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REVIEW

Hereditary nephrotic syndrome: a systematic approach for genetic testing and a review of associated podocyte gene mutations

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Abstract Several genes have been implicated in genetic forms of nephrotic syndrome occurring in children. It is now known that the phenotypes associated with mutations in these genes display significant variability, rendering genetic testing and counselling a more complex task. This review will focus on the recent clinical findings associated with those genes known to be involved in isolated steroid-resistant nephrotic syndrome in children and, thereby, propose an approach for appropriate mutational screening. The recurrence of proteinuria after transplantation in patients with hereditary forms of nephrotic syndrome will also be discussed.

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Introduction

The annual incidence of idiopathic nephrotic syndrome (NS) in children in the USA and in Europe has been estimated to be 1–3 per 100,000 children, with a cumulative prevalence of 16 per 100,000 children [1, 2]. About 90% of patients are steroid responsive [3], with a favorable long-term prognosis. The remaining 10% of children who do not respond to corticosteroids are particularly at risk for extrarenal complications of NS and the development of end-stage kidney disease (ESKD), which occurs in 30–40% of children with steroid-resistant NS (SRNS) after a follow-up of 10 years [4–6]. It has been suggested that steroid-sensitive NS, as well as a subset of SRNS, particularly those with a response to immunosuppressive agents and/or recurrence of proteinuria after kidney transplantation, have an underlying immune defect that may imply an unrecognized proteinuric circulating factor whose production seems to follow T cell dysfunction [7]. Some authors have found that the serum of patients with focal segmental glomerulosclerosis (FSGS) increases glomerular permeability to albumin when incubated with rat glomeruli in vitro [8, 9]. In contrast to these immune forms of NS, recent studies have shown that inherited structural defects of the glomerular filtration barrier are responsible of a large proportion of SRNS cases, thereby emphasizing the crucial role of the podocyte in the pathogenesis of glomerulopathies. Indeed, mutations in genes highly expressed in podocytes have been found in two thirds of patients presenting with SRNS in the first year of life [10]. To date, mutations in seven genes (*NPHS1*, *NPHS2*, *CD2AP*, *PLCE1*, *ACTN4*, *TRPC6*

and *INF2*) [11–17] have been implicated in different forms of nonsyndromic SRNS. Nephrin, podocin and CD2AP, encoded by *NPHS1*, *NPHS2* and *CD2AP*, respectively, are the main structural elements of the slit diaphragm [18]. Nephrin, a transmembrane protein of the immunoglobulin superfamily, interacts through its C-terminal part with podocin, a harpin-like scaffolding protein. Nephrin also interacts with CD2AP, an adapter protein found on the surface of T-cells and natural killer cells. PLCε1, encoded by *PLCE1*, is a phospholipase that catalyses the hydrolysis of membrane phospholipids to generate the second messenger molecules inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG), thereby initiating intracellular pathways of cell growth and differentiation [19]. Additionally, PLCε1 interacts with IQGAP-1 [16], a podocyte cell junction-associated protein and interacting partner of nephrin implicated in cell adhesion [20]. *TRPC6* encodes the calcium channel TRPC6, which is localized in the membrane lipid supercomplex together with podocin and regulates mechanosensation sensed at the slit-diaphragm, while *ACTN4* and *INF2*, encoding α-actinin-4 and a member of the formin family of actin-regulating proteins, respectively, are both involved in cytoskeletal dynamics.

Syndromic forms of SRNS, which are far less frequent, may be due to mutations in genes coding transcriptional factors (*WT1*, *LMX1B*) [21, 22], glomerular basement membrane components (*LAMB2*, *ITGB4*) [23, 24], lysosomal (*SCARB2*) [25] and mitochondrial (*COQ2*, *PDSS2*, *MTTL1*) [26–30] proteins or a DNA-nucleosome restructuring mediator (*SMARCAL1*) [31]. However, mutations in the *WT1* gene, encoding the Wilms tumour 1 protein and typically leading to Denys–Drash syndrome (male pseudohermaphroditism, progressive glomerulopathy and Wilms tumour) or Frasier syndrome (male pseudohermaphroditism, progressive glomerulopathy and gonadoblastoma), can also cause isolated SRNS. In addition, mutations in *LAMB2*, encoding laminin-β2 and implicated in Pierson syndrome (a rare autosomal recessive disorder characterized by microcoria and other complex ocular abnormalities in association with NS of congenital onset), have been found in one family with isolated congenital NS (CNS).

MYH9, a podocyte-expressed gene encoding nonmuscle myosin IIA, has been identified as the disease-causing gene of the rare giant-platelet disorders, which may comprise Alport-like syndrome manifestations [32]. However, the particular interest of *MYH9* resides in its role as a susceptibility gene in the development of kidney diseases. Indeed, it has recently been shown that common *MYH9* genetic variants, although not directly pathogenic, confer a greater risk of FSGS and ESKD in African Americans [33–35].

Mutations in *NPHS1* are responsible for most of cases of CNS [11], while mutations in *NPHS2* are responsible for most of the early-onset SRNS cases [12]. Recent findings, however, including the identification of *NPHS1* mutations in childhood-onset SRNS [36] or the implication of the *NPHS2* p.R229Q variant in adult-onset NS [37], have broadened the spectrum associated with mutations in these genes. With the rapidly increasing number of genes known to be implicated in NS and the significant phenotypic variability observed, genetic testing is now a more complex task which needs to be based on different clinical information, including the type of renal histological lesions. Indeed, the approach taken during genetic testing will be different depending on the identification of diffuse mesangial sclerosis (DMS), a particularly severe renal lesion characterized by mesangial expansion and sclerosis that evolves toward obliteration of the capillary lumen and contraction of the glomerular tuft, or FSGS, a lesion characterized by sclerosis and foot process effacements in only some of the glomeruli and a part of each entire glomerulus.

This review will focus on the recent clinical findings associated with those genes known to be involved in isolated SRNS and, thereby, propose an approach for appropriate mutational screening. It has to be stressed that corticotherapy was not attempted in a significant number of patients with very early-onset NS and in patients already in ESKD at the time of the first medical evaluation, as the probability that they would have been refractory to therapy was high. Nevertheless, in order to enhance the clarity of our review, these patients are also classified as SRNS cases in this paper. Finally, the controversial issues concerning the recurrence of proteinuria post-transplantation in patients with hereditary forms of NS will be discussed.

Nonsyndromic steroid-resistant nephrotic syndrome

Congenital nephrotic syndrome

Congenital nephrotic syndrome has been arbitrarily defined as the occurrence of NS in patients <3 months of age, although congenital actually means that the disease is present from birth. The most common type of CNS is congenital nephrotic syndrome of the Finnish type (CNF), a recessively inherited disorder characterized by massive proteinuria detectable at birth, a large placenta, marked edema and characteristic radial dilatations of the proximal tubules. These histological lesions are detected more frequently after 3 months of age, but they have also been identified in fetuses [38–41]. They are also considered to be an inconstant feature. Mesangial expansion and capillary obliteration are also evident in a significant proportion of CNF cases [42, 43]. It has been shown that hypoperfusion of glomerular and tubulointerstitial capillaries and rarefaction of the latter may explain the rapid development of

fibrosis in these patients [41]. The incidence of CNF in Finland has been estimated to be 1 in 8,200 live births [44], and a high incidence has also been reported among the Old Order Mennonites in Lancaster, Pennsylvania [45]. *NPHS1* has been identified as the major gene involved in CNF [11] with the Fin-major (p.L41fsX91) and Fin-minor (p.R1109X) mutations accounting for 78 and 16% of the mutated alleles among Finnish patients, respectively [11]. The Fin-major and Fin-minor mutations have rarely been found in other ethnic groups. Among non-Finnish cases with a CNF phenotype, the *NPHS1* mutation detection rate approaches 66% [46]. To date, more than 140 different *NPHS1* mutations have been identified, comprising nonsense, missense, frameshift insertion/deletion and splice-site mutations.

It has now become clear that not all *NPHS1* mutations cause severe CNS or a severe clinical course. In one study involving patients bearing the p.R1160X *NPHS1* mutation in the homozygous state (resulting in a truncated protein lacking the C-terminal 82 amino acids implicated in the interaction with podocin), about half of the patients had a milder phenotype, in that although they presented with severe NS in the first 3 months of life, their further renal course was relatively benign with spontaneous partial or complete remission in childhood [47]. Histological findings, where available, were consistent with CNF. The clinical variability was apparently influenced by gender, as the majority of the mildly affected cases were female.

Although *NPHS1* is the main gene that has been identified in patients presenting NS in the first 3 months of life, it has also been shown that CNS may be caused by mutations in several other genes, including *NPHS2*. Mutations in this latter gene have been detected in patients with a classically severe CNF phenotype [47] and have been shown to account for up to 51% of all mutations in Central European patients with CNS [10]. It has to be pointed out that the median age at onset of CNS in the latter study was relatively late (median age 4 weeks), which may induce a bias toward a greater prevalence of *NPHS2* mutations instead of *NPHS1* mutations. Indeed, in our large worldwide cohort of CNS patients, which mostly presented proteinuria in the first few days of life, *NPHS1* mutations were detected in more than half of all cases (unpublished data). Mutations in the *PLCE1* gene [16, 48–50], and the *WT1* gene [48, 51–55] have also been detected in patients presenting isolated CNS with DMS on renal histology; mutations in these two genes will be discussed in more detail in the next section.

These observations suggest that, for patients presenting nonsyndromic CNS, the *NPHS1* gene should be tested first in those presenting NS shortly after birth as well as in those with typical proximal tubular radial dilatation. Molecular analysis of *NPHS2* should be the next step whenever mutations in *NPHS1* are not detected. Patients presenting later in the congenital period (particularly if renal biopsy shows FSGS or minimal glomerular changes) should probably be initially screened for *NPHS2* mutations, followed by *NPHS1*. In cases for which renal histological findings are available and DMS is determined, genetic testing of the *WT1* and *PLCE1* genes should initially be performed.

Infantile nephrotic syndrome and childhood nephrotic syndrome

The term infantile NS has been proposed for patients that develop NS between the ages of 4 and 12 months. *NPHS2* mutations are responsible for most of these cases [12], and they have also been found in a significant proportion of patients with childhood-onset SRNS. Mutations in this gene occur in about 40% of familial and 6–17% of sporadic SRNS cases (Table 1) [55–59]; patients typically present NS from birth to 6 years of age and reach ESKD before the end of their first decade of life [10, 55–57, 59, 60]. Renal histology of such patients reveals either minimal glomerular changes (if biopsied early) or FSGS. The identification of mutations is important as it may enable the treating physicians to avoid (or discontinue) prescribing immunosuppressive therapies for these patients, sparing them the significant side-effects associated with these drugs. Indeed, considering that access to genetic testing is now easier than it has been in the past and that sequencing of the *NPHS2* gene is relatively fast as it comprises only eight exons, screening for *NPHS2* mutations in patients presenting the renal phenotype described above should be performed prior to the initiation of additional—potentially deleterious—therapy. To date, more than 100 pathogenic *NPHS2* mutations and 25 variants of unknown significance have been reported, including a full spectrum of protein-truncating nonsense and frameshift mutations, splice-site variants and missense changes, involving all coding exons. Patients with frameshift, nonsense or the homozygous p.R138Q missense mutations manifest symptoms at a significantly earlier age [57, 59]. The p.R138Q mutant, which accounts for up to 32% of all mutant alleles, is retained in the endoplasmic reticulum (ER), where it essentially functions as a null allele and fails to recruit nephrin to lipid rafts [61, 62]; this may explain the phenotype severity associated with this mutation. In contrast, the p.R229Q variant has been shown to lead to late-onset NS [37, 63], when found in association with one pathogenic *NPHS2* mutation. This variant represents the most frequently reported nonsynonymous *NPHS2* variant in Caucasians and is particularly common among Europeans, in whom the observed frequency of heterozygotes ranges from 0.03 to 0.13 [55, 59, 63–66]. In vitro studies have

Table 1 Rate of detection of two pathogenic *NPHS2* mutations in SRNS cases

References	Sporadic cases (*n*)	Percentage	Familial cases (*n*)	Percentage	Age at onset (years)	Age at ESKD (years)
Caridi et al. [56]	14/120	11.7	–	–	2.3	9.2
Weber et al. [59]	11/172	6.4	31/81[c]	38.3	3.4	–
Berdeli et al. [60]	41/254	16.1	9/32	28.1	3.8	9.4
Hinkes et al. [57][a]	64/381[b]	16.8	9/23	39.1	2.6	–

ESKD, End-stage kidney disease

[a] Subgroups of patients in this study had been included in the analysis by Ruf et al. (2004) [55] and Hinkes et al. (2007) [10]

[b] Represents families with only one affected member

[c] Defined as families with either two or more affected children, or one (or more) affected individual in consanguineous families

demonstrated decreased binding of the p.R229Q mutant protein to nephrin, providing a likely explanation for its pathogenic role [63].

It has recently been shown that mutations in *NPHS1* also account for a nonnegligible proportion of infantile and childhood-onset SRNS cases. Two studies found *NPHS1* mutations in 7–14% of the patients presenting SRNS at least 3 months after birth [age at onset of NS in mutated patients 0.5–8 years (mean 3 years) and 0.7–27 years (mean 8 years), respectively] [36, 67] with minimal glomerular changes, FSGS or mesangioproliferative lesions on renal biopsies. These percentages are, however, overestimated as these studies included patients for which mutations in the *NPHS2* gene were excluded. The presence of at least one "mild" mutation likely explains the later onset and milder course of the disease among these cases. As such, missense mutants retaining their abilities to traffic in the cell properly, a splice-site mutation allowing some correct splicing and a protein-truncating mutation involving only the very C-terminal end of the protein may be designated as "mild" because partial function of nephrin is maintained. Similarly, NS with spontaneous partial remissions and repeated relapses concurrently with respiratory infections has been described in two siblings bearing nephrin mutations [68]. Both infants, who presented NS at birth and at 10 months of age, respectively, were compound heterozygous for the p.C265R and p.V822M mutations with minimal glomerular changes observed on the renal biopsy. The p.C625R mutant protein was predominantly trapped within the ER, while the p.V822M variant protein reached the plasma membrane, probably explaining the milder phenotype seen in these patients. Modifier genes or environmental factors may also play a role in renal phenotype variability, as the same two *NPHS1* mutations (p.R827X and p.R976S) have been identified in one adult patient diagnosed with FSGS at 27 years of age with unimpaired renal function after 2 years of follow-up [67] and in one patient with infantile-onset NS [36].

In addition to *NPHS2* and *NPHS1*, *PLCE1* is involved in some infantile and childhood-onset SRNS cases and is the main gene causing DMS. *PLCE1* mutations have been detected in 28.6% of families with isolated DMS [48], with the clinical onset of reported cases of DMS varying from few days of life to 4 years of age [16, 48–50] and all patients having truncating mutations. The results of one study suggested that homozygous *PLCE1* missense mutation (found in only two siblings) may lead to a milder phenotype of FSGS with a relatively late age at onset of proteinuria (in this study, 8.8 years and 2.0 years, respectively). However, in our cohort, truncating or missense mutations were detected in both DMS and FSGS patients, leading to a similar renal evolution (in press). Nevertheless, *PLCE1* mutations remain an infrequent cause of FSGS: a Dutch study did not find *PLCE1* mutations in 19 cases of childhood-onset FSGS [69] nor were mutations in this gene found in 69 families (median age of disease onset 26 years, range 1–66 years) with idiopathic or hereditary FSGS [70].

WT1 mutations may account for about 9% of patients with nonfamilial isolated SRNS [54], and they have been identified in patients with isolated DMS, with a clinical onset varying from a few days of life up to 2 years of age, as well as in isolated FSGS (1–14 years of age) [48, 51, 54, 71–74]. Almost all cases were those of phenotypically female patients, and the mutations occurred mainly in exons 8 and 9, which code for zinc finger domains 2 and 3, respectively [54].

Based on these observations, *NPHS2* followed by *NPHS1* remain 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). However, the probability of identifying a *PLCE1* mutation in this patient category is low, and the molecular analysis of this large 33-exon gene is expensive and time-consuming; thus, these factors should be taken into account in the decision on genetic screening. In cases of isolated DMS, *PLCE1* is the most frequently involved gene

but, for cost-effective reasons, genetic testing of exons 8 and 9 of *WT1* (especially in phenotypically female patients) may be performed prior to *PLCE1*.

Mutations in the *CD2AP*, *ACTN4* and *TRPC6* genes have been anecdotically reported in young patients, precluding any suggestion of systematic mutational screening of these genes in children—unless there is an autosomal dominant familial history of FSGS, which would warrant the molecular analysis of the two latter genes. To date, the clear pathogenic implication of *CD2AP* in NS has been shown in only one study: a homozygous mutation (p.R612X) was identified in a 10-month-old nephrotic child who presented global glomerular sclerosis on renal biopsy; the truncated protein displayed a dramatic reduction of actin binding efficiency in vitro [75]. Heterozygous expression of the *CD2AP* mutation in both parents did not lead to any kidney pathology. Nevertheless, the insignificant role of recessive *CD2AP* mutations has been emphasized in a recent study that did not find homozygous *CD2AP* mutations in a cohort of 42 children (35 families) with SRNS for whom the *NPHS1*, *NPHS2*, *PLCE1* and *WT1* mutations had been previously excluded [76]. On the other hand, carrying a heterozygous mutation in the *CD2AP* gene has been reported to be a predisposing factor towards developing FSGS. To date, five different *CD2AP* heterozygous mutations have been identified in pediatric (and adult) FSGS patients [14, 69, 77], associated with reduced expression of *CD2AP* or defective CD2–CD2AP interaction in lymphocytes and down-regulation of CD2AP, nephrin and podocin glomerular expression on kidney biopsies. Unfortunately, as complete segregation data are not available in all of these cases, neither penetrance nor inheritance can be assessed with certitude. In addition, heterozygous *CD2AP* mutations have been found in clinically unaffected patients. Therefore, there is still some doubt surrounding the direct causal link between heterozygous *CD2AP* mutations and FSGS; it is possible that CD2AP haploinsufficiency has a role in the susceptibility to develop glomerular disorders instead [14]. Mutations in *ACTN4* and *TRPC6* have been implicated in the rare autosomal dominant FSGS and account for approximately 4 and 6% of familial FSGS, respectively [78, 79]. Patients with *ACTN4* mutations usually present proteinuria in their teenage years or later, with a slow progression to ESKD in their fifth decade of life [13, 80]. Similarly, disease onset in patients with *TRPC6* mutations has initially been reported to vary between 17 and 57 years [15, 81]. However, de novo *ACTN4* mutations have been reported in three children (two families) from 3 to 5 years of age who presented rapid progression to chronic kidney disease/ESKD [78, 82]. We have detected a de novo *TRPC6* missense mutation in one patient that presented NS secondary to FSGS at 6.5 years of age and reached ESKD few months later (unpublished data), while *TRPC6* missense mutations have also recently been identified in two FSGS patients with disease onset at 7 and 9 years of age, respectively; the first patient showed a partial response to cyclosporine A and mycophenolate mofetil (MMF), and the mutation was also found in her asymptomatic 40-year old father [79, 83]. Therefore, similarly to *CD2AP* heterozygous mutations, *TRPC6* may contribute to glomerular disease in a multi-hit setting. Finally, mutations in *INF2* have also been found very recently in 11 families presenting moderate proteinuria and FSGS lesions in early adolescence or adulthood, thereby explaining about 12% of familial FSGS cases with an apparent autosomal dominant inheritance [17]. If the significant role of *INF2* as a genetic cause of NS is confirmed by further studies, screening for mutations in this gene will also need to be recommended in the near future in patients with an autosomal dominant familial history of FSGS.

A summary of the mutational screening approach suggested in patients with nonsyndromic SRNS is given in Fig. 1.

Genetic counselling

Genetic counselling should be offered to any patient and his/her family affected with an inherited disorder. Information that needs to be shared will vary according to the mutations found. Therefore, clinicians should be aware of the specific aspects that should be discussed with patients, particularly in cases involving mutations in the *NPHS2*, *WT1* and *PLCE1* genes.

In SRNS patients with *NPHS2* mutations who are considering having children, genetic testing for the p.R229Q variant, which is found in a high frequency in some populations, should be proposed to their asymptomatic spouses. Indeed, if the p.R229Q variant is identified in the spouse, there is up to a 50% risk of disease (a juvenile or adult-onset form of SRNS) transmission to their progeny. In addition, heterozygous carriers, such as patient siblings, also have a non-negligible risk of disease transmission to their children if the spouse carries the p.R229Q variant.

Special considerations also apply for patients bearing mutations in the *WT1* gene. Although most of the *WT1* mutations are de novo, XX female patients with isolated DMS/FSGS secondary to the *WT1* mutation have normal genital development and may become pregnant; therefore, these patients have a 50% risk of transmitting the mutated gene to their children. The phenotype of the progeny will depend on its karyotype; a 46 XY child may develop complete Denys–Drash syndrome or Frasier syndrome, while a 46 XX child will not present ambiguous genitalia. A case report well illustrates the clinical implications of familial *WT1* mutation transmission: the index case's

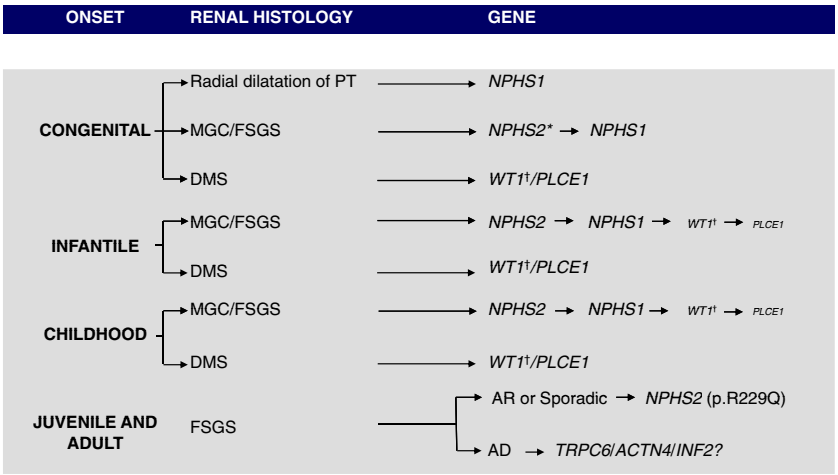

Fig. 1 Genetic approach in children with isolated steroid-resistant nephrotic syndrome. *Asterisk* In patients with minimal glomerular change/focal segmental glomerulosclerosis (*MGC*/*FSGS*) who present in the congenital period, those with nephrotic syndrome (NS) onset very shortly after birth should probably be screened first for *NPHS1* mutations followed by *NPHS2* mutations. *Cross* Only nonsyndromic forms of NS associated with *WT1* mutations are included. Most of the *WT1* mutations in patients with isolated steroid-resistant NS (SRNS) have been found in phenotypically female patients. *PT*, proximal tubules, *DMS* diffuse mesangial sclerosis, *AR* autosomal recessive, *AD* autosomal dominant

mother had typical FSGS (clinical onset at 6 years of age) with normal external and internal genitalia, while the index case presented with early DMS and XY pseudohermaphroditism [73]. Therefore, a female bearing a *WT1* mutation and planning to be pregnant should be informed that her children may develop a phenotype significantly different (and more severe) from her phenotype.

Variability in the renal phenotype should also be discussed with families in which *PLCE1* mutations are present. Indeed, it has been suggested that *PLCE1* mutations are not always sufficient to cause DMS [49] as a same homozygous truncating mutation has been found in an asymptomatic father and his affected children with DMS. We have also identified three asymptomatic adults from three unrelated families bearing homozygous mutations with at least one affected sibling haploidentical to the unaffected cases (in press). We may speculate that modifier genes or environmental factors play a role in the renal phenotype variability observed in individuals bearing *PLCE1* mutations. These observations need to be considered in the ensuing discussion with the patients on renal prognosis.

Finally, prenatal diagnosis should be offered to families with a known risk for severe NS, such as CNS, and to patients for whom elevated measurements of alpha-fetoprotein (AFP) in the maternal serum and amniotic fluid have been detected. In the first scenario, it has to be stressed that although results can be obtained relatively quickly when the causative mutations have previously been identified in the family, prenatal genetic testing may be more time-consuming in other cases. This highlights the importance of referring these families to a geneticist—ideally before conception or, if not possible, very early during pregnancy. An early genetic consultation may not only provide the background for a discussion of the risk of disease transmission and ethical questions related to medical termination of pregnancy (MTP), it may also allow the appropriate diagnostic procedures (trophoblastic biopsy or amniocentesis) to be planned within an adequate timeframe. In the second scenario involving families in which elevated AFP have been unexpectedly found, genetic counselling is also required as this finding may suggest CNS secondary to nephrin mutations (assuming that anomalies such as fetal anencephaly or omphalocoele have been ruled out). However, this diagnosis needs to be confirmed by mutation analysis prior to considering MTP as AFP elevations have been observed in *NPHS1* heterozygous fetal carriers and in a fetus with Denys–Drash syndrome [84, 85].

Recurrence of proteinuria after renal transplantation

Most patients with genetic forms of NS are resistant to immunosuppressive agents [10, 55, 86] and are therefore at high risk of ESKD [4–6]; renal transplantation may be the treatment of choice in these cases. In patients with SRNS requiring transplantation, the recurrence of NS in kidney grafts raises significant concerns as it is observed in approximately 30–50% of FSGS cases [87–89]. However, 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 [55, 59, 78, 90–95], 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. In a study of cases with *NPHS1* Fin-major/Fin-major mutations (mutations leading to the absence of nephrin in the native kidney), recurrence occurred in 25% of patients at a mean time of 12 months post-transplantation (range 5 days to 48 months), and antinephrin antibodies were detected in almost half of these cases [96]. Because recurrence in these patients may be considered as an immune process against the "neo-antigen" nephrin present in the graft, it is not surprising that treatment with steroids, cyclophosphamide and plasmapheresis may lead to remission; however, the percentage of graft loss remains significant [96, 97]. On the other hand, antipodocin antibodies have never been found [59, 90, 91]. As podocin is a harpin-like protein with two intracellular ends but no extracellular domain, one may speculate that antibodies against this "hidden" protein cannot be produced. This hypothesis may also explain the absence of post-transplantation recurrence in nephronophtisis or cystinosis, two diseases for which the involved proteins are only intracellular. In addition, renal transplantation should stimulate the production of antibodies only when mutations lead to the absence of the encoded protein (or at least a part of it) in the native kidney. Therefore, the development of antibodies cannot explain the recurrence of proteinuria in some patients with *NPHS2* missense mutations as well as in the reported cases of two children with either a missense (p.W54R) *ACTN4* mutation [78] or a splice-site (IVS9 + 4C>T) *WT1* mutation [95].

Data on the risk of proteinuria recurrence in patients with podocin mutations may be confusing as patients without true pathogenic mutations were included in some studies. In the reports of Bertelli et al., post-transplantation recurrence was stated to occur in five of 13 (38%) patients with homozygous (n=9) or heterozygous (n=4) podocin mutations compared to 15 of 40 (44%) non-*NPHS2* FSGS patients [91]. However, among the five patients described as having *NPHS2* mutations and recurrence, three had only heterozygous variants for which a pathogenic role is very unlikely (the Polyphen software program predicted the p.S221T variant would be benign, and the p.P20L variant is clearly a polymorphism [55]). When only patients with two pathogenic *NPHS2* mutations are taken into account, the risk of recurrence significantly decreases, thereby being more in agreement with the low recurrence rate of 3–8% reported in other studies [55, 59] (Table 2). Finally, it has also to be stressed that several etiologies, including drug side-effects, may explain the occurrence of proteinuria after transplanta-

Table 2 Recurrence of proteinuria on renal graft in patients with homozygous or compound heterozygous *NPHS2* mutations

References	Mutation	Recurrence, *n* (%)	Timing of recurrence PT	Biopsy-proven recurrence	Treatment	Outcome	Anti-podocin antibody	Permeability factor (P_{alb})
Bertelli et al. [91]	p.R138Q-p.R138Q	2/9 (22.2)	10 days	ND	PL + Cycl	Good	Negative	High[e]
	p.R138Q-p.R138Q		300 days	FSGS	PL + Cycl	Good	ND	High[e]
Ruf et al. [55]	p.L347X-p.L347X[a,b]	2/24 (8.3)	7 days	ND	Pred Pulse, ↑CsA	Good	ND	ND
	p.L347X-p.K126N		ND	ND	ND	ND	ND	ND
Weber et al. [59]	p.R138X-p.R138X[a]	1/32 (3.1)	2 years	FSGS	ND	↓GFR	Negative	ND
Höcker et al. [94]	p.R138Q-IVS 4-1 G>T[a]	Case report	10 years[c]	FSGS	Reswitch from sirolimus to CsA	Good	ND	Normal[f]
Becker-Cohen et al. [90]	p.R138X-p.R138X	Case report	4 years	FSGS	PL	↓GFR[d]	Negative	Normal

PT, Post-transplantation; PL, plasmapheresis; FSGS, focal segmental glomerulosclerosis; Cycl, cyclophosphamide; CsA, cyclosporine A; GFR, glomerular filtration rate; ND, not determined

[a] Living-related donor (mother)

[b] A part of the data related to this case was published in the article from Billing et al. [92]

[c] Temporal association with conversion from CsA to sirolimus

[d] No clinical improvement on PL. Proteinuria decreased significantly after PL was discontinued. Estimated GFR decreased from 80 ml/min/1.73 m^2 in the first years after transplantation to 45 ml/min/1.73 m^2

[e] High P_{alb} was noted for these patients in the article from Carraro et al. [93]

[f] The measurement took place after reintroduction of CsA

tion, even in patients with inherited SRNS. One possible example is the reported case of an 18-year-old female patient, compound heterozygous for *NPHS2* mutations, with recurrence 10 years post-transplant after conversion from cyclosporine A to sirolimus; however, one may also argue that proteinuria was induced by sirolimus and/or that the FSGS lesions documented on the graft biopsy were secondary to the long course of cyclosporine administration [94]. Considering these cases as "true" recurrence may lead to overestimation of the risk.

Altogether, the risk of recurrence in patients bearing two pathogenic *NPHS2* mutations is low. The absence of a clear explanation for post-transplantation recurrence in these cases raises interesting pathophysiological questions that should be addressed in further studies. Indeed, the determination of high serum glomerular permeability activity in two patients with homozygous *NPHS2* mutations at the time of recurrence episodes may suggest the additional potential role of a circulating permeability factor in some cases of hereditary SRNS [93]. Further studies on the impact of donor-dependant genetic factors on renal graft function are also warranted, as it has been suggested that variants in the donor *NPHS2* promoter may affect podocin expression, thereby determining the outcome of proteinuria [98, 99], and that potential donor-recipient *MYH9* genotype interactions may influence the occurrence of NS after pediatric kidney transplantation [100].

Finally, before considering living related donor transplantation in cases with inherited SRNS, particular issues should be discussed with the families. It is worth reporting that three patients (bearing the *NPHS2* p.L347X [55, 92], p.R138X [59] and p.R138Q-IVS 4-1 G>T [94] mutations, respectively, in the homozygous or compound heterozygous state) receiving a kidney from their respective mother, who was an obligatory healthy heterozygous carrier, presented recurrence of NS after transplantation. Despite recurrence, renal outcome was favourable in most of these cases. One may speculate that the graft may be more susceptible for the late development of FSGS and that the donor with one kidney may be at risk of developing FSGS. However, the number of recurrence cases (which remains low in the literature) should be compared to the total number of transplants performed before clear guidelines are drawn on living related donor transplantation in genetic forms of SRNS.

In conclusion, genetic disorders account for most of the cases of NS that start within the first year of life and a considerable proportion of those with childhood-onset NS. It is important to differentiate between NS due to an underlying genetic defect and other cases of SRNS, as the pathophysiology, clinical course and response to therapy are different. The identification of mutations may also modify the approach taken to counselling patients, particularly on the risk of recurrence after renal transplantation. Although the clinical course is dependant on the gene(s) involved, significant phenotypic variability has been demonstrated in patients bearing mutations in the same gene and even in patients bearing the same mutation. Therefore, a systematic step-wise approach for appropriate mutational screening is required in patients with SRNS. When a hereditary disorder is suspected, at least three main criteria should be considered in order to better determine the appropriate(s) gene(s) to test: (1) age at onset of NS, (2) presence of extrarenal abnormalities and (3) type of renal histological lesions. Careful clinical and biochemical investigations, including a search for ocular abnormalities, ambiguous genitalia/male pseudohermaphroditism, skeletal abnormalities, neurological symptoms and thrombocytopenia, need to be performed prior to the initiation of genetic testing. The country of origin of the patients may also orient the genetic testing; indeed, while *NPHS1* mutations are more frequently found in Finland, the prevalence of *NPHS2* mutations in SRNS cases is higher in Europeans and Turkish patients than in Asian children [57, 101]. Nevertheless, for some patients with SRNS, genetic testing will fail to detect mutations in the known genes involved in NS. Although an immune etiology or a complex genetic inheritance is probably responsible for most of these cases, the ongoing identification of novel gene loci in families with SRNS indicates that there are additional genetic causes of SRNS that still need to be identified. Further promising strategies, including high-throughput sequencing and copy number analysis-based strategies, may lead to the identification of novel genes in the near future.

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