expression of aquaporin-2 and the V_2 receptor resulting in a decrease of water permeability in the collecting duct [65] that is supposed to amplify the polyuria. Therefore, the beneficial effect of indomethacin on urinary sodium wasting and polyuria might be better figured out by the inhibition of the prostaglandins pathway on tubular structures than by a mild or a moderate decrease of the glomerular filtration rate [66, 67]. However, prescribers must be aware that necrotizing enterocolitis and death have been reported following the use of indomethacin in the neonatal period [55, 58, 68–70].

5.4. Potassium-Sparing Drugs. Many drugs are susceptible to block the secretion of potassium in the collecting duct and to prevent potassium depletion. Those that have been used in Bartter syndrome are modamide, the antagonist of the Epithelial Sodium Channel, spironolactone, the antagonist of mineralocorticoid receptor, and angiotensin-converting enzyme inhibitors. Unfortunately, all these drugs impair the stimulation of the sodium reabsorption in the collecting duct and are susceptible to worsen the urinary sodium wasting and the chronic contraction of the extracellular volume [25, 71, 72].

6. Unresolved and New Questions

6.1. Cysts. The development of renal cysts have been reported in patients with Bartter syndrome, of whom one had a mutation in the *CLCNKB* gene [61, 73]. The mechanism remains unveiled.

6.2. Chronic Renal Failure. Renal fibrosis has been reported in a series of 12 patients, of whom four had a decreased glomerular filtration rate. No statistical correlations could be established between the indomethacin dose and the percentage of altered interstitial surface area [74]. By contrast, significant relationships have been evidenced between the severity of urinary sodium wasting and the level of the glomerular filtration rate suggesting that the extent of renal fibrosis might be related to the chronic contraction of the extracellular volume and hypovolemia. Consistently, the development of chronic renal failure is a frequent feature in patients with a barttin mutation in parallel to severe chronic

extracellular dehydration [53, 75]. In addition, chronic renal failure has also reported in patients who developed proteinuria and lesions of focal segmental glomerulosclerosis superimposed to a Bartter syndrome [76, 77]. Finally, end stage renal failure is rare but has been yet reported [78].

6.3. Secondary Diabetes Insipidus. Hypernatremia with spontaneous diluted urine below 100 mosmol/Kg and failure to concentrate urine over 200 mosmol/Kg has been reported without any mutation in the genes of the V_2 receptor and aquaporine-2 [57]. Diluted final urine may be paradoxical in Bartter syndrome while the Henle loop is a major site to dilute urine. One should recall that the first half of the distal convolution is also water impermeable while aquaporine-2 is never coexpressed along with the sodium-chloride transporter [79] and that the distal convolution may consequently account for by a nonnegligible part of the diluting urine capacity [80]. Nevertheless, the failure of the principal cell of the collecting duct to appropriately transport water when strongly and continuously activated by angiotensin-2 and aldosterone remains to be understood.

6.4. Cholelithiasis. A case of cholelithiasis has been reported recently and attributed to alkalosis and dehydration that favour precipitation of calcium carbonate in biliary ducts [81].

7. Conclusion

In conclusion, renal physiology allows a rational approach to understand the biological features of Bartter syndrome and supports sodium chloride supplementation as the most appropriate treatment to control both the extracellular volume and the potassium depletion. On a basic point of view, it is noteworthy that the preservation of the sodium pool (and the balance of the extracellular volume) is a higher priority of the system than the protection against the potassium depletion, itself a priority upon the hydrogen balance.

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Research Article

Depressive Symptomatology in Children and Adolescents with Chronic Renal Insufficiency Undergoing Chronic Dialysis

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This paper presents a descriptive study, using the Birleson Scale to determine the frequency of depressive symptomatology in children and adolescents with chronic renal insufficiency (CRI) undergoing hemodialysis (HD) and chronic peritoneal dialysis (CPD). There were 67 patients (40 female and 27 male) with a mean age of 14.76 ± 2.71 years, duration of illness ≥3 months, 43 (64.18%) patients with CPD and 24 (35.82%) undergoing HD. The frequency of high occurrence, low occurrence, and absence of depressive symptomatology was 10.45% ($n = 7$), 43.28% ($n = 29$), and 46.27% ($n = 31$), respectively; all of the seven (100%) patients with high occurrence of depressive symptomatology were female ($P = 0.04$), and none of these (0%) had a friend to confide in ($P = 0.03$). Depressive symptomatology in patients with CPD was associated with a lower weekly K_t/V compared to those without depressive symptomatology (2.15 ± 0.68 versus 2.52 ± 0.65; $P = 0.01$). There was no association with patient age, caregiver, time and dialysis type, anemia, bone disease, nutritional or financial status, origin, schooling, or employment.

1. Introduction

Depression is a public mental health problem that affects children, adolescents, and adults, negatively impacting their personal, academic, social, and family lives [1]. In recent years the prevalence of depression has increased worldwide, and, at the same time, the presentation age has decreased. According to the World Health Organization (WHO), this makes it the medical condition with the fourth highest loss of life years due to premature death or years living with a severe and chronic incapacity [1].

The definition of depression is a mood alteration characterized by a depressed state of mind, decreased enjoyment and concentration, lack of interest, a feeling of disability, guilt, and hopelessness, insomnia, reduced appetite, and suicide ideation. The earlier it is present, the greater the risk of suicide, substance abuse, and behavioral problems; for this reason it is very important to diagnose and treat quickly [2].

The prevalence of depression is about 3–5% in children and around 8% in adolescents, with a female predominance [3, 4]. The presence of depressive symptoms is about 15%. These frequencies increase in patients with a chronic disease [5].

Depression is the most common mental alteration in patients with terminal chronic renal insufficiency, which is neither diagnosed nor studied [5]. Livesley found that the frequency of anxiety, personality disorder, and depression in patients with chronic dialysis was significantly higher than in healthy individuals [6]. Lopes and colleagues found a 20% prevalence of depression in 5256 patients and 13.9% in 9382 patients, both undergoing chronic hemodialysis [7, 8]. Bakr and coworkers compared children undergoing chronic hemodialysis with children not undergoing chronic hemodialysis, finding that 52.6% of the hemodialysis patients had psychiatric alterations, and of these 10.3% had depression. The prevalence of depression changes according to the studied population and the diagnostic method [9, 10].

Different studies show an association between depression, decrease in length of life, and adherence to therapy, with an increase of 15 times the suicide risk and an increasing number of hospitalizations. This makes it an independent risk factor predicting mortality, and one with a significant

Table 1: Correlation of high occurrence of depressive symptoms (score above 21 according to Birleson Scale) and absence of depressive symptomatology (score below 13) with clinical, demographic, and social variables.

Variable	High occurrence of depressive symptoms		Absence of depressive symptoms		P
	n	%	n	%	
Gender					
Female	7	100	19	61.29	0.04
Male	0	0	12	38.71	
Provenance					
Lima	5	71.43	17	54.84	0.82
Other cities	2	28.57	14	45.16	
Attending school					
Yes	1	4.35	14	60.87	0.2
No	6	13.64	17	38.64	
Relationship age/level of education					
Yes	4	57.14	13	41.94	0.18
No	3	42.86	18	58.06	
Are you currently employed?					
Yes	0	0	4	80	0.49
No	7	11.29	27	43.55	
Socioeconomic level					
Extreme poverty	3	9.38	15	46.88	0.4
No extreme poverty	4	17.39	8	34.78	
No poverty	0	0	8	66.67	
Family					
Two-parent home	4	8.51	19	40.43	0.28
One-parent home	2	15.38	8	61.54	
Others	1	14.29	4	57.14	
Anemia					
Yes	6	11.11	1	7.69	0.16
No	22	40.74	9	69.23	
Renal osteodystrophy					
Yes	2	28.57	22	70.97	0.16
No	5	71.43	9	29.03	
Dialysis type					
Hemodialysis	2	8.33	13	54.17	0.65
Peritoneal dialysis	5	11.63	18	41.86	
Peritonitis					
Yes	1	5	8	40	0.47
No	4	17.39	10	43.48	
Nutritional status					
Eutrophic	0	0	4	100	0.4
Mild chronic malnutrition	2	16.67	6	50	
Moderate chronic malnutrition	2	8.7	9	39.13	
Severe chronic malnutrition	3	10.71	12	42.86	
K_t/V					
Acceptable	5	71.43	23	74.19	0.45
Not acceptable	2	28.57	8	25.81	
Friend to confide in					
Yes	0	0	17	54.84	0.03
No	7	100	14	45.16	

Table 2: Correlation of depressive symptoms (score above 13 according to Birleson Scale) and no depressive symptoms with clinical and demographic variables.

Variable	Depressive symptoms $\bar{x} \pm \sigma$	No depressive symptoms $\bar{x} \pm \sigma$	$P < 0.05$
Patient's age	14.67 ± 2.89	14.10 ± 2.78	0.33
Age of patient's guardian	43.38 ± 7.0	40.16 ± 7.44	0.1
Dialysis time	1.91 ± 1.47	2.47 ± 2.41	0.25
Hematocrit (%)	26.52 ± 6.88	28.28 ± 6.44	0.14
Parathormone (pg./mL)	277.37 ± 245.11	309 ± 385.86	0.68
K_t/V hemodialysis	1.45 ± 0.19	1.39 ± 0.28	0.43
K_t/V peritoneal dialysis	2.15 ± 0.68	2.52 ± 0.65	0.01

impact on the patient's quality of life, greater than biological or therapy factors [8, 11].

The principal objective of this study was to determine the frequency of levels of depressive symptoms in children with terminal chronic renal insufficiency undergoing chronic hemodialysis and chronic peritoneal dialysis and further to ascertain whether there was a relationship with clinical, demographic, or social factors. We used The Modified Birleson Scale validated in Peru [10]. The adaptation of the original test, Birleson Scale made by Peterson Birleson in England in 1981, used in several studies, was made, and it was evaluated for expert council. It has discharge trustworthiness.

2. Materials and Methods

The investigation was a descriptive, case series study. We evaluated children with terminal chronic renal insufficiency undergoing dialytic treatment for three months, at the dialysis unit of the nephrology service of the Cayetano Heredia Peruvian University.

We selected 84 patients with an average age of 14.76 ± 2.71 years, from which we excluded 14 because they were under 8 years old, two who had Down's syndrome, and one who declined to participate in the study. Sixty-seven patients (40 female and 27 male) fulfilled the inclusion criteria (age between 8 and 18 years, duration of illness ≥3 months, no chronic comorbidities, accepted consent and assent, stable clinically with no psychiatric or psychological comorbidity diagnosed at that time, and knowledge of writing). 43 (64.13%) chronic peritoneal dialysis patients and 24 (35.82%) chronic hemodialysis patients were selected.

The Birleson Scale was used to determine the presence of depression and depressive symptoms, being the value 13 as the cut-off score, where a score of 13–21 evidenced the presence of depressive symptoms and a value of more than 21 demonstrated depression. While the Birleson Scale has been used in disease states, it still has not been validated in ESRD patients, and therefore high scores are suggestive but not diagnostic of clinical depression. For this reason, those variables were recategorized as high and low occurrence of depressive symptomatology in terms of depression and depressive symptoms, respectively, as well as, an absence of depressive symptoms if the score was <13 and the presence of depressive symptoms ≥13.

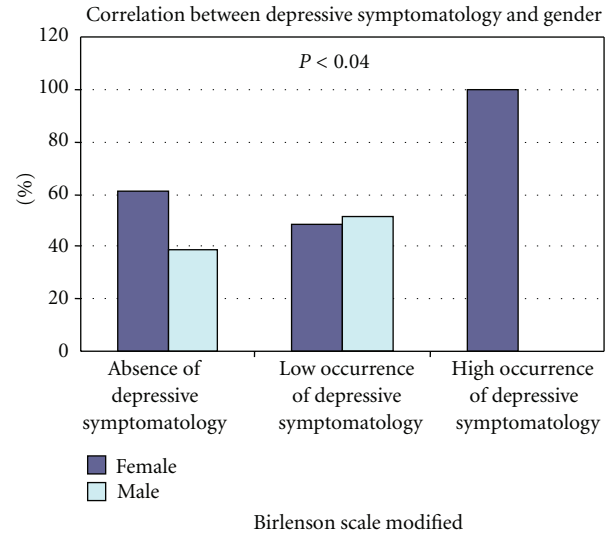

Figure 1: Correlation between depression symptomatology and gender.

Clinical data were collected from the patient charts and sociodemographic data from the patients and their caregivers.

Statistical Procedure. The statistical program Stata v.10 was used for the data analysis. Chi^2 and Student's t were employed to determine statistical significance for continuous and categorical variables, and variance analysis was used to compare means.

3. Results

The frequency of high depressive symptomatology was 10.45% ($n = 7$), low depressive symptomatology 43.28% ($n = 29$), and no depressive symptoms 46.27% ($n = 31$). Of the children with high occurrence of depressive symptomatology, five (11.63%) were undergoing chronic peritoneal dialysis and two (8.33%) were undergoing hemodialysis. All (100%) of the children with high occurrence of depressive symptomatology were female ($P = 0.04$), and none of the seven (0%) had a friend to confide in ($P = 0.03$) (Table 1 and Figure 1). The occurrence of depressive symptomatology in patients with CPD was associated with a lower weekly K_t/V compared to those with absence of depressive symptomatology (2.15 ± 0.68 versus 2.52 ± 0.65; $P = 0.01$) (Table 2).

Twenty (83.33%) of the children undergoing hemodialysis were from Lima, and 25 (58.14%) of the children undergoing chronic peritoneal dialysis were from a province outside the capital ($P = 0.02$). Of the patients undergoing chronic peritoneal dialysis, 35 (81.40%) had both parents, but five (11.63%) had only one parent, giving a $P = 0.027$ (Table 3).

We found some relationship between high occurrence of depressive symptomatology and feelings, as follows: not

Table 3: Features according to dialysis types.

Variable	Total	HD		DP		P
	%	n	%	n	%	
Birleson Scale						
High occurrence of depressive symptomatology	10.45	2	8.33	5	11.63	0.65
Low occurrence of depressive symptomatology	43.28	9	37.5	20	46.51	
Absence of depressive symptomatology	46.27	13	54.17	18	41.86	
Provenance						
Lima	56.71	20	83.3	18	41.86	0.002
Other cities	43.28	4	16.7	25	58.14	
Family						
Two-parent home	70.14	12	50	35	81.4	0.027
One-parent home	19.40	8	33.3	5	11.63	
Others	10.46	4	16.7	3	6.98	

Table 4: Correlation of depressive symptoms with satisfaction with their physical appearance, studies, economic status, and friendship.

Satisfaction with themselves	Very	Somewhat	Not very	P
Physical appearance				
Absence of depressive symptomatology	48.39%	6.45%	45.16%	0.041
Low occurrence of depressive symptoms	41.38%	10.34%	48.28%	
High occurrence of depressive symptoms	0%	42.86%	57.14%	
Studies				
Absence of depressive symptomatology	35.48%	9.68%	54.84%	0.001
Low occurrence of depressive symptoms	17.24%	24.14%	58.62%	
High occurrence of depressive symptoms	0%	85.71%	14.29%	
Economic status				
Absence of depressive symptomatology	32.26%	16.13%	51.61%	0.001
Low occurrence of depressive symptoms	0%	34.48%	65.52%	
High occurrence of depressive symptoms	0%	71.43%	28.57%	
Friendship				
Absence of depressive symptomatology	58.06%	12.9%	29.03%	0.001
Low occurrence of depressive symptoms	37.93%	13.79%	48.28%	
High occurrence of depressive symptoms	0.00%	85.71%	14.29%	

very happy with their physical appearance (4, 57.14%) $P = 0.04$, somewhat with their studies (6, 85.71%) $P = 0.001$, somewhat unhappy with their financial status (5, 71.43%) $P = 0.001$, and somewhat unhappy with their friends (6, 85.71%) $P = 0.001$ (Table 4).

According to the Modified Birleson Scale (Table 5), children with high recurrence of symptomatology answered "always" the following questions: Number 21 (85.7%, $n = 6$) and Number 5, 10, 12, 16, 17, 19, and 20 (100%, $n = 7$). Children with low recurrence of symptomatology answered all questions in a random way, including the questions Number 10 and 21.

The presence of high and low occurrence of depressive symptomatology was not related to the patient's age, caregiver's age, dialysis period, presence of anemia, bone disease, nutritional status, financial situation, origin, schooling, or employment.

4. Discussion

Having a chronic disease during childhood confers a large risk of developing a psychiatric disorder, and chronic renal disease is not an exception: it is a significant stressor with a psychological and social impact on the children and their family [10].

In this study, we found a general frequency of 53.73% for high and low depressive symptomatology, 10.45% for high depressive symptomatology and 43.28% for low depressive symptomatology, of whom 11.63% were undergoing peritoneal dialysis and 8.33% hemodialysis.

Bakr et al. [9] analyzed 19 children with chronic renal insufficiency undergoing predialysis and 19 children with terminal chronic renal insufficiency undergoing dialysis, finding a 52.6% prevalence of psychiatric disorders, 18.4% of adaptation disorders, 10.3% of depression, and 7.7% of neurocognitive disorders. However, in children with terminal chronic renal insufficiency, depression was as high as 15.8%; the authors also found a higher prevalence in dialysis patients (68.4%) than in predialysis patients (36.8%). In addition, Fukunishi and Kudo [12] reported that 17 (65.4%) of 25 children with terminal chronic renal insufficiency undergoing peritoneal dialysis had psychiatric disorders; however, they did not analyze the depression frequency. Wass and colleagues [13] found that of 26 British children who were receiving hemodialysis at home, five (19.2%) had a psychiatric disease. Another study, by Garralda and colleagues, showed that psychiatric alterations such as depression are common in children and adolescents (22 children and

Table 5: Modified Scale of Birleson and frequency of symptoms of the 67 patients; it is presented as an original sample, and it has been literally translated in order to have a better understanding of depressive symptomatology. Always have a score of 2, sometimes of 1, and never of 0.

Questions	Equivalent in English	%		
		Always	Sometimes	Never
(1) Las cosas me gustan, me interesan como antes	I like and am still interested in the same things as before	34.32 ($n = 23$)	59.70 ($n = 40$)	5.97 ($n = 4$)
(2) Duermo muy bien	I sleep well	64.18 ($n = 43$)	31.34 ($n = 21$)	4.47 ($n = 3$)
(3) Me dan ganas de llorar	I feel like crying	11.94 ($n = 8$)	55.22 ($n = 37$)	32.83 ($n = 22$)
(4) Para adolescentes: me gustan salir com amigos. Para niños: me gusta salir a jugar	For adolescents: I like going out with my friends. For children: I like going out to play	38.80 ($n = 26$)	35.82 ($n = 24$)	25.37 ($n = 17$)
(5) Me gustaria escapar, salir corriendo	I would like to run away	10.45 ($n = 7$)	29.85 ($n = 20$)	59.70 ($n = 40$)
(6) Me duele la barriga, cabeza y otros sítios de mi cuerpo	My stomach and my head hurt, as well as other parts of my body	5.97 ($n = 4$)	41.79 ($n = 28$)	52.23 ($n = 35$)
(7) Tengo ganas para hacer las cosas	I have a desire to do things	37.32 ($n = 25$)	50.74 ($n = 34$)	11.94 ($n = 8$)
(8) Disfruto de la comida	I enjoy eating food	55.22 ($n = 37$)	38.80 ($n = 26$)	5.97 ($n = 4$)
(9) Puedo defenderme por mi mismo	I am able to defend myself	43.28 ($n = 29$)	38.80 ($n = 26$)	17.91 ($n = 12$)
(10) Pienso que no vale la pena vivir	I feel that life is not worth living	16.42 ($n = 11$)	28.35 ($n = 19$)	55.22 ($n = 37$)
(11) Soy bueno para las cosas que hago	I am good at the things I do	47.76 ($n = 32$)	41.79 ($n = 28$)	10.44 ($n = 7$)
(12) Me molesto y me irrito por cualquier cosa	I feel bothered and irritated with everything	23.88 ($n = 16$)	55.23 ($n = 37$)	20.89 ($n = 14$)
(13) Disfruto lo que hago tanto como lo hacia antes	I still enjoy doing things as I did before	43.29 ($n = 29$)	47.76 ($n = 32$)	8.95 ($n = 6$)
(14) Me he vuelto olvidadizo y distraído	I have become forgetful and listless	7.46 ($n = 5$)	55.22 ($n = 37$)	37.32 ($n = 25$)
(15) Tengo sueños horribles	I have nightmares	4.47 ($n = 3$)	35.82 ($n = 24$)	59.70 ($n = 40$)
(16) Pienso que haga lo que haga no lograre conseguir lo que deseo o que las cosas no van a cambiar	I think that it does not matter what I do; I will never be able to accomplish the things I want to accomplish; things are never going to change	31.34 ($n = 21$)	31.34 ($n = 21$)	37.32 ($n = 25$)
(17) Me siento muy solo	I feel very lonely	11.94 ($n = 8$)	37.31 ($n = 25$)	50.74 ($n = 34$)
(18) Puedo alegrarme facilmente	I become happy easily	41.79 ($n = 28$)	50.74 ($n = 34$)	7.46 ($n = 5$)
(19) Me siento tan triste que me cuesta trabajo soportarlo	I feel so sad that I can hardly stand it	20.91 ($n = 14$)	28.35 ($n = 19$)	50.74 ($n = 34$)
(20) Me siento muy aburrido	I feel very bored	16.42 ($n = 11$)	58.21 ($n = 39$)	25.37 ($n = 17$)
(21) Pienso muy serio en la muerte o en matarme	I seriously think about death and killing myself	8.95 ($n = 6$)	19.41 ($n = 13$)	71.64 ($n = 48$)
Total				

adolescents with terminal chronic renal insufficiency and 22 predialysis children and adolescents with chronic rena insufficiency, compared to healthy children) and that these increase in those whose renal disease is more severe [14].

Children with terminal chronic renal insufficiency have growth retardation and develop secondary sexual characteristics, bone deformities, multiple scars, and so forth. As a consequence of bone dystrophy, uremia, and the treatment, the children look and feel different from other children, thus increasing the risk of psychiatric problems such as depression [15].

Before puberty, depression is more frequent in males, while in the postpuberty period it predominates in females. This is consistent with our results, where all of the children with high occurrence of depressive symptomatology were female and their average age was 14.76 ± 2.71. This differs from other studies that did not find any relationship with gender [4, 10].

Peritoneal dialysis patients without depressive symptomatology had a better K_t/V value than patients with depressive symptomatology, which was significant ($P = 0.01$). We could infer that depression as comorbidity is a negative clinical factor. Another study did not show any relationship with K_t/V [16]. There is evidence that suboptimal dialysis increases mortality in patients undergoing peritoneal dialysis and hemodialysis with cardiac, cerebral, and other comorbidities. A K_t/V that diminishes by 0.1 weekly is associated with a 5% increase in relative death risk [8, 17],

and suboptimal dialysis contributes to the risk of depression [11, 18–20]. This finding needs to be clarified in further studies.

None of the children with high occurrence of depressive symptomatology had a friend to confide in ($P = 0.03$), which agrees with other studies. This confirms that social support contributes to the patient's quality of life and decreases the number of hospitalizations in children with chronic renal insufficiency [21].

When asked *How content or happy are you with your physical appearance, studies, financial status, and friends? ($P < 0.05$)*, they answered "more or less (content or happy)" or "fairly (content or happy)." This confirms the presence of low self-esteem related to high occurrence of depressive symptomatology, as the response "more or less" might be considered "fair" because patients tend to deny depression as a social defense [8, 10].

We know that the stress and impact of disease generate a distressing and downcast reaction and/or pain in children and their families. This is where the depressive symptoms predominate and then develop to depression or other psychiatric changes [22]. It is for this reason that it is recommended that all children with depression, depressive symptoms, and suicidal ideation according to the Modified Birleson Scale or high and low depressive symptomatology according to the recategorization should undergo psychiatric interview to confirm their probable diagnosis. As we know, depression is a clinical diagnosis and the Modified Birleson Scale gives us information about the presence of depressive symptoms, not clinical depression itself. It can be used as a tool to help us to be aware of any possible disorder, which would then need to be confirmed by a psychiatrist. Future research needs to validate self-reported questionnaires in patients with chronic disease like ESRD patients. A few studies show an increased frequency of depression diagnosis after psychiatric interview compared with frequency of possible depression after self-completion of a diagnostic scale [23]. Such individuals should be candidates for treatment, since there is evidence that social, psychological, and pharmacological interventions reduce mortality, morbidity, and treatment withdrawal [24], and improve the quality of life [11, 25] associated with depression in patients with or without comorbidities [26]. Given this, diagnosis of depression should always be included in screening of pediatric patients with terminal chronic renal insufficiency [8].

5. Conclusion

The frequencies of high and low occurrence of depressive symptomatology were 10.45% and 43.28%, respectively. The clinical and sociodemographic factors related to high occurrence of depressive symptomatology were female sex, having or not having a friend to confide in, and dialysis dose.

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Review Article

Molecular and Genetic Basis of Inherited Nephrotic Syndrome

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Nephrotic syndrome is an heterogeneous disease characterized by increased permeability of the glomerular filtration barrier for macromolecules. Podocytes, the visceral epithelial cells of glomerulus, play critical role in ultrafiltration of plasma and are involved in a wide number of inherited and acquired glomerular diseases. The identification of mutations in nephrin and other podocyte genes as causes of genetic forms of nephrotic syndrome has revealed new important aspects of the pathogenesis of proteinuric kidney diseases and expanded our knowledge of the glomerular biology. Moreover, a novel concept of a highly dynamic slit diaphragm proteins is emerging. The most significant discoveries in our understanding of the structure and function of the glomerular filtration barrier are reviewed in this paper.

1. Introduction

The ultrafiltration of plasma during primary urine formation is one of the central function of the human kidney. Normal filtration function of the glomerulus depends on the structural and functional integrity of the filtration barrier, that is the primary target of several inherited and acquired glomerular disorders, characterized by nephrotic syndrome (>3.5 g protein/day) and rapid progression to end-stage renal disease (ESRD).

The glomerular filtration barrier, responsible for the size and charge-selective properties of renal filter, is composed of three separate layers: the fenestrated endothelium, the glomerular basement membrane (GBM), and the podocyte foot processes layer. Recent studies have emphasized the role of podocytes as a key cell type involved in the mechanisms responsible for proteinuria and glomerular damage [1–3]. Podocytes are injured in many forms of human and experimental glomerular diseases, including congenital nephrotic syndromes, minimal change disease (MCD), focal segmental glomerulosclerosis (FSGS), membranous glomerulopathy, diabetes mellitus, and lupus nephritis [1, 3, 4]. In fact, the majority of glomerular diseases are characterized by alterations in the molecular composition of the slit diaphragm (SD) and a reorganization of foot process structure with fusion and effacement. The major causes leading to foot process effacement and proteinuria are (i) abnormalities in the GBM or podocytes-GBM interactions; (ii) impaired formation of the slit diaphragm area; (iii) alterations of the actin cytoskeleton and associated proteins [1, 5–7].

A better understanding of the molecular properties of GBM, podocytes and the slit diaphragm is critical to develop novel therapeutic strategies for patients with glomerular disease and to prevent end-stage renal insufficiency.

Mutations in different podocyte proteins can target the function of the podocyte through distinct pathologic mechanism by affecting the structure of the slit diaphragm, by directly or indirectly perturbing the intricate podocyte

cytoskeleton, by breaking cell-matrix interactions, or by blocking important signaling pathways. All these mechanisms result in a common final disease pathway characterized by podocyte foot processes effacement, proteinuria, and ultimately disruption of the glomerular filter (Figure 1).

The most significant discoveries in our understanding of the structure and function of the glomerular filtration barrier and related diseases are summarized in this paper.

2. Podocyte Structure and Development

Glomerular podocytes are highly differentiated cells with a complex cytoarchitecture. They have a voluminous cell body, long primary processes and regularly spaced, interdigitated foot processes that completely enwrap the glomerular capillaries (Figure 1). The interdigitated foot processes of neighboring podocytes cover the GBM and form a narrow filtration slit connected by an electron dense structure, called the slit diaphragm (SD), a zipper-like structure with a 40 nm diameter, according to the model proposed by Karnovsky and Ainsworth [8]. Podocytes are polarized epithelial cells with a luminal or apical and a basal cell membrane domain. A well-developed cytoskeleton accounts for the unique shape of the cells and the maintenance of the processes. The apical membrane of foot processes is equipped with a negatively charged surface coat, primarily made up of podocalyxin. This protein is critical for formation and preservation of cellular architecture: its absence causes immature glomeruli with flattened podocytes. The first marker of podocyte development in vertebrates is the restriction of *WT1* expression to a subset of cells within the renal vesicle [9, 10]. Several other transcription factors are expressed in the early podocytes, including podocyte-expressed 1 [11], forkhead box C2 (*Foxc2*) [12], kreisler (*Mafb*) [13], the forkhead domain transcription factor *Mf2* [14], and the Lim domain protein *Lmx1b* [15].

WT1 is probably the best studied of the transcription factors expressed in podocytes. *WT1* encodes a protein with four zinc fingers that can bind to both DNA and RNA [16, 17]. In the fetal kidney, WT1 is expressed in metanephric mesenchyme, renal vesicles, and developing podocytes. In adult life, the WT1 expression is restricted to podocytes. In maturing glomeruli, WT1 expression increases while the PAX2 expression is downregulated. The homeobox PAX2 gene encodes for a transcription factor expressed early during development and essential for conversion of metanephric blastema to renal vescicole. Downregulation of PAX2 appears as a prerequisite to allow podocyte differentiation which is governed by WT1. Both WT1 and PAX2 knockout mice lack kidneys, suggesting the critical role of these transcription factors in metanephric development. Mutations in PAX2 gene are associated with renal coloboma syndrome and isolated renal hypoplasia. The WT1 expression is altered in both congenital and acquired human diseases. In particular, the WT1 expression is lost in podocytes of collapsed glomeruli. Dominant mutations in WT1 are associated with the Denys-Drash and Frasier syndromes, characterized by glomerulopathy, mesangial sclerosis, male pseudohermaphroditism, and nephroblastoma. In these patients, the WT1 abnormal expression is associated with increased expression of PAX2 [18, 19].

POD1 (also known as epicardin and capsulin) encodes a basic helix-loop-helix transcription factor that is expressed early in mouse kidney development, and subsequently in the primitive podocytes of S-shaped bodies [20, 21]. Kreisler (MAFB) encodes a basic domain leucine zipper (bZip) transcription factor of the MAF subfamily and is expressed in mouse podocytes of capillary loop-stage glomeruli [22]. It also has an important role in hindbrain segmentation. *Pod1* and kreisler mutations in mice result in similar phenotypes: glomerular development is arrested at the single capillary loop stage [20, 22], and the podocytes remain as columnar-shaped cells that have lost their lateral cell-cell attachments but remain fully adhered to the GBM without any foot processes. Thus, *Pod1* and kreisler are required just prior to the time when podocytes would normally begin migrating around the capillary loops and assembling foot processes. *Pod1* is expressed in kreisler mutant podocytes, indicating that kreisler is likely to act either downstream or in a separate pathway from *Pod1* [22].

Foxc2 was identified during a screen for genes with enriched expression in mouse glomeruli [12]. It belongs to the forkhead domain family of putative transcription factors and is expressed in podocytes. In *Foxc2* mutant mouse kidneys, mesangial cells cluster at the base of the glomerular stalk, podocyte foot processes, and endothelial fenestrations are absent, and dilated capillaries are observed, similar to the other phenotypes discussed above [12].

LMX1B, a Lim homeobox gene, is another important transcription factor, regulating the expression of multiple genes which are critical for podocyte differentiation and function. Homozygous Lmx-1b knockout mice have reduced numbers of podocyte foot processes, absence of typical slit diaphragms, and glomerular basement membrane abnormalities, but they express near-normal levels of nephrin, synaptopodin, ZO-1, and GBM laminins. Mutations of the human Lmx-1b gene are responsible for the nail-patella syndrome, an autosomal dominant disease with skeletal abnormalities, frequently associated with glomerulopathy [24].

3. Podocyte-GBM Interactions

Proteinuria, the most common clinical manifestation of glomerular diseases, is invariably associated with podocyte foot process effacement, flattening, and retraction. To maintain the complex foot process architecture, the adhesion of the podocytes to the GBM is controlled by the expression of several adhesion proteins. The foot processes are fixed to the GBM via $\alpha_3\beta_1$-integrin and dystroglycan (DG) complex. The $\alpha_3\beta_1$-integrin binds to fibronectin, collagen IV, and laminin of GBM, and it is essential for maturation of podocytes, as shown by the loss of foot processes development in α_3-deficient mice. The dystroglycan complex is connected to podocyte actin cytoskeleton (Figure 1) through urotrophin, and its expression is reduced in MCD but not in membranous nephritis and FSGS [25–27].

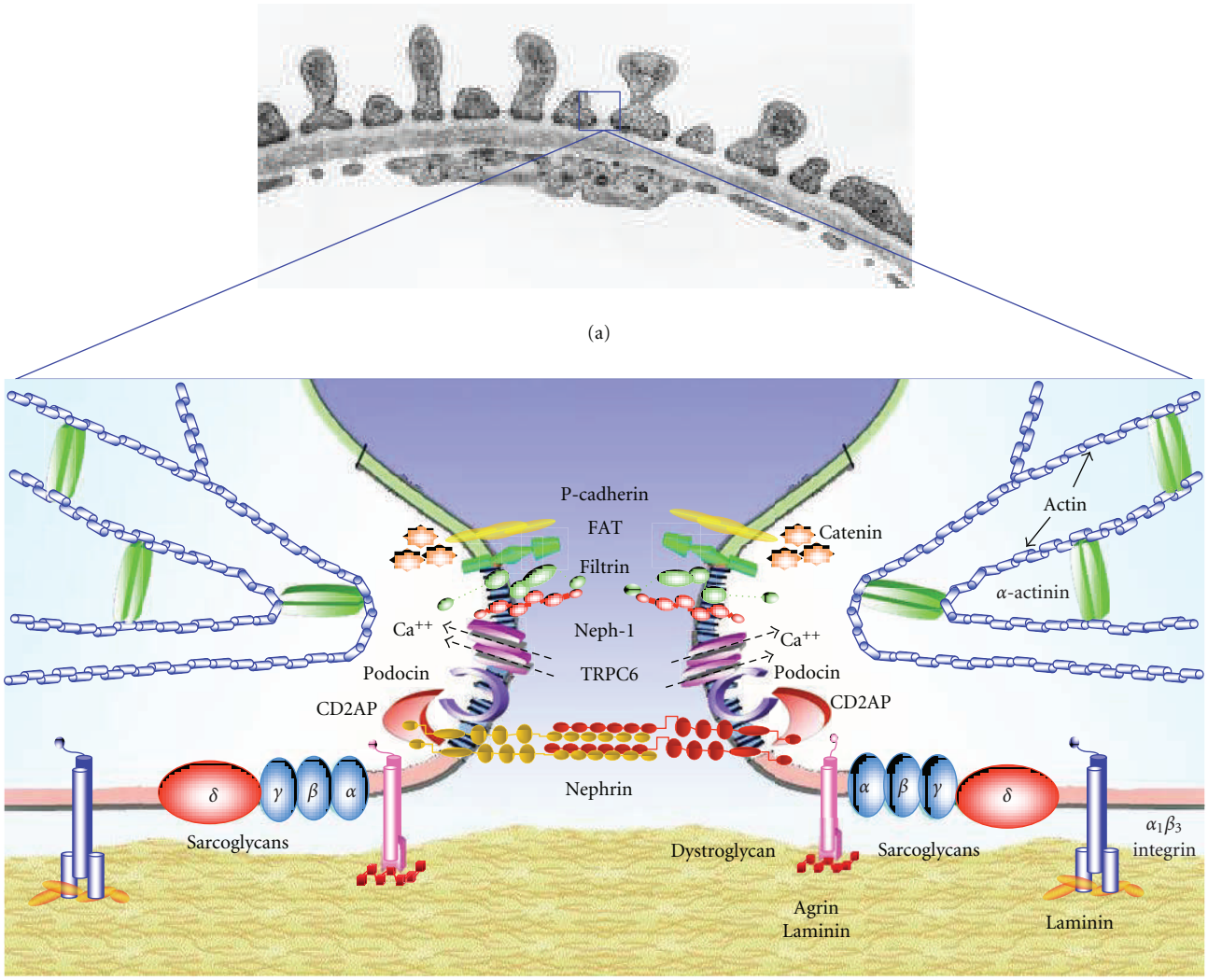

Figure 1: (a) Low-power view of glomerular filtration barrier in situ. The glomerular filter consists of three components: porous endothelium, glomerular basement membrane, and podocyte foot processes with the interposed slit membrane. Figure is transmission electron microscopy from a rat. Magnification: *B*, x~48,000. (b) Schematic drawing of the molecular equipment of the podocyte foot processes, similar to the area marked in Figure 1(a). See text for further explanations, (modified from [23]).

Podocyte detachment leaves the denuded GBM, and this may play an important role in the FSGS pathogenesis: podocytes have not proliferative capacity and cannot repopulate denuded areas, and a scar is formed by parietal ephitelial cells [7].

The glomerular basement membrane (GBM), responsible for the charge-selective property of glomerular filtration barrier, is organized as a highly cross-linked network of specific extracellular matrix proteins, such as type IV collagen, fibronectin, laminin, nidogen, and heparansulfate proteoglycans (HSPGs). The flexibility and dynamism of the GBM requires a constant turnover. In the adult glomerulus, the podocytes continue to add and assemble GBM components and secrete matrix modifying enzymes [43].

Genetic modifications of structural GBM proteins, such as type IV collagen, cause Alport syndrome (AS), a hereditary nephropathy associated with deafness [30]. Thickening, basket-wave splitting, and rarefaction of the GMB have been reported in other hereditary nephritis with Döhle-like inclusions in polymorphonuclear cells and/or thrombocytopenia with giant platelets. This condition is known as Alport-like syndrome or Fechtner syndrome (FTNS), when Döhle-like bodies are associated with macrothrombocytopenia (MTCP) and Epstein syndrome (EPTS) when no leukocyte inclusions are present. Recently, it has been shown that mutations in MYH9, the gene encoding for nonmuscle myosin heavy chain IIA (NMMHC-IIA), are responsible for Fechtner syndrome, Epstein syndrome, and other two MTCPs (Sebastian syndrome and May-Hegglin anomaly) without renal, ocular, or hearing defects (Table 1).

In the glomerulus, MYH9 mRNA and its protein are highly expressed by podocytes and colocalized with actin and

Table 1: Glomerular inherited diseases.

Disease	Gene locus	Gene	Exons (n°)	mRNA (kb)	Protein	Animal model	References
Autosomal dominant renal coloboma syndrome (RCS)	10q24.3–q25.1	PAX-2	11	3.5 kb	Paired box gene 2 (PAX-2), transcription factor	PAX2−/− knockout mice: lack kidneys.	[28]
(i) Denys-Drash and Frasier syndromes (ii) Wilms tumor	11p13	WT-1	9	3 kb	Wilms' tumor 1 (WT1), transcription factor	WT1−/− knockout mice: lack kidneys.	Rose et al.; *Cell*, 1990.
Nail-patella syndrome	9q33.3	LMX-1B	8	1.1 kb	M homeobox transcription factor 1, beta	LMX1B−/− knockout mice: reduced numbers of podocyte foot processes, absence of SD and GBM abnormalities.	[29]
Alport syndrome (AS)	Xq22.3	COL4A5	51	6.4 kb	Type IV collagen, alpha 5 chain	Canine X-linked hereditary nephritis: transcription of COL4A5 gene was reduced by a factor of 10 in the affected dog.	Barker et al.; *Science*, 1990 [30]
(i) May-Hegglin anomaly (ii) Sebastian syndrome (SBS) (iii) Fechtner syndrome (FTNS) (iv) Epstein syndrome (EPTS)	22q12.3–13.1	MYH9	40	7.2 kb	Nonmuscle myosin heavy chain IIA (NMMHC-IIA)	—	[31]
Minimal change disease (MCD)	3p21	DAG1	3	5.4 kb	Dystrophin-associated glycoprotein 1	DAG1−/− mice: developmental abnormalities.	[32]
CNS of Finnish type (CNF)	19q13.1	NPHS1	29	4.3 kb	Nephrin	NPHS1−/− knockout mice: nephrotic syndrome, perinatal lethality, and effacement of podocyte foot processes.	Kestilä et al.; *Molec Cell*, 1998 [33].
Steroid-resistant NS (SRNS)	1q25–q31	NPHS2	8	1.8 kb	Podocin	—	Boute et al.; *Nature Genet*, 2000 [34].
Focal segmental glomerulosclerosis (FSGS)	6p12	CD2AP	18	4.6 kb	CD2-associated protein	CD2AP−/− knockout mice: compromised immune function and death of massive proteinuria shortly after birth.	Kim et al.; *Science*, 2003 [35].
Focal segmental glomerulosclerosis (FSGS)	19q13	ACTN4	21	2.9 kb	α-actninin-4	ACTN4−/− mice: progressive proteinuria, glomerular disease, death by several months of age.	Kaplan et al.; *Nature Genet*, 2000 [36].
Focal segmental glomerulosclerosis (FSGS)	11q21-q22	TRPC6	13	4.5 kb	Transient receptor potential channel 6	—	Winn et al.; *Science* 2005 [37]. Reiser et al.; *Nat Genet* 2005 [38].
Unknown	1q21–q25	KIRREL o NEPH1	14	9 kb	Nephrin-like 1 (NEPH1)	NEPH1−/− knockout mice: nephrotic syndrome, perinatal lethality, and effacement of podocyte foot processes.	Donoviel et al.; *Molec Cell Biol*, 2001 [39].
Unknown	19q13.1	NLG1/NEPH3	15	2.5/3.5 kb	Filtrin	—	Ihalmo et al.; *Biochem Biophys Res Commun*, 2003 [40]. Sellin et al.; *FASEB J*, 2003 [41].
Clear cell renal carcinoma	4q34-q35	FAT	24	14.7 kb	FAT tumor suppressor homolog 1 (Drosophila)	FAT−/− knockout mice: perinatal lethality.	[42]

α-actinin, suggesting that NMMHC-IIA could be an important component of the podocyte actin-myosin contractile apparatus and play a central role in maintaining capillary wall integrity [44–48].

4. The Slit Diaphragm Complex

Our knowledge of the molecular and structural composition of podocyte slit diaphragms have been improved in the past few years. The discovery of several novel slit diaphragm proteins, including nephrin, podocin, Zonula Occludens 1 (ZO-1), CD2-associated protein (CD2AP), P-cadherin, catenins, FAT1, Neph1-3, densin, and TRPC6 [33–38, 40, 41, 49, 50], has helped to characterize the region of the SD as a critical locus of podocyte function (Figure 1). Moreover, mutations in the genes encoding for slit diaphragm proteins have been linked to a variety of inherited and sporadic glomerular diseases (Table 1).

The first protein located at the SD domain is nephrin, codified by *NPHS1* (19q13.1), the gene responsible of congenital nephrotic syndrome of the Finnish type (CNF), a rare autosomal recessive disease with a highest incidence in Finland [51–53]. Nephrin is a large transmembrane protein of 1241 amino acids, belonging to the immunoglobulin (Ig) superfamily, with eight extracellular Ig-like motifs, a fibronectin type III-like, a transmembrane, and an intracellular domain [54, 55].

Nephrin is a critical structural component of the slit diaphragm complex: both CNF patients with severe NPHS1 mutations and knockout mice fail in foot process and slit diaphragm development and exhibit severe proteinuria already in *utero* [56].

Recently, Donoviel et al. identified *NEPH1*, a novel nephrin-like protein, that localizes to the slit diaphragm and causes congenital nephrotic syndrome in knockout mice. NEPH1 belongs to a family of three closely related proteins (NEPH1, NEPH2, NEPH3) with a common domain architecture [39].

Ihalmo et al., independently, identified NEPH3 gene, which they called NLG1 (nephrin-like gene 1). NLG1 or NEPH3 was localized to chromosome 19q13.1, immediately adjacent to the NPHS1 gene, and encodes a type I transmembrane protein, termed *filtrin*, which contains an extracellular region with five tandem immunoglobulin-like domains, a transmembrane region, and a cytoplasmic domain with a proline-rich region.

Another important protein of the slit diaphragm complex is *podocin*, an integral protein, homologous to the band-7 stomatin family [34]. Podocin is codified by NPHS2 (1q25–1q31), the gene responsible for autosomal recessive steroid-resistant nephrotic syndrome [34, 57, 58]. Due to its structural similarity to stomatin, podocin is predicted to have a hairpin-like membrane topology, with both NH_2- and COOH-terminal intracellular domains. Podocin expression is restricted to podocytes. Podocin localizes to the podocyte foot process membrane and accumulates in an oligomeric form in lipid rafts of the slit diaphragm. Podocin associates with CD2AP and nephrin via its COOH-terminal domain (Figure 1). These findings suggest that podocin may have a crucial role in the assembly of the SD complex, and, similar to the role of stomatin in erythrocytes, it may act as a scaffolding protein [23, 59, 60].

A complex of nephrin, podocin, and CD2AP seems to be indispensable to maintain the structural integrity of the SD. CD2AP, initially described as a protein involved in T-cell activation, contains a coiled coil domain and three Src homology 3 (SH3) domains, which serve as attachment sites for other proteins. In CD2AP, knockout mice immune function was compromised and they died of massive proteinuria shortly after birth, suggesting an important role in glomerular function. Knockout mice presented flattened podocytes, mesangial cell hyperplasia, and extracellular matrix deposition. Mice with CD2AP haploinsufficiency developed glomerular changes at 9 months of age and had increased susceptibility to glomerular injury by nephrotoxic antibodies or immune complexes. Interestingly, some glomerular lesions of these mice exhibited a phenotype similar to human FSGS [61]. Kim et al. screened a population of 30 African Americans with idiopathic FSGS and 15 African Americans with HIV-associated FSGS for changes in CD2AP. Six distinct DNA variants, absent in control subjects, were detected in 10 of 45 patients. One nucleotide variant, altering the exon 7 splice acceptor site, was predicted to alter the expression of CD2AP. These findings and others implicate CD2AP as a determinant of human susceptibility to glomerular disease [35, 62–64].

Nephrin, podocin, and CD2AP are pivotal for slit diaphragm structural organisation, suggesting that these proteins could participate in a common cell-signaling pathway.

Huber et al. have demonstrated that both nephrin and CD2AP interact *in vivo* with the p85 regulatory subunit of phosphoinositide 3-OH kinase (PI3K). PI3K is the first protein demonstrated to interact with the cytoplasmic surface of SD protein complex *in vivo*. Nephrin and CD2AP recruit PI3K to the plasma membrane and, together with podocin, stimulate PI3K-dependent activation of the serine-threonine kinase, AKT. They demonstrate that nephrin-induced AKT mediates phosphorylation of several target proteins in podocytes. Although the importance of this signalling is not fully understood, it is interesting that one target of nephrin-CD2AP-induced phosphorylation is Bad, a proapoptotic protein of the Bcl-2 family; its phosphorylation and inactivation protect podocytes against apoptosis, suggesting that the nephrin-CD2AP-mediated AKT activity can regulate complex biological programs. These findings reveal a novel role for the slit diaphragm proteins and demonstrate that nephrin, CD2AP, and podocin proteins, in addition to their structural functions, initiate PI3K/AKT-dependent signal transduction in glomerular [65, 66].

In addition to these characterized podocyte proteins, the slit diaphragm area contains several other components, including P-cadherin, Zonula Occludens 1 (ZO-1), FAT, and densin. P-cadherin is associated with signalling proteins α-, β-, γ-catenins. It colocalizes with the zona occludens associated protein (ZO-1), a member of the membrane associated guanylate kinase (MAGUK) family. FAT is a large cadherin homologue, localized to the slit diaphragm

domain. FAT knockout mice exhibit perinatal lethality. The relation between P-cadherin, FAT, and nephrin is not known [67]. Densin is a new podocyte protein that belongs to the LAP protein family, characterized by leucin-rich repeats and PDZ domains. LAP proteins are involved in maintenance of cell shape and the apical-basal polarity, thus densin may be necessary for maintenance of podocyte polarity [50]. Finally, two recent studies by Winn et al. [37] and Reiser et al. [38] have identified six families with autosomal dominant hereditary FSGS caused by six different mutations in the gene encoding TRPC6, a nonselective cation channel. These mutations lead to a kidney disease with late onset and a variable rate of progression to FSGS. TRPC6 is a member of the transient receptor potential (TRP) family of nonselective cation channels [68, 69]. TRP channels have been implicated in different biological functions such as cell growth, ion homeostasis, mechanosensation, and phospholipase C-dependent calcium influx. Calcium, as a second messenger, affects many of these cellular functions. Will et al. have reported, in all affected members of a New Zeland family with autosomal dominant FSGS, a TRPC6 missense mutation, p.P112Q, within the first ankyrin repeats of the protein. $TRPC6^{P112Q}$ mutation increased peak calcium concentrations after stimulation with diacylglycerol and also potentiated angiotensin-II-mediated calcium signaling in HEK293 cells. The authors have speculated that the enhanced calcium signaling conferred by the $TRPC6^{P112Q}$ mutation might disrupt glomerular cells function or cause apoptosis and amplify injurious signals triggered by ligands such as angiotensin II, that promote kidney injury and proteinuria [37]. These findings are also consistent with the results reported by Reiser et al.: two out of five mutant proteins exhibited larger current amplitudes that wild-type TRPC6 channels. Using immunofluorescence and immunoelectron microscopy, Raiser et al. found that TRPC6 protein is enriched in podocytes and localized in podocyte foot processes near the slit diaphragms; moreover, TRPC6 interacts with nephrin and podocin but not with CD2AP [38, 69]. Moreover, we have recently demonstrated that *TRPC6* mutations can also be detected in children with early onset and sporadic SRNS and described for the first time a *de novo TRPC6* mutation in a severe form of pediatric collapsing glomerulosclerosis [70].

Thus, TRPC6 might belong, together with nephrin and podocin, to a signaling platform located at the slit diaphragm domain, suggesting a possible involvement of TRPC6 channels in regulating the dynamics of foot processes and slit diaphragm (Figure 1).

5. The Slit Diaphragm Genes and Congenital Nephrotic Syndromes: Genotype/Phenotype Correlation

The discovery of mutations in the genes coding for slit diaphragm proteins in patients with inherited nephrotic syndrome (NS) has been a breakthrough in both molecular and clinical research of glomerular diseases [71–78]. Moreover, mutations in the slit diaphragm genes have been reported in sporadic cases [79, 80]. There is growing evidence that the presence of slit diaphragm gene defects has a great importance in clinical practice of nephrotic patients; in fact, the identification of mutations in nephrotic patients might allow the avoidance of unnecessary treatments, might permit the prediction of absence of recurrence after transplantation, and might allow for the provision of prenatal diagnosis to families at risk [81].

The best characterized inherited nephrotic syndromes are congenital nephrotic syndrome of the Finnish type (CNF) and Steroid Resistant Nephrotic Syndrome (SRNS), due to mutations in *NPHS1* and *NPHS2* genes, respectively. However, recently genes related to steroid-sensitive nephritic syndrome (SSNS) were also identified; finally, podocyte dysfunction is also seen as a component of several inherited multiorgan syndromes.

5.1. Congenital Nephrotic Syndrome of the Finnish Type (CNF). CNF is an autosomal recessive disorder frequent in Finland (1 : 10,000), but it has also been described in various ethnic groups throughout the world [82]. The disease develops *in utero*, and a severe nephrotic syndrome, resistant to steroids or immunosuppressive drugs, is present from birth. Infants are premature with low birth weight and large placenta. Renal biopsy specimens show mild mesangial hypercellularity and extensive effacement of foot processes. Microcystic dilations of proximal tubules are common but not specific. Nutritional status and statural growth are poor, and children are highly susceptible to bacterial infections and thromboembolic complications. In patients with CNF who progress to ESRD between 3 and 8 years of age, the only long-term and life-saving treatment is renal transplantation [51, 52].

The CNF gene (NPHS1), encoding for nephrin, has been mapped to chromosome 19q13.1 by Kestilä et al. using the positional cloning approach [33]. In Finnish patients, two main NPHS1 gene defects, Fin-major (c.121delCT) and Fin-minor (p.R1109X), were found in over 94% of the CNF cases, suggesting the existence of two founder effects [53]. Recurrence of proteinuria after transplantation, as a result of the development of antinephrin antibodies, occurs in 20% of the patients with Fin-major/Fin-major genotype, which leads to the absence of nephrin in native kidney [83]. Outside Finland, CNF constitutes the commonest type of congenital nephrotic syndrome, but the exact incidence is unknown. Several non-Finnish cases emulate the classically severe clinical phenotype seen in Finland, and a variety of NPHS1 mutations distinct from Fin-major and Fin-minor have been detected [49, 74–76]. However, an unexplored area remains the milder disease phenotypes, with occasional remission of proteinuria [77]. It seems that NPHS1 mutations causing a total absence of nephrin expression and a complete flattening of foot processes are responsible for a severe, therapy-resistant form of nephrotic syndrome; while patients with NPHS1 mutations causing only partially defective nephrin may still have slit diaphragms and respond to therapy [73].

To date, about 173 different mutations have been reported both in Finnish and non-Finnish patients. These

mutations include small deletions, insertions, nonsense, missense, splice site, and promoter variations, and they are distributed throughout the gene, emphasizing a functional requirement for both extracellular and intracellular domains. A surprisingly large number of NPHS1 mutations are missense resulting in single amino acid substitutions, all located at the extracellular domain, particularly within immunoglobulin domains "hot spot" [84].

Nephrin is a signaling molecule, which stimulates mitogen-activated protein kinases. Nephrin-induced signaling is greatly enhanced by podocin, which binds to the cytoplasmic domain of nephrin. Mutational analysis suggests that abnormal or inefficient signaling through the nephrin-podocin complex contributes to podocyte dysfunction and proteinuria [65].

5.2. Steroid Resistant Nephrotic Syndrome (SRNS). Steroid-resistant NS is characterized by an autosomal recessive transmission, onset of proteinuria between 3 months and 5 years, resistance to steroid treatment, rapid progression to ESRD, absence of recurrence after renal transplantation, and absence of extrarenal disorders. Minimal changes on early biopsy specimens and FSGS at later stages are observed [58]. The causative gene, *NPHS2*, encoding for podocin, was mapped to 1q25–q31 by positional cloning approach [34].

NPHS2 mutations were first described in children with familial steroid-resistant idiopathic nephrotic syndrome. More than 116 pathogenic mutations have been found to segregate with the disease [85–87]. These mutations alter the expression of the gene or the structure of the protein. Two mutations, the R138Q and the R138X, were recurrent: the first one was observed in patients originating from Germany or The Netherlands, and the second one in families with Israeli-Arab descent. The R138Q podocin is retained in the endoplasmic reticulum and loses its ability to recruit nephrin in lipid rafts [88].

Podocin mutations have also been reported in patients with congenital or infantile nephrotic syndrome. Schultheiss et al. [89] found NPHS2 gene mutations in 11/27 (41%) patients with congenital nephrotic syndrome and NPHS1 gene mutations in 15/27 (55%) patients. Caridi et al. [90] reported an infantile steroid-resistant nephrotic syndrome associated with FSGS in three children with a homozygous haplotype in which two mutations are present in cis (P20L and R168H). Tsukaguchi et al. analyzed NPHS2 gene in 30 FSGS families with adolescent or adult onset. In six of these families, the affected subjects were compound heterozygous for R229Q amino acid substitution, which has an allele frequency of 3.6% in control population. Using *in vitro* translated podocin and purified nephrin, it was found that nephrin bound poorly to R229Q podocin; these data suggest that the R229Q mutation alone is, probably, insufficient to cause FSGS but it might enhance susceptibly to renal injury in association with a second NPHS2 mutation or variants in other genes, such as nephrin. However, the clinical relevance of the R229Q variant is unknown [91]. Pereira et al. [92] found that R229Q polymorphism was associated with a 2.77-fold increased risk of presenting microalbuminuria. It remains to be demonstrated whether this polymorphism is a risk factor for developing end-stage renal disease.

NPHS2 mutations have also been reported in 10 to 33% of sporadic steroid-resistant NS, which represents a frequent cause of ESRD in children [57, 79]. Ruf et al. [86] studied 152 patients with sporadic FSGS and found that 32 (21%) had homozygous or compound heterozygous mutations. Weber et al. [87] found a lower mutation rate of 6.4% in 172 patients with sporadic steroid-resistant nephrotic syndrome.

Podocin mutations are restricted to steroid-resistant patients. In a recent study, no podocin mutations were found in 124 children with steroid-responsive nephrotic syndrome, confirming the results of Frishberg et al. and Caridi et al. [57, 78, 86]. The identification of podocin mutations in sporadic cases of steroid-resistant nephrotic syndrome is important for therapeutic decisions and genetic counseling. None patients with sporadic steroid-resistant NS and podocin mutations had complete remission following cyclosporine or cyclophosphamide treatment; only few patients presented a partial remission after cyclosporine therapy, but long-term benefit of this treatment is not documented, and cyclosporine may be nephrotoxic in patients with persistent proteinuria. Thus, steroid-resistant patients should be tested for podocin mutations before giving immunosuppressive therapy. Rapid screening of these patients for NPHS2 mutation is possible because of the small size of the gene. However, it should be remembered that not all familial cases of steroid-resistant nephrotic syndrome are linked to NPHS2 gene, indicating that other genes remain to be identified. This is important to understand therapy results and for a possible multicenter therapeutic trials.

Finally, Koziell et al. [77] detected NPHS2 mutations in two patients with typical CNF in whom NPHS1 mutations were not found and mutations in both NPHS1 and NPHS2 genes were found in four cases with congenital FSGS (digenic inheritance). These data, confirmed successively by other studies [87, 89], indicate an epistatic gene interaction, resulting in a rare example of multiple allelic hits, and provide the first evidence for a functional interrelationship between nephrin and podocin. These findings demonstrate the genetic heterogeneity of congenital nephrotic syndrome and the absence of genotype/phenotype correlations. Congenital nephrotic syndrome may also be due to WT1 mutations and diffuse mesangial sclerosis. Currently, three genes are associated with congenital nephrotic syndrome: NPHS1, NPHS2, and WT1 [79, 93].

5.3. Steroid-Sensitive Nephrotic Syndrome (SSNS). Whereas gene identification has furthered the understanding of pathomechanisms in steroid-resistant nephrotic syndrome (SRNS), not even a gene locus is known for SSNS. Total genome linkage analysis was performed in a consanguineous SSNS kindred, 11 patients, to identify a gene locus for SSNS. Homozygosity mapping identified a locus for SSNS on chromosome 2p12–p13.2 [94].

This locus is not responsible for the disease in all SSNS families, demonstrating that, like SRSN, this phenotype is also genetically heterogeneous. In fact, other authors

reported an extended SSNS Bedouin family with a high rate of consanguinity. The clinical presentation and steroid response of its 11 affected individuals were similar to those of sporadic SSNS, but it was not linked to any of the presently known chromosomal loci nor predicted to be caused by mutation in any one of a list of genes associated with nephrotic syndrome [95].

5.4. Diffuse Mesangial Sclerosis. Children with diffuse mesangial sclerosis appear normal at birth, with a normal birth weight and without placental enlargement. The nephrotic syndrome may be present at birth or even suspected *in utero* by the finding of an elevated plasma alpha-fetoprotein level in the mother or the discovery of large hyperechogenic kidneys [96]. Abnormalities in the PLCE1 gene, which encodes phospholipase C epsilon, appear to cause isolated diffuse mesangial sclerosis. In one study of 12 children from 6 families with the disease, homozygous truncating gene mutations in PLCE1 were found in eight children [97]. Phospholipase C epsilon is a member of the phospholipase family of enzymes that catalyzes the hydrolysis of polyphosphoinositides resulting in generation of second messengers (e.g., inositol-1,4,5-triphosphate), which are involved in cell growth and differentiation. A pathogenetic role for PLCE1 in glomerular development was supported by findings of disruption of the glomerular filtration barrier and edema in a PLCE1 knockout zebrafish model. How a PLCE1 gene defect results in changes in the glomerular nephrotic syndrome is unknown. One possible explanation is that phospholipase C epsilon interacts with GTPase-activating protein, which is known to interact with the slit diaphragm protein, nephrin. Perturbations of this normal interaction would have a downstream effect including the subsequent interaction of GTPase-activating protein with nephrin.

6. Syndromic Disease

6.1. WT1 Mutations. The WT1 gene encodes a transcriptional factor of the zinc finger protein family that is involved in kidney and gonadal development. WT1 has been localized to chromosome 11q13; it consists of ten exons and generates four different isoforms resulting from alternative splicing [98]. After birth, WT1 protein expression is restricted to renal podocytes where it probably contributes to maintain an adult differentiation. Germline heterozygous WT1 mutations have been extensively reported in the literature as the cause of Denys-Drash (DDS) and Frasier (FS) syndromes that are characterized by nephrotic syndrome, genitalia anomalies, and pseudohermaphroditism. Renal findings in DDS are predominantly characterized by diffuse mesangial sclerosis of early onset and rapid evolution to end-stage renal failure, while FS usually presents slow progressive focal segmental sclerosis (FSGS). WT1 mutations associated with nephrotic syndrome are restricted to exons 8 and 9, that represent a sort of hot-spot that may be easily investigated. The three major studies [99–101] overall confirm an incidence of WT1 mutation in patients under 18 years around 6-7%. A most remarkable finding is that, in young females, this incidence is higher (10–12%) and probably becoming the most frequent inherited cause of nephrotic syndrome under 18 years in this sex cohort. Moreover, we have demonstrated that WT1 splice mutations are not rare in females under 18 years with SRNS, frequently in absence of phenotype change typical of Frasier syndrome. In adults and children with SDNS, screening analysis is of no clinical value. WT1 hot spot mutation analysis should be routinely done in children with SRNS; if the molecular screening anticipates any further therapeutic approach, it may modify the long term therapeutic strategy [101].

6.2. LMX1B Gene. LMX1B gene mutations are associated with autosomal dominant nail-patella syndrome, a condition displaying dysplastic nails, hypoplastic patellae, and glomerulopathy with proteinuria and hematuria [102]. Its phenotype is highly variable, and the main pathologic finding is an altered GBM. LMX1B gene is a transcription factor that plays an important role in glomerular development, regulating the transcription of multiple genes integral for proper glomerular basement membrane formation and/or glomerular podocyte differentiation and function [103]. LMX1B binds to the putative enhancer sequence of COL4A4, the gene for alpha-4 chain of collagen type IV [103].

6.3. LAMB2 Gene. LAMB2 gene mutations are associated with Pierson syndrome, an autosomal recessive syndrome characterized by congenital nephrotic syndrome with histologic lesions of diffuse mesangial sclerosis and ocular malformations (microcoria, abnormal lens with cataracts, and retinal abnormalities) [104]. LAMB2 gene encodes the laminin beta 2, a protein abundantly expressed in the glomerular basement membrane where it plays a role in anchoring and in the development of podocyte foot processes [105]. LAMB2 mutations have also been found in patients with congenital nephrotic syndrome and either no or less severe ocular abnormalities.

6.4. CD151 Deficiency. Recent work in humans has shown that the tetraspanin CD151 is essential for the function of the kidney, as mutations in *CD151* have been identified in three patients presenting with hereditary nephrotic syndrome leading to end-stage renal failure, pretibial bullous skin lesions, sensorineural deafness, and thalassemia. CD151 is part of the tetraspanin family of proteins that are ubiquitously expressed, membrane-embedded proteins that share a similar structure, and form dynamic complexes with each other and with integrins. Mice deficient in CD151 develop proteinuria, FSGS, and kidney failure [106].

6.5. SMARCALI Gene. Immunoosseous dysplasia is a rare autosomic recessive disorder that presents with spondyloepiphyseal dysplasia, renal dysfunction, and T-cell immunodeficiency [107]. This syndrome is caused by mutations in SMARCALI gene that encodes for a widely expressed protein involved in the chromatin remodelling. The renal involvement is characterized by proteinuria, FSGS, and renal failure.

6.6. Beta4 Integrin Mutation. The occurrence of congenital nephrotic-range proteinuria secondary to focal segmental glomerulosclerosis has been reported in an infant with epidermolysis bullosa and pyloric atresia (EB-PA) [108].

EB-PA is an autosomal recessively inherited disease manifesting in the neonatal period with blistering of the skin and mucous membranes, as well as congenital gastrointestinal abnormalities including esophageal, gastric, or duodenal atresia. Both lethal and nonlethal forms have been described. The condition is caused by mutations in the a6 and b4 integrin genes, which are expressed in the hemidesmosomes of stratified epithelia. Most cases of EB-PA are associated with mutations in the b4 gene, ITGB4, located on the long arm of chromosome 17 [109]. Mutations in the a6 gene, ITGA6, located on the long arm of chromosome 2, are less frequent [110].

In the case reported by Kambham et al., a novel mutation in exon 31 of the b4 integrin gene, ITGB4, was identified. Authors proposed that the b4 integrin gene mutation led to expression of a dysfunctional protein important in the maintenance of normal glomerular permselectivity and podocyte integrity. The development of nephrotic-range proteinuria, without full nephrotic syndrome, is consistent with the role of b4 as a minor podocyte integrin. The failure to detect proteinuria more frequently in EB may relate to early mortality and the unique effect of this novel b4 integrin mutation on podocyte function.

6.7. Renal Disease and Mitochondrial Genetics. Mitochondrial diseases can give rise to various syndromes or association, namely, neurologic and neuromuscular diseases, cardiac, renal, hepatic, hematological and endocrinic, or dermatological presentations. Renal dysfunction associated with mitochondriopathies is generally a rare event. The most frequent renal symptom is proximal tubular dysfunction with a more or less complete de Toni-Debre-Fanconi Syndrome [111]. A few patients have been reported with tubular acidosis, Bartter Syndrome, chronic tubulointerstitial nephritis, or nephrotic syndrome. Any mode of inheritance can be observed: sporadic, autosomal dominant or recessive, or maternal inheritance [111].

Three cases that presented with central and peripheral nervous system involvement and with NS secondary to FSGS in the first decade of life were associated to ubiquinone deficiency [112]. More recently steroid-resistant NS or neonatal renal failure has also been described in patients who bore inherited *COQ2* mutations [113]. Taken together, these data allow identification of a new entity within the category of mitochondrial cytopathies, characterized by inherited *COQ2* mutations, proliferation of dysmorphic mitochondria, and primary glomerular damage. This new entity may be defined as "*COQ2* nephropathy," because the kidney seems to be a primary target in some patients presenting with isolated renal symptoms [113]. *COQ2* mutations cause a renal disease that is characterized by variable renal lesions and widespread proliferation of dysmorphic mitochondria in glomerular cells. The clinical picture can be heterogeneous, and neuromuscular symptoms may complicate the course of the disease. Early recognition of this new entity may be crucial, because clinical symptoms can improve after ubiquinone supplementation, and neurologic complications may be prevented [114].

FSGS lesions have already been associated with mutations in the mitochondrial genome (3243A3G in the tRNALeu(UUR) gene), which may cause isolated glomerular disease [114–116]. Podocyte damage secondary to inherited mitochondrial dysfunction may cause visceral cell depletion, accumulation of extracellular matrix, and ultimately sclerosis of the glomerular tuft. In other cases, the same mitochondrial disease seems to trigger epithelial cell proliferation (in particular podocyte proliferation), associated with GBM collapse. Whereas increased apoptosis of podocyte cells may explain the mechanisms underlying FSGS formation in mitochondrial cytopathies [117], it remains unclear why in some cases the pathway taken by injured podocytes leads to proliferative lesions [118].

Mitochondrial dysfunction and altered mitochondrial gene expression have also been documented in patients with NS secondary to nephrin mutations [119, 120] suggesting that, regardless of the initial insult, mitochondria play an important role in podocyte metabolism and may be actively involved in the pathophysiology of various forms of NS.

6.8. Limp2 Gene. Lysosomal integral membrane protein type 2 (LIMP-2), the product of the SCARB2 gene (MIM_602257), is a member of the CD36 superfamily of proteins [121]. The absence of this protein in mice causes urinary and neurological alterations, associated with impaired vesicular trafficking and distribution of apically expressed proteins [122]. A deficiency in LIMP-2 resulting from a nonsense mutation in the SCARB2 gene has been recently described in humans [123]. When in a homozygous state, the mutation was associated with progressive myoclonic epilepsy without intellectual impairment and a nephrotic syndrome with strong accumulation of C1q in capillary loops of the kidney, whereas healthy parents were heterozygous for the mutation. The main clinical features are nephrotic syndrome, normocytic normochromic anemia, and thrombocytopenia.

The histological analysis of the medullar zone in renal material revealed extensive tubular alterations with isometric vacuolization in distal and collecting tubules and the presence of granular material in cortical tubules without inflammatory infiltration deposits [122].

Berkovic et al. [124] described LIMP-2 mutations in three patients with action myoclonus-renal failure syndrome. Action myoclonus-renal failure syndrome (AMRF [MIM 254900]) is a lethal inherited form of progressive myoclonus epilepsy associated with renal failure. It typically presents at 15–25 years with proteinuria evolving into renal failure or with neurological symptoms (tremor, action myoclonus, seizures, and later ataxia). The renal pathology is of focal glomerulosclerosis, sometimes with features of glomerular collapse. The disorder was mapped to 4q13–21, and microarray-expression analysis identified SCARB2/Limp2, which encodes a lysosomal membrane protein, as the likely candidate. Mutations in SCARB2/Limp2 were found

in all three families used for mapping and subsequently confirmed in two other unrelated AMRF families. The mutations were associated with lack of SCARB2 protein. The heterogeneous pathology in the kidney and brain suggests that SCARB2/Limp2 has pleiotropic effects that may be relevant to understanding the pathogenesis of other forms of glomerulosclerosis or collapse and myoclonic epilepsies. However, mutations in SCARB2 might account for unsolved cases of progressive myoclonus epilepsy (PME) without renal impairment, especially those resembling Unverricht-Lundborg disease (ULD) [125]. Finally, contrary to earlier proposals, LIMB 2 mutations share no features with Charcot-Marie-Tooth disease both at the clinical and neurophysiological levels [124, 125].

7. Podocyte Cytoskeleton and Familial FSGS

The major processes of podocytes have an abundant and dynamic cytoskeleton composed mainly of actin-rich microfilaments, containing several actin-associated proteins, such as myosin, synaptopodin, and α-actinin. Podocyte damage and proteinuria can result from cytoskeletal alterations too, rather than direct alterations in slit diaphragm proteins.

Kaplan et al. found linkage to chromosome 19q13, in three families with clear evidence of autosomal dominant inheritance of FSGS, with late onset. They analyzed the NPHS1 gene, located in this interval, and found no mutations associated with this disorder. By BLAST analysis, they considered ACTN4, encoding α-actninin-4, as candidate gene, and a mutational screening was performed in affected individuals of families. α-actninin-4, an actin-filament cross-linking protein (Figure 1), is highly expressed in glomerular podocytes and involved in nonmuscle cytoskeletal function. ACTN4 missense mutations were identified in affected members of each family. Mutant α-actinin-4 protein binds F-actin more strongly than wild-type α-actinin-4. These data suggest that mutations in ACTN4 gene might cause an increased affinity for actin filaments, and podocyte actin cytoskeleton may be altered in this group of patients. Interestingly, α-actinin-4 deficiency not only causes recessive glomerular disease, but also increases cellular mortality [36]. Thus, lesions which compromise cytoskeletal functions of podocyte appear to result in a slowly progressive loss of podocyte, the hallmark of FSGS.

Consistent with this hypothesis are also the results reported by Winn et al. and Reiser et al. in two recent works, in which a familial late onset form of FSGS, linked to chromosome 11q and caused by mutations in the gene encoding TRPC6, was described [37, 38]. A failure in the receptor-mediated influx of Ca^{++} through mutated TRPC6 protein, a nonselective cation channel, might underlie the new 11q-linked FSGS. Cytoplasmatic calcium concentrations are tightly regulated to prevent cellular damage. The authors speculate that mutated TRPC6 channels might disrupt glomerular homeostasis and/or cause podocyte apoptosis. The onset of kidney disease linked to mutations in TRPC6 gene occurs at a relatively advanced age. There are two possible explanations of this finding: podocytes express several other TRPC channel subtypes, so late onset of disease might be caused by compensation for impaired TRPC6 function by other channels; in addition, TRPC6 mutations might cause only a minor damage in podocyte function and lead to irreversible alterations in the presence of a second glomerular insults [69, 126]. However, to better understand the exact function of TRPC channels in podocytes and their role in familial and acquired forms of FSGS, additional studies should be performed.

Recently, Brown et al. found heterozygous mutations in the formin *INF2* gene segregating with FSGS in 11 (12%) of 93 families with age at diagnosis and ESKD varying from 11 to 72 years and 13 to 67 years, respectively [127]. This finding has been successively confirmed by Boyer et al. in a cohort of 54 families with a glomerular proteinuric disorder of apparent AD inheritance and documented FSGS in at least one affected member. Missense *INF2* mutations were found in nine families (28 patients), translating to a detection rate of 16.7% [128].

INF2 is a member of the formin family of actin-regulating proteins that accelerate actin polymerization [129]. To date, 15 mammalian formin genes have been identified, among which are the best studied diaphanous-related formins (DRF): mDia1, mDia2, and mDia3. In the C-terminal half, DRF proteins contain the forming homology domains FH1/FH2 and the diaphanous autoregulatory domain (DAD) region, whereas the diaphanous inhibitory domain (DID) is localized at the N-terminal half [4]. Interestingly, all of the 13 INF2 mutations associated to FSGS lie within the DID region of the protein [127, 128], and six of them are localized in the corresponding INF2 region of a mDia1 DID subdomain interacting with IQ motif-containing GTPase-activating protein (IQGAP1) [128]. IQGAP1 has been identified as a Dia1-binding protein that is necessary for its subcellular location [130]; it is also involved in actin cytoskeleton dynamics [131] and has been shown to interact with the podocyte proteins nephrin [132] and PLCE1 [133]. Although, the exact mechanism explaining how mutations in the INF2 gene may lead to a proteinuric phenotype remains unclear, and these first observations reinforce the idea of podocytes as dynamic structures that are extremely sensitive to alterations in the spatial or temporal regulation of the actin cytoskeleton. Thus, *INF2* seems to be a major gene of AD FSGS. Screening for *INF2* mutations, at least in exons 2 to 4, encoding the DID domain, should be strongly considered in patients with an AD familial history of FSGS, even before *ACTN4* and *TRPC6*.

8. The Other Side of the Moon: Immunopathogenesis of Idiopathic Nephritic Syndrome

Although recent genetic approaches have elucidated the disease pathogenesis through the discovery of several podocyte genes mutated in distinct forms of hereditary nephrosis, the molecular basis of minimal change nephritis syndrome and FSGS with relapse remains still unclear. In this setting, the immune system seems to play a critical role in the active

phase of this disease through disturbances involving several cell subsets, mainly T cells.

MCNS may be a systemic disorders of T-cell function and cell-mediated immunity [134]. Nowadays, it is quite clear that MCNS is the most common kidney disease associated with primary immunological disorders and that the sensitivity to steroid and immunosuppressive therapy is an important argument in favour of the immune origin of the MCNS [135]. Recent reports suggest a clonal expansion of CD8+ T cells expressing the memory T-cell marker, CD45RO [136]. and of CD4+ T cell expressing the CD25 antigen, the IL-2 receptor chain in long-lasting, active disease. Moreover, also the native immune system seems to be involved in MCNS, probably through the signalling pathway of the NF-κB [137]. It has been shown that peripheral mononuclear cells, including T cells, exhibit high NF-κB binding activity involving the p50/p65 complexes during relapses, which returned to basal levels during remissions [138]. There is a T-cell commitment towards a Th2 phenotype in MCNS that might explain why these patients often display a defect in delayed-type hypersensitivity response, suggesting an abnormal Th1-dependent cellular immunity [135].

Moreover, a second set of signals mediated by co-receptors is needed to promote T-cell proliferation, lymphokine secretion, and effector functions. In this setting, C-mip is as new discovered gene of unknown function, which is initially identified in T lymphocytes of patients with MCNS [139]. c-mip interferes at different levels of cell signaling and in particular is involved in the Th2 signaling pathway [140].

An hypothesis unifying T-cell disorders and podocyte dysfunction has recently been proposed by Zhang and coworkers [135]. The functional alterations allowing to nephritic proteinuria could result from the downregulation of transduction pathways playing a key role in slit diaphragm function such as the nephrin-mediated pathway. It is possible that the circulating factor is somehow linked to the NF-κB pathway and that podocyte and immune cells might share the same molecular defect [135].

9. Conclusions

Recent molecular studies have allowed a better understanding of structure and function of glomerular filtration barrier.

Nephrin seems to be the main member of the slit diaphragm, where it forms a zipper-like ultrafilter structure; podocin and CD2AP, instead, have probably the function to connect the cytoplasmic domain of nephrin to cytoskeleton and lipid rafts of the SD. Moreover, the latter findings on ACTN4, TRPC6, and INF2 proteins suggest an important role of cytoskeletal dynamics in the normal maintenance of podocyte function.

Nephrotic syndrome is a genetically heterogeneous condition. In general, recessive mutations in *NPHS1*, *NPHS2*, and *PLCE1* are associated with more severe disease with earlier onset proteinuria and ESRD presenting in infancy and throughout childhood, although some milder cases have also been noted. By contrast, dominant mutations in *ACTN4*, *TRPC6*, and *INF2* are associated with milder disease with later onset proteinuria in the second decade and ESRD in the third and fourth decades of life.

This new important discoveries may provide useful guidance to the clinicians in deciding whether a course of immunosuppressive drug treatment is appropriate. However, additional molecular genetics and *in vivo* studies should be improved to apply this new knowledge for the development of comprehensive molecular diagnostic test and new mechanism-based therapeutic tools.

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Review Article

Nephrotic Syndrome in Children: From Bench to Treatment

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Idiopathic nephrotic syndrome (INS) is the most frequent form of NS in children. INS is defined by the association of the clinical features of NS with renal biopsy findings of minimal changes, focal segmental glomerulosclerosis (FSGS), or mesangial proliferation (MP) on light microscopy and effacement of foot processes on electron microscopy. Actually the podocyte has become the favourite candidate for constituting the main part of the glomerular filtration barrier. Most cases are steroid sensitive (SSINS). Fifty percents of the latter recur frequently and necessitate a prevention of relapses by nonsteroid drugs. On the contrary to SSINS, steroid resistant nephrotic syndrome (SRINS) leads often to end-stage renal failure. Thirty to forty percents of the latter are associated with mutations of genes coding for podocyte proteins. The rest is due to one or several different circulating factors. New strategies are in development to antagonize the effect of the latter.

1. Introduction

Nephrotic syndrome (NS) is an illness consisting in leakage of proteins in urine, resulting in life threatening conditions due hypovolemia, hypercoagulation, and infection. The annual incidence of NS in children in the USA and in Europe has been estimated to be 1–7 per 100,000 children, with a cumulative prevalence of 16 per 100,000 children [1–3]. Nephrotic syndrome in children can be classified according to 3 three groups [3]: secondary, congenital and infantile, and idiopathic.

Secondary nephrotic syndrome is defined as nephrotic syndrome associated with well-defined diseases that are inflammatory (e.g., lupus nephritis, acute postinfectious glomerulonephritis, IgA nephropathy, Henoch-Schönlein purpura, etc.) or not (e.g., Alport syndrome, focal sclerosis due to reduced nephronic mass resulting from renal scarring, etc.).

Congenital and infantile NSs are occurring before the age of one year and are mostly associated with infections (e.g., syphilis, toxoplasmosis, etc.) or with mutations of genes coding for podocytes proteins and are steroid resistant.

Idiopathic nephrotic syndrome (INS) is the most frequent form of NS in children representing more than 90 percent of cases between 1 and 10 years of age and 50 percent after 10 years of age [1]. INS is defined by the association of the clinical features of NS with renal biopsy findings of diffuse foot process effacement on electron microscopy and minimal changes (called minimal change disease (MCD)), focal segmental glomerulosclerosis (FSGS), or diffuse mesangial proliferation (DMP) on light microscopy [4]. Most patients have histologic findings of MCD. The vast majority of patients with MCD (>90 percent) respond to glucocorticoid therapy whereas only 50 percent of those with DMP and 30 percent of those with FSGS are expected to do so [5]. Clinical findings at presentation differentiate children with MCD from those with other glomerular pathology [1]. The latter include: age younger than six years of age, absence of hypertension, absence of hematuria, normal complement levels, and normal renal function. However, onset of nephrotic syndrome in the first year of life, particularly in the first three months of life, is more likely to be due to a gene mutation and to be resistant to glucocorticoids [6].

It is therefore actually generally admitted that a course of glucocorticoids should be given without previous kidney biopsy when the illness has started after the age of one year whereas the upper age limit to do so is generally considered to be 10 years since only 10 percent of patients under 10 years old are steroid resistant in comparison with 20% for the totality of patients less than 18 [4].

2. The Slit Diaphragm

The INS pathophysiology has been attributed in the past mainly to structural abnormalities and a loss of anionic charges of the glomerular basal membrane (GBM) leading to proteinuria. Actually the podocyte has become the favourite candidate for constituting the main part of the glomerular filtration barrier. The latter is highly specialized, terminally differentiated cells with cytoplasmic extensions, the so-called foot processes anchored on the GBM, forming the slit diaphragm (SD) which is essential in retaining proteins inside the lumen of capillary loop.

Genetic studies of hereditary forms of NS have led to the identification of proteins playing a crucial role in slit-diaphragm signalling, regulation of actin cytoskeleton dynamics, maintenance of podocyte integrity, and cell-matrix interactions. The latter have been recently reviewed [7]. Structural elements of the SD (nephrin, podocin, and CD2AP) and actin cytoskeleton (a-actinin-4) control podocyte differentiation and survival, cell polarity, and cytoskeletal dynamics. Podocyte and glomerular development are critically regulated by the transcription factor *WT1* and phospholipase C 11 (PLC11) mediated signals. The calcium channel TRPC6, which localizes in membrane lipids supercomplex along podocin, regulates mechanosensation sensed at the SD, whereas the structural component of the GBM, laminin-b2, is essential for podocyte cell-matrix interactions. Podocyte integrity may also be affected by derangements in proteins involved in varied subcellular processes including the mitochondrial respiratory chain, DNA restructuring and repair, and lysosomal function. Finally, identification of novel genetic determinants in glomerular disease, such as high-risk haplotypes in the *MYH9* gene, may also explain the increased risk of some adult patients to glomerular injury.

3. Mutations of Genes Coding for Podocytes Proteins

INS secondary to mutations of genes coding for podocytes proteins is typically steroid resistant.

Excellent reviews on this topic [6–9] including the following systematic approach for genetic testing by the group of Antignac [8] have been recently published. A mutation of *NPHS1*, encoding nephrin and responsible for the Finnish-type congenital nephrotic syndrome, is found in most patients presenting with a nephrotic syndrome in the first 3 months of life. The second most frequent mutation in that age group concerns *NPHS2*, which encodes podocin and is particularly frequent in Central Europe. Finally some patients less than 3 months of age present with a mutation of the *WT1* (also often associated with the Denys-Drash and the Frasier syndromes characterized by gonads and genital abnormalities) and/or the PLCE1 genes, particularly when the renal biopsy shows diffuse mesangial sclerosis. In the group of patients starting the illness between 4 and 12 months (infantile nephrotic syndrome) and later on (childhood nephrotic syndrome), *NPHS2* followed by *NPHS1* is the first genes to be tested in nonsyndromic patients presenting SRNS associated with minimal glomerular changes/FSGS in the infantile or childhood period. In the remaining patients with the same histological lesions, genetic testing for *WT1* mutations (exons 8 and 9 in phenotypically female patients) should be performed, while screening for PLCE1 mutations may be considered in some cases (mainly in familial cases). Mutations in the *CD2AP*, *ACTN4*, *TRPC6*, and *INF2* have also been found anecdotally in childhood SRNS ([10] and for review, [7–9]).

Steroid resitant nephrotic syndrome accompanying several rare syndromes is also reported. A mutation of LAMB2, which encodes laminin beta 2 (a component of the glomerular basement membrane), is responsible for Pierson syndrome. Other rare syndromal conditions are mitochondrial disorders (gene *NA* coding for nonprotein tRNA), Nail-patella syndrome (gene *LMX1B* coding for LIM homeobox transcription factor 1 beta), Schimke immunoosseous dysplasia (*SMARCAL1* coding for SWI/SNF2-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a-like 1), Mandibuloacral dysplasia (*ZMPSTE24* gene coding for the zinc metalloproteinase STE 24), and Galloway-Mowat syndrome (gene *GMSI* coding for *GMSI*).

Genetic studies are indicated when steroid resistance has been demonstrated in order to withdraw immunosuppression if a mutation is present or in case of congenital nephrotic syndrome before starting any treatment since the great majority of cases is steroid resistant and since steroid treatment can be complicated by severe infectious in neonates [3].

The causes of NS recurrence after transplantation in FSGS cases (30–50%) have been recently reviewed and analyzed by the group of Antignac [8]. In contrast to patients with an immune form of NS, those with an inherited structural defect of the glomerular filtration barrier represent a subset of patients for whom the primary disease cannot a priori recur. Surprisingly, recurrence of proteinuria post transplantation has been reported in some patients bearing mutations in the *NPHS1*, *NPHS2*, *ACTN4*, and *WT1* genes (for a review, [8]), and the mechanism of recurrence remains unsolved for a significant proportion of these cases. The most often provided explanation for recurrence of NS is the development of antibodies against the "neoantigen" which is suggested by the fact that treatment with steroids, cyclophosphamide, and plasmapheresis may lead to remission; however, the percentage of graft loss remains significant (for a review, [8]). However, this eventuality is not frequent and genetic studies remain useful before transplantation to precise the risk of posttransplant recurrence and to help for the decision of living kidney donor or not.

4. Role of Immunity

INS that is not associated with a mutation of genes coding for podocyte's proteins is actually thought to be the consequence of an immunological dysfunction leading to a circulating factor that modifies the permeability of the glomerular filtration barrier.

In 1974, Shalhoub [11] proposed that MCNS was a disorder of lymphocyte function with increased plasma levels of a lymphocyte-derived permeability factor. This hypothesis was based on several clinical observations that suggested the involvement of the immune system in the pathogenesis of idiopathic NS, for example, the response to immunosuppressive drugs and the association with Hodgkin disease and with allergy.

The possible role of allergy has been reviewed by van den Berg and Weening [12]. Many reports have been published on patients who developed NS after having experienced allergic reactions to inhaled allergens, with vaccinations, food, and insect stings. Furthermore, the incidence of atopy was reportedly higher in patients with idiopathic NS than in healthy subjects, ranging from 17 to 40% in MCNS patients compared with 10–23% in age-matched control subjects. Allergy is associated with an elevated production of IgE by B-lymphocytes, and several investigators have reported an elevation of IgE in the serum of NS patients.

The role of circulating factor is particularly suggested by the following observations (for a review, [13]): (1) lmmediate recurrence of proteinuria after transplantation, (2) transfer of proteinuria to fetus, (3) efficacy of plasmapheresis and immunoadsorption in reducing proteinuria, (4) transfer of proteinuria to rat after injection of serum or plasma.

4.1. Role of T Lymphocytes and Cytokines. Valuable studies are difficult to obtain since homogeneous patients groups are necessary and it is often not the case since the duration of proteinuria varies at the time of presentation and some patients may have already started treatment. Hyperlipidaemia which is a common complication may activate the immune system, as shown by Lenarsky et al. [14].

Van de Berg and Weening [12] have recently reviewed the immunological role of T cells and cytokines in INS. Frank et al. [15] showed that CD8-positive T-cells of idiopathic NS patients are clonally expanded, which was not observed in healthy controls. Zhang et al. [16] showed high levels of NF-κB (nuclear factor κB) DNA-binding activity in T-cells from untreated MCNS patients during relapse compared with the MCNS patients in remission while treated with immunosuppressants. This points to activation of the T-cells in MCNS. Kimata et al. [17] studied the unstimulated production of cytokines by T-lymphocytes of MCNS patients and found an increased production of IL-13, whereas production of IL-4 was normal. An elevated expression of IL-13 mRNA was shown by Yap et al. [18] using a semiquantitative RT- (reverse transcriptase-) PCR technique. Using a subtractive cDNA library screening technique, Zhang et al. [16] reported differential expression of transcripts involved in the T-cell receptor-mediated complex signaling cascade and a decreased expression of IL-12 receptor 2 mRNA by PBMC in untreated MCNS patients during relapse compared with MCNS patients in remission. The latter studies reveal the involvement of T-cells in the pathogenesis of idiopathic NS and, more specifically, Th2-mediated immunity. Van de Berg and Weening [12] have studied, by quantitative real-time PCR, the expression of IL-1β, IL-1ra (IL-1 receptor antagonist), IL-2, IL-4, IL-5, IL-9, IL-10, IL-13, TNF-α, and IFN-γ by PBMC from patients with MCNS during relapse and remission and from a control group of patients with NS primarily caused by endogenous alterations within the glomerular filter, for instance, mutations in the genes encoding nephrin and podocin. Out of the cytokines studied, only the expression of IL-10 and IL-13 mRNA was significantly upregulated in relapsing MCNS patients when compared with MCNS patients in remission. The latter authors and others (for a review, [12]) have shown that podocytes constitutively express functional transmembrane receptor complexes for IL-4, IL-10, IL-13, and TNF-α. The possible role of IL-13 is also suggested by the NS rat model of Lai et al. [19]. IL-13 was overexpressed in Wistar rats through transfection of a mammalian expression vector cloned with the rat IL-13 gene, into the quadriceps by in vivo electroporation. The IL-13-transfected rats showed significant albuminuria, hypoalbuminemia, and hypercholesterolemia when compared with control rats. No significant histologic changes were seen in glomeruli of IL-13-transfected rats. However, electron microscopy showed up to 80% of podocyte foot process fusion. Glomerular gene expression was significantly downregulated for nephrin, podocin, CD 80, and dystroglycan. Immunofluorescence staining intensity was reduced for nephrin, podocin, and dystroglycan IL-4R alpha in IL-13-transfected rats compared with controls.

Abdel-Hafez et al. [20] suggest in review of the literature and of their own results that the relation between allergy and INS could be the stimulation by IL-13 of the expression of CD 80 on podocytes. They report that urinary CD80 levels are increased in patients with MCD during relapse and return to normal after remission. They also have preliminary evidence that the source of the CD80 is the podocyte because they found, by using immunohistochemical staining, that CD80 was expressed by podocytes in kidney biopsy specimens from patients with MCD in relapse.

The successful treatment of SRNS on native kidney or after kidney transplantation with anti-TNFα antibodies strongly suggests that this cytokine participates to the pathogenesis of some types of idiopathic nephrotic syndrome [21, 22]. It is also suggested by high levels of TNFα in patients with active disease and TNFα normalization with remission and by an animal model of NS that is controled by anti-TNFα agents (for a review, [21, 22]).

4.2. Role of B Lymphocytes. The beneficial treatment by rituximab, a monoclonal antibody directed against CD20, in difficult SSINSs suggest, a role for B cells in INS [23–25]. Sellier-Leclerc et al [25] underline that most arguments gathered by Shalhoub [11] in support of T cell dysfunction have a counterpart to supporting B cell dysfunction and that the contribution of B cells and the potential role of immunoglobulin chains in modifying the glomerular

permeability to protein in children with steroid-sensitive nephrotic syndrome have been repeatedly reported [25]. In addition, B cells may be involved through an unidentified antibody-independent pathway, that might be a control on T cells [26].

5. Possible Permeability Factors

Aside cytokines other factors have been suspected to be involved in the pathogenesis of INS. One of them consists in radical oxygen species (ROS) that have been largely studied by the group of Ghiggeri (for a review, [27]). Some experimental models of nephrotic syndrome result from substances as puromycine and adriamycin that induce oxidative stress in glomeruli. Furthermore the injection of H_2O_2 induces proteinuria in rats and NO prevents the increase of permeability to albumin induced by the production TNF alpha-induced O_2- production in an isolated rat glomeruli system [28]. Active focal segmental glomerulosclerosis is associated with massive oxidation of plasma albumin [27]. Recently, Bertelli et al. [29] demonstrated a 10-fold increase of ROS production by resting PMN in INS compared to normal PMN. When PMNs were separated from other cells, ROS increased significantly in all conditions while a near normal production was restored by adding autologous cells and/or supernatants in controls, vasculitis, and postinfectious glomerulonephritis but not in INS. The second finding was that the oxidative burst by PMN was regulated highly by T lymphocytes, mainly Tregs, by means of soluble factors and that this regulatory circuit was altered in INS.

The group of Moin Saleem has intensively studied the possible pathophysiological role of hemopexin (Hx) in INS [30, 31]. Hx is well described as a heme-scavenging protein. It is predominantly produced in the liver, and it increases in the acute phase reaction to inflammation or infection. Plasma-purified and recombinant Hx has been shown to have serine protease activity. It has been suggested that in normal conditions circulating Hx is inactive but under certain circumstances Hx becomes activated as a serine protease. Activated Hx has been shown to have dramatic effects on the glomerular filtration barrier. Kidney sections incubated with Hx have a reduction of the anionic layer and reduced sialoglycoproteins. In vivo, activated Hx induced reversible proteinuria in rats parallel to podocyte foot process effacement. Activated hemopexin is increased in children with minimal change nephrotic syndrome [31]. In vitro within 30 minutes of treatment with hemopexin, actin reorganized from stress fibers to cytoplasmic aggregates and membrane ruffles in wild-type podocytes [30, 31]. This process is nephrin dependent since it did not occur in nephrin-deficient podocytes and in cells that do not express nephrin and was inhibited by preincubation with human plasma. In addition, hemopexin led to a selective increase in the passage of albumin across monolayers of glomerular endothelial cells and to a reduction in glycocalyx. What remains to be elucidated is the primary events leading to the activation of Hx. A possibility resides in the inhibition of Hx inhibitors or in their leakage in urine. In the latter case, Hx activation should be only a secondary event depending on the increased permeability of the glomerular filtration barrier to proteins.

The group of Virginia Savin in US has studied and characterized the circulating factor in FSGS by analyzing the plasma of patients presenting with a posttransplant relapse (for a review, [13]). Those studies are based on standard methods of biochemical purification and analyses of molecular characteristics followed by gel electrophoresis and mass spectrometry. They have used a functional assay of permeability activity with isolated rat glomeruli that shows changes in the glomerular capillary permeability to albumin after incubation with the patient plasma or serum. This assay has made it possible to perform sequential purification steps and select fraction(s) with enhanced activity. Using galactose as an effective affinity material to enrich activity of FSGS plasma they reported that cardiotrophin-like cytokine factor 1 (CLC-1; encoded by *CLCF1*), a member of the interleukin 6 family, is present in the enriched fraction of FSGS plasma, and that CLC-1 increases glomerular P_{alb}, and its injection causes proteinuria in rats.

6. Treatment

Pathophysiological consequences of nephrotic syndrome as hypovolemia, acute renal failure, edema, hypercoagulation, and infections should be treated symptomatically.

The basis of treatment of INS by steroids was the early hypothesis of the implication of an immunological factor in the pathophysiology of the disease. The latter has also lead to use immunosuppressive drugs to prevent relapses. However, recent experiments using podocytes in vitro have shown that steroids and cyclosporine, aside their effect on the immunological system, may also act directly on the podocyte to stabilize its structure [32, 33].

Recovery of a normal permeability to proteins of the glomerular filtration barrier is rendered possible by steroids in the majority of cases. The Cochrane reviews that use severe criteria of quality to analyze results of clinical studies are very useful for the clinician.

6.1. Steroid Sensitive Idiopathic Nephrotic Syndrome. The treatment of the primary immunological cause of INS resides in the use of steroids. The latter allows the differentiation between steroid sensitive and steroid resistant nephrotic syndrome. Different protocols are used according to countries. Initial dosage of 60 mg/m^2/d prednisone or prednisolone pursued by alternate day administration to reduce side effects is a common feature. Steroids protocols are mostly differing by duration, way of tapering, and definition of steroid resistance. A 2007 Cochrane review [34] has analyzed the results of the literature concerning the best initial steroid treatment. The authors conclude that in their first episode of SSNS patients should be treated for at least three months with an increase in benefit for up to seven months of treatment. For a baseline risk for relapse following the first episode of 60% with two months of therapy, daily prednisone or prednisolone given for four weeks followed by alternate-day therapy for six months would reduce the number of children relapsing by 33%. However, it remains to be known if the

time of administration or the cumulative steroid dosage is the most important determinant. This question is addressed partly in a double blind RCT comparing the effect of the same cumulative prednisone dosage given during 3 or 5 months by a study actually going on at the University of Rotterdam (The Netherlands).

In case of frequently relapsing or steroid-dependent nephrotic syndrome, repeated and prolonged high dosage of prednisone might lead to severe side effects as growth retardation, osteoporosis, infections, diabetes, cataract, hypertension, hirsutism, and Cushing aspect. In order to avoid the latter, different nonsteroid treatments have been used. The 2008 Cochrane analysis of the already published results of the latter concludes that eight-week courses of cyclophosphamide and prolonged courses of cyclosporin and of levamisole reduce the risk of relapse in children with relapsing SSNS compared with corticosteroids alone [35]. They conclude also that clinically important differences in efficacy are possible and that further comparative studies are still needed. Furthermore side effects should be taken into account by the clinician. Infection, infertility, and possibly cancer at long term for cyclophosphamide and hypertension, hirsutism, and chronic renal failure for cyclosporine lead the clinicians to consider alternative treatments. A preliminary study comparing cyclosporine to mycophenolate mofetyl suggested that the latter could replace cyclosporine in the majority of cases [36]. Recently, the successful use of rituximab, a monoclonal anti-CD20 antibody, has been reported to prevent relapses in difficult steroid-dependent SSINS in several reports [23–25]. However, the latter should be confirmed in a RCT to better define efficacy, modalities (administration during relapse or after remission induction, used alone or in combination), indications, and safety. Presently, rituximab has to be considered as the last option, for patients who cannot be managed properly despite alternate day steroids, MMF, and anticalcineurins (because of ongoing relapses and/or side effects of drugs and/or noncompliance).

6.2. Steroid Resistant Idiopathic Nephrotic Syndrome. The last Cochrane review on this topic [37] reports that when cyclosporin was compared to placebo or no treatment, there was a significant increase in the number of children who achieved complete remission. Cyclosporin also significantly increased the number of children, who achieved complete or partial remission compared with IV cyclophosphamide. There was no improvement with other immunosuppressive agents. However the number of studies was small. More research is needed. The analysis of results of treatment of SRINS is complicated by the heterogeneity of steroid resistance definition according to protocols and countries. Another issue concerns the lack of studies considering the presence of mutations of podocytes proteins to explain different responses to treatment. It seems indeed important to distinguish the latter from SRINS possibly due to a circulating factor since any immunological treatment is most probably ineffective in the former. When no mutation is detected, a circulating factor might be suspected, particularly when a recurrence is observed after kidney transplantation. In that case, removal of the latter by plasma exchange can be effective but recurrence is often observed when treatment is interrupted. The future treatment strategy will imply the determination of the latter factor and the use of specific treatment to antagonize its effect. Based on this principle, RCTs studying the effect of galactose and anti-TNF Ab have already been initiated by the FONT study group [38]. Another approach used by the latter group is to antagonize the effect of TGFβ, a cytokine playing a major role in fibrotic processes [39].

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Review Article

Hemolytic Uremic Syndrome: New Developments in Pathogenesis and Treatment

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Hemolytic uremic syndrome is defined by the characteristic triad of microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure. In children, most cases of HUS are caused by Shiga-toxin-producing bacteria, especially Escherichia coli O157:H7. Common vehicles of transmission include ground beef, unpasteurized milk, and municipal or swimming water. Shiga-toxin-associated HUS is a main cause of acute renal failure in young children. Management remains supportive as there is at present no specific therapy to ameliorate the prognosis. Immediate outcome is most often favourable but long-term renal sequelae are frequent due to nephron loss. Atypical HUS represents 5% of cases. In the past 15 years, mutations in complement regulators of the alternative pathway have been identified in almost 60% of cases, leading to excessive complement activation. The disease has a relapsing course and more than half of the patients either die or progress to end-stage renal failure. Recurrence after renal transplantation is frequent.

1. Introduction

The hemolytic-uremic syndrome (HUS) is defined by the association of hemolytic anemia (low haptoglobin levels, high lactate deshydrogenase levels, and schistocytes), thrombocytopenia, and acute renal failure [1]. It is a common cause of acute renal failure in patients younger than three years who require acute dialysis. The renal histological lesion is glomerular thrombotic microangiopathy and in more severe cases, arteriolar thrombotic microangiopathy. Increased platelet aggregation and thrombi affect the microcirculation. Thrombotic microangiopathy may affect other organ systems, including the central nervous system [2].

HUS can be divided into two forms, typical and atypical HUS. Typical D^+ or shiga-toxin associated HUS is the most common form of HUS in children accounting for 90 percent of all cases. It usually occurs after a prodromal episode of diarrhea that is frequently bloody. In the majority of cases, HUS is associated Shiga toxin-producing enterohemorrhagic Escherichia coli (EHEC) or Shigella [3–7] and generally carries a good prognosis [6]. Atypical HUS (aHUS) is a heterogeneous disorder which is responsible for only 10 percent of cases in children [8, 9]. It is also referred to as nondiarrhea-associated HUS, D-HUS, or sporadic HUS. In addition to the usual absence of a diarrheal prodrome, children with aHUS may have an insidious onset, a relapsing course, or a progressive course leading to severe renal dysfunction [1, 8, 10]. Blood pressure is often markedly increased. There is a high risk of recurrence after renal transplantation.

2. Shiga-Toxin-(Stx-) Associated HUS

In the majority of cases, Stx-HUS is triggered by strains of EHEC that produce a Shiga toxin [3, 7, 11], but Shigella dysenteriae can also be involved [4, 12]. It usually occurs after a prodromal episode of diarrhea that is frequently bloody in nature. Five to 10 days after onset, HUS develops abruptly [13, 14]. Cases of HUS in children due to Shiga toxin-producing E. coli infections other than colitis (e.g., urinary tract infections) can occur [15]. Although these cases are not associated with diarrhea, the clinical course and pathophysiology are the same as the D+ HUS.

2.1. Epidemiology. Stx-HUS principally affects young children. The annual incidence of the disease in North America and Western Europe is about 2 to 3 per 100,000 children less than five years of age [6, 12]. Stx-HUS occurs most commonly in the summer months [11] and is more frequent in rural versus urban populations [7]. Most cases are sporadic, but outbreaks are often reported due to a common contaminated food or water source. The natural reservoir of EHEC is the digestive tract of healthy cattles Infection in humans occurs following ingestion of contaminated undercooked meat, unpasteurized milk or milk products, water, fruits, or vegetables [16]. Secondary human-to-human transmission is also a major risk factor for HUS [14, 17] and may be a concern in day-care centers or in sibships.

EHEC causes at least 70 percent of cases of postdiarrheal HUS in the United States, 80 percent of which are caused by E. coli O157:H7, but non-O157 strains are also involved. Although laboratory diagnosis of EHEC infection [18] can be made by stool culture, the bacteria are only present in the stools for a few days and, even if present, may not be detected by culture from stool samples. The rate of stool isolation is substantially higher in the first six days after onset of diarrhea. Direct detection of shigatoxin in stool is another diagnostic tool. Only 10–15% of children with E. coli O157 colitis eventually develop HUS [19] suggesting that, in addition to pathogen factors, host factors also contribute to its development. Shigella dysenteriae type 1-associated HUS occurs in India, Bangladesh, and southern Africa. Although the pathogenesis of disease is similar to that of HUS induced by O157 E. coli infection, the disease is usually more severe with an acute mortality rate of 15 percent and over 40 percent of patients developing chronic renal failure [12].

E. coli infection has become a major health care concern. Prevention recommendation should be provided to patients and their parents including meticulous hygiene when cooking and changing diapers, avoidance of undercooked meat and unpasteurized milk products in young children. Cattle vaccination could also help reducing the reservoir of E. coli. Promising results have been obtained in reducing the fecal shedding of E. coli O157:H7 and hide contamination following cattle vaccination [20–22]. But larger epidemiological studies are needed to assess a potential beneficial effect of cattle vaccination for food and environmental safety.

2.2. Pathophysiology. Shiga toxin-(Stx-) mediated injury to vascular endothelial cells in the kidney, brain, and other organs underlies the pathogenesis of HUS caused by EHEC. These potent cytotoxins are released in the gut by bacteria, enter the blood stream, and cause endothelial injury through the binding to the globotriaosylceramide (Gb3) receptor on the plasma membrane of target cells [23]. Gb3 is a sphingolipid receptor expressed on endothelial cells, podocytes, and proximal tubular cells in human. Stx binding to Gb3 leads to Stx internalization by receptor-mediated endocytosis and its retrograde transport to the endoplasmic reticulum. This triggers a cascade of signalling events, involving NF-κB activation, which induces apoptosis and the binding of leukocytes to endothelial cells [24]. Stx-activated endothelial cells become thrombogenic by a complex mechanism not yet fully unravelled [24]. It has been demonstrated that Stxs modulate the expression of endothelial cell adhesive molecules such as β3-integrin subunit, vitronectin receptor, PECAM-1, and P-selectin on the microvasculature [25] and induce the release of cytokines and chemokines by endothelial cells, implicated in platelet activation [26]. Moreover, upon Stx activation, endothelial Von Willebrandt factor has been shown to undergo conformational changes thereby mediating platelet adhesion on activated endothelial cells [25]. Additionally, Stxs stimulate *in vitro* the release of unusually large Von Willebrandt factor strings and impair their cleavage by the metalloprotease ADAMTS-13 [27]. Altogether, these endothelial cell dysfunctions associated to platelet activation may promote platelet adhesion on glomerular endothelial cells, thrombi formation, and glomerular occlusion in Stx-HUS. An *in vitro* study showed that in addition to directly damaging the kidney, Stxs also activate complement and delay the function of its inhibitor, factor H, on the cell surface, leading to indirect kidney damage [28].

Stxs include two major antigenic forms (Stx1 and Stx2) with different subtypes which differ in potency. Stx2a and Stx2d have been reported as the most potent [29]. Stx type-specific mechanisms of endothelial cell injury have been suggested in cellular models [30]. Bauwens et al. demonstrated that Stx1 causes both necrosis and apoptosis, whereas Stx2 induces almost exclusively apoptosis in human brain microvascular endothelial cell lines and inmacrovascular endothelial cells [30]. Free toxins have never been detected in the blood of HUS patients, but they have been found on the surface of polymorphonuclear leukocytes [31]. Purified Stxs alone are capable of producing HUS with glomerular thrombotic microangiopathy in large animal models such as baboons [32]. Conversely, rodents develop tubular lesions but no glomerular damage after Stx injection. This may be related to Gb3 expression differences among species. Indeed, Gb3 expressed on tubular cells is absent from glomerular endothelial cells and podocytes in mice [23]. Similarly, cattle which are the main vectors of EHEC lack Gb3 expression in their glomeruli. This may partly explain why they do not develop HUS [33]. This led to the hypothesis that different patterns of Gb3 expression with age could explain the predominance of HUS among young children. However, it was shown that Gb3 expression in the kidney and Stx1 binding to kidney structures does not vary with age [34]. Factors other than basal renal Gb3 expression, yet to be identified, account for the age-related incidence of Stx-HUS in EHEC infection. The increased frequency of antibodies to Stx1 and Stx2 suggests that preexisting immunity may play a role in age-specific host resistance to HUS [35].

2.3. Clinical Manifestations. Stx-producing E. coli causes a spectrum of outcomes ranging from asymptomatic carriage to diarrhea that may be uncomplicated or bloody to HUS. The diarrhea and associated gastrointestinal complaints in the prodromal phase of Stx-HUS may mimic those of ulcerative colitis, other enteric infections, and appendicitis. HUS is defined by the sudden onset of microangiopathic

hemolytic anemia with fragmented erythrocytes named schistocytes and negative Coombs' tests, thrombocytopenia, and acute kidney injury [11]. Despite the thrombocytopenia, there is usually no purpura or active bleeding. Whereas the degree of anemia or thrombocytopenia is unrelated to the severity of renal dysfunction, an increased white blood cell count is associated with a worse prognosis [36]. The hematologic manifestations of Stx HUS completely resolve usually within one to two weeks.

The renal involvement ranges from hematuria usually microscopic and proteinuria, to severe renal failure and oliguria that occur in one-half of cases. Hypertension is common. Dialysis is instituted to correct metabolic abnormalities when required. The short-term renal prognosis is generally favourable. However, the risk of renal failure 20 years after the recovery of Stx-HUS is not negligible, and renal histology showing a glomerular microangiopathy affecting >50 percent of glomeruli, arterial microangiopathy, and/or cortical necrosis is the best indicator of long-term prognosis [37, 38].

Stx-HUS commonly affects other organ systems [36]. Central nervous system involvement occurring in 20–50 percent of children with Stx-HUS is the most threatening complication associated with increased morbidity and mortality [2, 39, 40]. Patients may present with seizures, coma, stroke, hemiparesis, facial palsy, pyramidal or extrapyramidal syndromes, dysphasia, diplopia and cortical blindness [39]. Brain MRI typically reveals bilateral hypersignal on T2-weighted and hyposignal on T1-weighted images of basal ganglia, thalami, and brainstem sometimes extending to the surrounding white matter [41]. Additionally, MRI may display images of high blood pressure complications such as reversible posterior leukoencephalopathy syndrome and cerebral hemorrhage. In a large French multicenter series of 52 children with Stx-HUS and neurological involvement, nine patients died, 17 had mild to severe sequelae, and 26 fully recovered [2].

The whole digestive tract can also be involved. The more serious manifestations include severe hemorrhagic colitis, bowel necrosis and perforation, rectal prolapse, peritonitis, and intussusception [42]. Cardiac ischemia and dysfunction may occur. Transient and rarely permanent diabetes mellitus may occur [42]. Hepatomegaly and/or increased serum transaminases are frequently observed. The mortality rate has dropped to about 5%, usually due to neurologic or cardiac involvement [43]. Persistent oliguria (>5 days of anuria and >10 days of oliguria), dehydration, elevated white blood cells > 20,000 per mm^3, and hematocrit > 23 percent are risk factors for death [43] and long-term complications from HUS [44].

2.4. Therapy. Once a patient is infected with EHEC, attempts to prevent progression from the bloody diarrheal phase to the postdiarrheal phase of HUS have been unsuccessful. Antimotility drugs (such as anticholinergic agents and narcotics) do not reduce the progression to HUS due to EHEC infections, but in fact appear to increase the risk of subsequent development of HUS. In patients with EHEC, both retrospective and prospective observational studies report an increased risk of HUS with the administration of antibiotics during the bloody diarrheal phase [45]. In one retrospective review of 278 children with HUS, the use of antimotility drugs (anticholinergic agents and narcotics) was associated with an increased risk of subsequent development of HUS (OR 2.9, 95% CI 1.2 to 7.5) [46]. In another retrospective study of 91 patients, antimotility drugs increased the risk of central nervous system dysfunction (OR 8.5, 95% CI 1.7 to 42.8) [40]. Based upon these data, antibiotics and antimotility drugs should not be given to a child with confirmed or suspected E. coli O157:H7 infection. Increased volume expansion with intravenous isotonic saline during the diarrheal phase has been reported to attenuate but not prevent renal injury [6, 47].

Treatment of Stx-HUS includes supportive measures such as management of anemia, significant clinical bleeding, fluid and electrolyte disturbances, hypertension, and other extrarenal complications, together with renal replacement therapy required in 30–60% of cases [48]. Additionally, multiple modalities and/or agents have been utilized that are directed against the underlying or presumed pathogenic mechanisms of Stx-HUS. These include antithrombotic agents, plasma exchange and/or plasma infusion, tissue-type plasminogen activator, and oral Shiga toxin-binding agent. Although none of these agents have been shown to be efficacious, there may be a role for plasma exchange in patients with severe CNS involvement. The remaining agents which have not proven efficient are not recommended. Stx-receptor (Gb3) mimics and Stx-neutralizing (monoclonal) antibodies (STmAb) may be more promising. Although the first-generation Gb3-analog failed to ameliorate the disease course in a randomized placebo-controlled trial [49], advanced Stx receptor analogs and polymers, and peptide-based intracellular toxin inhibitors have been developed and could have more beneficial effects in the future [50–52]. STmAb injection 24 h after oro-gastric inoculation of Stx2-producing bacteria prolonged the survival of toxin-challenged mice [53]. This provided the rationale for the use of STmAb products for Phase 1 studies in patients in which a satisfactory tolerability was demonstrated [54]. Randomized controlled trials are needed to confirm the potential role of STmAb in patients with Stx-HUS. Eculizumab looks promising in severe cases with neurological involvement refractory to plasma therapy [55]. Finally, treatments targeting downstream events in the inflammatory or proapoptotic cascade induced by Stx could be a good option, yet to be developed.

3. Atypical HUS

An increasing number of genetic causes of aHUS have been described [1]. These include aHUS associated with mutation of genes encoding for regulators of the alternative complement pathway, Von Willebrand factor cleaving protease (ADAMTS13) deficiency, and intracellular defects of vitamin B12 metabolism. There are also cases of unknown etiology that are associated with an autosomal recessive and autosomal dominant inheritance.

3.1. Atypical HUS and Defective Complement Regulation. Mutations in the genes encoding complement proteins including C3, factors H, B, and I, and membrane cofactor protein (MCP) are associated with atypical HUS [56, 57]. It is estimated that approximately 60 percent of cases of aHUS result from mutations in these genes [56]. These mutations result in dysregulation of the complement system that leads to excessive complement activation and in endothelial damage.

The gene that is affected determines the clinical presentation and outcome [58, 59]. For example, patients with mutations of the gene for factor H (*CFH*) often progress to ESRD within the first year of presentation. By contrast, only few patients with MCP mutations progress to ESRD, although relapse is common [59]. Interestingly, only 50% of individuals with *CFH* or *CFI* or MCP mutation eventually develop aHUS, which mean that additional risk factors are involved in the development of the disease. Combined mutations of the genes encoding complement proteins are observed in 5% of patients. The association of *CFH* and MCP haplotypes with aHUS has been reported [60].

3.1.1. Factor H Mutations. The association between aHUS and mutations in *CFH* was first described by Warwicker et al. [61]. It was subsequently found that it is the most frequent genetic abnormality in patients with aHUS, accounting for 25 to 30% of cases [62–65]. Most mutations are heterozygous. Factor H, a serum complement regulatory protease, acts as a cofactor for factor I-mediated inactivation of C3b, competes with factor B for C3b binding, and accelerates C3 convertase decay. More than 70 mutations have been reported. Most mutations affect the short consensus repeats 19 and 20 of the protein. Most of them are missense mutations, which do not affect the levels of factor H and C3 but affect the C-terminal region, which is important for binding to C3b and anionic surfaces [66, 67]. Other mutations are located throughout the gene and are associated with low levels of factor H antigen. Interestingly, renal survival is higher in patients with low factor H levels compared with those with normal levels of factor H. Patients with homozygous mutations have very low levels of factor H antigen, low C3, low factor B, and low CH50. aHUS associated with factor H mutations may present during infancy or early childhood, or in adulthood [62, 63, 68–71]. The disease may be sporadic or clearly associated with a family history of disease. Hemolytic anemia is marked at disease onset and during relapses, with haptoglobin levels remaining low during the course of the disease. Hypertension is frequently severe. Progression to end-stage renal disease is often rapid, and there is a very high risk of recurrence after renal transplantation (75 to 90%) [72, 73].

3.1.2. Mutations in MCP (Membrane Cofactor Protein). Mutations in the complement regulatory protein MCP are observed in 5 to 10% of aHUS [58, 74–76]. MCP is the cofactor of factor I for the degradation of C3b and C4b. The mutation results either in a reduction of cell surface levels of MCP (most common) or an impaired function of MCP [75]. The disease is characterized by onset in childhood, a favourable renal outcome in most patients, frequent relapses and a low rate of recurrence in the renal allograft.

3.1.3. Factor I Mutations. Factor I is a serine protease which cleaves C3b and C4b in the presence of factor H and MCP. The frequency of CFI mutations in patients with aHUS varies between 3 and 10% [77]. Up to 15 different mutations have been identified [78]. Most patients have heterozygous mutations [79]. They result in either a quantitative defect or a qualitative defect of the protein. HUS often occur in early childhood, and progression to end-stage renal disease is observed in more than 50% of cases [80]. Other genetic anomalies are frequent and may explain a worse outcome in some patients. Recurrence occurs in 45 to 80% of patients, resulting in graft loss.

3.1.4. C3 Mutations. Heterozygous C3 mutations have been identified in patients with aHUS [81]. Fremeaux-Bacchi et al. reported 9 novel mutations in 14 patients with aHUS and persistently low serum C3 levels. Five of the seven C3 mutant proteins showed a reduced binding to MCP, possibly leading to a gain of function relative to complement activation.

The mean age at presentation was 6.5 years (range 8 months to 40 years). Seven of the 14 patients regained function after their initial presentation and four of these patients had recurrent disease. Among the 14 patients, there have been 12 renal transplantations, five of which have had recurrent disease.

3.1.5. Factor B Mutations. Mutations of complement factor B that either enhance the formation or delay the inactivation of C3bBb convertase have been reported in 1 to 3% of cases of aHUS [82, 83]. Progression to end-stage renal disease occurs in 70% of patients and all four renal transplantation reported failed because of HUS recurrence.

3.1.6. Thrombomodulin Mutations. Thrombomodulin is a cofactor in the initiation of the protein C anticoagulant pathway. In addition, it was shown that thrombomodulin accelerates the inactivation of C3b by factor I. In a study of 152 patients with atypical HUS, seven patients had six different heterozygous mutations of the *THBD* gene, which encodes thrombomodulin [84]. *In vitro* studies demonstrated that these mutations resulted in a decreased capacity to inactivate C3b, which may lead to excessive complement activation.

3.1.7. Antifactor H Autoantibody-Associated aHUS. The presence of autoantibodies against factor H is observed in 6 to 10% of cases, mainly in children, between 9 and 12 years. These antibodies interfere with the binding of factor H to the C3 convertase and are associated with a defective factor H-dependent cell protection [85, 86]. Most patients have a homozygous deletion of *CFHR1* and *CFHR3* genes [87–89]. The clinical course is characterized by a high frequency of relapses. Progression to end-stage renal disease is observed in 20 to 35% of cases [90]. Plasma exchange plus immunosuppressive therapy have been successful measures

in some patients with factor H antibodies [91]. Recurrence is observed when the autoantibodies are still present at time of transplantation [92].

3.1.8. Treatment. Intensive plasma exchange (40 to 60 mL/kg) is the first-line therapy for patients during the acute episode of aHUS. The response to plasma treatment appears to vary depending upon the affected complement component [58, 93]. In patients with either factor H or factor I deficiencies, about two-thirds of patients will remit with plasma therapy. In contrast, there has been no difference in remission rates with or without plasma therapy in patients with MCP deficiency. Patients with antifactor H autoantibodies should receive an immunosuppressive treatment in addition to plasma exchanges.

Eculizumab is a monoclonal antibody which binds to C5 and prevents the generation of C5a and the formation of the membrane attack complex. Eculizumab has been used in patients with aHUS on native kidney or recurrence of aHUS after transplantation with very encouraging results [94–97]. A prolonged therapy is more effective than a single dose. The delay between two injections should not exceed 14 days. The duration of therapy is still a matter of debate. A multicenter trial in children to establish the efficacy and safety of eculizumab in aHUS is ongoing.

Patients with mutations in genes for factor H, factor I, or C3 who fail to respond to plasma therapy and/or have recurrent disease are likely to progress to ESRD [98]. It is unclear whether renal transplantation is an appropriate intervention in these patients, because recurrence of disease occurs in 50 percent of the transplanted kidney, and graft failure occurs in 90 percent of those with recurrent disease [72, 99]. Genotyping for complement protein genes should be performed in patients who have atypical HUS and end-stage renal failure and are being considered for renal transplantation. Indeed, the risk of recurrence is dependent on the type of mutation; such genotyping is particularly important when living-related renal transplantation is being considered, so that cases in which *de novo* HUS occurs in the donor can be avoided. Most clinicians agree that living donor renal transplantation is contraindicated in patients with atypical HUS because of the high risk of disease recurrence after transplantation [1].

In 2007, a consensus conference recommended a combined liver-renal transplantation in aHUS cases in which renal transplantation is extremely unlikely to succeed [100, 101]. This would include patients with factor H or I deficiency who have lost an isolated kidney transplant due to disease recurrence or have a family member with the same gene mutation who lost a kidney transplant due to disease recurrence. The transplantation should be preceded by plasma exchange, and plasma should be infused intraoperatively and continued until liver function is adequate. Eculizumab is another option [94, 96].

3.1.9. Atypical HUS and Von Willebrand Factor Cleaving Protease Deficiency. Von Willebrand factor (VWF), a glycoprotein that carries factor VIII in the circulation, is required for platelet adhesion and platelet aggregation [102]. Large multimers of VWF are more effective than dimers for platelet adhesion and platelet aggregation. The large multimers do not normally circulate because they are cleaved by a specific protease, a metalloprotease synthesized by the liver. This protease is the thirteenth member of the ADAMTS family, or ADAMTS13.

Many cases of adult thrombotic thrombocytopenic purpura (TTP) are due to deficient activity of the VWF cleaving protease. Protease deficiency may be inherited by autosomal-recessive transmission or may be induced by an acquired autoantibody that inhibits protease activity [103, 104]. A few cases of antibody-induced VWF cleaving protease deficiency have been reported in children aged 1 to 11 years who presented with symptoms of TTP.

Most children with VWF-cleaving protease deficiency related to mutations in the ADAMTS13 gene present at birth with hemolytic anemia and thrombocytopenia. Renal involvement often occurs later in life and has a progressive course. Onset of disease up to five years of age has also been reported. Many develop symptoms of CNS involvement.

3.1.10. Atipical HUS and Defects of Vitamin B12 Metabolism. Vitamin B12 or cobalamin is the coenzyme of methionine-synthase (or methyl-transferase), which transforms homocysteine in methionine. It is also the coenzyme of methylmalonyl-CoA mutase, which is involved in the conversion of methylmalonyl CoA to succinyl-CoA [105]. Children affected with cobalamin C mutations have a functional defect of both methylmalonyl CoA mutase and methionine-synthase. This defect results in methylmalonic acidemia with homocystinuria.

Approximately 25 percent of reported cases also have atypical HUS [105]. The first symptoms, which are nonspecific, occur in the neonatal period and consist of anorexia, vomiting, failure to thrive, hypotonia, seizures, and lethargy [106, 107]. The first symptoms of HUS are observed between the end of the first month and three months of age with severe hemolytic anemia with schistocytes, which is accompanied by macrocytosis, frequent thrombocytopenia, hematuria, proteinuria, renal dysfunction of variable severity, and hypertension.

Amino acid and organic acid chromatography reveal a marked increase of homocysteine and a low level of methionine in the plasma, while urinary excretion of homocysteine and methylmalonic acid is very high. Therapy with hydroxycobalamin should be started as soon as possible [107].

3.2. Other Causes of Atypical HUS. HUS may occur during treatments with cytotoxic drugs, such as mitomycin C, bleomycin, or cisplatin [108, 109]. HUS has been described in patients with HIV infection [110, 111]. HUS has been observed in patients with systemic lupus erythematosus [112] as well as in those with a catastrophic antiphospholipid syndrome.

3.3. HUS after Renal Transplantation. HUS may be observed following renal transplantation either as a recurrent or a *de novo* disease [113]. More than 50% of patients with aHUS

have mutations in genes coding for regulatory factors of the complement system or antibodies against factor H. The risk of recurrence depends on the genetic defect. When the risk of recurrence is high, as in patients with mutations in genes coding for circulating regulators of complement, isolated renal transplantation is contraindicated given the high risk of recurrence, although the use of eculizumab may allow such procedure.

Drug-induced HUS has been observed in transplant patients treated with either cyclosporine or tacrolimus [114, 115]. It usually occurs during the first few months posttransplantation, a time when high doses of calcineurin inhibitors are being administered.

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Case Report

The Hyponatremic Hypertensive Syndrome in a Preterm Infant: A Case of Severe Hyponatremia with Neurological Sequels

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Objective. To report the irreversible severe neurological symptoms following the hyponatremic hypertensive syndrome (HHS) in an infant after umbilical arterial catheterization. *Design*. Case report with review of the literature. *Setting*. Neonatal intensive care unit at a tertiary care children's hospital. *Patient*. A three-week-old preterm infant. *Conclusions*. In evaluating a neonate with hyponatremia and hypertension, HHS should be considered, especially in case of umbilical arterial catheterization. In case of diagnostic delay, there is a risk of severe irreversible neurological damage.

1. Introduction

In the neonatal intensive care unit (NICU) population, hyponatremia is the most frequent encountered water and salt abnormality. With its broad differential diagnosis, it provides a challenge to the neonatologist. The most frequent causes are renal salt loosing through an immature kidney and the use of drugs such as diuretics [1]. Hypertension, however, is a relatively rare feature in children, especially in neonates, with an incidence in NICU's ranging from 0.7% to 3.2%, and renal arterial thrombosis following umbilical arterial catheterization as the leading cause [2, 3].

A rare clinical presentation of unilateral renal arterial stenosis is the hyponatremic hypertensive syndrome (HHS), characterized by activation of the renin angiotensin aldosterone (RAAS) system in the ischemic kidney, causing hypertension, and a counteracting effect on the other kidney, by different mechanisms leading to volume depletion and loss of electrolytes. This syndrome is caused by unilateral renal ischemia, due to stenosis or occlusion of a (branch of a) renal artery, and also occurs in a variety of other underlying disorders [4]. So far, only a few reports of HHS in children are available, with polydipsia, polyuria, enuresis, weight loss, volume depletion, and various neurological and behavioural symptoms as presenting symptoms [5].

We present a case of HHS in a preterm infant, with an extremely low sodium concentration, and discuss the difficulties encountered in treatment and the irreversible neurological sequels due to this potentially life-threatening metabolic disturbance.

2. Case Report

A preterm boy presented with extreme hyponatremia (plasma sodium of 101 mmol/L) at the 20th day after birth. He was born from a nulliparous woman at a gestational age of 31 weeks and 4 days after an uncomplicated pregnancy, followed by spontaneous rupture of membranes and antenatal corticosteroid administration. Apgar scores were 9 and 10 at 1 and 5 minutes, and the birth weight was 2080 grams. There were no complications during NICU stay over the first 3 days of life. An umbilical arterial catheter was inserted directly after birth, for the purpose of blood pressure monitoring, and removed after 3 days. Furthermore

an umbilical venous catheter and subsequently a peripheral central venous catheter were inserted for the purpose of parenteral feeding. Routine cerebral ultrasonography showed an image consistent with the gestational age and mild periventricular flaring. No diuretics were administered.

At the third day of life the boy was transferred in good clinical condition to a regional hospital. He gained weight (from 1900 grams at the 3rd day to 2100 grams at two weeks after birth). At the age of 3 weeks rejection to feeding (until this moment consisting of 150 mL/kg/day breast milk with breast milk fortifier), weight loss (to a minimum of 1960 grams), irritability, hyperthermia, and polyuria were noticed. Cerebrospinal fluid analysis showed 219 leukocytes/mm^3 with 12000 erythrocytes/mm^3, after a traumatic lumbar puncture, thus a meningitis could no be excluded and intravenous antibiotics were started. Intravenous fluids, with a total volume of 150 mL/kg/day, containing 8 mg/kg/min glucose and 5 mmol/kg/day sodium, were administered in the regional hospital for 2 days (before return to the NICU).

The plasma sodium level had declined, from 140 mmol/L nine days before, to 101 mmol/L. There were no sodium levels examined in the interval between, but the level at the onset of symptoms was established at 112 mmol/L retrospectively in plasma stored at the laboratory of the regional hospital. The boy returned to the NICU under suspicion of a syndrome of inappropriate antidiuretic hormone secretion (SIADH) associated with the assumed meningitis, with initiated fluid restriction and sodium supplementation considered to be the appropriate therapy. The body weight at that time was 2030 grams.

We saw a pale, irritated neonate with tachypnea, arterial hypertension (104/60 mmHg, mean 78 mmHg), opisthotonus, and abnormal synchronized extensions of arms and legs. Additional to the plasma sodium level of 101 mmol/L, laboratory analysis revealed a mild hypokalemia, hypochloremia, and hypomagnesemia, with normal calcium and phosphate levels (for detailed information on all important laboratory results, see Table 1). Plasma osmolarity was 219 mOsmol/kg, and urea and creatinine levels were normal. Blood gas analysis showed a respiratory alkalosis with normal bicarbonate. Infection parameters were low, but liver enzymes and lactate were elevated (in the blood drawn several shortly after the seizure). Furthermore an elevated plasma B-type natriuretic peptide (BNP) of 1228 pmol/L was found (in children, there are no validated data on normal values available). Urinalysis showed no leucocytes, mild hematuria, low sodium and potassium, with proteinuria, glucosuria, and a urine osmolarity of 129 mOsmol/kg. Cultures of blood, urine and cerebrospinal fluid revealed no microorganisms.

The combination of hyponatremia and hypertension (defined as a mean blood pressure of >2 standard deviations for age and weight, in this case >75 mmHg) was suggestive of renal pathology. Abdominal Doppler ultrasound showed a right renal arterial thrombosis, partially calcified, and an oedematous appearance of the left kidney. It was suggested that the symptoms of this neonate resulted from an HHS secondary to a renal arterial thrombosis.

Blood pressure levels further increased in the first hours to a maximum of 108/62 (mean 96) mmHg. We chose to carefully normalise the blood pressure with intravenous dihydralazine, causing the right kidney to become completely afunctional (as demonstrated with 99 m technetium MAG3 renography in combination with the findings on Doppler ultrasound as mentioned before). Furthermore we supplemented sodium (by intravenous sodium chloride). Plasma sodium levels rose to, relatively fast within the first hours, above 120 mmol/L, and more slowly within the next 24 hours to normal. The dihydralazine and sodium supplementation could be gradually stopped after a few days, after which a normal blood pressure and electrolyte levels were maintained without any additional therapy, also urinalysis returned to normal.

Within a few minutes after return to the NICU the boy developed convulsions, successfully treated with phenobarbital. Cerebral ultrasound and magnetic resonance imaging (MRI) showed extensive white matter abnormalities and the presence of a sinus thrombosis of the superior sagittal, straight, and transverse sinus. Additional genetic screening revealed a mutation in the methylenetetra-hydrofolate reductase (MTHFR) gene.

Table 1: Laboratory values in plasma and urine, measured in the patient at readmission to the NICU.

Laboratory measurement	Measured value	Normal values
Plasma		
Sodium (mmol/L)	101	136–146
Potassium (mmol/L)	3.0	3.4–6.0
Chloride (mmol/L)	70	99–108
Magnesium (mmol/L)	0.66	0.70–1.00
Calcium, ionized (mmol/L)	1.25	0.95–1.50
Phosphate (mmol/L)	1.26	1.25–2.10
Glucose (mmol/L)	6.4	3.6–5.6
Osmolarity (mOsmol/kg)	219	280–295
Urea (mmol/L)	3.4	3.0–7.5
Creatinine (μmol/L)	44	27–62
pH (arterial blood)	7.56	7.37–7.4
pCO_2 (mmHg)	26	35–45
Bicarbonate (mmol/L)	22.9	22–28
Base excess (mmol/L)	1.0	−3.0–3.0
Lactate (mmol/L)	3.6	0.0–2.2
BNP (pmol/L)	1228	(Adult) <30–120
Hb (mmol/L)	8.4	(Adult) 5.9–8.4
Ht (mmol/L)	0.34	0.41–0.50
Erythrocytes ($\times 10^{12}$/L)	3.94	3.20–4.80
Urine		
Sodium (mmol/L)	<10	Not available
Potassium (mmol/L)	19	Not available
Glucose (mmol/L)	16.1	Not available
Osmolarity (mOsmol/kg)	129	Not available
Protein (g/L)	>2.0	Not available
Creatinine (mmol/L)	1.5	Not available
pH	7.0	4.5–8.0
Erythrocytes (per μL)	60	0–10

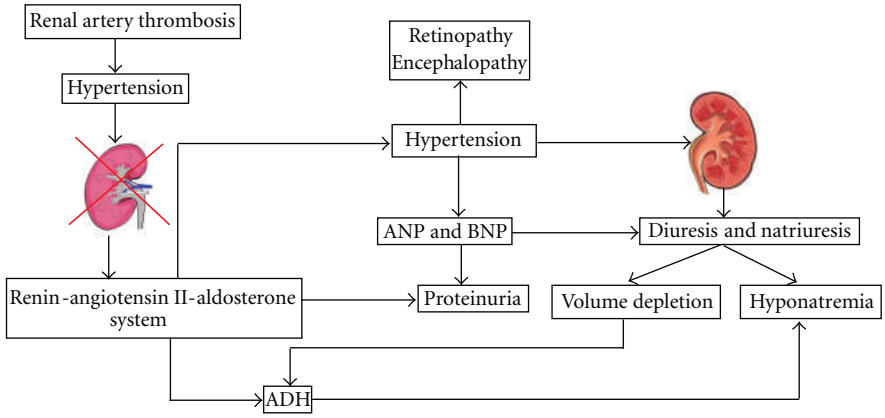

Figure 1: Suggested pathophysiology of the hyponatremic hypertensive syndrome, based on data from Nicholls 2006 [5].

Follow-up MRI at one and two months of age showed extension of the white matter abnormalities, with secondary haemorrhage, vacuolisation, and cyst formation. The gyration and myelinisation had increased, there were no signs of new ischemia, and all sinuses were recanalized. The child showed abnormal neurological behaviour (agitation, uncontrolled movements, and delayed motor development) at three months of followup. The parents of this child gave their informed consent to publication of this case report.

3. Discussion

In this case report, we describe a 3-week-old preterm boy, with extreme hyponatremia, hypertension and a dramatic neurological outcome, as a result of HHS following umbilical arterial catheterization.

The typical combination of symptoms in HHS was first described in 1952 in adults [6], and the term HHS was established by Brown et al. in 1965 [4]. The majority of adult patients are elderly women with atherosclerosis [7]. In children, the syndrome is not encountered frequently and in neonates it is even more rare, with renal arterial stenosis following umbilical arterial catheterization being one of the described causes [8–10], accompanied by renal microthrombi in sepsis [11] and an association with Dexamethasone use [7]. The syndrome has been described more often in preterm than in term infants [8–11] and sometimes showed a lethal course [12, 13]. The high incidence of hyponatremia (30%) reported in hypertensive neonates, suggests that HHS is probably more common than we think [14].

HHS is thought to be due to a complex interplay of different mechanisms, with unilateral renal hypoperfusion and a counteracting effect of the contralateral normal kidney as major hallmarks ("two-kidney-one-clip hypertension") [12] (Figure 1). The renal arterial thrombosis causes hypoperfusion of one kidney, which activates the RAAS system to cause hypertension. The contralateral nonstenotic kidney reacts to this hypertension by excreting water and sodium (pressure diuresis and natriuresis) [12, 15]. The hypertension additionally stimulates the cardiac atrial natriuretic peptide (ANP) and BNP to excrete more sodium and protein [16].

The resulting hypovolemia, probably together with an increased production of angiotensin II, stimulates antidiuretic hormone (ADH), further aggravating the hyponatremia. Furthermore, aldosterone causes renal potassium loss, which in turn stimulates renin secretion, causing a vicious circle [15]. Proteinuria, glucosuria, and hypercalciuria can also be present due to glomerular hyperfiltration in hypertension, increased renin activity, and probably even more extensive tubulointerstitial involvement [17].

In our case, the presence of an umbilical arterial catheter, and the finding of a mutation in the MTHFR gene (suggested to play role in homocysteine metabolism and enhancing atherosclerosis) were thought to be contributing factors in the development of the renal arterial thrombosis. At the moment of presentation at the NICU, our patient was thought to be already beyond the natriuretic phase of HHS (probably the severe hyponatremia finally resulted in sodium retention, explaining the low urine sodium) and most likely ADH had been turned on in reaction to the volume depletion. The initial sepsis-like presentation was retrospectively interpreted as the result of a combination of hypovolemia and central nervous system disturbances.

The prematurity was probably a major contributing factor leading to the severe outcome in this patient. This could have led to more extreme hyponatremia because preterms have a relatively low sodium intake, reduced tubular sodium reabsorption, and decreased glomerular filtration rate, impairing free water excretion [18]. Furthermore, recognizing the nonspecific clinical symptoms of HHS can be very difficult in a neonate. This diagnostic delay can result in long-lasting untreated hyponatremia and hypertension.

The symptoms of HHS disappeared after normalising the blood pressure with a vasodilator agent (dihydralazine), causing the ischemic kidney to become nonvital by totally abrogating the blood flow, destroying the juxtaglomerular cells, and hence stopping renin production. There was a gradual decline in blood pressure in this patient, different from the potentially dangerous fall which can be seen with angiotensin-converting enzyme (ACE) inhibitors [19]. With severe volume depletion, cautious repletion is needed, which can probably also reduce arterial pressure by suppression of RAAS [20].

Correction of longstanding hyponatremia should be managed carefully, to minimise the risk of developing cerebral shrinking [21]. Final therapy of the underlying renal arterial stenosis was not necessary in this case, but can be achieved by balloon angioplasty, renal artery stenting, or uninephrectomy. However, in small children the first mentioned options can be technically impossible.

The remarkable irreversible neurological features in this case are most likely to be the consecutive effect of a hypertensive and hyponatremic encephalopathy, aggravated by a diminished cerebral circulation due to hypovolemia and a sinus thrombosis. Furthermore the convulsions could also have led to irreversible damage to the vulnerable preterm brain. Previous case reports in older children mainly mention reversible neurological symptoms, and even reversible findings on computer tomography or MRI associated with HHS [19, 22–24] and only one infant dying from massive cerebral haemorrhage [13].

We realise that there are few limitations in the description of this case. First, there is a lack of (clinical and laboratory) information about the period before the patient represented with the severe hyponatremia. Unfortunately, no detailed information on water balance or 24-hour urine volumes during the period in which the patient developed the hyponatremia was available. As it is especially cumbersome to collect such data in newborns, this is very rarely done. Second, no data on plasma ADH, rennin, and aldosterone are available to confirm our suggested diagnosis.

This case was meant to describe the complex pathophysiology of HHS, and the possible misleading clinical features in a neonate. Furthermore we want to underline the risk of severe irreversible neurological damage when there is a diagnostic delay. We think that in evaluating a neonate with severe hyponatremia, HHS should be considered, especially if following umbilical arterial catheterization.

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Review Article

Urinary Angiotensinogen as a Biomarker of Nephropathy in Childhood

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While most circulating angiotensinogen (AGT) is synthesized in the liver, the kidneys also produce AGT. Recently, we reported that urinary AGT is mainly originated from AGT. Using newly developed human AGT ELISA, we measured urinary AGT levels in chronic glomerulonephritis (GN) patients and patients with type 1 diabetes in childhood. Urinary AGT level was positively correlated with diastolic blood pressure, urinary albumin, urinary protein levels, and urinary occult blood in chronic GN patients. Furthermore, urinary AGT level was significantly increased in chronic GN patients not treated with renin-angiotensin system (RAS) blockers compared with control subjects. Importantly, patients treated with RAS blockers had a marked attenuation of this increase. Also, urinary AGT level was significantly higher in patients with diabetic nephropathy in the premicroalbuminuric phase than in control subjects. These results suggest that urinary AGT reflects intrarenal RAS status in chronic GN and may be an early marker of diabetic nephropathy.

1. Introduction

The renin-angiotensin system (RAS) plays a critical role in arterial pressure and sodium homeostasis [1]. Angiotensin II (Ang II) is the most powerful biologically active product of the RAS [2]. Recently, the focus of interest in the RAS has shifted toward the role of the local/tissue RAS in specific tissues [2]. Angiotensinogen (AGT) is the only known substrate for renin that is a rate-limiting enzyme of the RAS. Because the level of AGT is close to the Michaelis-Menten constant for renin, not only renin levels but also AGT levels can control RAS activity, and AGT upregulation may lead to elevated angiotensin peptide levels and increased blood pressure [3]. Recent studies of experimental animal models and transgenic mice have documented AGT involvement in the activation of the RAS and development of hypertension [4, 5]. Genetic manipulations that lead to AGT overexpression have consistently been shown to cause hypertension [6, 7]. In human genetic studies, a linkage has been established between the AGT gene and hypertension [8]. Enhanced intrarenal AGT mRNA and/or protein levels have also been observed in multiple experimental models of hypertension including Ang II-dependent hypertensive rats [9–12], Dahl salt-sensitive hypertensive rats [13, 14], and spontaneously hypertensive rats [15], as well as in kidney diseases including diabetic nephropathy [16–20], IgA nephropathy [21, 22], and radiation nephropathy [23]. Thus, AGT plays an important role in the development and progression of hypertension and kidney disease [2, 24]. Recent studies showed that urinary excretion rates of AGT provided a specific index of intrarenal RAS status [2, 25, 26]. This paper explores recent findings concerning the use of urinary AGT as a potential biomarker of intrarenal RAS status in childhood nephropathy.

2. Intrarenal RAS

The role of RAS in blood pressure regulation and sodium and fluid homeostasis is well recognized [2]. The biologically active peptides that are formed from AGT include Ang II and Ang 1–7. The balance between the vasoconstricting actions of Ang II, mediated by the AT_1 receptor, is countered by the

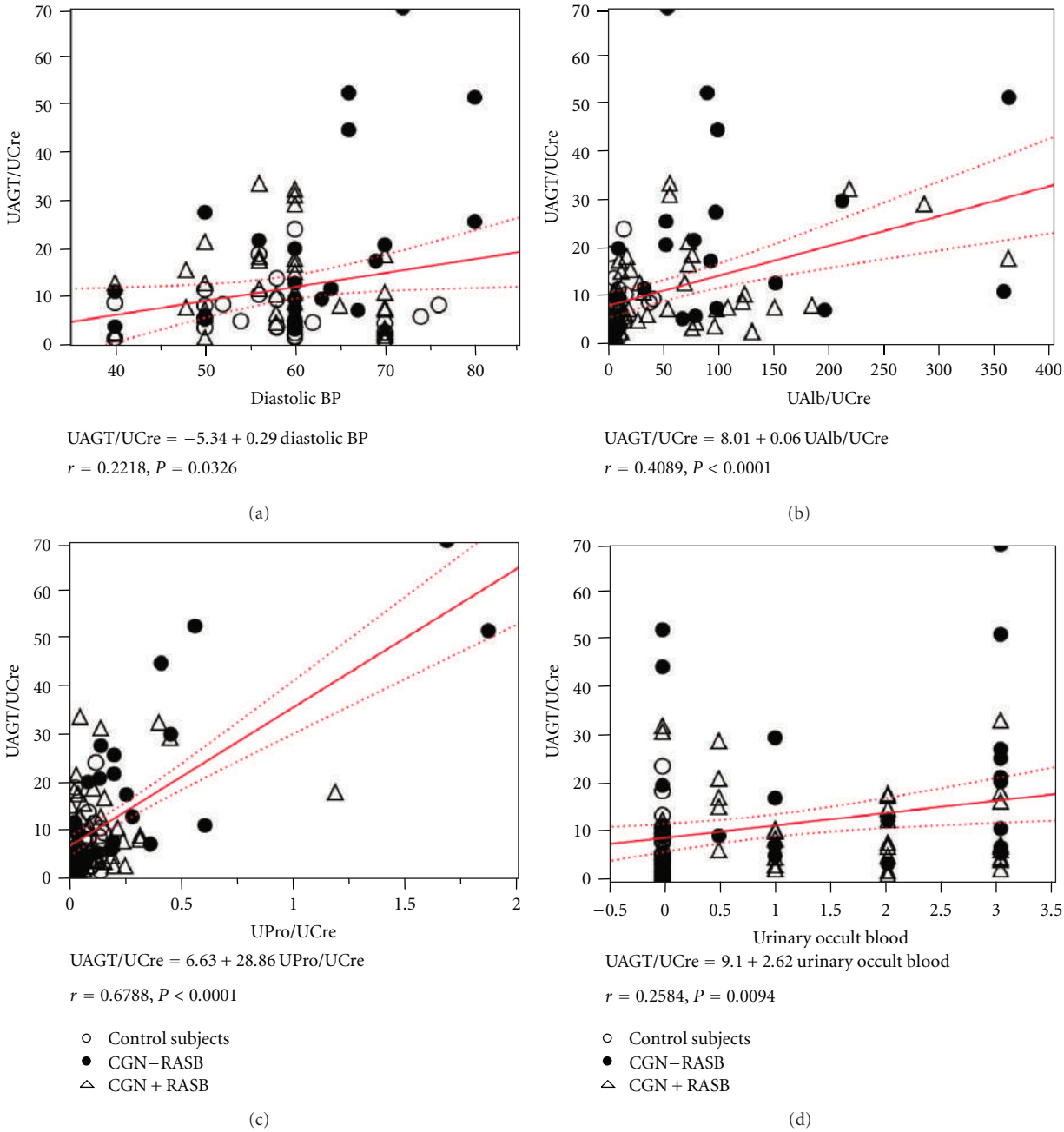

Figure 1: Single regression analyses for urinary AGT-creatinine ratio with diastolic blood pressure (a), urinary albumin-creatinine ratio (b), urinary protein-creatinine ratio (c), and urinary occult blood index (d), respectively, in chronic glomerulonephritis (CGN) patients with/without renin-angiotensin system blockade (RASB) and in control subjects. Cited from Am J Nephrol 2010; 31: 318–325 by Urushihara et al. [47].

vasodilating actions of Ang II, mediated by the AT_2 receptor [27], and Ang 1–7 acting on the G protein-coupled receptor Mas [28]. Formation of Ang II is dependent upon the substrate availability of AGT and Ang I and the activities of renin, ACE, ACE2, and ACE-independent enzymatic pathways including serine proteases, tonin, cathepsin G, trypsin, and kallikrein. The actions of Ang II are determined by signaling via AT1 and AT2 receptors and the putative Ang 1–7 receptor Mas [29].

Local/tissue RAS in specific tissues has become the focus of much recent interest [30]. Emerging evidence has demonstrated the importance of tissue-specific RAS in the brain [31], heart [32], adrenal glands [33], and vasculature [34, 35] as well as the kidneys [24]. In particular, renal RAS is unique because all of the components necessary to generate intrarenal Ang II are present along the nephron in both interstitial and intratubular compartments [2, 29]. The presence of AGT gene transcription in the proximal tubules

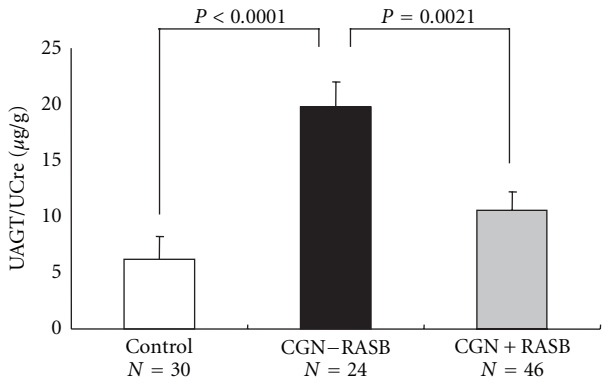

Figure 2: Urinary AGT-creatinine ratio in chronic glomerulonephritis patients (CGN) with/without renin-angiotensin system blockade (RASB) and in control subjects. Cited from Am J Nephrol 2010; 31: 318-325 by Urushihara et al. [47].

has been shown using *in situ* hybridization [36]. AGT mRNA is expressed primarily in the proximal convoluted tubules and proximal straight tubules, with small amounts in glomeruli, vasa recta, and renal vasculature [37]. Renal AGT protein is abundant in the proximal convoluted tubules [38]. Strong positive immunostaining for AGT protein has been reported in proximal convoluted tubules and proximal straight tubules, and weak positive staining in glomeruli and vasa recta; however, distal tubules and collecting ducts are negative [9]. The AGT synthesized in the kidney is secreted into the lumen leading to Ang I generation. Low but measurable renin concentrations have been detected in proximal tubule fluid in rats [2].

Renin mRNA and renin-like activity have been demonstrated in cultured proximal tubular cells [39]. The brush border membrane of proximal human kidney tubules expresses abundant levels of ACE mRNA [40] and protein [41]. ACE has also been measured in proximal and distal tubular fluid but is greater in proximal tubule fluid [42]. Therefore, all the major components required to generate Ang II are expressed within the kidneys [2, 24].

3. Urinary AGT as a New Biomarker of Intrarenal RAS Status

Recently, urinary AGT excretion rates were reported to provide a specific index of intrarenal RAS status in Ang II-dependent hypertensive rats [9, 11, 12]. Moreover, urinary AGT levels were reported to reflect intrarenal Ang II activity associated with increased risk of renal function deterioration in chronic kidney disease patients [25]. A direct quantitative method to measure urinary AGT using human AGT enzyme-linked immunosorbent assays (ELISA) was developed [43], which indicated significantly increased urinary AGT levels in hypertensive patients not treated with RAS blockers compared with normotensive subjects. Importantly, patients treated with RAS blockers exhibited a marked attenuation of this AGT increase [26]. These data prompted us to measure urinary AGT in chronic GN patients and patients with type 1 diabetes in childhood.

4. Urinary AGT Reflects Intrarenal RAS Status in Chronic Glomerulonephritis

Chronic glomerulonephritis (GN) resulting in substantial renal damage is frequently characterized by relentless progression to end-stage renal disease. Renal Ang II, production of which is enhanced in chronic GN, can elevate the intraglomerular pressure, increase glomerular cell hypertrophy, and augment extracellular matrix accumulation [44]. ACEi and/or ARB are often administered to patients with proteinuric nephropathies [45, 46]. This may reflect the relatively short-term nature and small sample size of these studies but may also be an indication that factors other than Ang II play an important role in progression of renal disease.

Previously, we examined glomerular AGT expression and its correlation with expression of other RAS components and levels of glomerular injury in samples from patients with IgA nephropathy and minor glomerular abnormalities [22]. Immunohistochemistry showed that AGT was highly expressed in nephritic glomeruli affected by IgA nephropathy compared with glomeruli affected by minor glomerular abnormalities. Levels of glomerular AGT protein were well correlated with levels of glomerular Ang II, transforming growth factor-β (TGF-β), α-smooth-muscle actin, glomerular cell number, and glomerulosclerosis score. These data suggest that activated glomerular AGT expression is likely involved in elevated local Ang II production and, thereby, may contribute to increased TGF-β production and development of glomerular injury in IgA nephropathy.

Based on these findings, a newly developed human AGT ELISA was used to elucidate urinary AGT levels in chronic GN patients [43]. To demonstrate that urinary AGT reflects intrarenal RAS status in chronic GN patients during childhood, 100 urine samples from 70 patients with chronic GN and 30 normal control subjects were recruited [47]. All patients had normal kidney function, and their background renal diseases were IgA nephropathy ($n = 26$), purpura nephritis ($n = 24$), lupus nephritis ($n = 8$), focal segmental glomerulosclerosis ($n = 7$), and non-IgA mesangial proliferative GN ($n = 5$). Urinary AGT-creatinine ratios did not correlate with sex, age, height, body weight, body mass index, systolic blood pressure, serum sodium levels, serum potassium levels, serum creatinine levels, estimated glomerular filtration rate (eGFR), urinary fractional excretion of sodium, or plasma AGT levels. However, urinary AGT-creatinine ratios significantly positively correlated with diastolic blood pressure (Figure 1(a); $r = 0.2218$, $P = 0.0326$), urinary albumin-creatinine ratios (Figure 1(b); $r = 0.4089$, $P < 0.0001$), urinary protein-creatinine ratios (Figure 1(c); $r = 0.6788$, $P < 0.0001$), and urinary occult blood (Figure 1(d); $r = 0.2584$, $P = 0.0094$).

Increased intrarenal AGT immunoreactivity was previously reported in IgA nephropathy patients and found to be significantly positively correlated with urinary protein-creatinine ratio [21]. Increases in protein-creatinine ratio generally reflect the severity of renal disease [48]. Therefore, taken together, these data suggest that urinary AGT levels may be a marker of the severity of chronic GN. This perspective is supported by recent clinical studies [25, 49]. As shown

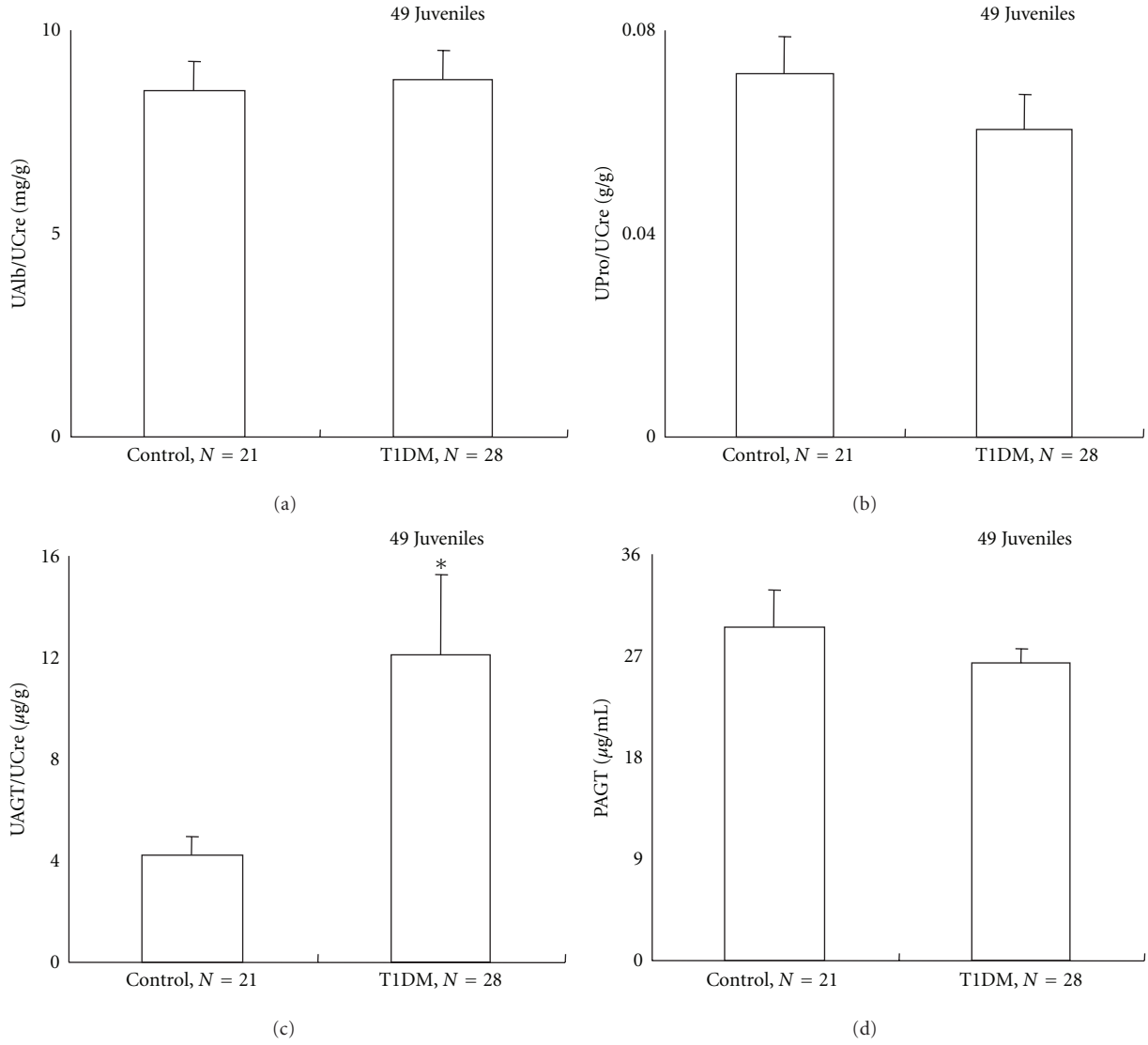

Figure 3: Urinary albumin-creatinine ratio (a) and urinary protein-creatinine ratio (b) were not increased in patients with type 1 diabetes (T1DM) compared to control subjects, suggesting that these patients were in the pre-microalbuminuric phase of diabetic nephropathy. However, urinary AGT-creatinine ratio was significantly increased in these patients compared to control subjects (c). Importantly, the AGT increase was not observed in plasma (d). Cited from Am J Med Sci 2009; 338: 478–480 by Saito et al. [56].

in Figure 2, urinary AGT-creatinine ratios were significantly increased in chronic GN patients not treated with RAS blockers ($19.79 \pm 3.70\,\mu$g/g) compared with control subjects (6.22 ± 0.98, $P < 0.0001$) [47]. Importantly, the use of RAS blockers attenuated this increase (10.58 ± 1.23, $P = 0.0021$). These data suggest that urinary AGT can be used to assess the efficacy of RAS blockade in reducing intrarenal RAS activity.

Although most circulating AGT is produced and secreted by the liver, the kidneys also produce AGT [2]. Intrarenal AGT mRNA and protein have been localized to proximal tubule cells, indicating that intratubular Ang II could be derived from locally produced and secreted AGT [36, 38]. The AGT produced in proximal tubule cells appears to be secreted directly into the tubular lumen, in addition to producing its metabolites intracellularly and secreting these into the tubular lumen [50]. Proximal tubular AGT concentrations in anesthetized rats have been reported in the range of 300–600 nM, which greatly exceeds the free Ang I and Ang II concentrations in tubular fluid [24]. Plasma AGT seems unlikely to filter across the glomerular membrane at high levels due to its molecular size (50–60 kDa), further supporting the concept that proximal tubular cells secrete AGT directly into the tubules [51].

To determine if circulating AGT is a source of urinary AGT, human AGT was infused into hypertensive and normotensive rats [14]. However, human AGT was detectable in plasma but not detectable in the urine of rats, indicating limited glomerular permeability and/or tubular degradation. These findings support the hypothesis that urinary AGT is derived from the AGT produced and secreted by the proximal tubules and not from plasma. In agreement with this concept, plasma AGT levels were not correlated

with urinary AGT-creatinine ratios in this study. Moreover, plasma AGT levels were unchanged across the three groups even though urinary AGT-creatinine ratios demonstrated significant differences. Therefore, AGT in urine seems highly likely to originate from AGT in the kidney, not from AGT in plasma. These data suggest that urinary AGT is a potential novel biomarker of the intrarenal RAS status in chronic GN. The efficacy of RAS blockade to reduce the intrarenal RAS activity can, thus, be confirmed by measurements of urinary AGT excretion rates.

5. Urinary AGT as a New Biomarker of Intrarenal RAS Status in Type 1 Diabetes

Microalbuminuria is the most commonly used early marker of diabetic nephropathy [52], and diabetic nephropathy is thought to be a unidirectional process from microalbuminuria to end-stage renal failure [53]. However, recent studies in type 1 diabetic patients demonstrate that a large proportion of diabetic nephropathy patients revert to normoalbuminuria and that one-third of these patients exhibit reduced renal function even in the microalbuminuria stage [54]. Urinary inflammatory markers are thought to be high in microalbuminuric type 1 diabetic patients with diminished renal function but not in those with stable renal function. However, no single marker has been sufficient to represent the whole panel [55]. Therefore, a more sensitive and more specific marker for diabetic nephropathy would be highly advantageous.

To demonstrate whether urinary AGT levels can be dissociated from urinary albumin or protein exertion rates in type 1 diabetic juveniles, early-phase studies were performed in type 1 diabetic juveniles ($n = 34$) and sex- and age-matched control subjects ($n = 21$) [56]. Since the primary focus of the study was comparing the characteristics of normoalbuminuric patients with type 1 diabetes with those of control subjects, 6 microalbuminuric patients with type 1 diabetes (urinary albumin-creatinine ratio >30 mg/g) were excluded. Consequently, urine and plasma samples from 28 diabetic patients were analyzed ($n = 49$ total juveniles). No patients received treatment with RAS blockers. Neither urinary albumin-creatinine ratios nor urinary protein-creatinine ratios were significantly increased in these type 1 diabetic patients compared to control subjects (urinary albumin-creatinine ratio: 8.8 ± 0.7 mg/g versus 8.5 ± 1.1 mg/g, $P = 0.8450$; urinary protein-creatinine ratio: 0.060 ± 0.010 g/g versus 0.070 ± 0.010 g/g, $P = 0.3231$), suggesting that these patients were in the premicroalbuminuric phase of diabetic nephropathy (Figures 3(a) and 3(b)). However, urinary AGT-creatinine ratios were significantly increased in these patients compared to control subjects ($12.1 \pm 3.2\,\mu$g/g versus $4.2 \pm 0.7\,\mu$g/g, $P = 0.0454$) (Figure 3(c)). Importantly, AGT was not increased in plasma ($26.3 \pm 1.3\,\mu$g/ml versus $29.5 \pm 3.3\,\mu$g/ml, $P = 0.3148$) (Figure 3(d)). These data indicate that urinary AGT levels are increased in type 1 diabetic subjects and that increased urinary AGT levels precede increased urinary albumin levels. Thus, urinary AGT levels may serve as a very sensitive early marker of intrarenal RAS activation and may be one of the earliest predictors of diabetic nephropathy in diabetic patients [56].

6. Conclusion and Future Prospects

Recent findings indicate that urinary AGT is increased in chronic GN patients, and treatment with RAS blockers suppresses urinary AGT. The efficacy of RAS blockade in reducing intrarenal RAS activity can be confirmed by measurement of urinary AGT in chronic GN patients. Although the relatively small sample size is a potential limitation, this study demonstrated a statistically significant relationship between urinary AGT and diastolic blood pressure, urinary albumin/protein levels, and occult blood in patients with chronic GN. We recognized that a larger multicenter, randomized control study is required to extend the clinical applicability of these observations. A prospective study to determine the relationship between the effect of RAS blockade on urinary AGT and urinary albumin/protein would be helpful in assessing the clinical significance of the decrease in urinary AGT associated with RAS blockade. These research projects will establish a novel diagnostic test to identify those chronic GN patients most likely to respond to a RAS blockade, which could provide useful information to allow a mechanistic rationale for a more educated selection of an optimized approach to treatment of chronic GN patients. Increased urinary AGT levels are also observed in patients with type 1 diabetes, and this increase precedes an increase in urinary albumin levels, suggesting that urinary AGT may function as an early marker of diabetic nephropathy. Randomized clinical trials have been projected to establish novel diagnostic tests to identify those patients most likely to respond to RAS blockade. These trials could provide information useful to developing a mechanistic rationale for improved selection of optimized treatments in patients with chronic GN or type 1 diabetes in childhood.

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Review Article

Renal Mitochondrial Cytopathies

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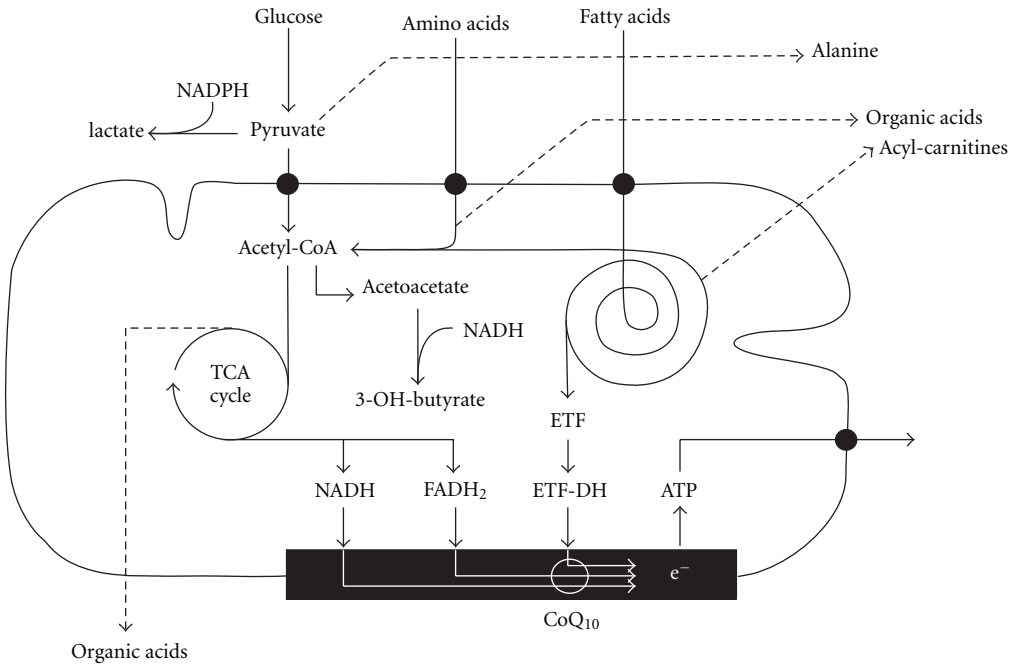
Renal diseases in mitochondrial cytopathies are a group of rare diseases that are characterized by frequent multisystemic involvement and extreme variability of phenotype. Most frequently patients present a tubular defect that is consistent with complete De Toni-Debré-Fanconi syndrome in most severe forms. More rarely, patients present with chronic tubulointerstitial nephritis, cystic renal diseases, or primary glomerular involvement. In recent years, two clearly defined entities, namely 3243 A > G $tRNA^{LEU}$ mutations and coenzyme Q10 biosynthesis defects, have been described. The latter group is particularly important because it represents the only treatable renal mitochondrial defect. In this paper, the physiopathologic bases of mitochondrial cytopathies, the diagnostic approaches, and main characteristics of related renal diseases are summarized.

1. The Mitochondrial Respiratory Chain

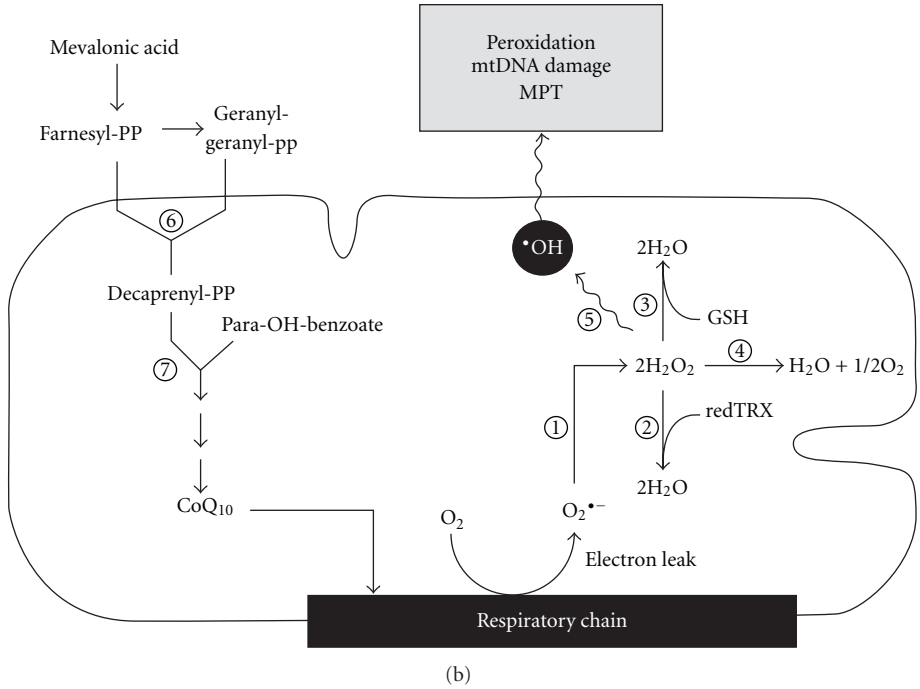
Mitochondria exert multiple roles in cells; in addition to ATP synthesis through oxidative phosphorylations (OXPHOS), they are at the crossroad of numerous metabolic pathways, contribute to heat production, and control cell-cycle/apoptosis and regulation of several anaplerotic reactions [1, 2]. OXPHOS occur within the respiratory chain (RC) that is composed of four protein complexes (complexes I–IV). These proteins transfer electrons and protons across the inner mitochondrial membrane generating the electrochemical gradient for ATP synthesis, which is performed by the ATP synthase (complex V) (reviewed in [1–4]). Within the mitochondrial RC, ubiquinone or coenzyme Q10 (CoQ_{10}) plays a crucial role in accepting and shuttling electrons (Figure 1) [5]. CoQ_{10} is a ubiquitous lipophylic vitamin-like substance that is found in high concentrations in tissues with elevated energy turnover (heart, brain, liver, and kidney). In humans, CoQ_{10} is comprised of a quinone group and a tail of 10 isoprenyl units (Figure 1). It is endogenously synthesized through a multienzyme mitochondrial complex [6], that is encoded by at least 16 genes (PDSS and COQ genes) (Figure 1). CoQ_{10} is also a cofactor for several dehydrogenases, a modulator of the mitochondrial permeability transition pore (MPT) that acts as a gating channel for apoptosis, a cofactor for pyrimidine biosynthesis, and an important antioxidant [7]. Approximately 0.2% of oxygen molecules are not reduced into water during OXPHOS and form reactive oxygen species (ROS) that can be converted by the Fenton reaction into highly reactive hydroxyl radicals ($^{\bullet}OH$), causing oxidative damage of mitochondrial DNA (mtDNA), peroxidation of lipids and proteins, and activation of the MPT [1, 2] (Figure 1). Accumulation of free radicals plays a crucial role in the physiopathology of many mitochondrial diseases [8]. In normal conditions, ROS are scavenged by superoxide dismutase, catalase, glutathione peroxidase, and thioredoxin peroxidase (Figure 1); CoQ_{10}, vitamin C, vitamin E, and other small molecules also contribute to the mitochondrial defense arsenal against oxidation [1, 5].

2. Genetics of Mitochondrial Diseases

mtDNA is composed of a 16,569 bp circular string that encodes for 37 genes, including all 22 tRNAs, 2 rRNA



(a)



(b)

Figure 1: Schematic representation of different metabolic processes involved in cellular energy metabolism. (a) shows the interrelationships between the oxidative pathways of glucose, aminoacid, and fatty acid, which lead to ATP production in the mitochondrial RC enzymes (black box). White lines illustrate the electron flow through CoQ_{10} in the RC. Dashed lines indicate substrates that generate abnormal metabolites in mitochondrial cytopathies (i.e., lactate, alanine, organic acids, and acyl-carnitines). (b) shows the enzymatic cascade for the biosynthesis of CoQ_{10} from mevalonic acid (left) and the principal mitochondrial enzymes with antioxidant function (right): (1) superoxide dismutase, (2) thioreductase, (3) glutathione peroxidase, (4) catalase, (5) Fenton reaction, (6) prenyl diphosphate synthetase subunit 1 and 2 (*PDSS1* and *PDSS2*), and (7) CoQ biosynthesis enzymes (*COQ2–COQ8*).

subunits, and 13 structural proteins of the RC; the remaining 75 proteins that compose the RC and other structural or functional mitochondrial proteins (more than 1000) are encoded by nuclear genes [3, 9]. In particular and relevant to several mitochondrial disorders, the biogenesis of the RC requires a number of assembly factors that are encoded by nuclear genes; although these proteins are not structural components of mature RC complexes, they control correct folding and maturation of protein subunits, and the delivery and insertion of prosthetic groups into the holoenzymes of the RC [10]. Mitochondria are transferred from mothers to their progeny in the oocyte; therefore, genetic diseases involving mitochondrial genes follow a maternal inheritance. The first mutations in mitochondrial genes have been reported in the late 1980s [10–13]. Since then, a large number of mutations in mtDNA have been reported and are collected in the MITOMAP human mitochondrial genome database (http://www.mitomap.org/). Their prevalence in the general population may be as high as 1-2 : 10000 live births [14]; mutations can be maternally inherited or sporadic. In addition, mtDNA disorders can follow a Mendelian pattern of inheritance if they are secondary to mutations of nuclear genes that control mtDNA copy number or integrity [15]. As opposed to nuclear genes, the genetic information encoded by mtDNA is present in hundreds of copies per cell; mutations may affect all mtDNA copies (*homoplasmy*) or only a portion of the mtDNA endowment of cells (*heteroplasmy*). Symptoms of patients with heteroplasmic mutations depend on the relative proportion between mutated and wild-type mtDNA copies [8]. Cell dysfunction generally occurs when the proportion of mutated mtDNA exceeds a given threshold; tissues with high metabolic rates such as brain, skeletal muscles, heart, and renal tubules are particularly exposed [3]. The degree of heteroplasmy is also variable from oocyte to oocyte, causing significant differences in disease expression among siblings [15].

Mutations in nuclear DNA may affect genes involved in mtDNA maintenance and replication, mitochondrial protein synthesis, protein subunits of individual complexes (they are common for complex I, but rare for other RC complexes), and assembly factors [1, 3]. Despite Mendelian inheritance, high variability in the clinical expression also characterizes nuclear mutations. Phenotypes associated with individual genes, however, tend to be more homogeneous; SURF1 mutations, for example, cause Leigh syndrome, SCO2 mutations are always associated with cardiomyopathy, complex I deficiencies (regardless of the gene involved) tend to present with isolated encephalopathy, and tubulopathy is a common feature of BCS1L mutations. Differences among patients with mutations in the same gene can be ascribed to the severity of individual mutations, degree of residual activities, modulating genes, or to the redundancy of the system [8].

3. Clinical Symptoms of Mitochondrial Cytopathies

Nearly all organs can be affected in mitochondrial cytopathies, resulting in very heterogeneous clinical presentations. Skeletal muscles are very frequently affected (myopathy, hypotonia, and exercise intolerance). Exercise intolerance is a common complaint that is often mislabeled as "psychogenic," "chronic fatigue syndrome," or "rheumatic fibromyalgia" [15]. Central nervous symptoms develop over time in most patients; virtually all types of neurological symptoms have been described in these disorders, including apnea, hypotonia, lethargy, psychomotor regression, ataxia, stroke-like episodes, hemiparesis, spasticity, seizures, dementia, leukodystrophy, myoclonus, cortical blindness, migraine, polyneuropathy (sensory and/or motor), and neurogenic bladder. Sensorineural deafness and cardiac diseases (myocardiopathy, arrhythmias, and heart block) are also commonly observed but may remain subclinical and should always be excluded. Endocrine complications include diabetes mellitus, hypoparathyroidism, hypothyroidism, hyporeninemic hypoaldosteronism, and growth hormone deficiency. Gastrointestinal symptoms may be related to liver dysfunction, to intestinal dysmotility (vomiting, diarrhea, and pseudoobstruction), or malabsorption. Hematological signs include sideroblastic anemia, neutropenia, and thrombocytopenia. Many patients have ocular problems, including progressive external ophthalmoplegia, ophthalmoparesis, pigmentary retinal degeneration, ptosis, cataract, optic atrophy, and blindness. Finally, various skin and hair lesions have also been described [16].

From the renal stand point, patients may present with signs of tubulopathy, proteinuria, nephrotic syndrome, tubulointerstitial nephritis, cystic kidney disease, myoglobinuria, or renal failure (see below).

Although the above list of symptoms highlights the extreme variability of phenotypes, a major characteristic of these diseases is the progressive involvement of different organs over time.

To date, more than 40 clinical syndromes have been described, based on the association of different symptoms [1, 3].

4. Diagnostic Approaches to Mitochondrial Cytopathies

When suspecting a mitochondrial defect, the first step is generally to measure serum lactate, which is frequently elevated. In oligosymptomatic renal diseases, serum lactate may be normal, but urine lactate is generally elevated. Brain lactate can also be directly measured in the cerebrospinal fluid or estimated by brain MR spectroscopy. If lactate levels are normal, further genetic studies are not usually recommended. The diagnostic workup requires a combination of different approaches, including biochemistry and enzymology analyses, molecular genetics, pathology (histology, histochemistry, and electron microscopy), and neuroradiology studies.

Measurement of urine organic acids by gas-chromatography/mass spectrometry (GC-MS) represents a helpful tool for diagnosing mitochondrial cytopathies (Figure 2). Impaired RC activity causes the accumulation of reduced NADH/NADPH promoting the conversion of acetoacetate into 3OH-butyrate in the mitochondrion and the conversion

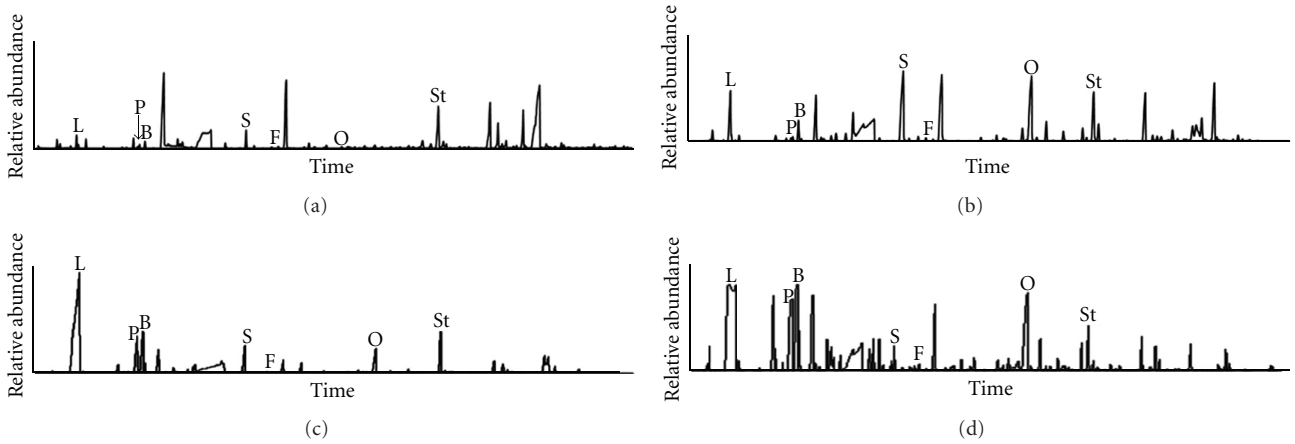


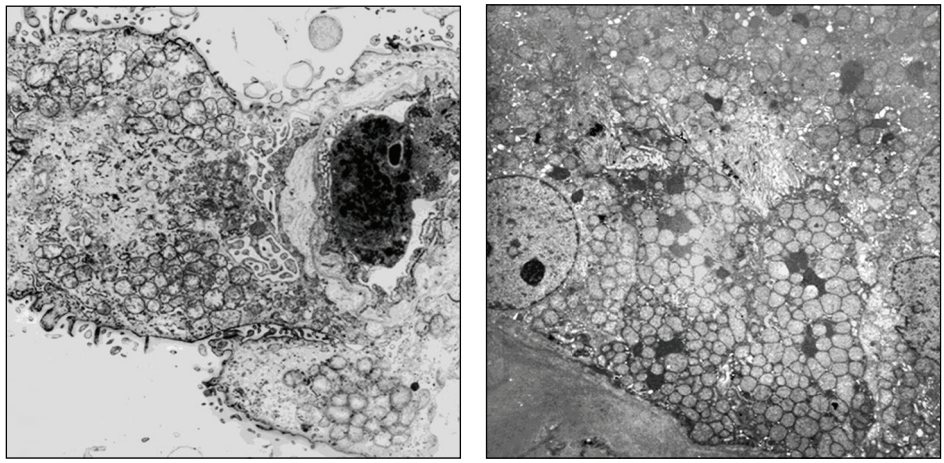
Figure 2: Urine organic acids chromatograms in mitochondrial cytopathies. (a) Control patient. The other panels show the urine chromatograms of their patients with a mitochondrial cytopathy presenting with a generalized tubular defect (b), with De Toni-Debré-Fanconi syndrome (c), or with congenital nephritic syndrome (d). Note the marked increase in lactate (L) excretion in all 3 patients, which was associated with increased urinary excretion of 3-OH-butyrate (B) and 5-oxoproline (O). Other metabolites such as pyruvate (P), succinate (S), and fumarate (F) may also be found in excess.

of pyruvate into lactate in the cytosol (Figure 1). These compounds are often observed in excess in urines, in association with intermediary products of the Krebs cycle (e.g., 2-ketoglutarate, fumarate, malate, or succinate). In some cases, specific profiles of urinary organic acid in combination with abnormal patterns of blood acylcarnitines allows the diagnosis of specific defects, such as ethylmalonic encephalopathy (ETHE) or SUCLA2-, SUCLG1-, and TMEM70-related diseases [17–20]. In addition, low cytosolic ATP impairs the activity of the γ-glutamyl cycle, which generates glutathione using the energy provided by the hydrolysis of ATP. We have observed in several cases of renal mitochondrial cytopathies increased urinary excretion 5-oxoproline, the upstream metabolite of the γ-glutamyl cycle; this finding is not specific of mitochondrial dysfunctions but is highly evocative of a mitochondrial defect if found in conjunction with high urinary excretion of 3OH-butyrate and lactate. Further indications may be obtained by quantitative analysis of plasma aminoacids, which typically shows high alanine (Figure 1) and/or low citrulline levels [21]. These tests require specialized laboratories, but represent first-line analyses allowing to investigate mitochondrial cytopathies with minimal invasiveness.

Further investigations usually require to obtain tissue samples; the general rule is to perform tests on samples collected from the most affected organs. However, in some cases, this approach may be unreasonably aggressive, and studies can be performed on cultured fibroblasts. Measurement of the RC complexes in the kidney, for example, may require an open surgical biopsy to obtain enough material. Similarly, CoQ_{10} determination has been traditionally performed on skeletal muscle [22], which is an invasive procedure in infants. Fortunately, the metabolic defects observed in skeletal muscles, heart, liver, or kidneys are generally present in fibroblasts, which can be easily expanded and shipped to specialized laboratories for diagnosis. In addition and contrary to other biopsy specimens, treatment of metabolic defects (CoQ_{10} biosynthesis defects, e.g.) can be started before obtaining a skin biopsy, because oral supplementations do not influence the results of analyses performed on cultured fibroblasts [23, 24].

Several methods have been developed to assess the RC activity in tissues (reviewed in [25]). Polarographic studies are usually used to measure oxygen consumption in the presence of oxidative substrates such as pyruvate, glutamate, malate, or succinate. Spectrophotometric studies allow to measure the activity of each RC complex; in addition, they allow to test the combined activity of [complex II + complex III] that depends on CoQ_{10}. When the combined activity of [complex II + complex III] is markedly lower than the activity of each complex tested separately, results strongly suggest a ubiquinone biosynthesis defect [26].

In tissues sections, the activity of mitochondrial enzymes, such as cytochrome *c* oxidase (COX) and succinate dehydrogenase (SDH), can be easily assessed with histochemistry techniques [3, 27–29]. These assays are routinely performed on frozen muscle biopsy specimens but can also be applied to other tissues, such as the renal cortex. Because COX is encoded in part by mtDNA, and SDH is entirely encoded by nuclear genes, these studies can demonstrate heteroplasmy by showing cells with high SDH activity secondary to compensatory mitochondrial proliferation and low COX activity [30]; in other cases, they may show a more diffuse decrease in the activity of both enzymes. Electron microscopy, when available, generally demonstrates abnormal mitochondria, proliferation of mitochondria, or mitochondria depletion (Figure 3). Depletion of mitochondria is particularly apparent in proximal tubular cells, which are very rich in these organelles; mitochondrial proliferation in podocytes of patients with steroid-resistant nephritic syndrome (SRNS) is very evocative of a CoQ_{10} defect.



(a) (b)

Figure 3: Electron microscopy studies. Two examples of intense proliferation of abnormal mitochondria (swollen mitochondria with simplified or absent mitochondrial cristae) in a podocyte of a patient with a CoQ_{10} biosynthesis defect (a) and in the proximal tubular cells of a child with severe De Toni-Debré-Fanconi syndrome (b).

5. Renal Mitochondrial Diseases

Kidney involvement is more frequently reported in children than in adults [31]. Several renal diseases have been reported over the past 2 decades, including tubular disorders, chronic tubulointerstitial nephritis, cystic renal disease, and glomerular diseases [31]. In addition, two distinct entities that have primarily glomerular involvement have been identified; these include mtDNA mutations in the $tRNA^{LEU}$ gene and CoQ_{10} biosynthesis defects.

This paper is restricted to mitochondrial cytopathies that affect primarily OXPHOS. However, inherited disorders of mitochondrial fatty acid oxidation can also present with renal involvement. In a large series of 107 patients, Saudubray et al. have reported a tubulopathy and transient renal failure in more than 25% of cases [32]. Since massive rhabdomyolysis represents a frequent event during episodes of acute metabolic decompensation, acute renal failure is generally secondary to myoglobinuria in these patients. Transient renal tubular acidosis has been observed in carnitine palmitoyltransferase type 1 deficiency in combination with Reye-like syndrome [33]. Deficiency of carnitine palmitoyltransferase II causes a neonatal onset lethal multiorgan disease with cystic kidney dysplasia associated with dysmorphic features, central nervous system malformations, liver failure, and cardiomyopathy [34, 35]. Similar findings can be observed in glutaric aciduria type II (or multiple acyl-CoA dehydrogenase deficiency), an autosomal recessive defect of mitochondrial energy metabolism [36]. In both conditions, cystic kidneys can be detected prenatally or at birth through routine ultrasonography examination, showing hyperechoic and enlarged kidneys. These abnormalities are also observed in Zellweger syndrome and other disorders of peroxisomal β-oxidation suggesting that, regardless of the subcellular localization of the biochemical defect, abnormalities of fatty acid oxidation can lead to abnormal organogenesis.

5.1. Tubular Defects. Proximal tubular cells are very rich in mitochondria. Not surprisingly, the most frequent renal tubular finding is a proximal tubular defect, which has been reported in more than 60 patients; of these, 39 have been summarized by Niaudet and Rotig in 1997 [31]; 21 additional patients could be identified in the literature [37–42], including a large Spanish cohort reported by Martín-Hernández et al. in 2005 [43]. In approximately one-third of patients, the tubulopathy corresponded to overt De Toni-Debré-Fanconi syndrome. The remaining patients had more restricted and generally less overt tubular losses and presented with proximal renal tubular acidosis, glycosuria, hyperphosphaturia, and/or aminoaciduria. Nearly all patients had extrarenal symptoms, although cases of isolated tubulopathy have been reported [38, 39], indicating that serum and urine lactate should be investigated in all patients presenting with idiopathic De Toni-Debré-Fanconi syndrome [43]. From a biochemical stand point, the most frequent findings are complex III and/or complex IV defects, followed by complex I defects. From a genetic stand point, all types of mutations have been reported, but large mtDNA deletions are particularly frequent. Tubular involvement is a relatively frequent feature in severe, neonatal-onset RC defects with autosomal recessive inheritance and multisystem involvement; among genes associated with this phenotype are COX10, BCS1L, RRM2B, MRPS22, and SARS2 [44–48]. Symptoms were present in the neonatal period in one-third of patients and in 80% of cases by 2 years of age [31]. Renal biopsies, when available, showed chronic tubulointerstitial changes with damaged proximal tubular epithelia; electron microscopy often showed proliferation of abnormal mitochondria (Figure 3) [31, 37]. Few patients with a Bartter-like phenotype have also been reported [30, 49]. Finally, severe hypomagnesemia is often mentioned in the descriptions of patients with mitochondrial tubulopathies [50].

Of notice, abnormal renal tubular findings remain subclinical (or are overlooked because of the prominence of neurological symptoms) in nearly 2/3 of patients with tubulopathy [43].

When approaching patients with a mitochondrial tubulopathy, clinicians should keep in mind that mitochondrial damage can also be secondary to other causes, including metabolic diseases (tyrosinemia type I, e.g., [51]), drugs (ifosfamide, e.g., [52]), or toxic agents, in particular heavy metals (cadmium, e.g., [53]). A De Toni-Debré-Fanconi syndrome secondary to antimitochondrial antibodies in two patients with primary biliary cirrhosis has also been described [54].

5.2. Chronic Tubulointerstitial Nephritis and Cystic Diseases. Rare cases presenting with chronic renal failure secondary to tubulointerstitial nephritis, without evidence of a primary tubular defect, have been reported; they all had extrarenal symptoms [55–57]. Cystic renal changes have also been rarely described [58–60].

5.3. Sporadic Cases of Glomerular Involvement. Sclerotic glomerular lesions are often described in renal mitochondrial diseases and are probably secondary to tubular and tubulointerstitial lesions. At least nine patients presenting with primary glomerular lesions have been described in the literature [31, 58, 61–63]. They were generally diagnosed with proteinuria and/or hematuria. Some presented with congenital nephrotic syndrome. All patients had or developed over time neurological symptoms (encephalomyopathy); most patients progressed to chronic renal failure if they survived their extrarenal symptoms. The renal pathology, when available, was consistent with focal segmental glomerular sclerosis (FSGS); by EM, depletion or proliferation of abnormal mitochondria in glomerular cells has been described.

5.4. Renal Disease in $tRNA^{LEU}$ Gene Mutations. The 3243 A > G point mutation in the leucine tRNA gene is the most prevalent mtDNA defect. This mutation was initially described in children with MELAS syndrome (myopathy, encephalomyopathy, lactic acidosis, and stroke-like episodes) [2, 3]; however, investigations of mothers that carried the same mutation showed that the clinical spectrum can be more restricted to diabetes mellitus, deafness, and/or neuromuscular symptoms [2, 64]; nearly 1% of the diabetic population carries this mutation [65]. In 1997, a large systematic screening of diabetic patients with a history of maternally inherited diabetes and/or sensorineural hearing loss showed a disproportional number of patients with end-stage renal failure secondary to a proteinuric renal disease in this subset of patients. Since then, at least 27 cases (and their relatives) have been described [64–72]. Patients with deafness and proteinuria can also be misleadingly diagnosed with Alport syndrome; a total of 90 Alport patients have been screened in two studies for a MELAS mutation, allowing to identify 2 misdiagnosed cases [65, 69].

Overall, 2/3 of reported patients are females; diabetes and/or deafness is generally present in the proband's mother and other family members [65, 67–69]. The age at diagnosis ranges from 14 to 50 years. The prevalent renal pathology finding is consistent with FSGS. Four cases of chronic tubulointerstitial nephritis and one case presenting with cystic kidney disease have also been described [64, 72]. A peculiar vasculopathy with hyalinosis of small arteries and myocyte necrosis has been noticed in 2 reports [66, 67].

All patients had high-urinary protein excretion; nephrotic syndrome developed in approximately one-third of cases. Proteinuria generally began in the second or third decade of life, with the youngest patient diagnosed at the age 5 [64]. Most patients were hypertensive; two female patients developed pre-eclampsia [66]. Chronic or end-stage renal failure developed within 10 years in approximately 50% of cases.

Nearly 80% of patients had sensorineural deafness or diabetes mellitus at diagnosis; some, in particular younger patients, developed these symptoms during followup [64–72]. Other reported findings include neuromuscular symptoms, retinal dystrophy, and cardiomyopathy. Serum lactate are generally normal.

5.5. CoQ_{10} Biosynthesis Defects. Primary CoQ_{10} deficiencies deserve a special place among mitochondrial renal defects because they represent the only treatable mitochondrial disorder. They were first reported in 1989 in association with myopathy and encephalopathy [22], and later with cerebellar ataxia [73].

The link between CoQ_{10} and renal disease was established in 2000 when three siblings were diagnosed with progressive encephalopathy and SRNS; neurological symptoms improved significantly after treatment with oral ubidecarenone [74]. Two additional siblings that developed SRNS at 1 year of age were reported in 2005 [75]. The first child developed progressive encephalomyopathy and stroke-like episodes at 18 months but improved after oral CoQ_{10} therapy, while the younger sister, who was diagnosed following her brother disease, was treated immediately after developing proteinuria and never developed neurological symptoms [76]. Mutations in the *COQ2* gene were identified in these two siblings as the first report of a genetic defect associated with primary CoQ_{10} deficiency [77]. *COQ2* encodes for *parahydroxybenzoate polyprenyl transferase*, the enzyme that joins the polyprenoid tail to the quinone group of CoQ_{10} [78] (Figure 1). *COQ2* mutations have been found in four additional patients, all presenting with congenital or early-onset SRNS [26, 79].

Mutations in two other genes involved in the biosynthesis of CoQ_{10} have been identified in patients with similar clinical features, namely, in the *PDSS2* gene (1 patient) [80] and in the *COQ6* gene (11 patients from 5 different kindreds) [81]. Symptoms always began within the first years of life; SRNS was the presenting symptom in most cases, and unless treated, renal disease progressed to end-stage renal failure within few years. Other clinical features included deafness and encephalomyopathy in *COQ6* patients; severe forms with neonatal onset may also present with liver failure and severe lactic acidosis [26, 79].

Other genes required for CoQ_{10} biosynthesis can present with different phenotypes: *COQ9* mutations, for example

(1 patient), cause a severe multisystem disorder with a renal tubulopathy, but no apparent glomerular involvement [82]; patients with mutations in the *COQ8* or *PDSS1* have no apparent renal disease [78, 83, 84].

The renal pathology varies from focal segmental glomerulosclerosis to collapsing glomerulopathy [26, 85]; electron microscopy generally shows numerous dysmorphic mitochondria in the cytoplasm of podocytes [26, 85].

Our understanding of CoQ_{10} has largely benefitted from the availability of the *kd* mouse model that recapitulates the renal phenotype of many CoQ_{10}-deficient patients. These animals were described in the early 1970s [86], but their genetic defect was identified only in 2008, when it was shown that they harbor a homozygous mutation in the *PDSS2* gene [87]. Kidneys are normal at birth and develop progressive interstitial nephritis associated with focal segmental glomerulosclerosis or collapsing glomerulopathy; most animals progress to end-stage renal disease by 4–8 months of age and die of renal failure [88]. Glomerular podocytes play a central role in this animal model, while tubular dilatations and interstitial nephritis represent a downstream consequence of the glomerular disease, as demonstrated by conditional knock-out experiments of the *PDSS2* gene in podocytes or tubular cells [87]. Furthermore, it has been shown that dietary supplementation with CoQ_{10} prevents proteinuria and renal changes in mutant mice [89].

To date, the pathogenesis of CoQ_{10} deficiency remains unclear. In particular, the prevalence of lesions in glomerular cells, which are less energy dependant than tubular cells, raises the possibility that cell damage may not be entirely related to the role of CoQ_{10} in bioenergetics. Silencing the *COQ6* gene in cultured podocytes for example, results in increased apoptosis [81]. In addition, growth of CoQ_{10}-deficient fibroblasts can be corrected by uridine, suggesting that impairment of nucleotide metabolism (CoQ_{10} is required for the biosynthesis of pyrimidines) may also play a role in the pathogenesis of these disorders [24]. The important role of CoQ_{10} as an antioxidant may also be responsible for glomerular damage; an inverse relationship between the severity of CoQ_{10} deficiency and ROS production has been demonstrated in patient's fibroblasts [90, 91]; this hypothesis, however, is not substantiated by *in vitro* data showing that quinone analogues such as idebenone, which are good antioxidants but cannot rescue the mitochondrial respiratory defect, are probably not effective in the treatment of these diseases [74, 92]. CoQ_{10}-deficient cells also display increased autophagy [93]. Finally, a number of studies in *kd* mice indicate that environmental factors are important in the development and progression of renal disease. For example, it has been shown that calorie restriction dramatically increases survival of these animals, while protein restriction has no effect [94]; other studies have shown that placing mice in a germ-free environment slows disease progression, underscoring the complexity of factors that are involved in the pathophysiology of CoQ_{10} renal defects [95].

Regardless of the mechanisms underlying CoQ_{10} defects, one of the most important aspects is the clinical response to oral supplementations. Initial reports failed to show benefits on renal lesions because patients had already advanced kidney disease [74, 75]. Conversely, when treatment was initiated immediately after the onset of renal symptoms in one girl, prompt reduction of proteinuria was observed; this patient has normal renal function nearly 8 years after starting treatment [76]. A similar response has also been documented in 2 patients with *COQ6* mutations [81]. Empirically, CoQ_{10} doses of 30–50 mg/Kg/day have been used, but appropriate pharmacokinetic studies are lacking [76, 81], whereas PDSS2 mutant mice were treated with doses of 200 mg/Kg/day [89]. Different pharmaceutical formulations are commercially available; aqueous or oleous suspensions should probably be preferred to tablet preparations that contain crystalline forms of CoQ_{10} [96].

In summary, current evidences indicate that ubiquinone treatment should be started very early to prevent the development of irreversible lesions, especially in brain and kidneys [76]. A suspicion of a CoQ_{10} deficiency should always arise in patients with early-onset SRNS, especially when podocyte mitochondrial abnormalities are observed by electron microscopy, in the presence of lactic acidosis or when neurologic or muscular symptoms are present.

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Review Article

(Pro)renin Receptor in Kidney Development and Disease

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The renin-angiotensin system (RAS), a key regulator of the blood pressure and fluid/electrolyte homeostasis, also plays a critical role in kidney development. All the components of the RAS are expressed in the developing metanephros. Moreover, mutations in the genes encoding components of the RAS in mice or humans are associated with a broad spectrum of congenital anomalies of the kidney and urinary tract (CAKUT). These forms of CAKUT include renal papillary hypoplasia, hydronephrosis, duplicated collecting system, renal tubular dysgenesis, renal vascular abnormalities, and aberrant glomerulogenesis. Emerging evidence indicates that (pro)renin receptor (PRR), a novel component of the RAS, is essential for proper kidney development and that aberrant PRR signaling is causally linked to cardiovascular and renal disease. This paper describes the role of the RAS in kidney development and highlights emerging insights into the cellular and molecular mechanisms by which the PRR may regulate this critical morphogenetic process.

1. Introduction

1.1. Brief Overview of Kidney Development. During embryogenesis, the nephric duct (ND) is formed from the intermediate mesoderm on embryonic (E) day E22 in humans and E8 in mice [1]. The ND extends caudally and induces adjacent intermediate mesoderm to form two transient kidney types, pronephros and mesonephros. The pronephros degenerates in mammals, whereas the mesonephros involutes in females, but gives rise to male reproductive organs [1]. On 5th week of gestation in humans (E10.5 in mice), the caudal portion of the ND forms an epithelial outgrowth called the ureteric bud (UB). The metanephric kidney arises from two embryonic tissues: the UB and the metanephric mesenchyme (MM) [2, 3] (Figures 1(a)–1(d)). UB grows out from the ND, elongates, invades the MM, and then branches repeatedly within the mesenchyme to form the renal collecting system (the ureter, pelvis, calyces, and collecting ducts) [3–5]. Linear arrays of inner (medullary) collecting ducts converge centrally to form the papilla. Distal ureter subsequently translocates from the ND to fuse with the bladder which originates from the urogenital sinus (Figures 1(e)–1(g)) [6, 7]. Terminal UB tips induce surrounding MM-derived nephron progenitors to condense and then differentiate into nephrons (from the glomerulus to the distal tubule), thus forming the metanephric kidney (Figures 1(a)–1(d)) [3, 4].

1.2. Wnt Signaling in Metanephric Kidney Development. Metanephric kidney development depends on reciprocal interactions of transcription and growth factors expressed in the metanephric mesenchyme, stroma and the UB [3, 8, 9]. The wingless (Wnts) are secreted glycoproteins fundamental for proper kidney development. Wnt ligands bind to extracellular domain of frizzled (Fz) seven trans-membrane domain receptors and, in some cases, the low-density lipoprotein (LRP) 5 and 6 coreceptors to activate distinct intracellular signaling cascades [10–12]. Wnt signaling regulates metanephric kidney development *via* canonical or noncanonical signaling pathways [13]. Binding of Wnt to its receptor leads to accumulation of β-catenin in the cytoplasm followed by translocation to the nucleus and interaction with the T-cell factor/lymphoid-enhancing factor (Tcf/Lef) family of transcription factors to regulate gene transcription [14]. In addition, β-catenin is an important component of cell-cell adherens junctions and interacts with the actin cytoskeleton [15]. The noncanonical Wnt signaling pathway consists of the planar cell polarity/convergent extension (PCP/CE)

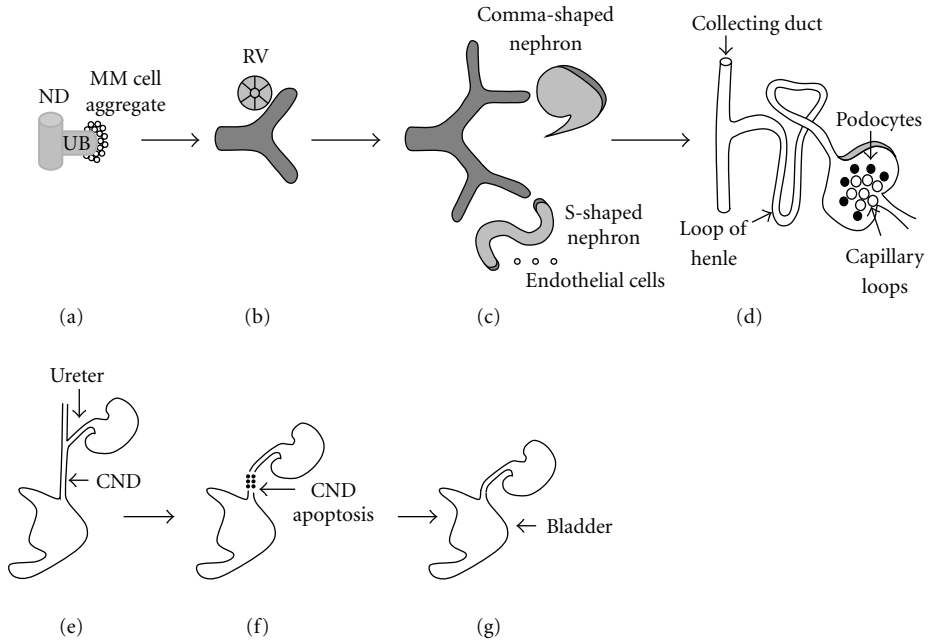

Figure 1: Schematic representation of normal development of the kidney and urinary tract. (a): Invasion of the metanephric mesenchyme (MM) by the ureteric bud (UB) on weeks 5-6 of gestation induces MM cells to aggregate around the UB tip. (a)–(c): UB outgrowth from the nephric duct (ND), its subsequent iterative branching (branching morphogenesis), and continuous condensation of the MM cells around emerging UB tips are induced primarily by reciprocal interactions among glial-derived neurotrophic factor (*GDNF*), its receptor *c-Ret,* and coreceptor *GFRα1*. (b): MM cell aggregates undergo mesenchymal-to-epithelial transformation (MET) to form the renal vesicle (RV) on weeks 6–36 of gestation. (c): RV elongates along the proximal-distal axis to form comma-shaped and then S-shaped nephron. Distal ends of S-shaped nephrons fuse with UB-derived collecting ducts, whereas proximal clefts form glomeruli. Endothelial cells migrate into the proximal cleft. UB branching occurs on weeks 6–22 of gestation. Formation of nascent nephrons and their patterning occur on weeks 6–36 of gestation. (d): Patterning of the S-shaped nephron and UB result in formation of mature nephron which contains glomerulat capillary tuft, podocytes, proximal tubule, loop of Henle, distal tubule, and collecting duct. (e). Ureter becomes patent and common ND (CND) fuses with cloaca on weeks 4-5 of gestation. (f): Apoptosis of the CND accounts for the positioning of the ureter (derived from proximal UB) in proximity of the urogenital sinus on weeks 5-6 of gestation. (g): Ureter fuses with the bladder by 6 weeks of gestation (with kind permission from Springer Science + Business Media: [82]). Please see text for details.

pathway and the Ca^{2+}-releasing pathway [13]. PCP controls polarization of cells within the plane of the tissue, whereas CE directs intercalation of cells in an epithelial sheet to form a longer and narrower strip of the tissue [16]. Several Wnts are expressed in the discrete domains of the developing mouse kidney and play a critical role in proper metanephric organogenesis. *Wnt6*, *Wnt7b*, *Wnt9b,* and *Wnt11* are expressed in the UB [17–19]. *Wnt4* is expressed in the MM and *Wnt2b* in the cortical stroma [17, 20]. Of the Wnts expressed in the metanephros, *Wnt2b*, *Wnt4*, *Wnt7b,* and *Wnt9b* activate canonical pathway. Wnt signaling is essential for UB branching, nephrogenesis, and medullary patterning. Available data suggest that UB signals to the MM by secreting Wnt9b, a soluble growth factor, which acts *via* the canonical β-catenin to induce expression of fibroblast growth factor 8 (*FGF8*), LIM homeobox 1 (*Lhx1*) and *Wnt4* in the MM [18, 21]. In turn, *Wnt4* induces MM cells to undergo mesenchymal-to-epithelial transformation (MET) and differentiate into the nephron epithelia [21]. Genetic inactivation of *Wnt9b* or *Wnt4* in mice leads to arrest of nephrogenesis at renal vesicle stage, and deficiency of *Wnt9b* causes severe defects in UB branching [18, 21]. UB-specific inactivation of β-catenin, the central effector of the canonical *Wnt* signaling pathway, causes aberrant UB branching and premature differentiation of collecting duct cells and results in renal hypodysplasia [22, 23]. In addition to directing UB branching and nephrogenesis *via* the canonical pathway, Wnt9b acts *via* a noncanonical Wnt pathway to induce PCP in UB-derived collecting ducts. Available evidence suggests that longitudinally oriented cell division (OCD) leads to collecting duct elongation without a change in diameter. In conditions in which collecting ducts dilate to form cysts (e.g., polycystic kidney disease), OCD is randomized, leading to a progressive increase in collecting duct diameter [24]. Mice that lack *Wnt9b* exhibit dilated collecting ducts, aberrant OCD of the collecting duct cells and develop renal cysts postnatally [25]. *Wnt7b*-mutant mice do not form renal medulla and papilla [26]. These defects are likely due to aberrant OCD and decreased survival of the medullary collecting duct cells [26].

2. The Renin-Angiotensin System

The renin-angiotensin system (RAS) plays a fundamental role in the regulation of arterial blood pressure,

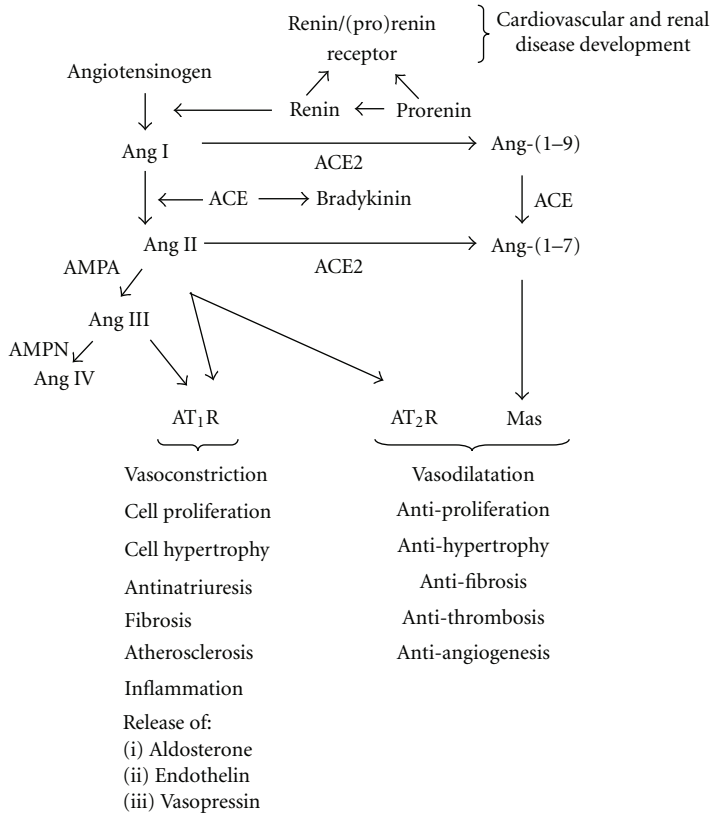

Figure 2: The Renin-Angiotensin System. Ang: angiotensin II. ACE: angiotensin-convertin enzyme, ACE2: angiotensin-converting enzyme 2, AMPA: aminopeptidase A. Please see text for details.

fluid/electrolyte homeostasis, and kidney development [27]. In the RAS, renin cleaves angiotensinogen (AGT) to generate Ang I [Ang-(1–10)] (Figure 2). Ang I is converted by angiotensin-converting enzyme (ACE) to Ang II [Ang-(1–8)], the most powerful effector peptide hormone of the RAS [28–30]. Ang II acts *via* two major G protein-coupled receptors (GPCR): AT_1R and AT_2R [29]. Most of hypertensinogenic and sodium-retaining actions of Ang II are attributed to the AT_1R [31]. In contrast to the AT_1R, the AT_2R elicits vasodilation, promotes renal sodium excretion, and inhibits proliferation in mesangial cells [32–34]. ACE2 is a homologue of ACE which is abundantly expressed in the kidney and acts to counterbalance ACE activity by promoting Ang II degradation to the vasodilator peptide Ang-(1–7) [35, 36]. Ang-(1–7) acts *via* the GPCR Mas encoded by *Mas* protooncogene to oppose Ang II-AT_1R-mediated effects [37, 38]. Renin is synthesized in juxtaglomerular cells of the afferent arterioles of the kidney as preprorenin [39]. The human renin gene encoding preprorenin is located on chromosome 1 [40]. Cleavage of a 23 amino acid signal peptide at carboxyl terminus of preprorenin generates prorenin. Prorenin is then converted to active renin by cleavage of 43-amino acid N-terminal prosegment by proteases [41, 42]. The kidney secretes both renin and prorenin into the peripheral circulation. Plasma levels of prorenin are approximately 10-fold higher than those of renin [43].

In addition to cleaving AGT, renin binds the (pro)renin receptor (PRR) [44, 45]. The PRR protein is a seven transmembrane domain receptor encoded for by the *ATP6AP2* (*ATPase-associated protein2)*/*PRR* gene (*subsequently referred to as PRR*) located on the X chromosome in humans [44]. The PRR protein exists in three forms: (1) A full-length 35–39 kDa form composed of 3 domains: extracellular, a single transmembrane, and a cytoplasmic, (2) A 28 kDa soluble form found in plasma and urine, and (3) A truncated form containing transmembrane and cytoplasmic domains [42, 45]. In addition to proteolytic activation *via* cleavage of the prosegment by proteases, prorenin may be activated by binding to the PRR and undergoing a conformational change that does not require removal of the prosegment [44].

2.1. Expression of the RAS Components in the Developing Metanephros. The developing metanephros expresses all the components of the RAS [46–48]. In the fetal mouse kidney, renin mRNA is first detected on E14.5 by *in situ* hybridization in a few scattered foci of cells [46]. By E15.5, renin is widely expressed in branches of the renal artery, interlobar, and arcuate arteries. In the human kidney, renin mRNA is detected at the vascular pole of the glomerulus and in arteries located next to glomeruli [48]. With fetal maturation, renin expression shifts to its mature localization in the juxtaglomerular cells [46, 48]. Studies in renin knockin

reporter mice have demonstrated that renin-producing cells may originate from the mesenchyme at E11-E12 before vessel development has occurred [49]. Ontogeny studies have demonstrated that renal renin synthesis is highly activated during early postnatal development in rodents [47]. Because immunoreactive Ang II levels are higher in the fetal and newborn than adult mouse kidney [50, 51], renin is considered to be the rate-limiting factor for Ang II generation during metanephric development. In the adult rat kidney, PRR mRNA and protein are expressed in the collecting duct and the distal nephron [52]. In the CD, the PRR is most abundant at the apical surface of type A intercalated cells where it colocalizes with the vacuolar H^+-ATPase [52]. In addition, PRR immunoreactivity is also detected in the podocytes, renal mesangial, vascular smooth muscle, and endothelial cells [53–55]. Even though PRR is expressed in *Xenopus* pronephros [56], the expression of the *PRR* gene and PRR protein during metanephric development remains to be determined.

2.2. Effect of Pharmacological or Genetic Interruption of the RAS on Kidney Development. Treatment of several animal species or humans with ACE inhibitors or AT_1R antagonists during gestation or postnatal metanephrogenesis leads to renal tissue dysplasia [57]. A decrease in the number and size of glomeruli, delay in glomerulogenesis, a reduction in the number and length of the renal arteries accompanied by arterial thickening, tubular dilation and a hypoplastic papilla are observed [58]. Nephrotoxic effects of ACE inhibitors and AT_1R antagonists in humans include oligohydramnios and anuria [59, 60]. *AGT*-, *renin*-, *ACE*-, or AT_1R-deficient mice exhibit virtually identical phenotypes characterized by vascular hyperplasia, hydronephrosis, hypoplastic medulla, and papilla [61–66]. Functionally, *Renin*-, *ACE*-, and AT_1R-null animals are polyuric and have a reduced ability to concentrate urine [63–65]. Deletion of the AT_2R in mice causes ectopic UB budding from the nephric duct, duplicated collecting systems, and hydronephrosis [67]. Mutations in the genes encoding for *AGT*, *renin*, *ACE,* or AT_1R in humans are associated with renal tubular dysgenesis (RTD) [68, 69]. Kidneys of patients with RTD demonstrate reduced number of proximal tubules, collapsed collecting ducts, enlarged glomeruli, and thickened arteries [69]. Attempts to generate knockout mice with global deletion of the *Prr* failed [70]. Even though this observation precludes so far determination of the specific role for the PRR in kidney organogenesis, it also indicates that the PRR is essential during development. Targeted inactivation of the *Prr* gene in mouse cardiomyocytes causes cardiac tissue fibrosis, cardiomyocyte apoptosis, and death within the first several weeks of postnatal life from heart failure [71].

2.3. Signaling Pathways Downstream of the PRR. Even though the cytoplasmic domain of the PRR has no intrinsic kinase activity [48], *in vitro* studies demonstrate that binding of the PRR by renin or prorenin leads to activation of mitogen-activated protein (MAP) kinases such as Erk1/2 or p38 in renal mesangial and vascular smooth muscle (VSMC) cells and induces phosphorylation of phosphatidylinositol 3-kinase/Akt (PI3K/Akt) in human embryonic kidney (HEK293T) cells [72, 73]. The effect of the PRR on Erk1/2 phosphorylation in human monocytes is independent of Ang II or transactivation of the EGF receptor [74]. In contrast, induction of Erk1/2 and Akt phosphorylation by recombinant rat prorenin in cultured rat VSMCs is independent of Ang II, but dependent on phosphorylation of EGF receptor [75]. Since these changes are blocked by the *PRR* siRNA, activation of the EGF receptor, Erk1/2, and Akt in VSMC depends on PRR. The importance of the PRR-dependent Erk1/2 activation in brain development is evident from the observations that hypomorphic mutation in the *PRR* is causally linked to the absence of Erk1/2 phosphorylation and the development of X-linked mental retardation in humans [76].

2.4. Role of the PRR in Kidney and Cardiovascular Disease. A direct pathological role of the PRR in renal injury and cardiovascular disease is suggested by the findings of glomerulosclerosis, proteinuria, and elevated blood pressure in rats with ubiquitous transgenic overexpression of the human *PRR* (Table 1) [91]. Targeted overexpression of the *Prr* in the rat vasculature under the control of the mouse smooth muscle myosin heavy chain gene causes mild hypertension after six months of age [77]. Although transgenic overexpression of the prorenin, a major ligand for the PRR, in rats does not cause renal fibrosis, it leads to myocardial hypertrophy, proteinuria, and hypertension [92, 93]. Of interest, since hypertension is controlled by ACE inhibition, it may be due to the increased formation of Ang II [92, 93]. Double-transgenic mice that overexpress human prorenin in the liver and human angiotensinogen in the heart display a selective increase in Ang I content in the heart (but not the plasma) as compared to the single-transgenic mice [94]. These results suggest that circulating prorenin is taken up by tissues where it can contribute to the local synthesis of Ang peptides and tissue damage. Expression of the PRR mRNA and protein is increased in the myocardium and renal tubular, VSMC, and endothelial cells in rats with congestive heart failure due to coronary ligation [54]. Moreover, treatment of spontaneously hypertensive rats (SHRs) with a synthetic peptide that blocks prorenin binding to the PRR reduces renal and cardiac fibrosis [78, 95]. These data demonstrate that the PRR may contribute to the pathogenesis of heart failure and kidney tissue damage. An important role for the PRR in the pathogenesis of hypertension in humans is supported by the findings that a polymorphism in the *PRR* gene is associated with a high blood pressure in men (IVS5+169C>T) and left ventricular hypertrophy in women (+1513A>G) [80, 81].

Although rare cases of human hypertension or CAKUT are due to mutations in single genes, the contribution of the genetic determinants in the vast majority of subjects with high blood pressure or CAKUT remains unknown [82, 96]. If common diseases such as hypertension or nonsyndromic cases of CAKUT are due to multiple gene variants with small effects, large study samples are needed to identify them.

Table 1: Role of (pro)renin receptor (PRR) signaling in kidney development and disease.

Model	Renal phenotype	Cardiovascular phenotype	Other phenotype	References
Global overexpression of human *PRR* in the rat	Glomerulosclerosis Proteinuria	Elevated blood pressure	Unknown	[54]
Targeted overexpression of human *PRR* in rat vasculature	Unknown	Elevated blood pressure and heart rate	Unknown	[77]
SHR rats treated with PRR blocker	Attenuation of renal fibrosis	Attenuation of cardiac fibrosis	Unknown	[78]
Mice with targeted deletion of *Prr* in cardiomyocytes	Unknown	Cardiac tissue Fibrosis Cardiomyocyt opoptosis	Unknown	[71]
Xenopus embryos treated with anti-*PRR* morpholino	Unknown	Unknown	Short body axis Impaired CE Small head Short tail Defects in eye pigmentation	[79] [56]
Genetic polymorphism of *PRR* in humans	Unknown	Hypertension in men LVH in women	Unknown Unknown	[80] [81]
Hypomorphic *PRR* mutations in humans	Unknown	Unknown	X-linked mental retardation Epilepsy	[76]

SHR: spontaneously hypertensive rats, CE: convergent extension, LVH: left ventricular hypertrophy, *PRR*: human PRR gene, PRR: human PRR protein, *Prr*: mouse *PRR* gene.

Within the last few years, several genome-wide association studies (GWAS), in which thousands of common genetic variants are analyzed for disease association, identified significant association of a limited number of genes with primary hypertension [97]. Further studies are needed to understand how the implicated genes contribute to such a complex multifactorial disease as primary hypertension. Despite identification of significant association of hypertension with variants of *renin*, *ACE* and *AGTR1* genes in a study by Zhu et al. [98], the results of RAS gene-association studies are inconsistent [99]. With respect to CAKUT, broad phenotypic spectrum of renal system anomalies and variability in genotype-phenotype correlation demonstrate that pathogenesis of CAKUT is a complex process that depends on interplay of many factors [82, 100]. It is likely that well-powered studies utilizing total human exome capture and next-generation sequencing will identify single-gene defects leading to CAKUT.

2.5. Potential Roles for the PRR in Kidney Development. The mechanisms by which the PRR may regulate kidney development may involve changes in the expression of genes or transcription factors that are critical for metanephric organogenesis, physical interaction between the PRR and other receptors or proteins with established roles in renal ontogeny or function, activation of the intracellular signaling pathways, or other mechanisms (Figure 3).

2.5.1. Interaction with the Promyelocytic Zinc Finger Transcription Factor (PLZF). PLZF is a nuclear phosphoprotein which belongs to the POZ/zinc-finger family of transcription factors and is encoded by the *Zfp145* gene. *Zfp145*-null mice exhibit aberrant expression of *Hox* genes, defects in limb, and axial skeletal patterning, whereas the kidney phenotype of these mice was not described [101]. Several observations support potential role for PLZF in kidney development. For example, *Hox* (*Hox11*) genes are necessary to specify the metanephric kidney identity from the intermediate mesoderm [102]. In patients with acute promyelocytic leukemia, PLZF fuses with the retinoic acid receptor α (RARα) and recruits histone deacetylase 1 (HDAC1) to render retinoic acid-target genes unresponsive to retinoic acid, an active form of vitamin A [103]. Both HDAC1 and retinoic acid are essential for embryo development. Genetic inactivation of *HDAC1* in mice results in embryonic lethality before E10.5 due to severe proliferation defects [104], whereas *RAR*-mutant mice display renal dysplasia [105]. Concerning the RAS, inhibition of HDAC activity induces renin expression in the embryonic kidney [106, 107]. PRR interacts with the PLZF protein in HEK293 cells *in vitro* [73]. Moreover, treatment of HEK293 cells with renin causes nuclear translocation of PLZF followed by recruitment of PLZF to the promoters of the *PRR* and *PI3K-p85α* genes. This leads to repression of *PRR* transcription and induction of *PI3K* gene expression [73]. Notably, inhibition of PI3K/Akt blocks UB branching induced by glial-derived neurotrophic factor (GDNF)/rearranged during transfection (Ret) ligand-receptor pair or by Ang II [85, 86].

In addition, PLZF inhibits transcription of *c-kit* in $CD34^+$ hematopoietic progenitor cells (HPC) and in spermatogonia [83, 108]. Functionally, increased *c-kit* expression is necessary to sustain differentiation of these cells. *Zfp145*-null mice exhibit depletion of the proliferative spermatogonial compartment in the testis due to deregulated expression of *c-kit*, which controls a tight balance between spermatogonial self-renewal and differentiation [108]. c-kit is a receptor tyrosine kinase (RTK) for the stem cell factor (*SCF*), a key regulator of HPC proliferation, survival, and differentiation [83]. Notably, *c-kit* is expressed in the interstitial cell and angioblasts of the developing metanephros [84]. Moreover, antagonism of *c-kit* RTK activity inhibits UB branching and reduces the number of nephrons and renal angioblasts [84].

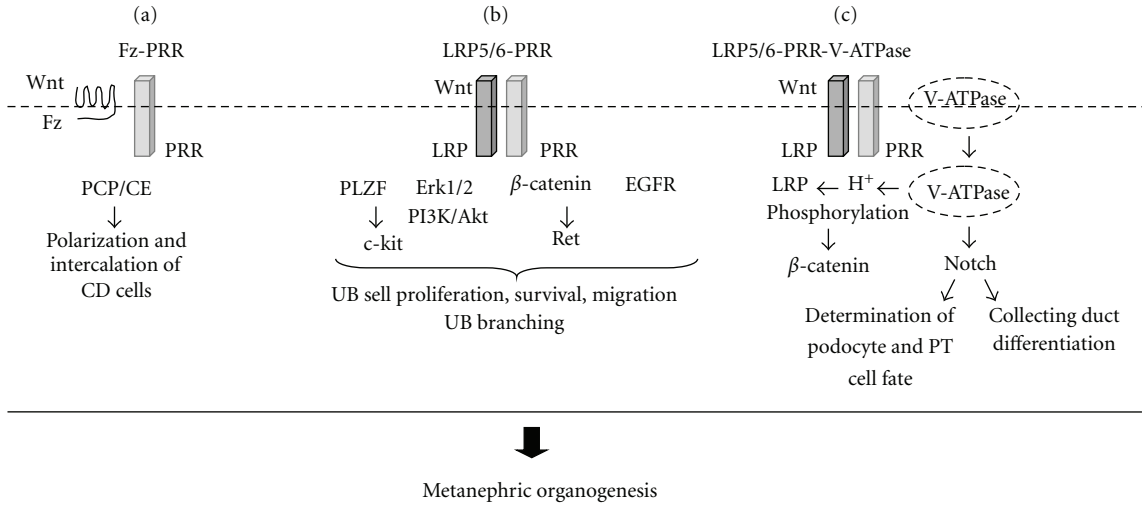

Figure 3: Proposed mechanisms mediating the effect of the (pro)renin receptor (PRR) in metanephric kidney development. (a): PRR interacts with Wnt receptor frizzled (Fz) to regulate polarization and intercalation of collecting duct (CD) cells *via* planar cell polarity (PCP)/convergent extension (CE) Wnt signaling pathway [16, 25, 79]. (b): PRR may regulate UB branching by (1) inhibiting *c-kit* transcription *via* promyelocytic zinc finger transcription factor (PLZF) [73, 83, 84] (2) induction of Erk1/2, PI3K/Akt or epidermal growth factor receptor (EGFR) phosphorylation [52, 72, 73, 75, 85–87], and (3) interaction with LRP5/6 Wnt coreceptor leading to activation of *β-catenin* and *Ret* gene expression [22, 23, 56]. (c): PRR interacts with LRP5/6 and V-ATPase to form a complex at the plasma membrane. Following endocytosis, V-ATPase generates a gradient of H^+ ions that is essential for LRP5/6 phosphorylation and activation of β-catenin [56]. V-ATPase stimulates Notch signaling [88]. Notch acts to define the podocyte and proximal tubular cell fates [89] and regulates differentiation of the CD cells [90].

Since PLZF represses *c-kit* expression, loss of PLZF can cause dysregulation of the differentiation of *c-kit*-positive progenitors of renal interstitial cells and lead to renal hypodysplasia.

2.5.2. Interactions with the Canonical Wnt/β-Catenin Signaling. Another mechanism by which the PRR may control metanephric development is by the regulation of the canonical Wnt/β-catenin signaling. Inhibition of the *PRR* by small inhibitory RNA (siRNA) in HEK293T cells *in vitro* or by the *PRR* antisense morpholino in *Xenopus* embryos *in vivo* decreases luciferase reporter activity stimulated by canonical Wnt signaling [56]. The PRR binds to Fz8 and LRP6 in HEK293T cells transfected with the *PRR* expression vector. Activation of LRP6 and β-catenin signaling depends on extracellular, but not on cytoplasmic or transmembrane, domain of the PRR [56]. Given that canonical Wnt signaling is critical in metanephric organogenesis [22, 23], it is conceivable that direct interaction between the PRR and LRP6 plays an important role in the activation of canonical Wnt signaling *via* β-catenin to regulate kidney development. This possibility is supported by the observations that mutations in *LRP4*, which is known to antagonize LRP6-mediated activation of canonical *Wnt* signaling, are associated with CAKUT in humans with Cenani-Lenz syndrome (OMIM# 604270) [109].

2.5.3. Interactions with Noncanonical Wnt/PCP Signaling. In addition to its function in canonical Wnt signaling, PRR modulates Wnt/PCP pathway of the noncanonical Wnt signaling. Treatment of *Xenopus* embryos with anti-*PRR* morpholinos causes a short body axis and a broader expression domain of the notochord marker *Xnot*, a hallmark of impaired convergent extension movements [79]. Aberrant convergent extension (lateral intercalation) of the collecting duct and renal tubular cells may be causally linked to polycystic kidney disease [25]. In addition, *Drosophila* PRR interacts biochemically with Fz in HEK293T cells [79]. The *Drosophila* Fz receptor is required for PCP. Collectively, PRR mediates both the Wnt/PCP and the Wnt/β-catenin signaling pathways. These effects of the PRR are independent of renin [56, 79].

2.5.4. Interactions with V-ATPase. PRR may regulate kidney development or function *via* the V-ATPase (Figure 3). V-ATPase is a multiprotein complex localized in the kidney in intracellular organelles and at the plasma membrane of the intercalated collecting duct cells. Its major function is to pump protons to promote endocytosis [110]. Mutations in the genes encoding the kidney-specific isoforms B1 or A4 of V-ATPase in humans are responsible for inheritable forms of distal renal tubular acidosis, a disease characterized by elevated H^+ concentrations in the plasma due to the impaired renal excretion of acid [111, 112]. Critical role for V-ATPase in development is evident from the observation that mutations in the genes encoding subunits C or D of V-ATPase in mice result in embryonic lethality [113, 114]. Mutations in subunits *B1* or *A3* of V-ATPase in mice cause metabolic acidosis and osteopetrosis, respectively [115, 116]. Variability in defects observed in these mice indicates that different subunits of V-ATPase have different functions and that subunits A3, A4, and B1 may be important in bone

remodeling or differentiation of collecting duct cells involved in acid-base homeostasis.

In vitro studies demonstrate that the endogenous PRR binds to the endogenous V-ATPase subunits ATP6V0D1 and ATP6V0C in HEK293T cells [56]. Injection of a dominant-negative V-ATPase subunit E in *Xenopus* embryos synergizes with anti-*PRR* morpholino in inhibition of Wnt signaling. Phosphorylation of LRP6, which correlates with LRP6 activation, is inhibited in mouse P19 embryonal carcinoma cells treated with *PRR*- or *V-ATPase* siRNA [56]. These findings indicate that the PRR and V-ATPase interact functionally to inhibit Wnt signaling during *Xenopus* embryonic development *in vivo*.

Genetic inactivation of the *Prr* in cardiomyocytes in mice decreases expression of the V(O) subunits of V-ATPase, resulting in deacidification of the intracellular vesicles [71]. Thus, the PRR is also essential for vacuolar H^+-ATPase assembly in murine cardiomyocytes *in vivo*. Mutations in *VhaAC39*, a V-ATPase subunit, in Drosophila are associated with the loss of Notch signaling [88]. Ligand binding to Notch receptor induces proteolytic cleavage and release of the intracellular domain of Notch which enters the cell nucleus to regulate target gene expression. Notch signaling exerts dual function during mouse metanephric development. Notch activity defines the podocyte and proximal tubular cell fates during segmentation of the S-shaped body [89]. In the collecting duct, Notch acts to increase the ratio of principal-to-intercalated cells and regulate urinary concentrating ability [90]. Thus, Notch signaling is required for the differentiation and functional maturation of the principal cells in murine renal collecting duct. Mutations in *Notch2* in humans result in Alagille syndrome (OMIM# 610205) [117]. The renal phenotype in Alagille syndrome is characterized by hypodysplasia. Since PRR is most abundant at the apical surface of type A intercalated cells of the collecting duct, where it colocalizes with the vacuolar H^+-ATPase, in the rat [52], the PRR may act in an autocrine fashion to regulate H^+ transport. During metanephric development, PRR may promote differentiation of H^+-secreting intercalated cells in the collecting duct. PRR located on the apical membrane of intercalated cells may be activated in a paracrine fashion by prorenin released by adjacent principal cells [118]. Additional evidence to implicate regulated intracellular acidification in mediating Wnt signaling is provided by the observations that *Nhe2*, a sodium/proton exchanger in Drosophila and a homologue to human *NHE3*, interacts genetically with *Fz* receptor to regulate Wnt/PCP signaling [119]. Together, these findings suggest that PRR cross-talks with V-ATPase in a renin-independent fashion to regulate both canonical Wnt/β-catenin and noncanonical Wnt/PCP signaling.

2.5.5. Regulation of Intracellular Signaling Pathways Critical for Metanephric Kidney Development. Another mechanism by which the PRR may modulate metanephric morphogenesis is *via* activation of downstream signaling pathways such as PI3K or Erk1/2 [72, 73]. This possibility is supported by an attenuation of Erk1/2 phosphorylation with *PRR* siRNA in collecting duct/distal tubule lineage Madin-Darby Canine Kidney (MDCK) cells *in vitro* [52]. An important role for Erk1/2 and PI3K in kidney development is demonstrated by the findings that inhibition of Erk1/2 decreases UB branching [120] and that antagonism of PI3K/Akt blocks directional migration of *Ret*-transfected MDCK cells in response to *GDNF in vitro* [85]. Critical role of GDNF and Ret in UB morphogenesis is evident from the findings that targeted inactivation of *GDNF* or *Ret* in mice results most frequently in bilateral renal agenesis due to a failure of UB outgrowth [121, 122]. Notably, tyrosine phosphorylation of the EGF receptor and activation of PI3K/Akt and Erk1/2 are also essential for Ang II-induced UB branching [86, 87].

3. Conclusions and Perspectives

The PRR is emerging as potential critical player in normal metanephric kidney development. It has become evident that the PRR has an evolutionary conserved role in bridging V-ATPases with canonical and noncanonical Wnt signaling. Acting *via* these or other intracellular pathways (e.g., Erk1/2, PI3K), the PRR may regulate segmentation of the proximal nephron and direct functional maturation of UB-derived collecting ducts. Even though significant progress has been made in defining the potential role of the PRR and specific contribution of PRR-dependent signal transduction in metanephric development, further evidence supporting the direct role for the PRR in metanephric organogenesis is needed. What are the perspectives for system-wide approaches to understand the role of the PRR in kidney organogenesis? In this regard, application of new genetic tools, such as conditional/tissue/cell-specific gene knockouts, genetic lineage tracing and fluorescent *in vivo* reporters of cell signaling, genome-wide analysis of gene regulatory networks (including epigenetic regulation) that control different aspects of kidney development (e.g., microarray, ChIP-Seq) should provide important insights in understanding molecular mechanisms by which the PRR may direct normal and abnormal metanephric kidney development. Defining molecular aberrations leading to CAKUT in animals and humans with mutations in the *PRR* will uncover biomarkers that can be used for early diagnosis or prevention of renal system anomalies in children. Finally, establishment of shared large biorepositories of patients encompassing a wide spectrum of CAKUT phenotypes for molecular, genetic, and translational studies will define clinically relevant mutations in the *PRR*, its ligands, and interacting genes. The evolution of our understanding of the cellular and molecular mechanisms by which intact PRR controls metanephric kidney development may provide targets for improved diagnosis and prevention of CAKUT in children.

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Review Article

Primary Hyperoxaluria

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Primary hyperoxalurias (PH) are inborn errors in the metabolism of glyoxylate and oxalate. PH type 1, the most common form, is an autosomal recessive disorder caused by a deficiency of the liver-specific enzyme alanine, glyoxylate aminotransferase (AGT) resulting in overproduction and excessive urinary excretion of oxalate. Recurrent urolithiasis and nephrocalcinosis are the hallmarks of the disease. As glomerular filtration rate decreases due to progressive renal damage, oxalate accumulates leading to systemic oxalosis. Diagnosis is often delayed and is based on clinical and sonographic findings, urinary oxalate assessment, DNA analysis, and, if necessary, direct AGT activity measurement in liver biopsy tissue. Early initiation of conservative treatment, including high fluid intake, inhibitors of calcium oxalate crystallization, and pyridoxine in responsive cases, can help to maintain renal function in compliant subjects. In end-stage renal disease patients, the best outcomes have been achieved with combined liver-kidney transplantation which corrects the enzyme defect.

1. Introduction

Hyperoxaluria may be either inherited or acquired. The primary hyperoxalurias (PH) are inborn error of metabolism resulting in increased endogenous production of oxalate leading to excessive urinary oxalate excretion. To date, three distinct hereditary enzymatic deficiencies have been linked to PH, namely, PH type 1 (PH1), type 2 (PH2), and type 3 (PH3), and there is evidence to speculate that further causes are yet to be identified. Due to marked hyperoxaluria, recurrent urolithiasis and progressive nephrocalcinosis are the principal manifestations of PH. As a result of kidney injury, glomerular filtration rate (GFR) declines leading to chronic kidney disease, and ultimately to end-stage renal disease (ESRD) and systemic involvement in PH1, the most severe type of PH. Despite recent improvement in disease spectrum knowledge, diagnostic procedure, and treatment strategies, PH1 still represents a challenging issue for both adult and pediatric nephrologists worldwide.

2. Physiopathology

Primary hyperoxalurias are inborn errors in the metabolism of glyoxylate and oxalate (Figure 1). They are characterized by an excessive production of oxalate, a metabolic endproduct. The most common type of PH, PH1 (MIM #259900) is caused by a deficiency of the liver specific, peroxisomal, pyridoxal phosphate-dependent enzyme alanine : glyoxylate amino transferase (AGT; EC 2.6.1.44) [1, 2]. The second type, PH2 (MIM #260000), is caused by a deficiency in glyoxylate reductase/hydroxypyruvate reductase (GRHPR; EC 1.1.1.26/79) [3–5], a cytosolic enzyme. The recently identified PH type 3 (MIM #613616) [6] is linked to the gene *DHDPSL*, encoding a mitochondrial enzyme, although

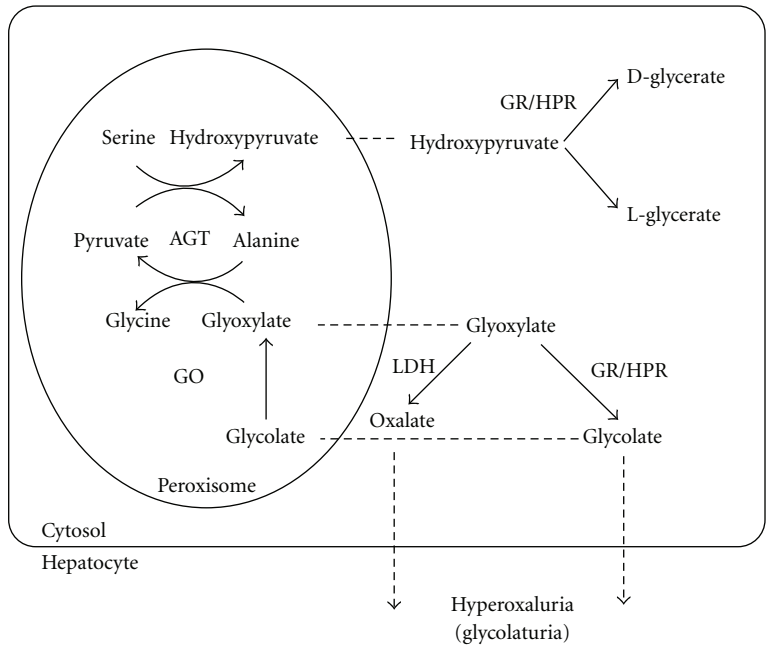


Figure 1: Reactions involved in oxalate, glyoxylate, and glycolate metabolism in human hepatocytes. Abbreviations: AGT alanine: glyoxylate aminotransferase; GR/HPR glyoxylate reductase/hydroxypyruvate reductase; GO glycolate oxydase; LDH lactate dehydrogenase.

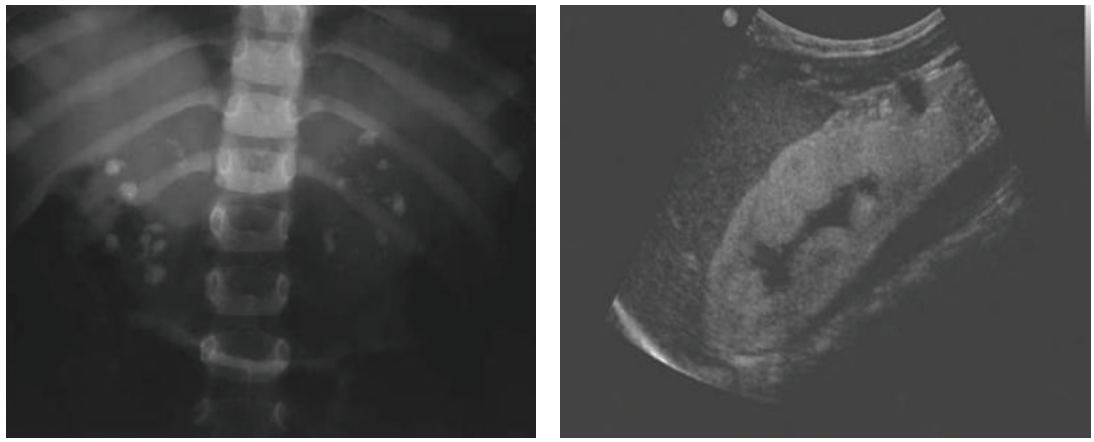
the metabolic reactions involved remain to be confirmed. All three are autosomal recessive diseases. AGT catalyses the transamination of glyoxylate to glycine while pyruvate is converted to alanine, whereas GRHPR catalyses the reduction of glyoxylate to glycolate [7]. The failure to detoxify glyoxylate in PH1 and 2 results in its conversion into oxalate by cytosolic lactate dehydrogenase. Since the main source of oxalate removal from the body is urine excretion, the deposition of the calcium oxalate salt, which is very poorly soluble, occurs primarily there either as urolithiasis along the urinary tract or in the kidney, or as interstitial deposition in the kidney leading to nephrocalcinosis [8]. The accompanying overproduction of glycolate in PH1 or L-glycerate in PH2 has no identified consequence. The crystal structures for both AGT and GRHPR have been solved, and some rationalization of the effect of mutations in the *AGXT* and *GRHPR* genes has been possible [9, 10].

3. Epidemiology

PH are rare autosomal-recessive inherited disorders. PH1 is the most common form of PH. The disease has an estimated prevalence ranging from 1 to 3 per million population and an estimated incidence rate of ~1 : 100,000 live births per year in Europe [11–13]. Higher rates are reported from inbred populations [14]. PH accounts for <1% of pediatric ESRD population in registries from USA, UK, and Japan [15–17]. In contrast, PH is more prevalent in countries where consanguineous marriages are common. Due to lack of registries, epidemiological information from developing countries primarily originates from major referral centers. Approximately 10% of Kuwaiti children and 13% of Tunisian children with ESRD have been reported to have PH [18, 19]. Incidence and prevalence of other types of PH are unknown but appear to be much lower than PH1.

4. Clinical Features and Oxalate Burden

Owing to the high urinary oxalate excretion, the urine become supersaturated for calcium oxalate (CaOx) resulting in the formation of crystals within the tubular lumen. PH1, therefore, manifests as severe urolithiasis and/or nephrocalcinosis (Figure 2). Progressive renal parenchyma inflammation and interstitial fibrosis due to nephrocalcinosis and recurrent urolithiasis cause renal impairment, which usually progresses to ESRD over time [8]. Once the GFR falls below 30–50 mL/min per 1.73 m^2, reduced renal excretion of oxalate by the kidneys together with continued overproduction by the liver lead to an increase plasma oxalate (Pox) that exceeds the supersaturation point for CaOx (Pox >30 μmol/l). Systemic deposition of CaOx, namely, oxalosis, can then occur in many organs such as bone, retina, skin, soft tissues, heart, vessels, and central nervous system (Figure 3). Severe systemic complications result in high morbidity and poor quality of life and, if treated late or untreated, early death. The bone compartment seems to be the main site for oxalate storage (15 to 910 in PH1 patients versus 2 to 9 μmol of oxalate per gram of bone tissue in non-PH1 ESRD patients) [20]. The skeleton can therefore store huge amounts of oxalate, even though the threshold of GFR at which this occurs is debated. Clinically, PH1 patients can experience severe bone pains and pathological fractures with low-trauma mechanism, as well as EPO-resistant anemia. Joints can also be damaged, with synovitis, chondrocalcinosis and oxalate deposits. Characteristics of oxalate osteopathy on X-ray include dense metaphyseal bands, submarginal



(a) (b)

Figure 2: Abdomen X-ray (a) and renal ultrasonography (b) showing urolithiasis and nephrocalcinosis in PH1.

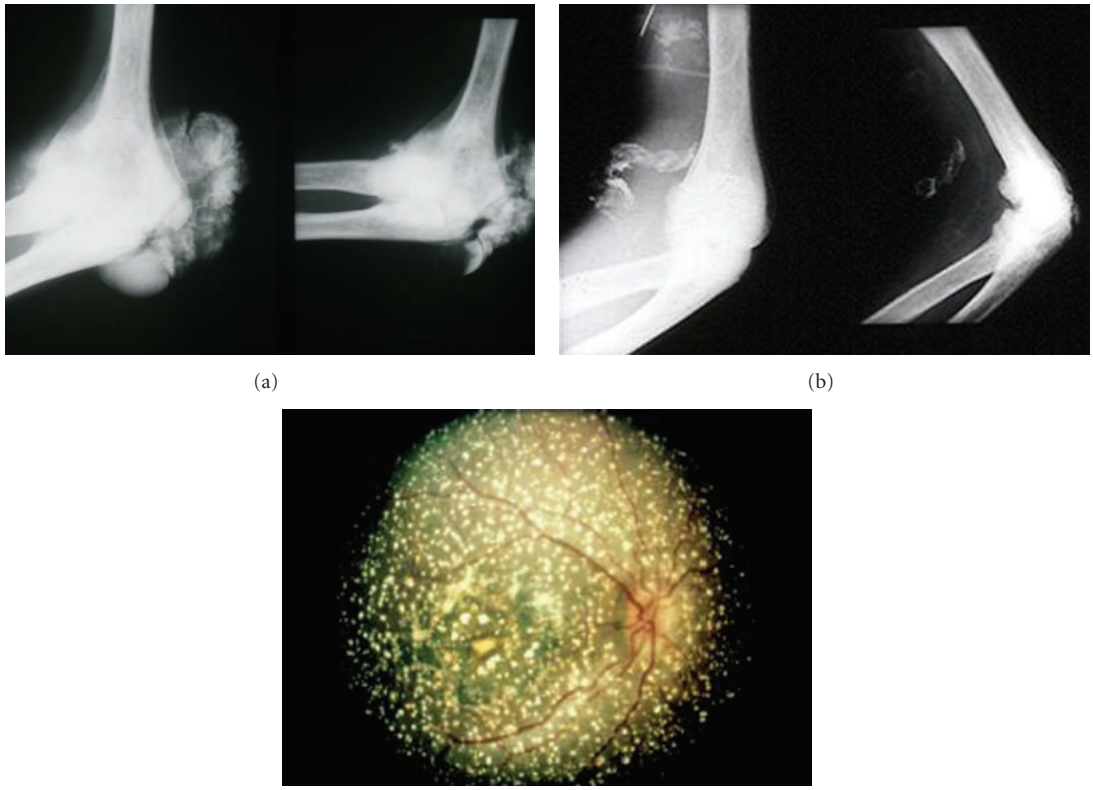
metaphyseal lucency, sclerosis of adjacent diaphysis, cystic bone changes, deformities, subperiosteal resorption, blurred trabecular pattern, radio-opaque rims in flat bones and epiphyseal nuclei, as well as increased bone density in vertebra and iliac crest [21, 22]. Symmetrical transverse lines of increased bone density at areas of rapid growth have been related to crystalline precipitation in cartilage calcification sites as well as in hematopoietic and other highly vascularized areas [23, 24]. Bone biopsies from the iliac crest can show specific features: oxalate crystals often surrounded by a granulomatous reaction corresponding to an invasion of bone surface by macrophages [20]. Instead of invasive bone biopsy, bone mineral density (BMD) measurement could be a valuable and noninvasive tool in determining and monitoring oxalate burden in PH1 [25]. Using a new bone imaging technique (e.g., high-resolution peripheral quantitative computed tomography, HR-pQCT), it has been shown that children with PH1 had decreased compartmental volumetric BMD and bone modifications with disorganized trabecular microarchitecture [26].

5. Diagnosis

5.1. PH1. The median age at initial symptoms of PH1 is 4 to 7 years in Europe and 13 years in Japan, ranging from birth to the sixth decade [13, 27, 28]. PH1 has variable presentation. The infantile form often presents as a life-threatening condition because of rapid progression to ESRD due to both early oxalate load and immature GFR; one half of the patients experience ESRD at the time of diagnosis and 80% develop ESRD by the age of 3 years [29]. Other presentations include recurrent urolithiasis and progressive renal failure in childhood or adolescence, late-onset form with occasional stone passage and sometimes ESRD as the first symptom in adulthood, diagnosis made after recurrence posttransplantation and presymptomatic individuals with a family history of PH1 [30]. Sonographic examination may show either cortical or medullary nephrocalcinosis [31]. Because of the rarity of disease, physicians are likely to encounter few or no PH patients during their practicing lifetime. The diagnosis of PH is, therefore, often delayed for years [12, 27, 32]. At time of diagnosis, a high proportion of patients (10–40%) had already reached ESRD [11–13, 27, 32, 33]. Overall, ESRD is reached by the age of 24 to 33 years in half of the patients with PH1 [27, 33]. The combination of both clinical and sonographic findings, that is, the association of stone passage, nephrocalcinosis, and renal impairment, is a strong argument for clinical diagnosis of PH1. Family history may bring additional information.

Crystalluria and infrared spectroscopy are of major interest for identification (qualitative) and quantitative analysis of crystals and stones (Figure 4), showing CaOx monohydrate crystals (type Ic whewellite) [34]. In patients with normal or significant residual GFR, concomitant hyperoxaluria (urine oxalate >1 mmol/1.73 m^2 per day, reference value <0.5) and hyperglycoluria (urine glycolate >0.5 mmol/1.73 m^2, reference value <0.5) are indicative of PH1, but some patients do not present with hyperglycoluria. The measurement of oxalate in a timed 24-hour urine collection corrected for body surface area is preferred for the diagnosis of PH [35]. Random urine oxalate/creatinine ratios can be useful to estimate oxalate excretion, particularly in infants, or patients with inability to provide complete 24-hour collection [36, 37]. Reference age-related values for oxalate and glycolate excretion have been established (Table 1). Urinary oxalate excretion may be falsely low in patients with decreased GFR because of oxalate retention and systemic deposition as calcium oxalate. In PH patients with GFR <30 mL/min per 1.73 m^2, Pox is usually >20–30 μmol/L, and in those with ESRD, Pox is >50 μmol/L. In ESRD patients, plasma oxalate (± glycolate)/creatinine ratio and oxalate (± glycolate) measurement in dialysate might also be helpful for screening [38].

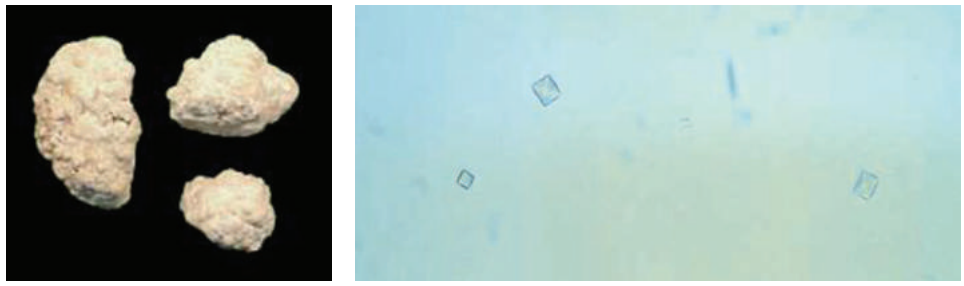
Until recently, liver biopsy to measure AGT catalytic activity has been essential for definitive diagnosis of PH1. As an alternative approach, genetic analysis of *AGXT* gene allows to detect mutations in most of suspected patients, thereby, supplanting the need for liver biopsy as a first



(a) (b)

(c)

Figure 3: Systemic involvement ("oxalosis") in PH1. Calcium oxalate deposition in the bones and joints (a), the vessels (b), and the retina (c).



(a) (b)

Figure 4: Calcium oxalate stones (a) and urine microscopic examination showing calcium oxalate monohydrate crystals (b) in PH1.

step [39]. In the presence of atypical presentation or in patients with no mutation identified, however, a definitive diagnosis requires AGT activity measurement in liver tissue. The *AGXT* gene is located on chromosome 2 (2p37.3). It is noteworthy that among more than 150 mutations responsible for PH1 found throughout the 11 exons of the *AGXT* gene [40], many, corresponding to almost 50% of the patients, cosegregate with a so-called minor allele, the most prominent feature of which is a proline to leucine change in position 11 [41]. The frequency of this minor allele is highest in the Caucasian population, and it acts in synergy with some of the mutations, in particular, the common p.Gly170Arg change [41, 42]. If most of the mutations in PH1 are "private" mutations, some mutations occur more commonly [39]. The most frequent mutation, p.Gly170Arg, is found in 20 to 40% of patients and is associated with significant residual catalytic AGT activity in liver biopsies [43]. Some mutations are found among specific ethnic groups, the most obvious example being the p.Ile244Thr mutation which is found in many patients of North African/Spanish origin [44]. Prenatal diagnosis can be performed from DNA obtained from crude chorionic villi or amniocytes, on the basis of a restricted analysis of exons including mutation(s) identified in the index case. Such a procedure allows the identification of fetuses affected or not with PH1. Independently of the diagnostic value,

Table 1: Reference age-related values for urinary oxalate, glycolate, and L-glycerate excretion (adapted from [8]).

Units	Normal values		
	Oxalate	Glycolate	L-Glycerate
mmol/1.73 m^2 per day	<0.5	<0.5	
μmol/l			<5
mmol/mol creatinine			
0–6 months	<325–360	<363–425	14–205
7–24 months	<132–174	<245–293	14–205
2–5 years	<98–101	<191–229	14–205
5–14 years	<70–82	<166–186	23–138
>16 years	<40	<99–125	<138

mutation analysis may bring information on pyridoxine responsiveness, on complex enzyme phenotype, and sometimes on clinical prognosis [27, 40]. Although PH1 usually shows marked inter- and intrafamilial heterogeneity [45–47], genotype-phenotype correlations have been described for some mutations [27, 48].

5.2. Other Types of PH. Symptoms onset in PH2 typically occurs in childhood. Although patients with PH2 are also at risk for ESRD and systemic oxalosis, they appear to form less stones, have a less pronounced nephrocalcinosis, and a lower incidence of ESRD over time than PH1 patients [49, 50]. In the presence of hyperoxaluria without hyperglycoluria, a diagnosis of PH2 should be considered, especially when AGT activity is normal. The diagnosis is based on increased urinary excretion of L-glycerate (reference values in Table 1), but the definitive diagnosis may require the measurement of GRHPR activity in a liver biopsy [51, 52] as some PH2 patients have normal level of L-glycerate in urine [53]. Genotyping can provide reliable diagnosis for PH2. The *GRPHR* gene is located on chromosome 9 (9q11) [5] and more than 15 mutations have been identified so far.

A few patients referred to as non-1 non-2 PH have been described. These patients have a phenotype similar to PH1 or 2 but no AGT or GRHPR deficiency. Recently, a third type of PH, PH3, caused by mutations in the gene *DHDPSL* has been characterized among non-1 non-2 PH patients [6].

In this paper, we propose an algorithm for the diagnosis and the conservative treatment of PH as illustrated in the Figure 5.

6. Treatment

6.1. Conservative Measures. Conservative measures should be initiated as soon as basal urinary oxalate measures have been completed and while renal function persists. Once ESRD has been reached, pyridoxine is the only stone treatment that should be pursued, if the patient is responsive. These measures apply to all types of PH with the exception of pyridoxine which is specific to some PH1 patients.

6.1.1. High Fluid Intake. The positive effect of high fluid intake has been proven in epidemiological and prospective intervention studies in stone formers [54]. In PH, the fluid intake recommended is >2 L/m^2 per day (up to 3 L/m^2 per day), distributed throughout the day and night. In small children and infants, a feeding or gastrostomy tube may be required. Special care should be taken in situations of fluid losses (diarrhea, vomiting, and fever) or limited oral hydration (surgery) and intravenous fluid intake instituted if necessary.

6.1.2. Diet. Oral calcium can bind oxalate in the bowels, and calcium restriction has been shown to result in higher oxalate intestinal absorption [55, 56]. Calcium intake should thus remain normal. A restriction in oxalate intake is of limited usefulness in PH patients as the main source of oxalate is endogenous. Excessive intake of vitamin C and vitamin D are to be avoided.

6.1.3. Inhibition of Calcium Oxalate Crystallization. Alkali citrate can reduce the urinary calcium oxalate saturation by forming complexes with calcium thus decreasing stone growth or nephrocalcinosis [57]. Potassium, or sodium, citrate at a dose of 100–150 mg/kg body weight per day (0.3–0.5 mmol/kg) is recommended. Pyrophosphate ions may decrease calcium oxalate crystallization although orthophosphate has never been evaluated in isolation of other treatments [58]. Moderate doses of elemental phosphorus 20–30 mg/kg body weight per day may be administered. There is as yet no evidence that probiotics, such as *Oxalobacter formigenes* despite its ability to metabolize oxalate [59], can significantly decrease urinary oxalate excretion in PH patients.

6.1.4. Pyridoxine. The cofactor of AGT is pyridoxal phosphate, one of the B6 vitamins. The administration of pyridoxine hydrochloride has been shown to be associated with a decrease in urinary oxalate in about 10–30% of patients with PH [60, 61]. The metabolic basis of pyridoxine responsiveness is unclear. All PH1 patients should be tested for pyridoxine responsiveness and treated until liver transplantation is performed, if responsive, even while undergoing hemodialysis. Studies have determined that a subset of patients with PH1, carrying 1 or 2 copies of AGT mutations (p.Gly170Arg or p.Phe152Ile), may be responsive to pharmacological doses of pyridoxine, though this list is not limitative [48, 62]. Responsiveness has been defined as a minimum 30% decrease in urinary oxalate excretion after a test period of a minimum 3 months at maximum dose [61]. The starting dose recommended is 5 mg/kg per day and can be increased to 10 mg/kg per day. No sensory neurotoxicity has been described in PH patients, and a dose of 500 mg per day is thought to be below toxic levels. Absorption of pyridoxine may vary between patients, and assessing plasma levels of pyridoxal phosphate may be useful, though therapeutic levels are not defined.

Added monitoring of the tolerance and efficacy of these measures may require evaluation of stone formation, and nephrocalcinosis by ultrasounds or X-ray, urinary pH and volume, urinary citrate, magnesium and oxalate excretion

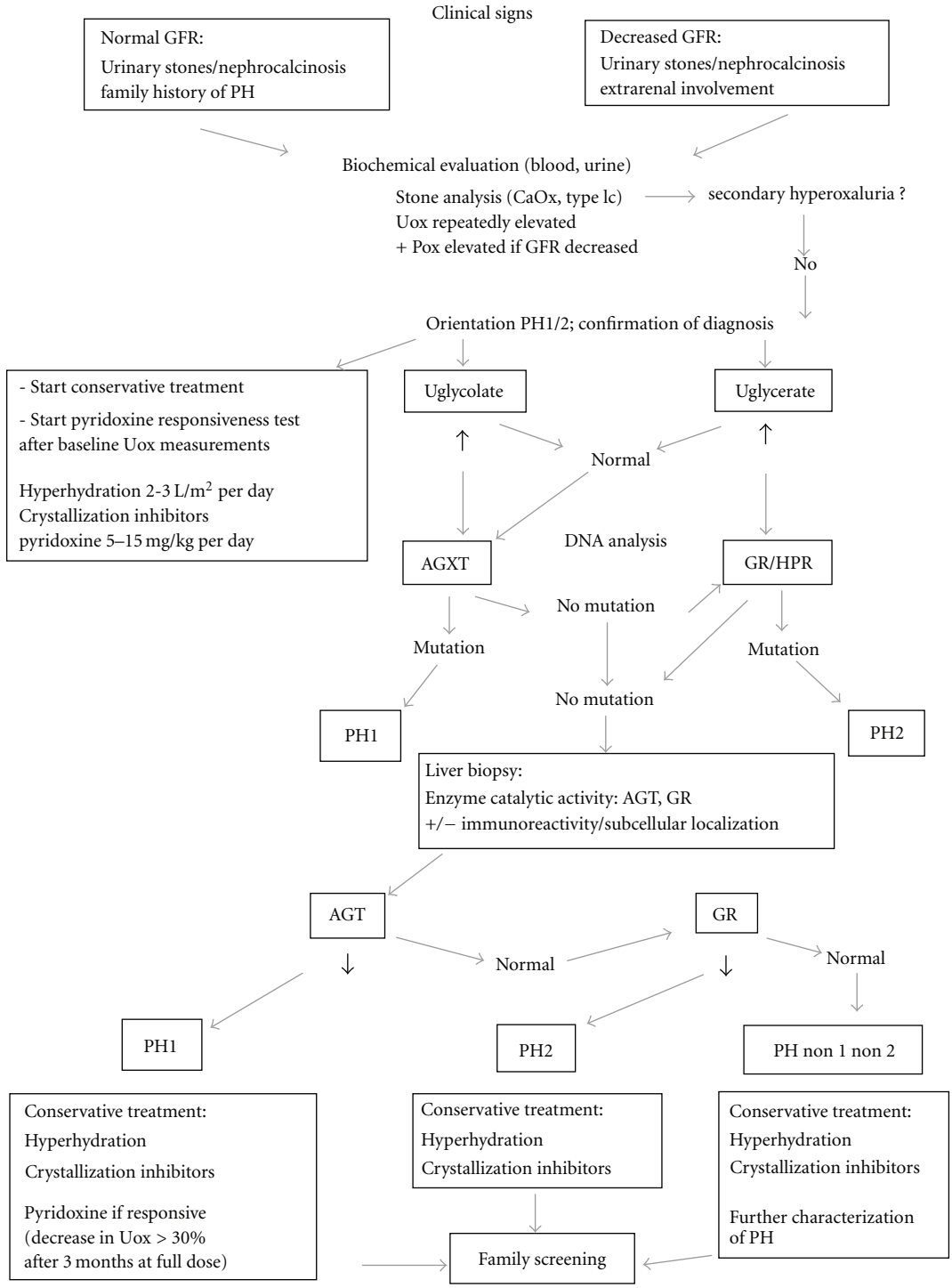


Figure 5: Proposed algorithm for the diagnosis and conservative treatment of primary hyperoxalurias. Abbreviations: PH: primary hyperoxaluria; GFR: glomerular filtration rate; Uox: urinary oxalate; Pox: plasma oxalate; CaOx: calcium oxalate; AGT: alanine:glyoxylate amino transferase; AGXT: AGT gene; GR/HPR: glyoxylate reductase/hydroxypyruvate reductase.

and supersaturation, and crystalline volume in the urines. In summary, an aggressive supportive management should be started as soon as the diagnosis of PH has been suspected and may improve renal survival provided compliance is optimal [63].

6.2. Urology. The treatment of stones should avoid open and percutaneous surgery because further renal lesions will alter GFR. The use of extracorporeal shock wave lithotripsy may be an available option in selected patients, but the presence of nephrocalcinosis may be responsible for parenchymal

Table 2: Suggestions for organ transplantation strategies in PH1 patients according to residual GFR (ml/min per 1.73 m^2), systemic involvement, and local facilities (from [68]).

Tx strategy	Combined simultaneous liver + kidney	Liver first Kidney as a second step	Isolated kidney	Isolated liver
HD strategy	Peroperative ± postoperative according to Pox and GFR	Standard HD following liver Tx aiming at Pox <20 μmol/L	Pre- and peroperative	Sometimes peroperative
40 < GFR < 60	No	No	No	Optional
20 < GFR < 40	Yes	No	(1) In developing countries (2) In very selected patients	No
GFR < 20	Yes	Yes	No	No
Infantile form (ESRD <2 years)	Yes	Yes (emergency)	No	No

Abbreviations: ESRD end-stage renal disease; GFR glomerular filtration rate; HD hemodialysis; Pox plasma oxalate; Tx transplantation.

damage. In patients with repeated renal colic, a ureteral JJ stent may be helpful for pain control and protection of renal damage. Bilateral nephrectomy may be proposed in patients on renal replacement therapy in order to limit the risk of infection, obstruction, and passage of stones.

6.3. Dialysis. Conventional dialysis is inadequate for patients who have reached ESRD, because it cannot overcome the ongoing oxalate production [64]. This results in continuing tissue deposition of oxalate and risk of organ damage. The removal of oxalate by hemodialysis exceeds that by peritoneal dialysis [65]. Better results may be obtained by combining daily high-flux hemodialysis and peritoneal dialysis or by long daily hemodialysis sessions [66, 67].

In summary, there are limited indications for dialysis in patients with PH1 [30]: (1) if diagnosis of PH1 has not been established, (2) in small children with infantile oxalosis waiting for organ Tx, (3) in preparation for kidney Tx, before or after liver Tx, in order to deplete oxalate from the body, (4) following isolated kidney or combined liver-kidney Tx with any delay in achieving optimal renal function, as a temporary adjunct in the case of high oxalate burden, or with transient loss of transplant function, (5) very exceptionally in older patients if the only alternative is no dialysis, (6) in developing countries; hemodialysis (or even less satisfactory, peritoneal dialysis) may be indicated as only a preference to absolute withdrawal of all therapy.

7. Organ Transplantation

Ideally, organ transplantation should be planned prior to the onset of ESRD and systemic oxalosis. The different options for organ Tx for PH1 according to residual GFR are summarized in Table 2 [68].

7.1. Kidney Transplantation. Kidney Tx allows significant removal of soluble Pox [69]. However, because the biochemical defect is in the liver, overproduction of oxalate and subsequent deposition in tissues continues unabated. The high rate of urinary oxalate excretion originates from both ongoing oxalate production from the native liver and oxalate deposits in tissues leading to high risk for disease recurrence. Isolated kidney Tx for PH1 is no longer recommended, because frequent recurrence leads to poor graft survival and patient quality of life. In Europe, the European Dialysis Transplantation Association (EDTA) registry reported poor results of isolated kidney Tx two decades ago with a 3-year graft survival of 17 to 23% according to donor source [70]. A United States Renal Data System (USRDS) analysis of 190 adults transplanted in the US between 1988 and 1998 showed superior death-censored kidney graft survival in combined liver-kidney Tx than in isolated kidney Tx, but no difference in patient survival [71]. More recently, data from 58 PH1 patients showed a death-censored kidney graft survival at 3 years of 95% with combined liver-kidney Tx versus 56% with isolated kidney Tx [72]. Proven pyridoxine responsive patients may benefit from isolated kidney Tx, provided pyridoxine is maintained, but the clinical evidence for this is still sparse. Isolated kidney Tx may be also regarded as a temporary solution in some developing countries before managing the patient in a specialized center for further combined liver-kidney procedure.

Isolated kidney Tx might be the treatment of choice in patients with PH2. Indeed, PH2 patients with kidney Tx alone appear to have a more favorable course than PH1 patients, and the benefit of liver Tx in such patients is still unclear.

7.2. Liver Transplantation. Since the liver is the only organ responsible for glyoxylate detoxification by AGT, the excessive production of oxalate will continue as long as the native liver is left in place. Therefore, PH1 can be cured only when the deficient host liver has been removed. Liver Tx is a form of gene therapy as well as enzyme replacement therapy as it will supply the missing enzyme in the correct organ (liver), cell (hepatocyte), and intracellular compartment (peroxisome).

In Europe, combined liver-kidney Tx has been the preferred approach in the past 25 years. A European PH1

Transplant registry reported 127 liver Tx including more than 100 liver-kidney Tx in 117 patients between 1984 and 2004 [73]. Results were encouraging with patient survival rates of 86, 80, and 69% at 1 year, 5 years, and 10 years, respectively. There were 13 kidney graft failures. Comparable results have been reported from the USRDS, with a patient survival above 80% at 5 years and a death-censored graft survival of 76% at 8 years post Tx [71]. Such a strategy can be successfully proposed to infants with PH1 [74]. Worldwide experience indicates that once perioperative mortality is avoided, combined liver-kidney Tx seems to be an acceptable treatment for PH1 [75–77]. The strategy of combined liver-kidney Tx may be influenced by the stage of the disease (Table 2). Simultaneous liver and kidney Tx is logical in patients with a GFR between 15 and 40 mL/min per 1.73 m^2, because, at this level, oxalate retention increases rapidly. A sequential procedure (first liver Tx, then dialysis until sufficient oxalate has been cleared from the body, followed by kidney Tx) may be proposed to ESRD patients, mainly infants with a long waiting time [78].

Preemptive isolated liver Tx might be the first option in selected patients before advanced chronic renal fail-ure has occurred, that is, at a GFR between 60 and 40 mL/min/1.73 m^2. In the Hamburg experience, 4 pediatric recipients with a GFR between 27 and 98 mL/min per 1.73 m^2 received a preemptive liver transplant, and 3 of them still have significant residual renal function after a median followup of ~12 years [79]. Another group reported good results at 5 years in 4 PH1 children who received a preemptive liver Tx with a mean pretransplant GFR of 81 mL/min/1.73 m^2 [80]. Such a strategy has a strong rationale but raises ethical controversies especially when the GFR is superior to 60 mL/min per 1.73 m^2. Indeed PH1 is the only peroxisomal disease without psychomotor delay due to cerebral involvement, and the conservative management of PH1 patients has significantly improved during the last 10 years; this may influence the role of preemptive liver Tx in such patients.

The choice of donor source may be based either on immunological bases (i.e., using the same donor for both organs) or on biochemical rationale (i.e., using a two-step procedure according to oxalate body store). Indeed most publication currently report on the use of cadaver donors but a living related donor may be considered under certain conditions [78].

7.3. Posttransplantation Reversal of Renal and Extrarenal Involvement. After combined liver-kidney Tx, urinary glycolate immediately returns to normal and Pox returns to normal before urine oxalate does [81]. Indeed, urinary oxalate can remain elevated for as long as several years post-Tx, due to the slow resolubilization of systemic CaOx deposition [72]. Therefore, recurrent nephrocalcinosis or renal calculi are still a risk and may jeopardize graft function. Thus, independent of the Tx strategy, the kidney must be protected against the damage that can be induced by heavy oxalate load suddenly released from tissues. Forced fluid intake (3–5 L per 1.73 m^2 per day) supported by the use of crystallization inhibitors is the most important approach. Pox, crystalluria, and CaOx saturation are helpful tools in renal management after combined liver-kidney Tx. The benefit of daily high-efficiency post-transplant hemodialysis is still debated and should be limited to patients with significant systemic involvement (Table 2). It will provide a rapid drop in Pox and, therefore, reduce the exposure of the transplanted organs to high Pox, but it may also increase the risk of CaOx supersaturation due to reduction in urine volume in the case of inappropriate fluid removal. However, posttransplant hemodialysis is mandatory in patients with acute tubular necrosis or delayed graft function.

8. Conclusion and Future Prospects

Patients with hyperoxaluria should be referred for diagnosis and management to reference centers with interest and experience in the conditions and access to the appropriate biochemical and molecular biological facilities. Major advances in biochemistry, enzymology, genetics, and management have been achieved during recent years. Improved knowledge of the disease, early and accurate diagnosis, before renal failure occurs, and aggressive supportive treatment are of critical importance for the prognosis. In (pre)ESRD patients, the greatest experience has been obtained with one-step combined liver-kidney transplantation. New insights into potential therapies including the restoration of defective enzymatic activity through the use of chemical chaperones and hepatocyte cell transplantation, or recombinant gene therapy for enzyme replacement [82–84], provide hope for curative treatments of primary hyperoxalurias in the future.

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Review Article

Peritoneal Dialysis Tailored to Pediatric Needs

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Consideration of specific pediatric aspects is essential to achieve adequate peritoneal dialysis (PD) treatment in children. These are first of all the rapid growth, in particular during infancy and puberty, which must be accompanied by a positive calcium balance, and the age dependent changes in body composition. The high total body water content and the high ultrafiltration rates required in anuric infants for adequate nutrition predispose to overshooting convective sodium losses and severe hypotension. Tissue fragility and rapid increases in intraabdominal fat mass predispose to hernia and dialysate leaks. Peritoneal equilibration tests should repeatedly been performed to optimize individual dwell time. Intraperitoneal pressure measurements give an objective measure of intraperitoneal filling, which allow for an optimized dwell volume, that is, increased dialysis efficiency without increasing the risk of hernias, leaks, and retrofiltration. We present the concept of adapted PD, that is, the combination of short dwells with low fill volume to promote ultrafiltration and long dwells with a high fill volume to improve purification within one PD session. The use of PD solutions with low glucose degradation product content is recommended in children, but unfortunately still not feasible in many countries.

1. Introduction

Peritoneal dialysis (PD) is increasingly applied around the globe; newborns and even preterm children with a body weight of as little as 1500 g are meanwhile included in the chronic PD program. Whereas initial prognosis is often determined by acute comorbidities, such as neonatal complications and diseases associated with hereditary syndromes, long-term outcome is essentially determined by adequate control of uremia-related sequelae, mainly bone mineral disease and cardiovasculopathy [1]. Dietary and life style changes are difficult to procure. Individually tailored, optimized PD regimes, considering specific pediatric aspects, are therefore essential to achieve an improved long-term outcome of patients with pediatric onset of dialysis.

2. Specific Pediatric Aspects

A salient feature of children is the rapid somatic and psychomotor development in the first years of life and during puberty. Growth rate reaches 20 cm during the first and 15 cm during the second year of life. Body length is doubled within four years. This requires careful and repeated adaptation of the PD regime to body size and of protein, energy, and mineral supply. Total body calcium content is 25 g in newborns and increases to 1 kg until adulthood. Insufficient calcium supply and hyperparathyroidism interfere with the growth plate mineralization process and potentially result in epiphyseal slipping and severe deformities. Thus, despite all concerns regarding cardiovascular calcifications, a positive calcium balance is mandatory in growing children. Energy supply should be 100% of the dietary reference intake adapted to age, body mass index (BMI) and physical activity, protein intake 100% (adapted to ideal body weight), and an additional compensation for dialytic protein and amino acid losses [2].

Body composition also differs considerably in children as compared to adults. Water content is 75% in newborns, 60% in adolescents, and only 50% in elderly man. 40% of CKD5d children have hypodysplastic kidneys associated with polyuria. Dehydration is more likely to occur, especially in association with gastroenteritis. On the other hand, infants with little urine output need much higher ultrafiltration (UF) rate per square meter body surface area (BSA) as

compared to adults to achieve adequate nutrition. Adequate nutrition is essential for normal physical and psychomotor development. In such children UF-related convective solute transport is considerable. While calcium may be supplied in sufficient amounts with calcium containing phosphate binders and high calcium dialysate concentrations, additional oral sodium chloride supply is often required to prevent a reduced body sodium content, hypotension, and associated neurological sequelae.

Successful insertion of a Tenckhoff catheter in newborns and infants is challenging, since the catheter is relatively larger and the peritoneal wall is thin and fragile. This readily explains the markedly increased risk of hernia and leakage in this age group [3, 4]. Moreover, rapid changes in body mass index and intraperitoneal fat mass and thus in intraperitoneal pressure occur during infancy [5] and further promote dialysis leak development.

In face of the good long-term prognosis of pediatric CKD5d patients as compared to adults [1, 6] with survival far into adulthood and the need of renal replacement therapy for many decades, the option to choose PD later in life should be maintained as long as possible. Prevention of peritoneal infections and inflammation and optimized PD biocompatibility are of particular importance to preserve long-term peritoneal membrane function.

3. Initiation of PD

To allow for sufficient healing of the PD catheter into the abdominal wall, early catheter implantation is advised. If possible, initiation of PD should be withheld for one to two weeks. Prophylactic herniotomy is often performed in newborns, omentectomy in most of the children to prevent catheter obstruction. Based on the clinical observation that catheter obstructions develop quite frequently even with curled catheters, the omentum should be removed as much as it is accessible during the insertion procedure. Preoperative treatment of constipation and adequate postoperative analgesia mitigate intraabdominal pressure peaks [7]. To safely increase the dwell volume postoperatively, intraperitoneal pressure (IPP) measurements should be performed [7]. An initial Peritoneal Equilibration Test (PET) is required to determine the optimal dwell time. In case these tests cannot be performed, PD should be started with 10 mL/kg body weight for 5–7 days after catheter insertion. The dwell volume should subsequently be increased to about 1100 mL/m^2 BSA within one week in children above 1 year and to 600 to a maximum of 800 mL in children below 1 year of age. BMI, organomegaly, and malnutrition increase the risk of hernia and leakage.

4. Defining Dwell Time According to PET

The PET is a standardized measure of the transperitoneal solute transport rates and ultrafiltration. It is performed with 1100 mL/m^2 BSA of PD fluid containing 2.5% glucose [8]. A short PET of 2 hours yields similar results as a 4-hour PET in children [9]. The biocompatibility of PD fluids does not impact on PET findings to a major extend [10, 11]. PET performed with 4.2% of glucose gives a more accurate estimate of UF and of sodium sieving. Sodium sieving is a measure of AQP-1 function and thus glucose driven free water transport, which mainly occurs during the early phase of a dwell [12].

The peritoneal permeability of the patient, as assessed by a PET, should impact on dialysis prescription, especially on dwell duration. A low transporter status in the PET indicates low purification rates and potential difficulties to achieve creatinine clearance (CrCl) and Kt/V targets but good ultrafiltration even with long dwell times. In these children, the number of cycles can be low whereas total daily dialysis duration should be long to achieve sufficient clearance rates. An additional long exchange may be required, for example, during day time in cycling PD patients with a high solute and toxin load.

High and especially very high solute transport rates in the PET imply good purification, which however is associated with rapid glucose absorption and thus dissipation of the osmotic gradient required for ultrafiltration. Short dwell times and increased peritoneal glucose exposure are usually required to achieve sufficient water removal. In particular anuric patients with rapid solute transport need a high dialysate glucose concentration and frequent exchanges. The high peritoneal glucose exposure induces progressive peritoneal neoangiogenesis and thus further acceleration of glucose uptake. A vicious circle ultimately results in ultrafiltration failure [13]. Icodextrin should be considered to halt peritoneal membrane degradation [14]. Of note, frequent short dwells with a high glucose concentration result in a high amount of free water transport, which predominantly reduces water overload but only to a smaller extent salt excess. An increase in dwell time and of total dialysis time per day may be required to normalize blood pressure.

In adults a higher transport status has been associated with a worse survival [15]. In children higher peritoneal transport kinetics have been associated with higher CrP and lower serum albumin levels [16] and with reduced longitudinal growth [17]. Patients presenting with a high transporter status at PD onset appear to be less suited for chronic PD [15, 18–20], this however has not been confirmed consistently [21, 22] and may rather be a reflection of comorbidity than a problem with PD per se [23–25].

5. Intraperitoneal Pressure: A Measure of Optimized Dwell Volume

Since body size changes rapidly in children, frequent adaptation of dwell volume is required. This can best be performed by determination of the intraperitoneal pressure. IPP gives an objective measure of the individual intraperitoneal filling and thus allows for an optimization of the individual dwell volume. The measurement is easy to perform, which provided a sufficient cooperation of the child [7, 27]. IPP increases with BMI and organ size [7] (Figure 1). The latter is especially relevant in children with organomegaly, for example, in children with autosomal recessive kidney disease.

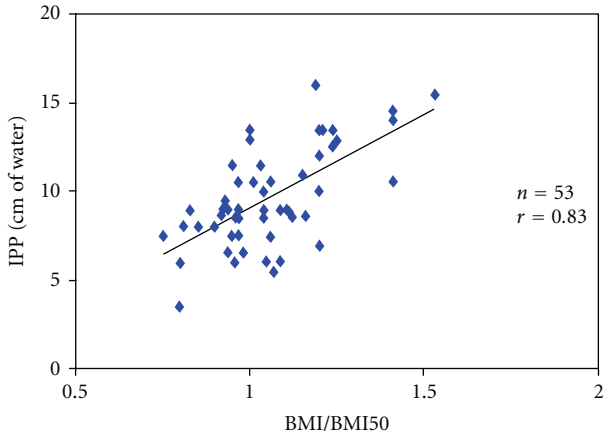

Figure 1: IPP is an individual patient characteristic, determined by BMI and dwell volume. The intraperitoneal pressure (IPP, y-axis) is positively correlated with the normalized body mass index (x-axis) within the general population. This correlation is even stronger than the correlation between dwell volume to IPP [5].

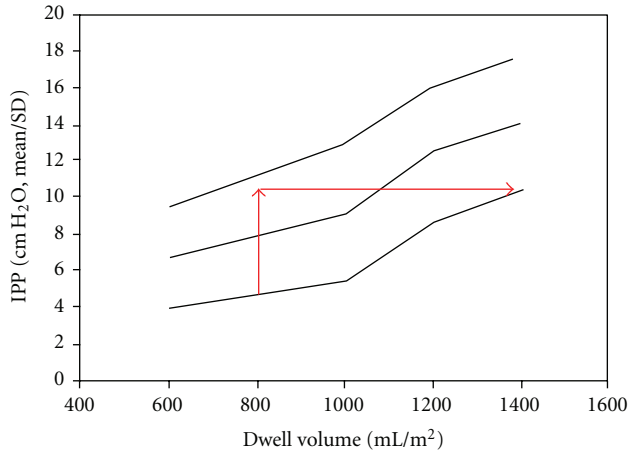

Figure 2: Intraperitoneal pressure (mean/SD) according to dwell volume in children on PD above two years of age [7, 26]. In infants IPP should be below 8–10 cm, in children below 13-14 cm H_2O. The red arrows give an example of how dwell volumes can be increased in patients with low IPP.

Likewise, abdominal pain and constipation increase IPP [7]. Pitfalls of the measurements include inadequate pressure transmission, which can be evaluated from the respiration-dependent oscillations of the fluid level, and a filled bladder. Biocompatible PD solutions reduce IPP by 15–20% [28]. Abdominal pain is not reported below 12 cm H_2O. IPP is usually acceptable up to 13-14 cm H_2O, which corresponds to a mean fill volume of 1400 mL/m^2 (Figure 2). In children below 2 years of age IPP should not be above 8–10 cm H_2O, that is, in most cases fill volume not above 800 mL/m^2 [29]. Otherwise the risk of hernia and leakage increases considerably in infants.

Of note, there are no clinical trials systematically evaluating the validity of IPP measurements. Extended clinical experience in pediatric dialysis centres, however, suggests that IPP is a suitable measure of abdominal filling and thus of the ratio of the wetted and functionally active peritoneal surface area to the anatomical surface area.

According to computed tomography analyses [30], the peritoneal surface area that is in contact with the dialysate is only 30 to 60% of the anatomic area. A larger fraction of the membrane can be recruited for exchange in the supine as compared to the upright position (which argues in favor of APD during the night) and with larger fill volumes. In fact, optimisation of the individual dwell volume may substantially increase the delivered dialysis dose. In the majority of children with an average fill volume of 800–1000 mL/m^2 BSA, an increase in fill volume of 30–50% is feasible (Figure 2). Such increases in fill volume should improve purification, for example, of phosphate and thus yield far reaching beneficial effects. Dialytic phosphate removal is highly correlated with total PD fluid turnover [31] and thus the cumulative phosphate load, a major cause of cardiovascular calcifications [1].

Calculations of the total pore area over diffusion distance ($A_0/\Delta X$) based on the three pore model of peritoneal transport in relation to the dwell volume support this notion.

Augmentation of the dwell volume from 800 to 1400 mL/m^2 increases $A_0/\Delta X$ and thus the peritoneal surface recruited for purification by 20% [29, 32]. Still, smaller fill volumes must be prescribed in infants, at initiation of dialysis, in patients with history of leaks or hernia, and in patients reporting discomfort.

Other potential beneficial aspects of repeated IPP measurements are the reduction in back filtration, which should improve the ultrafiltration rates per gram of glucose infused, and a lower incidence of hernia and dialysate leaks. These observations, however, await scientific reconfirmation in clinical trials. In adults IPP levels above 14 cm have been shown to increase the risk of peritonitis [33].

Since the peritoneal membrane, that is, the anatomic peritoneal surface area, is proportional to BSA and not to body weight, scaling the dwell volume must be performed according to BSA. Scaling according to body weight resulted in an inappropriately low dwell volume especially in infants and young children and thus in the false perception of a peritoneal membrane inherent hyperpermeability in this age group in the past [34, 35]. On the other hand, keeping the fill volume below the limits of IPP of 8–10 cm H_2O (800 mL/m^2) as suggested for the children below 2 years of age results in a functional hyperpermeability as compared to older children [34]. To achieve sufficient ultrafiltration in neonates and infants, more frequent and shorter dwells must be applied to compensate for the functional hyperpermeability with faster glucose absorption [35].

6. The Concept of Adapted PD (APD)

APD is classically prescribed as repeated exchanges, each of them having the same duration and fill volume [36]. As given above, modification of the dwell time impacts on dialysis efficiency, both in terms of UF and purification. The Accelerated Peritoneal Examination (APEX) time defined

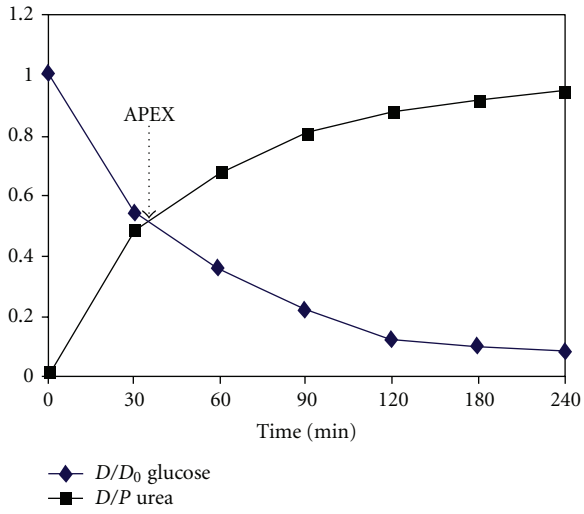

Figure 3: Illustration of APEX time, the crossing point of dialytic urea appearance, and glucose disappearance curve. APEX time indicates the optimal dwell time for ultrafiltration (normal range: 18 to 71 minutes).

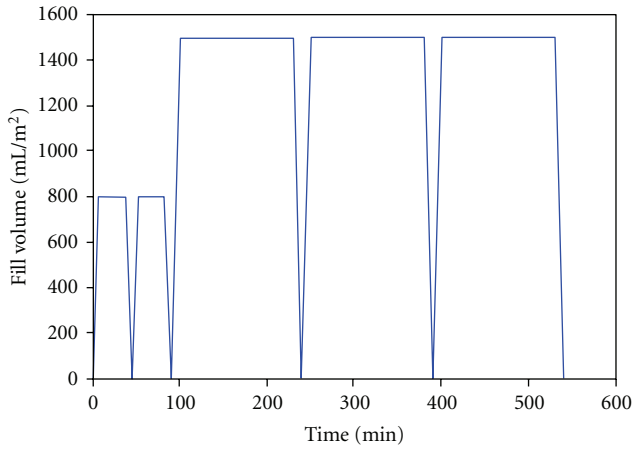

Figure 4: Example of an adapted PD session. Short dwells of 45 minutes of dwell time (i.e., individual APEX time) with a small fill volume (800 mL/m^2) favoring ultrafiltration by a high osmotic gradient at a low IPP are followed by long dwells (150 minutes; 3-4 times the APEX time) with a large fill volume (1500 mL/m^2), to promote uremic toxin removal.

by the crossing point of the urea and glucose equilibration curves obtained from a standardized PET has been proposed to be the optimal dwell time in terms of UF capacity [37, 38] (Figure 3). Likewise, modification of the dwell volume impacts on peritoneal surface area recruitment and thus ultrafiltration and purification capacity. A large intraperitoneal fill volume should favor convective and diffusive toxin removal. A very large fill volume with a too high intraperitoneal pressure, however, may result in back filtration and reduced net ultrafiltration [7]. Conversely, a smaller fill volume should favor UF, due to the lower intraperitoneal pressure [29, 38]. We propose to apply these principles by sequential exchanges with short and longer dwell-time and small and larger fill volumes as the concept of APD (Figure 4). The short exchanges with a smaller fill volume and a lower IPP to maximize UF should be based on the individual APEX time, the long dwells being three to four times the APEX time [38], and a higher fill volume with a still tolerable IPP to promote toxin clearance. IPP should not exceed 18 cm H_2O; close follow-up is required in children with IPP above 13 cm H_2O. This concept of individually assigned PD prescription based on the individual clinical tolerance and on IPP has been shown to allow for improved dialysis efficiency both in terms of blood purification and in terms of water and sodium removal and thus blood pressure control in children [39] and adults [40]. Of note, enhanced dialysis efficiency is achieved with the same total amount of dialysate delivered and within the same total daily dialysis time as compared to a conventional PD session.

7. Fluid Homeostasis

Structural and functional abnormalities of the heart are highly prevalent among paediatric PD patients, with oligoanuria and hypertension being independent predictors [16]. To achieve adequate sodium, volume and blood pressure control, repeated determinations of body weight, blood pressure, nutritional sodium supply and serum electrolyte concentrations, and eventually urine- and effluent electrolyte concentrations are required. Single-frequency bioimpedance analysis indicates intraindividual changes in hydration status [41, 42]. Margins of error, however, are large when total body water is predicted and the method is not yet broadly applied. Multiple-frequency bioimpedance analysis seems to be a promising method [43], but it is not yet sufficiently evaluated in children.

While infants with residual renal function may require additional sodium and water supply, dietary restriction of sodium and water intake is needed in many of the older children. The latter, however, is difficult to implement in the daily life. PD-associated water and sodium removal therefore plays a key role in maintaining euvolemia and normal body sodium content. In mice 50% of total UF occurs via aquaporin 1 (AQP-1) [44]. In humans treated with short dwells free water transport via AQP-1 is high, while water and convective solute removal via small pores predominates with long dwells. In adults 100 mmol of sodium are removed per litre of ultrafiltrate with a long, 4-hour dwell. About 80 mmol of sodium are removed per litre of ultrafiltrate during APD [45]. In children UF-associated sodium losses may even be high due to the relative lower fill volume per BSA and the associated functional hyperpermeability. Further sodium removal can be achieved with an additional day time exchange, in particular when icodextrin solutions are used [46].

All PD fluids currently available have a sodium concentration of 132–134 mmol/L. Reducing dialysate sodium concentration from 115 to 126 mmol/L and increasing glucose concentrations to up to 2.5% to maintain dialysate osmolality substantially increase sodium removal. Pioneering studies in adults have demonstrated promising effects on

blood pressure and fluid status [47, 48]. These PD solutions, however, have not yet been admitted to the market and have not yet been investigated in children.

Insufficient, membrane-related ultrafiltration may have the following causes:

(1) limited availability of membrane surface due to postoperative and post-infectious adhesions as well as fibrotic and sclerotic transformation of the membrane increasing the interstitial space,

(2) high lymphatic and extra lymphatic absorption rates, for example, due to a high IPP,

(3) impaired aquaporin 1 function, as suggested by the lack of sodium dipping,

(4) increased vascular surface area due to acute and chronic hyperperfusion observed with inflammation and neoangiogenesis.

Preventive measures include atraumatic operations, postoperative addition of heparin to the dialysate [49], and above all prevention of peritonitis episodes by implementing respective standards of care and semiautomated connection devices. Impairment of aquaporin 1 function and peritoneal membrane transformation are related to PD fluid bioincompatibility and treatment time [50].

8. Improving PD Biocompatibility

PD has been performed with first generation single chamber PD solutions for several decades. They, however, contain high amounts of glucose and toxic glucose degradation products (GDPs) and expose the patient to supraphysiological lactate concentrations at an unphysiologically low pH. These PD solutions impair peritoneal mesothelial cell function and local host defence [51, 52] and lead to profound alterations of PD membrane morphology and function within few years [50, 53].

Several new PD solutions have meanwhile been introduced, which have the potential to substantially improve PD therapy in children. Separation of the glucose at a very low pH in multichamber bags markedly reduces GDP formation by 50 to more than 90% [54, 55], depending on the manufacturer. The pH of these double and triple chamber solutions is neutral to physiological pH; the buffer compound consists of lactate, bicarbonate, or a mixture of both. Numerous in vitro and animal studies demonstrate an improved biocompatibility profile [52, 56, 57]. Lactate-related in vitro toxicity improves at a physiological pH but does not normalize [58, 59]. In humans, prospective randomized trials demonstrate similar solute transport and ultrafiltration capacity with multichamber as compared to conventional PD solutions [10, 11, 60, 61]. Effluent surrogate markers of biocompatibility improve [11, 60, 62, 63]; residual renal function is better preserved, provided that it is not very low, that is, below 2 mL/minutes$*1.73\,m^2$ [64, 65]. Switch from conventional to low GDP solutions results in a peritoneal washout of AGE [66, 67] and a 15% decline in systemic AGE levels in children [68] and adults [62]. Two large-scale registries demonstrate significant improvement of patient morbidity and mortality in adults using multichamber as compared to conventional fluids [69, 70]. In face of the plethora of positive scientific evidence, multichamber PD solutions have meanwhile been advocated as standard of care in children treated in countries where these solutions are available [71]. General recommendations with regard to the choice of specific multichamber PD solutions cannot be given at present. Noteworthy, the beneficial effects associated with the introduction of new PD solutions may also be related to the simultaneous introduction of semiautomated connection devices, reducing the risk of touch contamination and thus of infectious complications. Likewise, PD surveillance has improved with cycler-based chip systems, monitoring daily PD performance in detail. The impact of these innovations, however, has not yet been analysed systematically.

Icodextrin solution is another useful option, in particular in children with sodium and water overload. The GDP content is reduced, lactate concentration is 40 mmol/L, and pH is still 5.5. The transperitoneal absorption rate is much lower than that of glucose; 40% of the icodextrin molecules are absorbed within 12-hours [72]. Icodextrin is metabolized to maltose and its derivatives; a clinical impact of maltose accumulation has not yet been discerned. Icodextrin induces iso-osmotic, colloid osmotic ultrafiltration, which is aquaporin-1 independent; that is, sodium sieving does not occur. Thus, icodextrin fluid is particularly suitable for children with impaired AQP-1 function and insufficient UF. It is administered during a single long dwell per day, that is, the day time dwell with APD. Icodextrin fluid has been reported to increase sodium removal and improve hydration status [73], blood pressure, and left ventricular mass [74], independent of the underlying transporter status [75]. Limitations regard allergic skin reactions to icodextrin and exfoliative dermatitis, observed in up to 10% of the patients. In addition, aseptic peritonitis outbreaks due to transient contamination with bacterial membrane compounds have repeatedly been noted with icodextrin [76, 77]. Due to interference with the assays, falsely increased plasma glucose determinations and falsely reduced total alpha-amylase activity may be observed with icodextrin use.

Amino acids are another osmotic alternative to glucose. Amino acid-based PD solutions contain very low amounts of GDP [78] and allow for a phosphate-free amino acid supply. Experimental studies, however, do not unequivocally support the notion of improved biocompatibility [57, 79, 80]. Solute and water transport is similar as compared to conventional, high GDP fluids [81, 82]. The limited anabolic effects of the relatively expensive solutions and the usual achievement of adequate nutrition with enteral feeding thus far have prevented wider administration of amino acid-based PD fluids in children, although the concept is intriguing. They are not yet recommended for parenteral nutrition in malnourished children [71].

In conclusion, the biocompatibility of the new generation of PD solutions is substantiated by numerous experimental and clinical studies. They provide evidence for major local and systemic benefits justifying their use in children. The

associated increase in costs should be offset by reduced infectious complications [83, 84], improved long-term preservation of the PD membrane [57, 85], improved cardiovascular health [73, 85, 86], and improved long-term patient survival. Ultimate scientific evidence proving this assumption, however, is still missing. Large-scale randomized comparative trials are underway; an international pediatric peritoneal biopsy study evaluating the morphological and functional changes with standard and low GDP solutions is currently established.

9. Adequacy in Pediatric PD

Scientific data in children indicating that any of the measures of dialysis efficacy is predictive of well-being, morbidity, or mortality is absent and definition of PD adequacy is even more difficult than in adults. Adult targets of urea removal scaled to its volume of distribution (Kt/V) and clearance of creatinine have been implemented in pediatric PD. These measures, however, are a matter of debate. 2006 K/DOQI guidelines recommended a minimal total Kt/V of 1.8 [87]. To allow for sufficient dietary protein intake a Kt/V of 2.0 has been suggested for continuous ambulatory peritoneal dialysis (CAPD) patients [88]. Considering the much higher relative protein intake required in growing children per kg body weight [2], the Japanese Study Group of Pediatric PD even suggested a Kt/V of 2.5 or higher [89]. The adequate target Kt/V is yet unknown. At present a Kt/V of at least 2.1 and a total clearance of creatinine of 63 liters/week/1.73 m^2 are recommend in children on continuous cyclic peritoneal dialysis (CCPD). Other aspects such as the preservation of residual renal function are at least as important. This can be achieved by ACE inhibition and angiotensin receptor blockade [90], pediatric data however is scant, and a general recommendation on ACE and ARB use is not yet justified. In adults survival is closely related to residual renal function but not to Kt/V, provided that a lower limit of urea removal is maintained [91, 92]. In children growth correlates with renal but not with peritoneal solute clearance [93].

Kt/V is scaled to body weight, CrCl to BSA. Due to the relation of BW to BSA of 1 : 30 a ratio of Kt/V to CrCl of 1 : 30 has therefore been generally be recommended [36], even though small children and infants have a higher ratio of BSA to body weight. Kt/V targets are often easier to achieve than CrCl targets, in particular in anuric children, which require frequent cycles for ultrafiltration. Urea removal is more related to the number of cycles and dwell volume, CrCl more to PD duration [27]. Likewise, patients with a high peritoneal transporter status, either due to a hyperpermeable state of the peritoneum or due to a too low fill volume per BSA, have a relatively higher urea versus creatinine removal.

In view of these limitations, adequacy of pediatric PD is probably better described by the achievement of a normal water and electrolyte balance and thus normal blood pressure, by a minimal phosphate and toxin accumulation in a clinically asymptomatic child with growth and psychomotor development close to normal. This, of course, is difficult to achieve, especially in anuric children. A major step towards this goal is optimizing PD prescription by means of PET and IPP, allowing for individual adaptation of fill volume and dwell time. Too low fill volumes resulting in functional hyperpermeability and too large fill volumes resulting in back filtration, discomfort, and dialysate leaks can be avoided. Dwell time should be adapted to individual ultrafiltration and purification needs. This may be varied within one PD session. Administration of PD solutions with reduced local and systemic toxicity, consideration of the age specific needs of rapidly growing children, and continuous support of the families to reduce the burden of home dialysis therapy should further contribute to make adequate PD an achievable goal.

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Research Article

Acute Renal Replacement Therapy in Pediatrics

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Acute kidney injury (AKI) independently increases morbidity and mortality in children admitted to the hospital. Renal replacement therapy (RRT) is an essential therapy in the setting of AKI and fluid overload. The decision to initiate RRT is complex and often complicated by concerns related to patient hemodynamic and thermodynamic instability. The choice of which RRT modality to use depends on numerous criteria that are both patient and treatment center specific. Surprisingly, despite decades of use, no randomized, controlled trial study involving RRT in pediatrics has been performed. Because of these factors, clear-cut consensus is lacking regarding key questions surrounding RRT delivery. In this paper, we will summarize existing data concerning RRT use in children. We discuss the major modalities and the data-driven specifics of each, followed by controversies in RRT. As no standard of care is in widespread use for RRT in AKI or in multiorgan disease, we conclude in this paper that prospective studies of RRT are needed to identify best practice guidelines.

1. Introduction

Acute kidney injury (AKI) affects a significant proportion of critically ill children. Using a revised AKI definition, a recent study indicated that up to 10% of all children admitted to the pediatric intensive care unit (PICU) suffer some degree of kidney injury [1]. The kidney is central to numerous homeostatic mechanisms in the body including: (1) fluid balance, (2) acid-base balance, (3) erythropoiesis, and (4) vascular tone regulation. Aberrancies in kidney function, therefore, can negatively affect host survival. AKI is often recognized as a complication of multi-organ disease and independently increases the risk of mortality [2, 3]. Although formal and definite indications for use are lacking, RRT is employed for AKI, fluid overload, and sepsis. While revised definitions of AKI have aided in stratification of injury severity [4–6], controversy and uncertainty surrounds the use of RRT. It is unclear which patients are appropriate for therapy, which modalities should be used, what the triggers are for initiation, what "dose" should be prescribed, and how long treatment should continue.

The Prospective Pediatric Continuous Renal Replacement Therapy (ppCRRT) registry, a comprehensive and collaborative registry composed of thirteen select pediatric referral centers, was established in 2000 to prospectively evaluate clinical aspects of CRRT and recently reported an overall mortality of 43.1% for critically ill children placed on RRT [7]. This statistic underscores the need for urgent and broad-based prospective study of children placed on RRT. In this paper, we will lay a framework for understanding the rationale for RRT initiation, investigate the evidence for use of specific RRT modalities (Table 1), outcomes using these modalities, and also establish a purpose statement for the future of RRT study.

2. The Different Modalities of Renal Replacement Therapy

2.1. Peritoneal Dialysis. Peritoneal dialysis (PD) is the most widely available form of renal replacement therapy used in children. Almost all centers that care for acutely ill children

Table 1: Comparison of peritoneal dialysis (PD), intermittent hemodialysis (IHD), and continuous renal replacement therapies (CRRT).

Variable	PD	IHD	CRRT
Continuous therapy	Yes	No	Yes
Hemodynamic stability	Yes	No	Yes
Fluid balance achieved	Yes/No, Cycle dependent	Yes/No, Intermittent	Yes, pump controlled
Ease of use	Yes	No	No
Adequate nutrition delivery	variable	variable	Yes
Solute control	Yes	Yes	Yes
Ultrafiltration control	Variable	Yes	Yes
Anticoagulation	No	Yes	Yes
Acute ingestion removal	No	Yes	Variable
Continuous toxin removal	Variable	No	Yes
ICU nursing needs	Low	High	High
Patient mobility	No	Yes	No
Cost	Low	High	High
Vascular access need	No	Yes	Yes
Infection potential	Yes	Yes	Yes
Use in inborn-errors of metabolism	No	Yes	Yes

Adapted with permission from Walters et al. [8].

are capable of incorporating this technique into practice. PD is especially important for developing countries with limited resources.

Pro. The benefits of peritoneal dialysis include ease and quickness of catheter insertion, inexpensive cost compared to other modalities, and general tolerance with regards to hemodynamic stability. Percutaneously placed intraperitoneal catheters or permanent Tenckhoff catheters are used even in neonates, and catheter placement is relatively simple compared to the vascular access required for intermittent hemodialysis (IHD) or continuous renal replacement therapy (CRRT). Due to ease of tolerance, PD catheters have been prophylactically placed in neonates after cardiopulmonary bypass surgery felt to be at high risk for ischemic kidney injury [9]. Of global importance, particularly for areas with little technologic ability, PD can be performed manually and does not require a cycling device. Peritoneal dialysis fluid, either lactate based (USA) or bicarbonate based (outside USA), is nearly universally available. A major advantage of PD is absence of a need for anticoagulation. Functionally, PD is ideally suited for patients that have moderate illness and are poor candidates for modalities which require anticoagulation or large vascular access.

Con. Due to its relatively low clearance compared to IHD and CRRT, PD is ill suited for situations such as acute toxin ingestions, severe metabolic disturbances [10]. In cases of tremendous fluid overload, the rate of fluid removal of PD may not be rapid enough to prevent subsequent injury and morbidity. Manual PD can be labor intensive for the bedside practitioner, especially if the cycle frequency is high. Also, PD cannot be used in children with congenital abdominal malformations such as omphalocele, gastroschisis, and bladder exstrophy, or in patients with significant abdominal adhesions.

Evidence. Though rates of PD use have decreased in the PICU, it is still the most commonly used method of RRT in children in the world [11]. The use of PD is favored in the neonates who often have difficult vascular access, low tolerance to volume shifts, and thermodynamic instability [12]. In neonates with heart failure, PD provides a safe and adequate strategy for children and allows reasonable creatinine clearance rates [13]. The use of peritoneal dialysis is documented in children following cardiopulmonary bypass; Sorof et al. report no deaths or adverse hemodynamic effects during PD in 20 consecutive children immediately after cardiopulmonary bypass (CPB) [9, 13–17]. In a single center study, survivors after CPB had a shorter interval between the diagnosis of AKI and PD initiation than nonsurvivors (1.2 + 0.4 days versus 4.3 + 1.2 days) [17]. Numerous other reports demonstrate the safety and efficacy of PD after cardiopulmonary bypass including providing effective dialytic efficiency measurements ($Kt/V > 2.1$, where K is the clearance of urea, t is the time of dialysis, and V is a patient's total body water; goal for PD is >2.0/week) [15–19].

Opinion. PD is the most often used form of RRT in adults and children worldwide, owing to cost and ease of use [20]. In skilled hands, PD is highly effective at removing solute and fluid and can be tailored to patient needs. Practitioners should be mindful of the potential for PD if vascular catheter placement is problematic. This modality does not require anticoagulation and is ideal for neonates, children with inadequate vascular access, and children who can tolerate slower fluid removal and electrolyte correction.

2.2. Intermittent Hemodialysis. Intermittent hemodialysis (IHD) use requires center selective criteria of technical expertise and trained personnel and patient selective criteria of hemodynamic stability and vascular access.

Pro. The use of IHD is ideal for acute presentations of renal dysfunction or electrolyte imbalance. Because it offers the highest dose of dialysis in the shortest time frame, IHD is perfectly suited for disease processes which cause acute disruptions in homeostasis such as drug ingestions, tumor lysis syndrome, and hyperammonemia. Additionally, it allows for isolated ultrafiltration and titration of dialysis fluid solute concentration to correct metabolic disturbances such as hypernatremia. IHD is time limited, allowing for patient mobility, for example, to travel if needed for other diagnostic tests or procedures. In centers where continuous RRT is not available, some IHD machines permit slower and extended dialysis (SLED) which allows for gradual removal of solute and/or fluid [21].

Con. The technical requirements of IHD represent a major challenge to its use. IHD use is limited by the trained personnel available at a given medical care center and by the technical support available if trouble arises. Secondly, IHD requires vascular access in a large vessel. Though performed, this is especially problematic in children for whom obtaining access is a chronic problem [22]. Neonates may be unable to tolerate IHD simply because of inability to offer an appropriate cannula size to maintain adequate flow rates. Additionally, large IHD catheter placement can lead to vascular damage such as stenosis and thrombosis. Many nephrologists feel that for this reason, the subclavian veins should be avoided altogether [8]. Future chronic dialysis access needs may be greatly impeded by placement of temporary IHD catheters [23]. IHD use causes large volume shifts and may be unfeasible in patients that are hemodynamically unstable and those, such as small infants, who do not tolerate the volume shifts that occur during dialysis. Disequilibrium syndrome, secondary to rapid osmolar shifts, may complicate hemodialysis leading to seizures and cerebral edema, but it can be offset with short dialytic runs or with small doses of mannitol [24]. IHD requires anticoagulation, most commonly with heparin (although low-molecular-weight heparins and the alternative anticoagulants danaparoid, lepirudin, and citrate can be used in patients with heparin-induced thrombocytopenia). Finally, because it is time limited, IHD use does not lessen the fluid restrictions that are often placed on critically ill patients. Thus, using IHD requires diligent daily planning to balance UF with goal fluids, including boluses and blood products, and nutrition.

Evidence. Prior to the rise in popularity of continuous renal replacement therapy, intermittent hemodialysis was the modality of choice in patients requiring aggressive support for acute kidney injury. Other than for acute ingestions and electrolyte disturbances, the use of IHD has taken a backseat to CRRT in the intensive care setting requiring solute and fluid control. The adult SHARF 4 trials (Stuivenberg Hospital Acute Renal Failure), however, found no differences in mortality or renal recovery in 316 adults with AKI between IHD and CRRT [25]. Similarly, a meta-analysis of 43 studies comparing IHD to CRRT in adults reports that CRRT does not offer an advantage with regard to survival or long term dialysis dependence [26]. Pediatric data comparing IHD to other modalities in AKI is limited. In 2001, Bunchman et al. published data on 226 children receiving RRT. In this study, 81% of the 61 children receiving IHD survived compared to only 40% for hemofiltration; however, the former group received significantly less vasopressor support than the latter [27]. Retrospectively, the survival of pediatric AKI for IHD (73–89%) has been higher than those for CRRT (34–58%) [27].

Opinion. Intermittent hemodialysis is effective therapy for acute ingestions, toxins, and fluid overload. IHD carries risks for line placement, technical difficulty in small patients and neonates, and complications with rapid shifts in fluids and electrolytes. IHD is ideal for patients with acute electrolyte abnormalities and those that would benefit from and can tolerate high doses of dialysis in a short time frame [28].

2.3. Continuous Renal Replacement Therapy. The use of continuous dialysis for AKI is widespread in PICUs. Arteriovenous hemofiltration was first described in the late 1970s and the 1980s, including in infants [29]. CRRT became popular in pediatric AKI in part because venovenous filtration machines, which offset the need for patient perfusion-pressure-driven ultrafiltrate, eased the problem of low driving pressures in pediatric patients. Additionally, advances in CRRT technology allowed gravimetric and volumetric control of hemofiltration to allow for accurate ultrafiltration flows [30]. Still, while CRRT use expands, in both adults and pediatrics the risk-benefit ratio pits *known* risks to *theoretical* benefits. A major limitation to the standardization of CRRT use is the lack of prospective randomized data from controlled studies, evaluating the time of initiation, route of delivery, and efficacy. Retrospective data attempts to answer some of these questions.

Pro. The major advantage of CRRT is that it affords nearly continuous adjustment of ultrafiltration. This feature makes it well suited for patients with hemodynamic instability [31, 32]. CRRT (and IHD) allows for ultrafiltration to be independently regulated from solute removal. This confers great benefit for patients with sensitive electrolyte needs and fluid states. Finally, fluid delivery does not require restriction while CRRT is in use, affording increased freedom for nutrition. The preferred modalities of CRRT in children are likely affected by site-specific familiarity and local preference, and data are retrospective [8, 22, 31, 33].

Con. The primary drawback to CRRT use is its complexity. The level of technical expertise required is high and, as such, is not available at many centers worldwide. The need for vascular access can often complicate its use. Attaining adequate flow rates in neonates and small children can be

Table 2: Ideal Catheter size and patient size for CRRT.

Patient size	Catheter size	Site of insertion
Neonate	7 Fr	IJ/EJ, femoral
3–6 kilogram	7 Fr	IJ/EJ, femoral
6–10 kilogram	8 Fr	IJ/EJ, subclav, femoral
>10–20 kilogram	9 Fr	IJ/EJ, subclav, femoral
>20–30 kilogram	10 Fr	IJ/EJ, subclav, femoral
>30 kilogram	12 Fr	IJ/EJ, subclav, femoral

Fr: French; IJ: internal jugular vein; EJ: external jugular vein; subclav: subclavian vein. Adapted with permission from Goldstein [34].

difficult even despite recommended catheter size placement (Table 2). As in IHD, CRRT in small patients is complicated by the fact that a large percentage (10–15%) of blood volume may be extracorporeal at any given time. This amount of volume shift can lead to a high amount of thermodynamic instability. Bradykinin release syndrome (hypotension, mucosal congestion, and bronchospasm upon contact of patient blood with a hemofilter) is a problem with CRRT in infants using an AN-69 membrane circuit. Finally, anticoagulation may pose added complications to patients in septic shock and disseminated intravascular coagulation (DIC).

Evidence. CRRT offers minute to minute control of ultrafiltrate and affords practitioners the flexibility to use volume intense medications, blood products, and nutrition. CRRT offers better control of uremia compared to PD or IHD [35] and allows practitioners to control solute levels to targets, which is critical in cases such as children with increased intracranial pressure who require tight control of serum osmolarity [36]. However, no prospective study of CRRT exists in either the adult or the pediatric literature. Also, no controlled trial comparing the efficacy of PD to IHD to CRRT in pediatrics exists. A study comparing PD to CRRT demonstrated a significant survival difference with CRRT (85%) versus PD (43%), but critics have indicated that rigid tubing, manually exchanged fluid, and hyperglycemia in the PD group may have skewed the results [37, 38]. A recent multicenter adult study in Belgium indicated that, in 1303 patients, mortality was higher (58% versus 43%) for AKI patients in the RRT group than in conservative management group despite correction for severity of illness [39]. Unfortunately, clear evidence supporting or contraindicating the use of CRRT in pediatrics is lacking. Fortunately, through the ppCRRT, the study of pediatric RRT has progressed and more data emerges regarding the confounding variables associated with the risks and benefits of RRT. Some of these variables are discussed below.

Opinion. As center-specific technical expertise expands, the use of CRRT continues to also expand. Despite limited data indicating its superiority over PD or IHD, the ability to finely control fluid balance and electrolyte derangements makes CRRT an ideal option in the hemodynamically unstable patient. Additionally, the ability to provide optimal nutrition and deliver blood products and medicine while on CRRT (without worrying excessively about fluid shifts) is highly desirable. The tight control of such deliverables is paramount in the care of the critically ill pediatric patient and the primary reason why CRRT use expands. Institutional capability, technical familiarity, and ability to obtain adequate vascular access are barriers to its use, but efficacy in unstable patients is an attractive quality in this modality.

2.4. Controversies in Renal Replacement Therapy

Initiation of RRT. In the only randomized trial of early versus late initiation of RRT in critically ill patients [40], 106 ventilated, oliguric adults were randomized to either "high volume-early" (initiation 8 hours after stratification, 72–96 L hemofiltration in 24 hours), "low volume-early" (initiation 8 hours after stratification, 24–36 L hemofiltration in 24 hours), and "low volume-late" (initiation 36 hours after stratification, 24–36 L hemofiltration in 24 hours) of CRRT. Median duration of renal failure and 28-day survival were not significantly affected by timing of initiation. However, other data suggesting that early treatment is superior to late treatment is encouraging in patients who developed AKI following CPB [41–44]. There is also evidence suggesting that early RRT affords critically ill patients with a therapeutic strategy that goes beyond organ support. In other words, RRT may a serve a more direct, therapeutic role in ameliorating renal injury and improving the chances of "renal recovery." Renal recovery is variably defined in the literature, though most definitions used include the criterion of freedom from chronic RRT [45, 46]. The pediatric data regarding timing of initiation is largely retrospective and put into the context of triggers such as fluid overload and presence of sepsis.

Fluid Overload and CRRT. A retrospective study of 21 children receiving RRT for AKI suggested that the degree of fluid overload (FO) at time of RRT initiation was significantly lower in survivors than in nonsurvivors (16.4% versus 34%) [31]. In a larger study of 113 children with multiple-organ dysfunction syndrome (MODS) started on CRRT, median % FO was significantly lower in survivors compared to nonsurvivors (7.8% versus 15.1%), and mortality related to FO was independent of severity of illness [47]. Another study showed that, in 297 patients, % FO was again significantly lower in survivors versus nonsurvivors (12.5% versus 23.0%) [48]. In a prospective, uncontrolled, observational study DiCarlo initiated CRRT for ten children with ARDS after BMT regardless of presence of AKI and demonstrated an 80% survival rate [49]. Of the available retrospective evidence, the mortality for children started on CRRT is 10–57.1%. In select centers, the use of PD after cardiopulmonary bypass during the immediate postoperative period is routine [9]. The ppCRRT data suggests that a threshold may exist for initiation of CRRT based on FO to improve mortality, a threshold that appears to be independent of severity of illness [7]. The threshold, 10–15% FO, however, has yet to be prospectively tested. Further, it has yet to be demonstrated

that children placed on CRRT for AKI have better outcomes than those without such therapy.

Sepsis and CRRT. RRT for sepsis without AKI has been attempted in adults, with the thought that RRT could remove harmful circulating cytokines and inflammatory mediators, but trials have been underpowered and results regarding efficacy have been varied. While many inflammatory mediators can be removed using RRT, specifically CRRT, it is unknown how the amount removed compares to the serum concentration of such mediators and the significance of certain levels. No studies of RRT designed specifically to analyze its efficacy in pediatric sepsis, absent AKI, have been performed. A recent meta-analysis of the major adult CRRT studies, comparing the efficacy of "intensive-dose" CRRT (>40 mL/kg/hr) to "less-intensive-dose" CRRT (<25 mL/kg/hr), found no beneficial effect of intensive dose on mortality in sepsis and AKI [50]. While *in vitro* data continues to suggest that CRRT may offer benefit for immunomodulation in sepsis, clear clinical data are lacking. Accordingly, in adults and in children, the use of CRRT for immunomodulation is not currently recommended [51].

Access. While adequate venous access is paramount to effective IHD or CRRT, PD confers the advantage of not requiring large vessel access. Catheter placement in children increases risk for thrombosis and sclerosis, especially in the subclavian veins, and may be detrimental to obtaining future access. Data from the ppCRRT indicates that smaller catheters (5 and 7 French) and those placed in the femoral veins were associated with shorter circuit lives than larger catheters and those placed in the internal jugular veins [52]. Appropriate catheter sizes and placement are mutually dependent and can vary, but a starting framework is shown in Table 2 [30].

Anticoagulation. The absence of a need for anticoagulation is a major advantage for PD use. Heparin and citrate are the major anticoagulants used in IHD and CRRT; registry data reports equal circuit viability with both substances [53]. While citrate allows the patient to be free of heparin-induced systemic anticoagulation, it may require several additional solutions to be run concurrently with the circuit: anticoagulant dextrose solution A (ACD-A) [30] and a continuous calcium infusion (infused through separate central access) to prevent hypocalcemia which occurs when citrate binds ionized calcium. Higher citrate clearances or lower citrate delivery methods must be used to prevent citrate lock, defined as elevated serum total calcium but low serum ionized calcium, which results from increased citrate buffering of free calcium from decreased citrate metabolism.

Dose. The high dose of dialysis delivered in relation to time is an advantage of IHD over PD and CRRT. Much of the data regarding dose of RRT is retrospective and focuses on CRRT. An initial study reported mortality lessened with larger doses (≥35 mL/kg/hr) than with smaller doses (20 mL/kg/hr) [54]. However, a large recent study with meticulous documentation of *actual* doses received demonstrated no improvement in kidney function or mortality outcome in adults receiving high-intensity CRRT (35 mL/kg/hr) versus low-intensity CRRT (20 mL/kg/hr) or intermittent hemodialysis [55]. The few outcomes studies performed in pediatrics investigating the effects of RRT dosage and modality are retrospective. The ppCRRT in 2007 demonstrated no difference in overall outcomes based on modality or dose of CRRT used [56].

Modality of CRRT. As mentioned before, CRRT is offered in multiple modalities differing based on primary principle of filtration used (convection or diffusion). While the ppCRRT reported that 344 children on CRRT in 13 Centers in the United States used CVVHD (venovenous hemodialysis) (48%), CVVHDF (venovenous hemodiafiltration) (21%), CVVHF (21%) (venovenous hemofiltration), and SCUF (slow continuous ultrafiltration) (1%), most centers only offer one modality [57]. As the names imply, the modalities differ based on the ability to incorporate one or both methods of filtration (convection → filtration based on hydrostatic pressure, diffusion → dialysis, particles move based on concentration gradient). Some practitioners feel that CVVHD is the optimal method by which to remove small molecules in children, but this is most likely influenced by site-specific preference (or availability) [58]. Though not yet rigorously proven, filter life may also be affected by modality. In isolated studies, dialytic modes of CRRT offer longer filter survival (12 hours–48 hours) than purely filtration modes [59]. In the most recent ppCRRT data, more survivors had convective RRT than nonsurvivors (61.0% versus 43.0%) [48]. The lack of formal trials comparing modality in pediatrics limits any formal recommendation on use in relation to patient outcomes.

Outcomes. Limited prospective data is available to compare morbidity and mortality between the three dialytic modes. In adults, a study performed in a developing country analyzed PD versus CRRT in infection-related AKI and found that CRRT was significantly superior to PD in all end-points tested (reduction of creatinine, resolution of acidosis) [37]. In children, the data is quite limited, retrospective, and often limited to specific disease processes, such as cardiopulmonary bypass [60]. Data from the ppCRRT indicates 58% survival for all children placed on RRT, although high mortality rates (liver 69%, pulmonary 55%, and stem cell 55%) seen in select transplant populations and children under 10 kilograms influence the data significantly.

3. AKI, Renal Angina, and the Why

The use of renal replacement therapy in pediatrics continues to blossom. It is uncertain whether the risks of dialysis, both mechanical and technical, outweigh the perceived benefits (improved renal recovery or reduced mortality). At present, the only proper way to answer the question would be to conduct a prospective, randomized trial comparing the different RRT modalities to no invasive therapy, which would

be a trial loaded with ethical concerns. Further, if acute dialysis for AKI is actually beneficial, the critical *point* of intervention remains a mystery. Cardiac catheterization and coronary intervention therapy for myocardial infarction were optimized with the advent of troponins—tests made more reliable when run in patients with risk factors and signs of illness. Unfortunately, unlike chest pain, AKI does not hurt and the symptoms of AKI without fluid overload may be quite subtle. The phenotype of AKI in its earliest stage is unknown, which limits the applicability of biomarkers and detection strategies, and treatment efforts. Discovering the equivalent of angina for chest pain and myocardial infarction—renal angina—may strengthen biomarker utility, aid early AKI diagnosis, and possibly identify patients most likely to benefit from early renal replacement therapy [61]. Renal angina, defined as the composite of risk factors (i.e., high blood pressure, smoking for heart disease and shock, or sepsis for AKI) and subtle changes in renal function (small changes in creatinine clearance or fluid overload), may identify patients who are at the highest risk. Prospective trials comparing cohorts of patients and different modalities of RRT are needed to improve the delivery of care, and the ppCRRT registry aims to reach this goal.

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Research Article

Once-Daily Tacrolimus Extended-Release Formulation: 1 Year after Conversion in Stable Pediatric Kidney Transplant Recipients

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It is speculated that a once-daily dosage of immunosuppression can increase adherence and thereby graft survival. Until now, there have been no studies on once-daily use of Tacrolimus extended-release formulation (TAC-ER) in children following pediatric kidney transplantation. In 11 stable pediatric kidney recipients >10 years, efficacy, safety, and tolerability of a switch to TAC-ER were observed over one year. Adherence was determined by use of the BAASIS-Scale Interview and comparison of individual variability of Tacrolimus trough levels. Over the observation period, two acute rejections were observed in one girl with nonadherence and repeated Tacrolimus trough levels of 0 ng/m. Beside this, there were no acute rejections in this trial. TAC dose was increased in 3/11 patients and decreased in 2/11 patients within the course of the study. Six patients did not require a dose adjustment. All but one patient had a maximum of 1 dose change during therapy. Mean Tacrolimus dose, trough levels, and Glomerular filtration rates were also stable. Adherence, as measured by BAASIS-Scale Interview and coefficient of variation of Tacrolimus trough levels, was good at all times. It is concluded that conversion to Tac-ER is safe in low-risk children following pediatric kidney transplantation.

1. Introduction

Patient nonadherence to immunosuppressive therapy after kidney transplantation is an important factor contributing to acute rejection and decreased graft survival [1–5]. This is especially true for adolescents [1] who are at the highest risk of nonadherence. Fennell et al. indicated that approximately 50% of children with chronic diseases are nonadherent [6]. It has been shown that, following pediatric renal transplantation, nonadherence is one of the most important contributing factors for graft rejection and loss [7]. In renal transplantation in adults one study concluded that there was a statistically significant correlation between a once-daily dose of immunosuppression and better adherence [2]. However, until now, there has been no evidence that TAC-ER improves adherence to the immunosuppressive regimen. In other meta-analyses there is only limited evidence that once-daily dosing of a drug improves adherence to the immunosuppressive regimen in other chronic diseases [8, 9]. A once-daily dose of Tacrolimus extended-release formulation (TAC-ER) was safe and effective in a small cohort of children after liver transplantation [10]. No such data exists for children after kidney transplantation.

2. Patients and Methods

2.1. Study Design. This was an open-label, controlled prospective trial to determine the safety and efficacy of the use of one daily TAC-ER dose in children after kidney transplantation and to improve adherence. The study was performed in accordance with the ethics committee of Hannover Medical School. It was classified as a "noninvasive prospective trial" by the ethics committee according to German law that does not require registration. Therefore, ICH-GCP guidelines were followed partially as required: serious adverse events and adverse events were documented, but no external monitoring was implemented. Informed consent was given by parents and children. The study was conducted as an investigator initiated trial. Sponsor of the study was the Medical School of Hannover, Germany.

As this was the first trial with TAC-ER in children after kidney transplantation, a selected low-risk study group was

chosen: children >10 years with a minimum of one year since kidney transplantation and with stable renal function were eligible for the study. No acute rejections occurred within the year before the study. Immunosuppression consisted of either Cyclosporine A (CsA) and Mycophenolate Mofetil (MMF) ± Prednisolone or Tacrolimus (TAC), MMF ± Prednisolone. After conversion all participants were continued on MMF twice daily in addition to Tac-ER and Prednisolone once daily. The patients were studied from January 2008 to January 2009. Observation time was one year.

The study was a conversion study. At study initiation the dose of TAC-ER was calculated as the prior daily dose of Tacrolimus. In cases of CsA therapy, daily TAC-ER dose was calculated as follows: 6 mg/m^2 body surface area. Target Tacrolimus trough level was 5–7 μg/L. All patients were seen in the outpatient clinic one week after the conversion and then every 4 weeks for the rest of the study period. No surveillance biopsies were performed. Indication biopsies were mandatory in cases of increased creatinine of 15% over baseline.

In all patients the Basel assessment of adherence to immunosuppressive medication scale (BAASIS Scale) was done by interview at study initiation and one year later. The BAASIS Scale is a combination of the Siegal questionnaire [11] and the self-report on adherence of Walsh et al. [12]. The assessment was completed with the patients, not their parents.

The incidence of biopsy-confirmed acute rejection episodes, patient and graft survival rate was assessed throughout the study. Safety was assessed based on individual adverse events and the results of routine clinical laboratory tests and assessment of vital signs.

2.2. Patients. Twelve children (age > 10 years) were eligible for the study. One family refused consent to the study because of the stable situation of their child. Eleven children, mean age 14 ± 2 years, 4 female, 7 male, were included in the study. Complete demographics are given in Table 1. Ten patients were treated with TAC, MMF ± Prednisolone before study start and one with CsA, MMF, and Prednisolone.

2.3. Statistics. Primary endpoint of the study was an improvement of adherence as demonstrated by an increase of mean percentage of self-estimated compliance after Walsh et al. [12]. Sample size was calculated as follows: estimated mean percentage of adherence before switch 85%, after switch 95%, sample size 10 patients, standard deviation 7%, and α error 5%. Statistical Power was calculated as 93.9%.

In order to calculate the intraindividual variability of TAC trough levels, the last 5 trough levels before the switch and before the end of the first year after the switch measured every 4 weeks were used to calculate the individual coefficients of variation (CV = standard deviation/mean). Five outpatient trough levels with an interval of 4 weeks were used. No values were excluded. Based on these values, the mean coefficient of variation was calculated for both assessments. Values between different assessments were compared by paired t-test and those between patients by unpaired t-test. $P < .05$ was defined as statistically significant.

Table 1: Patient demographics.

Gender	Male 7, female 5
Mean age	14 ± 2 years
Immunosuppressant before switch	CsA/MMF: $n = 1$
	TAC/MMF: $n = 10$
Underlying disease	pANCA-positive vasculitis: $n = 2$
	Renal dysplasia: $n = 5$
	Nephronophthisis: $n = 2$
	Obstructive uropathy: $n = 1$
	Congenital nephrotic syndrome: $n = 1$
Living donation/cadaveric donation	4/7
Mean time from transplantation to switch	4.4 ± 2.6 years

Adverse events and serious adverse events were documented according to clinical practice guidelines.

3. Results

3.1. Tacrolimus Drug Exposure. Mean daily TAC dose was 4.8 ± 2.2 mg/m^2 before the switch and 5.1 ± 2.4 mg/m^2 (P = n.s.) one year later. Mean trough levels were 6.2 ± 2.0 ng/mL before the switch and 6.5 ± 0.9 ng/mL one year later. TAC dose was increased in 3/11 patients and decreased in 2/11 patients within the course of the study. Six patients did not require a dose adjustment. All but one patient had a maximum of 1 dose change during therapy. The adjunctive immunosuppressive therapies were not changed during the study.

3.2. Kidney Function. The mean GFR (abbreviated Schwartz 2009 formula [13]) was 56 ± 11 mL/min/1.73 m^2 at time of switch and 55 ± 11 mL/min/1.73 m^2 one year later (P = n.s.). There was no graft loss or patient death.

3.3. Acute Rejection. Indication biopsies due to an increase in s-creatinine were only performed in one 16-year-old girl. Both biopsies showed acute rejection, BANFF Grade Ia. In both, TAC levels were 0 ng/mL, and the patient reported not having taken TAC-ER due to psychosocial problems. Both acute rejection episodes were sensitive to steroid pulses. After multiple intervention by the psychosocial team, adherence increased and TAC levels were stable.

3.4. Intraindividual Variability of Tacrolimus Levels. The mean coefficient of variation of TAC trough levels was 0.27 ± 0.11 before the switch and 0.30 ± 0.19 one year later (P = n.s.).

3.5. BAASIS Scale. The results of the BAASIS-Scale Interview were comparable at both times of evaluation. Results of the important items are given in Table 2.

Table 2: Results of The BAASIS-Scale Interview concerning adherence in all patients before the switch to TAC-ER and one year later.

	Before switch to TAC-ER	One year after switch	*P*
Immunosuppression not taken	3/20: once monthly	3/20: once monthly	n.s.
Immunosuppression taken 2 hrs before or after prescribed time	3/20: once monthly 2/20: once every 2 weeks	2/20: once monthly 1/20: once every 2 weeks	n.s.
Dose of Immunosuppression changed without advice	0/20	0/20	n.s.
Mean percentage of self-estimated compliance after Walsh et al. [12]	94 ± 7%	93 ± 7%	n.s.

3.6. Serious Adverse Events. Two hospital admissions for 2 indication kidney biopsies in one 16-year-old girl and one admission in a 17-year-old boy due to gastroenteritis requiring i.v. rehydration took place. No other serious adverse events were documented during the study period.

4. Discussion

This data concludes that, in a selected cohort of pediatric kidney recipients, a switch from the standard formulation TAC to TAC-ER is safe. The acute rejection episodes taking place in one patient were due to nonadherence and interaction problems within her family. Despite this nonadherence, mean GFR remained stable, intraindividual variability of trough level was low, and TAC-ER was not discontinued in any patient. One dose adjustment of TAC-ER had to be performed in 45% of the patients.

In adults receiving antihypertensive medication, it has been shown that adherence can be increased from 59% to 83% when changing from a thrice-daily dose to a once-daily dose of medication [14]. These findings have been confirmed by Weng et al. [2] who used electronically measured adherence to immunosuppressive therapy. Wolff et al. studied the issue of medication adherence in 85 children with end-stage renal disease and found that, in 4% of the patients, nonadherence had been the primary reason for referral to psychosocial services [1]. They concluded that nonadherence is almost inevitable, often based on valid personal reasons. It has been shown that adherence and education influence the nephrologists' recommendation for transplantation [15]. In a meta-analysis, Dobbels et al. calculated a mean nonadherence rate of 35.2% in children after renal transplantation. They stated that adherence-enhancing interventions should consist of educational strategies, behavioural strategies, and strategies to increase social support [16]. Rianthavorn and Ettenger [7] described different methods of documenting medication nonadherence and stated standard deviation calculation for trough levels of TAC, as described by others [17], to be highly correlated with outcomes that suggested nonadherence, such as biopsy proven rejection. In this study the coefficient of variation of TAC trough levels was low even before study initiation and did not change thereafter in comparison to other immunosuppressive studies [18]. The girl who did not take her medication in the middle of the study period did not influence the low mean coefficient of variation, because for calculation purposes it was defined before study start that only trough levels before the switch and in the last months of the study period were used. In these two periods, TAC trough levels were stable in this girl.

The primary endpoint, showing superiority for adherence after switch to once-daily dosing, was not achieved within our study. The "good adherence" at the beginning of the study might explain why there was no more increase in adherence after the switch to TAC-ER. Obviously, there was a selection bias of adherent children in the study, only including children without acute rejection one year before the switch. However, this bias was accepted when designing the study, as stable patients for the first change to a new immunosuppressant was a requirement. In this study only children >10 years were included, because major differences in pharmacokinetics can be expected in children below this age.

Another aspect for the failure of this study to achieve more adherence may have been the combination of TAC-ER with MMF, which has to be administered twice daily. Future studies with TAC-ER in children should include teenagers with a history of nonadherence and also children <10 years. In a future study with a more high-risk population, careful pharmacokinetic monitoring should be mandatory. The possible positive effect of TAC-ER on adherence could be enhanced by immunosuppressive combinations needing only once-daily application for all agents. However, possible combinations such as TAC-ER and Sirolimus or TAC-ER and Azathioprine have not shown promising results in children until now and should not therefore be used in future studies.

In studies in adults, it could be demonstrated that the pharmacokinetic profile of TAC-ER is different compared to TAC. In several adults TAC-ER dose has to be increased after switch from TAC to TAC-ER to obtain the same exposure to Tacrolimus [19]. In this small pediatric cohort, dose had to be increased in 3/11 children. However, administration of TAC-ER in conversion studies in adults was also safe for the patients [20, 21].

It is concluded that TAC-ER can safely be used in a selected low-risk cohort of pediatric kidney graft recipients >10 years. In this selected study population, adherence could not be improved by once-daily administration of TAC.

Abbreviations

GFR: Glomerular filtration rate
TAC: Tacrolimus

TAC-ER: Tacrolimus extended-release
MMF: Mycophenolate Mofetil
CsA: Cyclosporine A
mTOR: Mammalian target of rapamycin.

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Review Article

Optimal Hemodialysis Prescription: Do Children Need More Than a Urea Dialysis Dose?

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When prescribing hemodialysis in children, the clinician should first establish an adequate regimen, before seeking to optimize the treatment (Fischbach et al. 2005). A complete dialysis dose should consist of a urea dialysis dose *and* a determined convective volume. Intensified and more frequent dialysis regimens should not be considered exclusively as rescue therapy. Interestingly, a recent single-center study demonstrated that frequent on-line HDF provides an optimal dialysis prescription, both in terms of blood pressure control (and therefore avoidance of left ventricular hypertrophy), and catch-up growth, that is, no malnutrition or cachexia and less resistance to growth hormone. Nevertheless, this one-center experience would benefit from a prospective randomized study.

1. Introduction

Over the past 20 years, children receiving dialysis have benefited not only from advances in modern technology, but also from improvements in clinical management. Nevertheless, conventional haemodialysis performed three times a week still results in an increased risk of cardiovascular morbidity and impaired growth in children [1, 2]. The "HEMO" study [3] found that the prescription of a high urea dialysis dose in hemodialysis performed three times a week in adults failed to result in an improvement in clinical outcome. Therefore, "a complete dialysis dose" should be prescribed, that is, not only a urea dialysis dose (a small solute diffusive clearance) [4] but also a convective dialysis volume (middle molecule uremic toxin clearance) [5]. At present, despite great interest and feasibility, hemodiafiltration is rarely performed [6–8]. However, the case for performing intensified and/or more frequent hemodialysis regimens in both children [9–13] and adults has been put forward [14–17]. All intensified treatment modalities, ranging from daily procedures to long intermittent hemodialysis sessions, clearly improve patient outcome in terms of mortality and morbidity. In children, daily on-line hemodiafiltration has even been found to promote catch-up growth [9, 18]. However, intensive and daily dialysis is rarely performed as a long-term dialysis regimen for "all patients" as it is often only considered as a rescue therapy [15].

2. Urea Dialysis Dose For Children

Urea kinetic modeling is generally accepted as a method for evaluating the dialysis dose prescribed [19], despite the fact that it is of limited value in the assessment of dialysis adequacy [4].

Quantification of dialysis is based on a urea dialysis dose, that is, Kt/V, (dialyzer urea clearance (K) multiplied by duration (t) of the dialysis session and divided by urea volume (V) of distribution. On-line urea clearance measurements, which are currently available on modern dialysis machines, make it easy to calculate the dialysis dose (Kt) and, thus, allow the clinician to calculate the Kt/V for each session, providing an estimation of V which is assumed to be equal to the total body water. Impedancemetry [20] is of clinical interest when estimating V, as it does not require blood sampling to be performed. However, it

does require the use and availability of a specific machine (BCM, Fresenius Medical Care). The use of anthropometric formulas to calculate "V" has been found to be less accurate especially in children [19]. An indirect method of calculating "V" can be achieved using the ratio $Kt/Kt/V$ [20], where Kt is obtained from the ionic dialysance provided by the dialysis monitor, and Kt/V is obtained from the second generation of Daugirdas formula [19], which requires blood samples to be taken before and after the dialysis session. The on-line urea Kt/V assessment performed by the dialysis monitor gives out a single pool Kt/V (spKt/V) [19], with a proposed target dose of 1.4 per dialysis session in anuric patients [19, 21]. Nevertheless, spKt/V does not take into account postdialytic urea rebound. Therefore, formulas for estimating the equilibrated Kt/V (eKt/V) are available [4, 19, 21]. Due to the need for postdialytic blood sampling, in practice spKt/V is a clinically acceptable way of monitoring HD adequacy, at least in a thrice-weekly hemodialysis regimen. In cases where hemodialysis is performed more frequently, standard Kt/V (stKt/V) should be calculated [22]. This formula integrates spKt/V and eKt/V in order to account for urea peaks and valleys. A dialysis dose based on body surface area (SAN-stKt/V) has been suggested to be a more accurate way of determining the urea dialysis dose required in children: a dose over 2.45 [23] should be considered to be an optimal dialysis dose, promoting catch up statural growth in children on chronic dialysis. Nevertheless, a complete dialysis dose [5] should be preferred to an urea dialysis dose, "only" a diffusive dialysis dose [5]. The "complete dialysis dose" is difficult to define and stays under discussion [4, 5]: beside the urea dialysis dose, a diffusive dialysis dose, a convective dialysis dose could be proposed [6, 15, 24], based on phosphate dialytic removal and on the achieved beta 2 microglobulinemia.

3. Convective Dialysis Dose: The Convective Volume per Dialysis Session

Uremic toxins can be categorized into three groups: free water soluble low-molecular-weight solutes such as urea, middle-sized solutes such as beta 2 microglobulin (β2 m), and protein-bound solutes such as p-cresol acid [24]. The phosphate ion is small, but not "free" as it is surrounded by water molecules; therefore, it has a clearance profile similar to that of the middle-sized molecules. The removal of uremic toxins by dialysis relies upon a combination of the diffusion process, convective mass transport, and membrane adsorption [25]. Haemodialysis (HD) provides optimal removal of low-molecular-weight uremic toxins such as urea due to the diffusive flow of solutes. Conversely, the convective flow of solutes which mainly occurs in hemofiltration (HF) and hemodiafiltration (HDF) results in the removal of middle-sized and large molecules. Convection and diffusion are presented as two separate phenomena, but in practice they are on a continuous interference. Nevertheless, the concept of a complete dialysis dose [5], which includes not only a urea dialysis dose, that is, Kt/V urea, but also a convective dialysis dose, that is, the convective volume achieved per dialysis session, may be of importance in terms of patient outcome—in particular resulting in an improvement in cardiovascular mortality [6, 26].

Hemodiafiltration is a combination of HD and HF performed during the same procedure, allowing a determined convective flow per dialysis session. The weight loss that arises when HD occurs only ensures a convective flow equal to the ultrafiltration rate, that is, a small convective volume. During conventional HD with high-flux filters, "internal" HDF occurs due to back filtration of the dialysate, providing only a slightly larger but nondetermined convective volume. Moreover, the dialysate used is not always a sterile, nonpyrogenic substitution fluid, and therefore may provoke systemic inflammation [28, 29]. Altogether, on-line hemodiafiltration, that is, substitution fluid prepared from ultrafiltration of the ultrapure dialysate, which is a safe routine replacement therapy [6, 7, 17, 30, 31], enables the use of a determined high convective volume throughout the dialysis session, impacting on both solute removal and clinical outcome. HDF offers improved control of phosphate when compared to both HD and high-flux HD [6, 7]. Predialysis β2 m serum levels are decreased during OL-HDF; the β2 m dialytic removal correlates with the convective volume achieved. High-efficiency HDF, as defined in adult patients in postdilution mode using a convective dose of more than 15 L per session, is superior to low-efficiency HDF both in terms of solute removal and patient outcome [6, 7]. As demonstrated by a recent single centre study [18], when performed on a daily basis, on-line HDF using a convective volume in pre dilution mode of 18 to 27 L/m^2 per procedure even promotes catch up growth in children. At present, it is not known if the different intensive hemodialysis regimens available are equivalent in terms of their growth promoting capacity [11, 21].

4. Hemodialysis Fluids Purity

The purity, in terms of microbial contaminants measured as colony forming units (CFU) and endotoxins units (EU), is recorded for the different fluids used in the dialysis procedure [7]. The purity should be carefully assessed in order to limit the risk of patient's systemic inflammation, in the clinical setting illustrated by the surrogate parameter of an elevated CRP. Standard quality dialysis fluid (bacterial count <100 CFU/ml; endotoxin units <0.50 EU/ml) is not appropriate for use with high-flux filters for HD, due to the internal convective flow, that is, the backfiltration. High-flux membranes require the use of ultrapure dialysate, that is, bacterial count <0.1 CFU/mL; endotoxin count <0.05 UI/mL. For "at risk" patients, the current European guidelines recommend the use of highly permeable membranes in HD [27]. This results in restricted "internal" HDF, as only a small nondetermined convective volume is used.

5. Blood Pressure Control, Cardiovascular Disease

Repeated conventional dialysis sessions have an additive effect which contributes, towards the cardiovascular risk

Table 1: Adequate hemodialysis prescription for children: more than a urea dialysis dose (adapted from [4, 5, 11, 19, 27]).

(i)	Hemodialysis should be performed in a pediatric dialysis center, in order to ensure optimal care and child development (nutrition, growth, education)
(ii)	A complete dialysis dose should be prescribed, not only a urea dialysis dose, that is, sp $Kt/V > 1.4$ in anuric patients, but also a high convective volume. Phosphate and $\beta 2$ m can be used as markers for the removal of "middle-sized uremic molecules."
(iii)	Prefer biocompatible materials where possible, that is, high-flux membranes which provide enhanced molecular permeability. High-flux membranes, especially in cases of high hydraulic permeability, require the use of ultrapure dialysate, that is, a bacterial count <0.1 CFU/mL and an endotoxin count <0.05 UI/mL.
(iv)	Control blood pressure and aim for prevention of cardiovascular sequelae such as left ventricular hypertrophy, left ventricular dysfunction, coronary artery calcifications, and vascular stiffness.
(v)	Ensure optimal nutrition, that is, limit malnutrition and cachexia in order to avoid muscle wasting and to promote growth and development.
(vi)	Propose an intensified hemodialysis regimen, that is, longer and/or more frequent dialysis in center or at home, not only for use as a rescue therapy.
(vii)	Deliver the highest standard of dialysis possible in all cases, that is, biocompatibility of the material used, monitor the purity of the dialysate, and use a controlled determined convective flow instead of an internal, small, nondetermined flow with backfiltration into the dialyzer.

profile in children, that is, morbidity and mortality. Adverse effects related to dialysis include: high blood pressure, left ventricular hypertrophy or impaired left ventricular function, elevated calcium/phosphate resulting in calcification of blood vessels (particularly vascular/coronnary calcifications), and systemic inflammation. The above list accounts for the vast majority of dialysis related risks to the cardiovascular system, contributing towards increased morbidity and mortality [2, 32].

In children on chronic conventional hemodialysis, hypertension is common. For example, 79% of children on HD in the United States have an elevated BP at the start of the dialysis session [33]. A further study found that Left ventricular hypertrophy (LVH) was worryingly common (82% of patients) in children at the initiation of dialysis, and remained frequent (82%) and severe (59%) after 2 years of maintenance conventional hemodialysis [34].

In children on conventional HD, LVH may be improved [35] by optimizing the sodium balance, for example, by strict dietary control of sodium intake, dry weight achievement, and supplementary dialysis time. Overall however, intensified hemodialysis regimens should not be disregarded in the hope of improving cardiovascular health [11], and they should, therefore, not be considered exclusively as rescue therapy [15].

6. Protein Wasting: Cachexia

Malnutrition is common amongst children on chronic haemodialysis. However, they are not only malnourished—which infers that dietary supplementation would be curative, but they are also cachexic (i.e., in a state of protein loss). Malnutrition and cachexia combined are two important factors which contribute towards the protein wasting seen in uremic patients [36]. Conventional hemodialysis performed three times a week requires patients to stick to a restrictive diet which limits the intake of protein, water, sodium, potassium, and phosphate, whilst at the same time maintaining an adequate calorie intake. Due to limited uremic toxin removal, chelators of phosphate and potassium are often prescribed-but these are also factors which contribute towards anorexia, that is, malnutrition. Conversely, if the dialysed child has a good appetite, he is at risk of increased weight gain between dialysis sessions and of raised blood phosphate or potassium levels and of major metabolic acidosis. In such circumstances, the duration of the dialysis procedure is often increased from 4 to 5 hours, and sometimes an additional dialysis session later in the week may be required. The child on long-term dialysis often considers these changes or adaptions to be a form of punishment, and he responds in turn by fasting and becoming increasingly less compliant and more opposed to the procedure. Aside from malnutrition, protein catabolism is an independent factor which plays a major role in the muscle wasting and poor linear growth that result despite rh GH therapy. Loss of protein stores, that is, more cachexia than malnutrition [37] is a multifactorial event, caused by: inflammation [38], acidosis, uremic toxin retention, and volume overload, that is, poor blood pressure control. Malnutrition and cachexia should be limited by more intensive and frequent hemodialysis. Interesting, online HDF performed on a daily basis has been found to reverse both cachexia and malnutrition and to promote catch up growth in children [18].

7. Conclusions

In children, an adequate hemodialysis prescription requires more than simply a urea dialysis dose (Table 1). If economically feasible, high-flux membranes should be used in combination with ultrapure disposable dialysate. Online hemodiafiltration, that is, substitution fluid prepared from ultrafiltration of the ultrapure dialysate, is a safe routine replacement therapy which has a positive impact not only on the removal of solutes such as phosphate or $\beta 2$ m, but also on clinical outcome. Intensified and more frequent hemodialysis regimens, that is, high-flux HD or

on-line HDF, provide adequate hemodialysis in children, and should, therefore, not exclusively be used as rescue therapy. Nevertheless, in young children with renal residual clearance, peritoneal dialysis remains an excellent choice of chronic at home dialysis modality.

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Review Article

Nephronophthisis: A Genetically Diverse Ciliopathy

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Nephronophthisis (NPHP) is an autosomal recessive cystic kidney disease and a leading genetic cause of established renal failure (ERF) in children and young adults. Early presenting symptoms in children with NPHP include polyuria, nocturia, or secondary enuresis, pointing to a urinary concentrating defect. Renal ultrasound typically shows normal kidney size with increased echogenicity and corticomedullary cysts. Importantly, NPHP is associated with extra renal manifestations in 10–15% of patients. The most frequent extrarenal association is retinal degeneration, leading to blindness. Increasingly, molecular genetic testing is being utilised to diagnose NPHP and avoid the need for a renal biopsy. In this paper, we discuss the latest understanding in the molecular and cellular pathogenesis of NPHP. We suggest an appropriate clinical management plan and screening programme for individuals with NPHP and their families.

1. Introduction

Nephronophthisis (NPHP) is an autosomal recessive cystic kidney disease and a leading genetic cause of established renal failure (ERF) in children and young adults [1]. NPHP literally means "disappearance of nephrons," which alludes to its histopathology, with interstitial fibrosis and corticomedullary cysts replacing normal renal tissue. The median age of an affected child with ERF is 13 years [2]. The incidence of NPHP varies worldwide; it was previously identified to range from 1 in 50,000 to 1 in 900,000 [3–5]; however, these figures are likely to underrepresent the true frequency, since molecular testing has diagnosed NPHP in adults presenting with advanced chronic kidney disease (CKD) [6, 7]. The prevalence of NPHP amongst the paediatric population with ERF is 5% in the USA [2] and 6.5% in the UK [8, 9].

Early presenting symptoms in children with NPHP usually develop at around 6 years of age and include polyuria, nocturia or secondary enuresis, polydipsia, and lethargy (secondary to anaemia) [10]. These features are a consequence of salt wasting and an inability to concentrate urine (<400 mosm/kg early morning urine), implicating dysfunction of the renal cortical collecting duct [11]. Renal ultrasound identifies normal or reduced kidney size, with increased echogenicity and corticomedullary cysts [2]. There is a less common infantile variant of NPHP in which children reach ERF by 3 years of age and have enlarged cystic kidneys on renal ultrasound [12]. Infantile NPHP is distinct from autosomal recessive polycystic kidney disease (ARPKD). There is a diffuse distribution of cysts within the kidneys of children with ARPKD, and it is more often associated with liver cysts and fibrosis [13]. A diagnostic renal biopsy of NPHP reveals a characteristic triad of tubular basement membrane disruption, tubulointerstitial nephropathy/fibrosis, and corticomedullary cysts [4, 14]. Increasingly, molecular genetic testing [15] is being utilised to diagnose NPHP and avoid the need for a renal biopsy [16].

NPHP is associated with extra renal manifestations in 10–15% of patients [1]. The most frequent anomaly is retinal degeneration; other associated features and disorders include cerebellar vermis hypoplasia (Joubert Syndrome (JS)), occipital encephalocele (Meckel-Gruber syndrome

(MKS)), hepatic fibrosis, situs inversus, bronchiectasis, and skeletal defects [1]. In addition to this apparent variability in the spectrum and severity of phenotype, NPHP is genetically heterogenous. To date mutations have been identified in 13 genes (Table 1) which collectively account for approximately 30% of patients [17]. The protein products of all of these genes localise on primary cilia and related structures (basal bodies, centrosomes), resulting in a unifying hypothesis that cystic kidney diseases are ciliopathies [18]. In this paper, we will discuss the latest understanding in the molecular and cellular pathogenesis of NPHP and suggest an appropriate management plan/screening programme for individuals and their families, particularly in view of the considerable clinical heterogeneity.

2. Molecular and Genetic Pathogenesis

NPHP is a recessive monogenic disorder [19], meaning that two mutations (homozygous or compound heterozygous) in a single gene are sufficient to cause disease [20]. Thirteen genes have been identified in affected families with NPHP (Table 1), and these genes currently allow 30% of patients with NPHP to be "solved" in terms of a molecular diagnosis. These genes have been identified using positional cloning strategies and homozygosity mapping in consanguineous families [20]. Subsequent localisation of all these encoded proteins, termed "nephrocystins," to the primary cilium/basal body led to recognition of NPHP as a ciliopathy [17]. Primary cilia are highly conserved, microtubule-based hair-like structures which extend from the apical surface of almost every epithelial cell. They function in order to detect extracellular cues and mediate cellular signalling pathways (discussed below) [13]. Ciliary genes are currently recognised as attractive candidates to evaluate when attempting to define the molecular cause of NPHP in the presently undiagnosed 70% of patients. With this in mind, combined homozygosity mapping, ciliopathy candidate exome capture, and parallel sequencing have recently been performed resulting in the successful identification of pathological mutations in the gene, serologically defined colon cancer antigen 8 (*SDCCAG8*), in families with NPHP [21]. *SDCCAG8* is synonymous with *NPHP10*. Also recently, the targeted screen of the ciliary gene *TTC21B* revealed that its protein product, IFT139, is essential for retrograde intraflagellar transport. IFT139 interacts with ciliopathy proteins BBS4 and BBS8, and pathogenic mutations in *TTC21B* were identified in patients with NPHP and more severe related ciliopathies [22].

Although the majority of currently known NPHP genes produce proteins which localise to primary cilia/basal bodies/centrosomes, recent identification of an NPHP-like locus in two affected families suggests that NPHP genes may not be exclusively ciliary [35]. Genome-wide homozygosity mapping identified pathogenic mutations in X-prolyl aminopeptidase 3 (*XPNPEP3*), or *NPHPL1* (NPHP like 1 gene), of which the protein product localises to mitochondria [35]. Although not currently identified in the primary cilium, XPNPEP3 may influence cilia function through enzymatic cleavage of associated ciliary proteins [35].

Whilst homozygous mutations in the *NPHP* genes can cause isolated NPHP, mutations in the same gene can be pleiotropic inducing a spectrum and variable severity of phenotypes. Similarly, it would appear logical that the type of mutation may influence the phenotype. For example, a missense mutation may cause isolated NPHP or Senior-Loken syndrome (SLS, retinitis pigmentosa), whilst a truncating mutation could cause MKS. Recently the effect of different mutations in *NPHP6* on clinical presentation has been eloquently reviewed [36]. Furthermore, some *NPHP* genes are more likely to be associated with certain extrarenal features. The concept of "modifier genes" has been recognised in patients with the related ciliopathy, Bardet-Biedl syndrome (BBS), where pathogenic mutations in more than one gene have been detected, implicating a role for oligogenicity [37]. Oligogenicity or triallelism, whereby a mutation in a third allele may exert an epistatic effect and modify the phenotype, has been described in NPHP [38]. A brief description of each of the NPHP genes, their encoded nephrocystin proteins, and any interacting protein partners is given below.

2.1. NPHP1 and Nephrocystin-1. *NPHP1* was the first NPHP gene identified and accounts for the majority (20–25%) of known cases of isolated NPHP [23, 24]. Recently adults presenting with signs of NPHP and ERF in two generations of a Turkish family with no known consanguinity suggested a diagnosis of a dominant cystic kidney disease such as medullary cystic kidney disease (MCKD) or perhaps a novel variant of dominant NPHP [7]. However, identification of homozygous mutations in *NPHP1* in all affected family members revealed a pseudodominant inheritance of unknown cause as a consequence of unidentified consanguineous relationships. The increased age at presentation with ERF is atypical and is hypothesised to be a consequence of currently unknown modifier genes [7]. *NPHP1* mutations can also cause retinal and cerebellar phenotypes leading to SLS and JS. Oligogenicity has been reported in families with NPHP, where mutations in both *NPHP1* and *NPHP3*, *NPHP1* and *NPHP4* [38], and *NPHP1* and the ciliopathy gene *Abelson helper integration site-1* (*AHI1*, the most frequently mutated gene in JS [39]) have been detected [40]. Mutations in both *NPHP1* and *NPHP6* have been identified in patients with NPHP-related ciliopathies including SLS and JS [41]. This evidence of genetic interaction, known protein-protein interactions between various nephrocystins, combined with an awareness of other ciliary proteins such as BBSome functioning as a complex [13], makes it highly likely that several of the nephrocystins form a functional supramolecular complex within cells [17, 20]. Nephrocystin-1 localises at cell-cell contacts including tight junctions, adherens junctions, and focal adhesions [42, 43]. Nephrocystin-1 has also been identified at the transition zone/base of the primary cilium [44]. Nephrocystin-1 interacts with various other proteins important in maintaining the cellular scaffolding or cytoskeleton including jouberin [45], ack1 [46], filamin A and B, tensin (actin binding), β-tubulin (microtubule structure), and protein tyrosine kinase 2B (PTK2B) [20].

Table 1: Mutated genes in nephronophthisis and associated extrarenal manifestations.

Locus	Gene	Chromosome	Protein	Mutation frequency [20]	Extrarenal features	Ref.
NPHP1	*NPHP1*	2q13	Nephrocystin-1	23%	SLS, JS,	[23, 24]
NPHP2	*INV*	9q31	Inversin	1-2%	SLS, HF VSD, situs inversus	[25]
NPHP3	*NPHP3*	3q22.1	Nephrocystin-3	<1%	SLS, HF, MKS, situs inversus	[26]
NPHP4	*NPHP4*	1p36.22	Nephrocystin-4 or nephroretinin	2-3%	SLS	[27, 28]
NPHP5	*IQCB1*	3q21.1	Nephrocystin-5 or IQ motif containing B1	3-4%	SLS	[29]
NPHP6	*CEP290*	12q21.32	Centrosomal protein 290	1%	LCA, SLS, JS, MKS, BBS	[30]
NPHP7	*GLIS2*	16p13.3	GLI similar 2	<0.5%		[31]
NPHP8	*RPGRIP1L*	16q12.2	RPGRIP1-like	0.5%	SLS, JS, MKS	[32]
NPHP9	*NEK8*	17q11.1	NIMA-related kinase 8	<0.5%	SLS	[33]
NPHP10	*SDCCAG8*	1q44	Serologically defined colon cancer antigen 8	<0.5%	SLS, BBS-like	[21]
NPHP11	*TMEM67*	8q22.1	Transmembrane protein 67	<0.5%	JS, HF, MKS	[34]
NPHPL1	*XPNPEP3*	22q13	X-prolyl aminopeptidase 3	<0.5%	cardiomyopathy, seizures	[35]
	TTC21B	2q24.3	Intraflagellar transport protein 139	<1%	JS, MKS, BBS, JATD	[22]

BBS: Bardet-Biedl syndrome; HF: hepatic fibrosis; JATD: Jeune asphyxiating thoracic dystrophy; JS: Joubert syndrome; LCA: Leber's congenital amaurosis; MKS: Meckel-Gruber syndrome; SLS: Senior-Loken syndrome; VSD: ventricular septal defect.

2.2. NPHP2/INVS and Inversin. Mutations in *NPHP2* are distinct because they cause infantile NPHP, characterised by an earlier presentation of ERF (at approximately 3 years of age), with enlarged kidneys on ultrasound. Additional clinical features include cardiac anomalies (situs inversus and ventricular septal defects (VSD)) [47]. Although *NPHP2* mutations are a rare cause of NPHP compared to *NPHP1*, there has been intense research regarding the molecular/cellular pathogenesis of inversin. Inversin is located in the primary cilium and other subcellular sites in a cell-cycle dependent manner [48]. A previous work has suggested that inversin acts as a switch between canonical and non-canonical (planar cell polarity) Wnt signalling [49]. When inversin is lost after *NPHP2* mutation, it was proposed that sustained canonical Wnt signalling led to cell proliferation and random oriented cell division [49]. However, recent experiments in the *inv* mutant mouse model of NPHP showed no difference in canonical Wnt signalling compared to controls [50]. In addition to nephrocystin-1, inversin interacts with calmodulin, catenins, β-tubulin [25], and anaphase-promoting complex 2 [48, 51].

2.3. NPHP3 and Nephrocystin-3. Mutations in *NPHP3* are again a rare cause of isolated NPHP; however, they can cause a broad spectrum of phenotypes as shown in Table 1. Nephrocystin-3 colocalises with nephrocystin-1 [26] and inversin [52] in primary cilia, adherens junctions, and focal adhesions [20]. The *pcy* mouse model of NPHP displays cystic kidneys and responded to treatment with aquaretic agents/vasopressin-2-receptor antagonists [53].

2.4. NPHP4 and Nephrocystin-4/Nephroretinin. Individuals with mutations in *NPHP4* most frequently have an associated retinal phenotype [54]. Nephrocystin-4 colocalises and interacts with nephrocystins 1, 3 and inversin in primary cilia and associated appendages, adherens junctions, and focal adhesions [20, 27]. Nephrocystin-4 also interacts with nephrocystin-8 [55, 56], α-tubulin, breast cancer antioestrogen resistance 1 (BCAR1), PTK2B [20], and the tight junction proteins PALS1/PATJ/Crb3 which are required for epithelial morphogenesis [57].

2.5. NPHP5/IQCB1 and Nephrocystin-5. NPHP5 mutations are associated with early onset retinal degeneration, SLS [29]. Nephrocystin-5 contains two IQ calmodulin binding sites; the significance of its interaction with calmodulin is unclear. It colocalises with nephrocystin-1 and nehrocystin-4 in the primary cilium, adherens junctions, and focal adhesions [29] and interacts with nephrocystin-6 [30, 58]. Nephrocystin-5 also complexes with the retinal ciliopathy gene retinitis pigmentosa GTPase regulator (RPGR) [29], explaining the frequent retinal phenotype.

2.6. NPHP6/CEP290 and Nephrocystin-6. Mutations in *NPHP6* cause a full spectrum of extrarenal features with no

apparent genotype-phenotype correlation [36]. It is the commonest genetic cause (21%) of isolated congenital retinal degeneration, Leber's congenital amaurosis (LCA) [59]. It was suggested that oligogenicity and the effect of modifier genes may account for some of the pleiotropy. Oligogenicity has been described in patients with homozygous *NPHP6* mutations and an additional heterozygous mutation in: *NPHP4* resulting in NPHP [36] or SLS [54], *NPHP11* causing BBS or MKS [60], and *AHI1* causing JS [41]. Oligogenicity has also been identified in patients with SLS and JS as a consequence of a homozygous mutation in *NPHP1* and heterozygous mutation in *NPHP6* [41]. NPHP6 interacts with and modulates the transcription factor ATF4, involved in cAMP-dependent renal cyst formation [30]. In addition to nephrocystin-5 [58], another protein interaction partner of nephrocystin-6 is coiled-coil and C2 domain protein (CC2D2A) [61]. In zebrafish models of combined *NPHP6* and *CC2D2A* knockdown, there is synergy of the renal cystic phenotype, suggesting an epistatic, disease-modifying effect [61]. *CC2D2A* mutations cause JS and MKS [62]; however, they have not been identified in patients with isolated NPHP [15, 61].

2.7. NPHP7/GLIS2 and GLIS2. NPHP7 is a rare cause of isolated NPHP [31]; its protein product is a Kruppel-like zinc-finger transcription factor, Gli-similar protein 2, which localises to the primary cilium and nucleus [31]. Interestingly, a *Glis2* knockout mouse model showed an upregulation of genes promoting epithelial-to-mesenchymal transition and histological features of NPHP including fibrosis [31]. This correlation of nephrocystin-7 with GLI transcription factors links the pathogenesis of NPHP to the Hedgehog (Hh) signalling pathway, which is essential for controlling tissue maintenance [63].

2.8. NPHP8/RPGRIP1L and RPGRIP1L. NPHP8 mutations more frequently cause extrarenal manifestations such as cerebello-oculo-renal syndromes, JS [32, 56], and MKS [56] than isolated NPHP. There appears to be some genotype-phenotype correlation with missense mutations causing LCA [64], whilst truncating mutations cause the more severe disorder MKS [56]. RPGRIP1L colocalises with nephrocystin-4 and nephrocystin-6 at basal bodies and centrosomes [56]. RPGRIP1L interacts with nephrocystin-1 and nephrocystin-4 [20].

2.9. NPHP9/NEK8 and NEK8. NPHP9 mutations are a rare cause of both infantile and noninfantile NPHP [33]. Oligogenicity has been identified with a pathogenic homozygous mutation in *NPHP5* and heterozygous *NPHP9* mutation, which may behave as a modifying gene, in an individual with SLS [33]. In some patients with heterozygous *NPHP9* mutations, a second recessive mutation has not been identified. Its protein product, never in mitosis A-related kinase 8 (NEK8), colocalises with various nephrocystins in primary cilia, basal bodies, and centrosomes and appears to be important in regulating the cell cycle [20]. NEK8 has been shown to interact with polycystin-2 (autosomal dominant polycystic kidney disease (ADPKD) protein), to regulate its expression and phosphorylation [65]. NEK8 may thus function in a protein complex with polycystin 1 and 2.

2.10. NPHP10/SDCCAG8 and SDCCAG8. SDCCAG8 was recently identified as *NPHP10* by homozygosity mapping, ciliopathy candidate exome capture, and parallel sequencing [21]. Twelve mutations were identified in ten families with NPHP-related ciliopathies, in particular SLS and BBS. Homozygous *SDCCAG8* mutations account for 3.3% of cases of SLS. Its protein product, serologically defined colon cancer antigen 8 (SDCCAG8), colocalises at centrosomes and cell-cell junctions with nephrocystin-5. SDCCAG8 and nephrocystin-5 colocalise in the transition zone of photoreceptors which is likely of functional significance and correlates with the phenotype of SLS. SDCCAG8 also colocalises with the retinal ciliopathy proteins RPGRIP and RP1. SDCCAG8 interacts directly with the protein oral-facial-digital syndrome 1 (OFD1) [21], although the functional significance of this is currently not clear, it is clearly of interest, as recessive mutations in *OFD1* are an X-linked cause of the NPHP-related ciliopathy JS [66].

2.11. NPHP11/TMEM67/MKS3 and TMEM67. Mutations in *NPHP11* are pleiotropic, having been identified in patients with NPHP and liver fibrosis, extending to patients with related ciliopathies including JS, MKS [34], and COACH syndrome (cerebellar vermis hypoplasia, oligophrenia (developmental delay), ataxia, coloboma, and hypotonia) [67]. Whilst oligogenicitiy has not been described, a patient with JS and an isolated heterozygous *NPHP11* mutation has been identified [68], suggesting that triallelism and the role of *NPHP11* as a modifier gene is possible. Transmembrane protein 67 (TMEM67) or meckelin localises to the membrane of primary cilia and diffusely at basal and basolateral cell surfaces [69]. TMEM67 interacts with several proteins including nesprin 2 [70], MKS1 [69], and TMEM216 [71] which are important in maintaining cellular structure and mitigating centrosome migration, which is essential for ciliogenesis.

2.12. NPHPL1/XPNPEP3 and XPNPEP3. NPHP-like 1 gene (*NPHPL1*) was recently identified in two consanguineous families with NPHP by genome-wide homozygosity mapping [35]. Additional extrarenal manifestations include cardiomyopathy and seizures. This is a novel discovery because it is the first NPHP gene identified whose protein product, X-propyl aminopeptidase 3 (XPNPEP3), does not localise to primary cilia, basal bodies, or centrosomes [35]. Instead, XPNPEP3 localises in mitochondria; however, it has been hypothesised that this enzyme may be able to interfere with cilia function by cleaving certain cilial proteins [17, 35].

2.13. TTC21B and IFT139. Mutations in *TTC21B* have recently been identified in families with isolated NPHP and extrarenal manifestations including the ciliopathy, Jeune

asphyxiating thoracic dystrophy (JATD) [22]. Interestingly, both causal mutations (homozygous or compound heterozygous) and modifier mutations (heterozygous) in *TTC21B* were identified in affected individuals. Oligogenicity was identified between *TTC21B* and several other ciliopathy genes. With regard to NPHP, triallelism was identified in a Turkish family with mutations in both *TTC21B* and *NPHP4*. The protein product of *TTC21B* is a retrograde intraflagellar transport protein IFT139, found in the primary cilium, and is essential for ciliary function.

3. Oligogenicity and Modifier Genes

Oligogenicity has been described above for *NPHP1, NPHP5, NPHP6, NPHP8, NPHP9, NPHP11,* and *TTC21B*. It has been hypothesised that oligogenicity may help to account for the intrafamilial variation in age of onset of ERF and severity of clinical features [38]. Current evidence fails to consistently identify a correlation between genotype and phenotype [17], therefore mutation analysis is required to identify the molecular cause. Making a molecular diagnosis often involves expensive and time-consuming mutation analysis. However, the results may be important when managing patients, to guide appropriate screening for potentially associated complications of the retina, cerebellum, liver and lungs. Understanding the natural history of NPHP and associated complications will hopefully improve following completion of the current clinical trial ongoing in France, which is evaluating the evolution of NPHP and related extrarenal manifestations in children (over 7 years of age) with a confirmed diagnosis of NPHP1–8 (excluding NPHP7) (see http://clinicaltrials.gov/ct2/show/NCT01022957?term=nephronophthisis&rank=1). The results of this study may facilitate understanding in characterising genotype/phenotype associations. Although NPHP-related ciliopathies are heterogenic disorders, the true frequency of oligogenicity remains uncertain. It is however interesting that mutation analysis of 18 NPHP associated ciliopathy disease genes (including 12 *NPHP* genes, *SDCCAG8* was not included) in 120 patients with NPHP and related ciliopathies, using DNA pooling and next generation sequencing, recently failed to identify any evidence of oligogenicity [15]. Remarkably, in 75% of patients in this cohort, no mutations were detected in the known candidate NPHP genes [15].

Since the molecular cause of NPHP remains unidentified in 70–75% of cases, it is anticipated that additional NPHP genes will be discovered. Whilst genes involved in the structure and function of the primary cilium are logical candidates to consider, indeed this approach has resulted in the recent successful identification of *TTC21B* [22], it is interesting that "noncilial" causal genes such as *XPNPEP3* [35] are now being identified. Additionally, genes implicated in maintaining the cell cytoskeleton and cellular junctions are likely involved. Whilst identifying causal genes is fundamental to determine the pathogenesis of NPHP, it is the tip of the iceberg and we need to understand the functional consequences of such mutations within tissues. We will now discuss current hypotheses for the cellular pathophysiology of NPHP.

4. Cystic Kidney Disease as a Ciliopathy

In 2005, realisation that polycystin-1 and polycystin-2 (protein products of ADPKD genes *PC1* and *PC2*) and the discovered nephrocystins (NPHP1–5) were all expressed in the primary cilium, basal body, and centrosomes led to consideration of the term "ciliopathy" as a unifying theory for cystic kidney disease [18]. A few years earlier, the concept of a ciliopathy, a disorder in which abnormal structure or function of cilia/centrosomes is associated with defective proteins encoded by mutated genes, was attributed to the multisystem disorder BBS [72]. In the intervening years, the primary cilium [73] had been extensively studied. Primary cilia are composed of an axoneme containing nine microtubular doublets which extend by a process of intraflagellar transport (IFT) and mediate the trafficking of signals between the extra- and intracellular environments [73]. Cilia are considered to be involved in mechanosensation of urinary flow in the renal tubules [74].

Normally, in healthy kidneys, the renal cortical collecting duct (CCD) concentrates urine by responding to vasopressin, which binds to vasopressin-2 receptors (V_2R). V_2R are coupled to adenylyl cyclase resulting in increased intracellular cyclic AMP (cAMP), leading to phosphorylation of aquaporin-2 water channels (AQP2), which mediate water reabsorption [11]. In NPHP, inability to concentrate urine is the earliest clinical feature and is unresponsive to desmopressin therapy [10]. In animal models of NPHP, vasopressin levels are elevated and thought to contribute to cystogenesis by upregulating cell proliferation [75]. Identification of V_2R in the primary cilium of renal epithelial cells [76] is consistent with the theory of cystic kidney diseases as ciliopathies. This also emphasises the importance of the primary cilium in water reabsorption which has recently been eloquently investigated in patients and renal epithelial cell culture models of the related ciliopathy BBS [77]. Understanding the pathogenesis of this urine concentration defect should help explain the success of vasopressin receptor antagonists in animal models of NPHP, where they induced a reduction in cAMP levels and caused regression of cysts [53].

In NPHP, in response to urinary flow, cilia are considered to alter expression of inversin and potentially influence Wnt signalling pathways and planar cell polarity [17, 78]. Originally, observations in mouse models established the link between primary cilia and cystic kidney disease: the *orpk* mouse model of ARPKD with a *Tg737* mutation (disrupting the protein polaris) has impaired ciliogenesis and renal cysts [79].

The extrarenal manifestations of NPHP including retinal degeneration and SLS, usually associated with *NPHP5* and *NPHP6* mutations, can be explained by ciliary dysfunction. Retinal photoreceptors contain a connecting cilium through which rhodopsin traffics along, mediating photosensation [18]. Both nephrocystin-5 and -6 are expressed in the connecting cilia of retinal photoreceptors and disrupted structure or function of these proteins interferes with rhodopsin transport, leading to retinal degeneration (17, 20).

Through their involvement in various cellular signalling pathways including Hh, calcium, and Wnt, cilia mediate several fundamental processes including cell cycle, proliferation, differentiation, and polarity [73]. Proposed mechanisms for renal cystogenesis include abnormal cell proliferation, fluid secretion, and disorientated cell division leading to cystic expansion rather than longitudinal tubular growth [78]. However, aberrations in the signalling pathways linking cilia to cystogenesis remain incompletely understood, complicated by sometimes contradictory evidence. A recent helpfulreview of mechanisms of NPHP discusses each gene [17]. Here we provide an overview, concentrating on the Wnt signalling pathway.

5. Wnt Signalling and NPHP

Wnt signalling involves several ciliary proteins including PC1, PC2, inversin, nephrocystin-3, jouberin, BBS1, BBS4, BBS6, OFD1, and HNF1β, to regulate cell proliferation and differentiation [80]. It is composed of two pathways: canonical (β-catenin dependent) and noncanonical (or planar cell polarity (PCP)); the functional branch appears to be determined in part by inversin acting as a switch at the base of the primary cilium [49]. Interestingly, both over activation [49, 81] and underactivation [82] of the canonical Wnt pathway have been identified in animal models of NPHP and JS, suggesting that unbalanced canonical Wnt signalling is damaging and mediates cystogenesis [80]. However, this theory has recently been refuted by studies in the *inv* mouse model of NPHP, where no change in canonical Wnt signalling was identified between cystic *inv* mutants and controls [50].

PCP describes the normal intrinsic organisation of cells in a tissue plane perpendicular to their apicobasal polarity [83]. PCP regulates kidney tubule development or recovery from injury by convergent extension and orientated cell division [78, 84], meaning that cells are spatially orientated and divide along an axis to maintain a constant tubule diameter whilst elongating [78, 85]. Several mouse models of cystic kidney disease provide evidence of aberrant PCP signalling [83, 86]. An excellent, comprehensive review of the role of Wnt signalling in cystic kidney disease has been published recently [80].

The other signalling pathway with particular relevance to understanding the pathophysiology of NPHP is the Hh pathway. An association with NPHP was realised when Hh effector proteins, GLI transcription factors, were noted to be related to GLIS2, the protein product of *NPHP7*. Loss of GLIS2 promotes epithelial to mesenchymal translation (EMT), fibrosis and apoptosis, and histological hallmarks of NPHP [31]. Thus although *NPHP7* is a rare cause of NPHP, its discovery has contributed important understanding to the signalling pathways involved.

6. Clinical Management of NPHP

6.1. Diagnosis and Investigations. In order to establish a clinical diagnosis of NPHP, a detailed history, clinical examination, including looking for extrarenal associations (abnormal eye movements, retinopathy, ataxia, polydactyly, and cardiac malformations) is required. A detailed family history must be taken both to facilitate diagnosis and to highlight other individuals who should be invited for review. Appropriate investigations include renal and liver function tests; urine concentration ability, renal and hepatic ultrasound, cerebral imaging if clinically indicated, and referral to an ophthalmologist (Figure 1) [16]. After genetic counselling, blood may be sent for genetic testing (Table 2) to genetic testing organisations (e.g., http://www.ukgtn.nhs.uk/ or http://www.eurogentest.org/) to seek a molecular diagnosis. Regular review is required to appropriately manage CKD/ERF, and individuals with extrarenal manifestations should be referred to appropriate colleagues and are ideally best managed in specialist clinics.

Table 2: NPHP genes available for testing via UK and European gene testing networks.

Gene	Laboratory
NPHP1	NE Thames, London
NPHP1	Glasgow, Scotland
NPHP1	Utrecht, Netherlands
NPHP1	Helsinki and Tampere, Finland
NPHP1	Malaga, Spain
NPHP1	Granada, Spain
NPHP1,2	Gosselies, Belgium
NPHP1,2	Brussels, Belgium
NPHP1–3	Aachen, Germany
NPHP1–4	Paris, France
NPHP1–4	Weißwasser, Germany
NPHP1–4	Tubingen, Germany
NPHP1–4, 7	Rostock, Germany
NPHP1–4, –9, NPHPL1	Barcelona, Spain
NPHP1–4, –9, NPHPL1	Oviedo, Spain
NPHP1–4, –9, NPHPL1	Leuven, Belgium
NPHP1–4, 6–9	Ingelheim, Germany

6.2. Treatment. Presently there is no cure for NPHP and related ciliopathies. Clinicians must focus on optimising the delivery of renal replacement therapy, ideally with renal transplantation where possible. However with a growing understanding of the pathophysiology of NPHP, the future is more hopeful. In recent years various drugs including vasopressin receptor antagonists [53], mTOR inhibitors (mammalian target of rapamycin) [87], triptolide [88] and roscovitine (cyclin-dependent kinase inhibitor) [89] have been shown to be effective in reducing renal cysts in animal models of NPHP and ADPKD. Many of these drugs are currently or have recently been involved in clinical trials in adult patients. Furthermore, large numbers of compounds which could be potential therapies are being screened in zebrafish [90] models of ciliopathies.

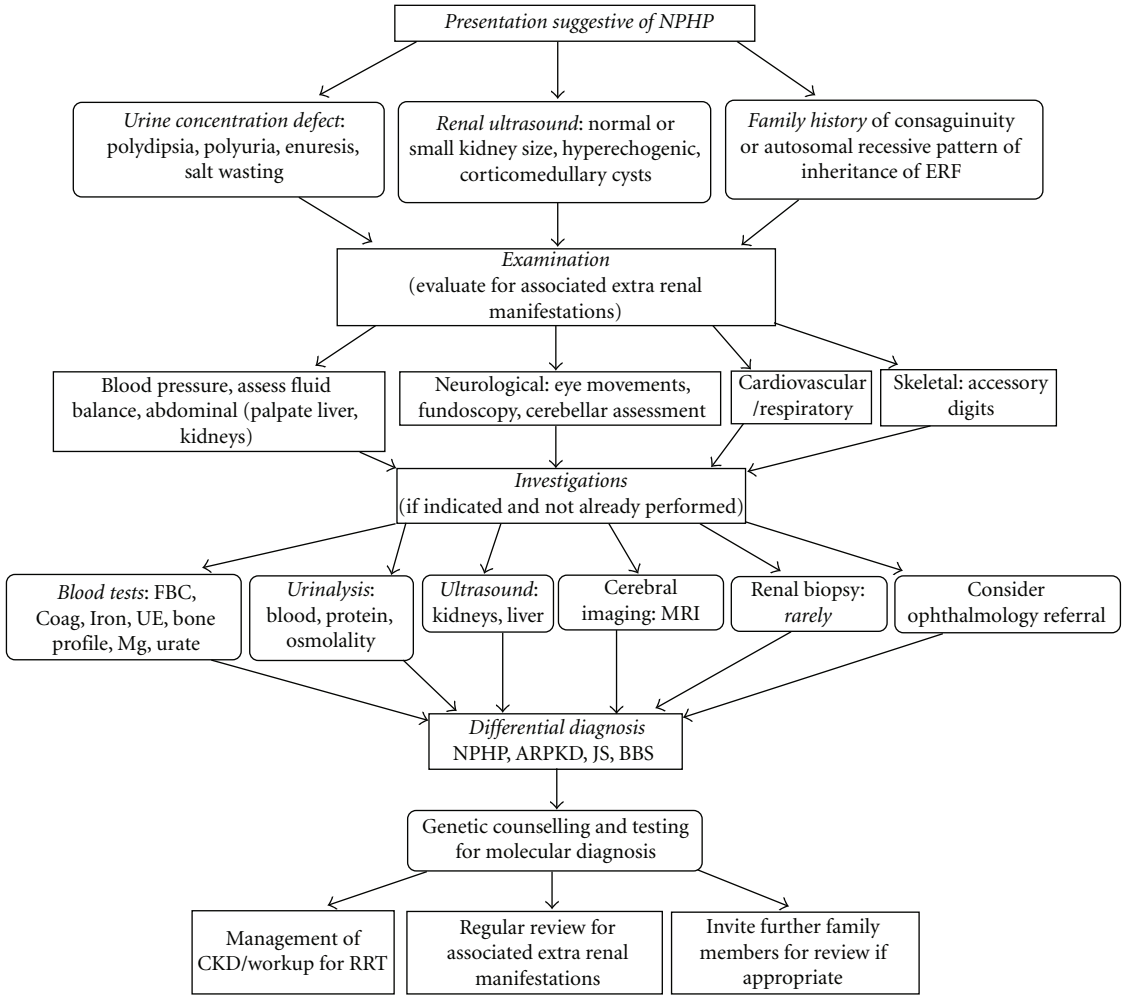

Figure 1: Diagnostic algorithm for suspected cases of NPHP. ARPKD: autosomal recessive polycystic kidney disease; BBS: Bardet-Biedl syndrome; CKD: chronic kidney disease; Coag: coagulation; ERF: established renal failure; FBC: full blood count; JS: Joubert syndrome; LFT: liver function tests; Mg: magnesium; MRI: magnetic resonance imaging; NPHP: nephronophthisis; RRT: renal replacement therapy; UE: urea and electrolytes.

7. Conclusion

Understanding of the molecular genetics of NPHP has advanced considerably over the last few years. We are increasingly aware of the heterogeneity, the pleiotropic nature of mutations, and oligogenicity, all of which contribute to the complexity of NPHP. Identification of further NPHP genes, many of which may be "ciliary," is warranted to provide further clues to its pathogenesis. This will require international multidisciplinary collaboration to sequence patient cohorts with no current molecular diagnosis and to screen candidate genes in animal and cell models. Acknowledgement that the genetic cause is unknown in 70% of NPHP cases, combined with awareness that a mitochondrial gene, *XPNPEP3*, has recently been identified to cause an NPHP-like phenotype, may encourage us to consider other nonciliary candidates, such as genes involved in cell-cell contacts and the cytoskeleton. Ultimately understanding the physiological outcomes at a protein and cellular level should facilitate identifying and developing novel therapeutic targets for affected patients.

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