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NPHS2 (Podocin) Mutations in Nephrotic Syndrome. Clinical Spectrum and Fine Mechanisms

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ABSTRACT

Nephrotic syndrome (NS) is the most frequent cause of proteinuria in children and is emerging as a leading cause of uremia. Molecular studies in families with recessive NS have led to the discovery of specialized molecules endowed in podocytes that play a role in proteinuria. This review focalizes the key position of podocin (NPHS2 gene) in this rapidly evolving field and furnishes a compendium to those involved in clinics and genetics of NS. Screening for NPHS2 mutations have been done in sporadic NS and familial cases with recessive inheritance, documenting a mutation detection rate of 45–55% in families and 8–20% in sporadic NS according to the different groups and considering all the clinical phenotypes. Almost 50 NPHS2 mutations have been reported and variants and/or non silent polymorphisms potentially involved in proteinuria were recognized. Personalized data on clinical aspects related to responsiveness to drugs, evolution to end stage renal failure and post-transplant outcome are reported. Functional studies and cell sorting experiments demonstrated retention in the endoplasmic reticulum of most mutants involving the stomatin domain. Pull-down experiments with the common R229Q polymorphism demonstrated an altered interaction with nephrin that affects the stability of the functional unit. Overall, data are here presented that underscore a major role of inherited defects of NPHS2 in NS in children (including a relevant impact in sporadic cases) and give the functional rationale for the association. A practical compendium is also given to clinicians involved in the management of NS that should modify the classic therapeutic approach. (***Pediatr Res* 57: 54R–61R, 2005**)

Abbreviations

CD2AP, CD2 associated protein
ESRF, end-stage renal failure
FSGS, focal segmental glomerulosclerosis
KO, knock-out mouse model
NPHS1, nephrin
NPHS2, podocin NPHS2
NS, nephrotic syndrome

Nephrotic syndrome (NS) is the most frequent glomerular disease in children (1) and the pathologic variant with focal segmental glomerulosclerosis is emerging as a leading cause of end stage renal failure. The annual incidence in children has been estimated to be 2.0 to 2.7 per 100.000 in USA with a cumulative prevalence of 16 per 100.000. Geographic or ethnic differences have been reported with a 6-fold greater incidence in Asian than in European children (2,3). It appears to be a clinical heterogeneous condition characterized by histologic variants (4–6) and different genetic backgrounds (7–9) with recessive and dominant inheritances. In the last few years, advances in molecular genetics of familial NS have led to the discovery of specialized molecules endowed in podocytes as responsible for proteinuria. They also have clearly indicated that the podocyte is the prevailing glomerular site for repulsion of proteins, a process that allows maintenance of big molecules (such as proteins with a molecular weight $>$ 40 kilo Dalton) in the vascular bed and ultra-filtration of water and solutes. Podocytes are specialized epithelia with a cell body and several polipoid cellular extra-flections that cover the outer surface of glomerular basement membrane. The inter-podocyte connection, called slit-diaphragm, is reminiscent of a tight junction with differentiated structure and functions. It presents an electron dense zipper-like structure composed of the extra-cellular components nephrin, nephrin homolog-1, Pcadherin and FAT (10–12) connected by other specialized structures (*i.e.*, podocin, CD2AP) to the main cell body (8,13–16). The discovery of mutations of the slit diaphragm components, in particular of podocin, in familial NS represented a break-through in the research of mechanisms of NS that overcome a pure genetic and clinical interest. There is now growing evidence that mutations of slit diaphragm proteins go far behind familial cases and frequently occur in sporadic NS (9,17–23). They

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include monogenic diseases due to mutations of the two major components of the slit, NPHS1 and NPHS2, and cases with triallelic digenic inheritance have been reported possibly contributing to worsen the clinical outcome (24,25). Clinical developments are suggesting a numeric relevance of inherited defects of the slit diaphragm that cannot be ignored by clinicians. This review focalizes the key position of podocin in this rapidly evolving field and furnishes a compendium to all those involved in clinical and experimental approaches to NS.

PODOCIN AS A KEY FUNCTIONAL COMPONENT OF THE SLIT-DIAPHRAGM

Podocin is a raft associated component of the glomerular foot-process membrane where it is localized at the insertion of the slit-diaphragm (8,14,15). It is an integral protein of 383 amino acids with a membrane domain forming a hairpin structure with two cytoplasmic ends at the C- and N-terminus. Podocin is a member of the stomatin family of proteins with unique expression in the kidney. It can form oligomers in the raft where, like a caveolin, forms a membrane invagination and recruits nephrin and CD2AP in these microdomains. In other words, oligomerization of podocin clusters nephrin and makes the slit-diaphragm assembly where CD2AP serves as an adapter to the overall network (13,15). The presence of intact podocin is, in fact, a prerequisite for nephrin transport to membrane and for podocyte intracellular signalling (26). Following this functional scheme it seems logical that alterations of the slit-diaphragm assembly lead to proteinuria and this occurs in both experimental models (27–29) and in human diseases (see the following sections on genetics of familial and sporadic NS). The phenotype in mice lacking podocin for engineered manipulation strongly supports this concept. They develop extensive podocyte lesions and proteinuria before birth and then die from uremia after a few days of life. It is noteworthy that NPHS2$^{-/-}$ mice do not develop FSGS but present pathologic features of diffuse mesangial sclerosis and tubular dilatation with vacuolization of epithelia more reminiscent of the human pathology due to the mutation of nephrin. At electron mycroscopy, NPHS2$^{-/-}$ mice present extensive effacement of foot process and lack podocin and nephrin. A comparable alteration of podocin-nephrin interaction was demonstrated by Huber *et al*. (26) utilizing cells transfected with intact nephrin and podocin R138Q and R138X mutants. Therefore, the molecular scaffold of the slit-diaphragm assembly disassembles when one component is removed. This appears to be the case in several proteinuric states in which the expression of most podocyte components is diminished as the result of foot process effacement (30–33).

NPHS2 MUTATION IN FAMILIAL AND SPORADIC NS

Clinical associations. Podocin is encoded by the gene NPHS2 that was discovered and localized at 1q25 by positional cloning in 2000 (8). Mutations of NPHS2 have been identified in several families with autosomal recessive-FSGS (AR-FSGS), accounting for most of familial NS with recessive inheritance, and in sporadic cases as well. At the time, based on enrollment in Europe, data have been published on NPHS2 screening in 671 patients with sporadic NS and in 205 familial cases with recessive inheritance (18–21). Most of the enrolled subjects were children with steroid resistant NS, but a few adults and patients with steroid dependence or sensitivity to cyclosporin were also analyzed. Moreover, studies on R229Q functional variant have contributed toward extending the interest in NPHS2 in marginal clinical conditions such as microalbuminuria and other proteinuric glomerular diseases (34,35). A synthesis of results is presented in Table 1 while descriptive details are given in Tables 2 and 3. Overall, results from the studies above demonstrate that NPHS2 mutations account for a significant part of all nephrotic patients roughly corresponding to a mutation detection rate of 45–55% in families with recessive traits and 8–20% in sporadic NS according to the different groups and considering all the clinical phenotypes. However, the incidence rate would increase if we consider only patients with poor response to steroids and/or showing a pathology of focal segmental glomerulosclerosis. It must be clearly stated that all cases with sporadic NS and NPHS2 mutation are, in fact, familial and this high incidence underscores a comparably high frequency of healthy carriers at least in the geographic areas where screenings were done. More than 50 NPHS2 mutations have been reported (details in Table 4) that occurred as homozygous or compound heterozygous combinations in 79 patients with a familial trait and 54 sporadic cases. Reported mutations involve the whole length of the gene and determine every kind of alteration, including missense, nonsense, and deletion. Studies so far published refer to European populations and report different distributions of mutations: R138Q was most frequently found in Germany and France (20,21) while the P20L variant was observed mainly in Italy (18) and Turkey (personal observation). One unique case involves the presence of heterozygous NPHS2 mutations associated with the R229Q variant that is a polymorphism with functional effects (see the section above). This association was found in 33 familial cases and in 2 sporadic NS (20,21,34). Clinical data are reported in Tables 2 and 3 and includes, when available, the age at onset of proteinuria, pathologic findings, response to drugs, incidence of ESRF, number of renal transplants and incidence of post-transplant recurrence.

Age at onset. Age at onset of proteinuria was rather variable in different reports, in general occurring before the 10th year with a strong prevalence of pediatric patients. A few cases with congenital or very early NS were described in all patient groups, including two families with double homozygous R168H and P20L mutations (21,36). The study by Ruf *et al*. (20) was the most exhaustive on this aspect and reported 14

Table 1. *Results of screening analysis for NPHS2 in 901 patients with nephrotic syndrome published between 2003 and 2004*

	Familial AR	Sporadic
Overall	230	671
Homozygous/compound heter	82	53
Heterozygous + R229Q	33	2
Heterozygous	7	14
Variants	–	11
Non-Silent SNPS	–	44
	122 (53%)	124 (18.6%)

Table 2. *Clinical data in patients with nephrotic syndrome and NPHS2 mutations*

	n	Age at onset (months)	Familial/ sporadic	Pathology FSGS/MCN/IgM	Drug Response St/CP/CsA	ESRF	Tx	Recurrence	NPHS2 Mut
Homozygous/Compound Heter									
Caridi *et al*. (18)	14	27 (1–100)	0/14	9/1/1	0/0/0	9	9	2	R138Q–R138Q R138Q–R138Q
Ruf *et al*. (20)	34	42 (4–192)	12/22	23/5/1	0/0/3*	24	18	1	L347X–L347X†
	14	congenital	8/6	7/2/0	0/0/1*	6	6	1	L347X–K126N
Weber *et al*. (21)	73	41.2	62/11	nd	nd	nd	32	1	R138X–R138X†
Heterozygous + R229									
Tsukaguchi *et al*. (33)	25	(100–250)	25/0	9/0/0	nd	13	nd	nd	–
Ruf *et al*. (20)	6	98 (36–264)	6/0	6/0/0	0/0/1	1	1	AR	R229Q–R291W
Weber *et al*. (21)	4	88.1	2/2	nd	nd	nd	nd	nd	–
Heterozygous									
Caridi *et al*. (18)	4	177 (19–408)	0/4	3/0/1	1/0/0	2	2	1	S211T
Ruf *et al*. (20)	5	37 (18–84)	2/3	2/1/0	2/0/2	0	0	–	–
Weber *et al*. (21)	12	147	5/7	nd	nd	nd	3	1	T326fsX345

Abbreviations: FSGS, focal segmental glomerulosclerosis; MCN, minimal change nephropathy; IgM, mesangial proliferative glomerulonephritis; St, steroids; CP, cyclophosphamide; CsA, cyclosporin; nd, non determined; AR, acute rejection. * Partial response. † Renal gift from the mother.

Table 3. *Clinical data in patients with nephrotic syndrome and NPHS2 variants and/or polymorphisms*

	n	Age at onset (month)	Pathology FSGS/MCN/IgM	Drug Response St/CP/CsA	ESRF	Tx	Recurrence	Reference
Variants								
P20L	h 6	18–720	3/0/1	2/0/1	2	2	2	18
	h 1	nd	0/1/0 ↑	1/0/0	0	–	–	20
	h 1	nd	nd	nd	1	1	1	21
A61V	h 2	nd	nd	nd	0	–	–	21
L172V	h 1	nd	nd	nd	0	–	–	21
Non-Silent SNP								
R229Q	H0/h11	47 (12–113)	4/1/2	5/0/7	2	2	1	18
	H2/h0	60–84	2/0/0	0/0/0	1	0	0	20
	H5/h13	nd	nd	nd	nd	1	1	21
E237Q	h 2	67–115	0/2/0	1/0/1	0	–	–	20
	h 2	nd	nd	nd	nd	1	1	21
A242V	h 1	25	0/1/0	1/0/0	0	–	–	18
	h 1	18	1/0/0	0/0/1	0	–	–	20
	h 7	nd	nd	nd	nd	nd	nd	21

Abbreviations: FSGS, focal segmental glomerulosclerosis; MCN, minimal change nephropathy; IgM, mesangial proliferative glomerulonephritis; St, steroids; CP, cyclophosphamide; CsA, cyclosporin; nd, non determined.

cases with congenital NS, 5 of which were familial. With respect to a possible genotype-phenotype correlation, in the paper by Weber *et al*. (21), some trends were apparent: 1) R138Q appears to be associated with early onset (12 ± 3 mo in 15 patients); 2) V180M and R238S are associated with late onset NS (129 ± 12 mo in 7 patients). Carriers of the association of R229Q variant with other NPHS2 mutations present a late onset of proteinuria, in general between the first and second decade of life.

Drug response. No drug response was reported with the exception of 4 partial responders to cyclosporin described by Ruf *et al*. (20); one presented congenital NS and no further details were available for the others.

Pathology. Personalized data on pathology background were available for 48 patients with homozygous or compound heterozygous NPHS2 mutations and for 15 with R229Q associated with another NPHS2 mutation. FSGS was the prevailing pathology feature present in 39 patients of the former group and in 15 of the R229Q/heterozygous NPHS2 group. Ten patients had an indication of 'milder renal changes' as epitomized by mesangial proliferation with IgM deposition in 8 cases and 2 with minimal changes. In 1 case there was an unspecific C3 deposition. Therefore, renal pathology is not suggestive of an inherited condition associated with NPHS2 mutations, this indication being in fully agreement with the current idea on the lack of correlation between renal alterations and clinical features.

End stage renal failure (ESRF). Personalized information on follow-up are available in 2 of the 4 studies published so far (18,20) that involve 34 sporadic patients with NPHS2 mutation and 10 familial cases. Twelve of the former group had progression to ESRF after 73 mo from the onset of proteinuria (range 6–155) while 12 patients did not reach the end point after a follow-up of 44 mo (range 6–166). Nine out of 10 familial cases reached ESRF after a follow-up of 76 mo (range 18–162) while no data are available for those who did not reach ESRF. Therefore data on follow-up of renal function are comparable for sporadic and familial NPHS2 and support the idea that this is a progressive condition that evolves to renal failure within the second decade of life.

Table 4. *43 Mutations and 6 functional variants identified from the discovery of the NPHS2 gene*

Exon	Nt Change	AA Change	Exon	Nt Change	AA Change
NPHS2 Mutations					
1	29insA	R10fsX69	5	587G>C	R196P
	85 G>A	A29T		622G>A	A208T
	104_105insG	G35fsX69		631 T>A	S211T
	274 G>T	G92C		705_713 del9	L236_R238del
	275-2 A>C	Splice		709 G>C	E237Q
2	304 G>A	E102K		714 G>T	R238S
	353 C>T	P118L	6	770G>A	G257E
	378 G>A	K126N		779 T>A	V260E
3	412 C>T	R138X		803 T>G	V268G
	413 G>A	R138Q	7	851 C>T	A284V
	419delG	G140fsX180		855_856delAA	Q285fsX302
4	435_436delA	R146fs		862 G>A	A288T
	467_468insT	L156fsX166		868 G>A	V290M
	467delT	L156fsX180		871 C>T	R291W
	479 A>C	D160G		872 G>A	R291Q
	502 C>A	R168S	8	929 A>T	E310V
	502 C>T	R168C		948delT	L347X
	503 G>A	R168H		964 C>T	R322X
	506 T>C	L169P		973C>T	H325Y
	514 C>G	L172V		976_977insA	T326tfX345
5	538 G>A	V180M		983 A>G	Q328R
	555delT	F185fsX186			
Exon	Nt Change	AA Change			
Functional variants					
1	59 C>T	P20L			
	182 C>T	A61V			
4	514 C>G	L172V			
5	686 G>A	R299Q			
	709 G>C	E237Q			
	725 C>T	A242V			

Abbreviations: Nt, nucleotide; AA, amino acids.

NPHS1-NPHS2 DIGENIC INHERITANCE

Triallelic digenic inheritance involving mutations of NPHS1 and NPHS2 has been described (24,25). Overall, 5 patients with homozygous NPHS1 mutations associated with R229Q heterozygous NPHS2 mutation and 4 patients with homozygous NPHS2 mutations associated with 1 heterozygous NPHS1 mutation have been described. The question is still debated whether a triallelic defect may adversely affect the phenotype.

NPHS2 HETEROZYGOUS CARRIERS

Simple heterozygous mutations were found in 17 patients (4 familial and 13 sporadic) that represent a minor, albeit significant, fraction of all patients with NS (Tables 2 and 3). Available clinical details require comment because they suggest differences in the presentation of NS in heterozygous carriers of NPHS2 mutations compared with patients with homozygous or compound heterozygous mutations. First, the age at onset of proteinuria was variable from a few months to several years (21 and 34 y in 2 patients from Italy); second, 3 out of 9 children for whom the clinical outcome was described had a good response to steroids and the other 2 responded to cyclosporin (18,20). Third, the outcome was ESRF in 5 of 10 patients that suggests a progressive trend also in these cases. In spite of a milder clinical phenotype, carriers of heterozygous mutations may present serious glomerular lesions including a typical picture of focal segmental glomerulosclerosis. Moreover, the histology analysis of glomeruli in an unique patient with heterozygous mutation (976_7insA) demonstrated profound alteration of podocin distribution in glomeruli (37) (see below).

The significance of heterozygous podocin mutations associated with NS is not clear so far, since FSGS associated with NPHS2 mutations is a recessive condition requiring a molecular defect on both alleles to determine a pathologic effect. It is also evident that heterozygous mutations are not always associated with proteinuria, the major example being parents of sporadic patients carrying homozygous mutations who are, by definition, healthy. While screening studies in relatives of affected patients are warranted to exclude sub-clinical proteinuria, a few pathogenic possibilities for a causative role of single NPHS2 mutations merit consideration. The first is the presence of another, albeit undiscovered, mutation in the podocin gene probably involving regulatory sequences or mutations in non coding regions. A second possibility is the concomitance of mutations involving one or more still undiscovered gene(s) that could produce an additive effect in the frame of a multigenic inheritance. A final and probably most reliable mechanism to explain proteinuria in heterozygous carriers is related to a sort of susceptibility to develop proteinuria conferred by the genetic milieu where other factors may represent the breaking event. The last mechanism has been investigated in a knock-out (KO) murine model for major podocyte genes CD2AP and NPHS2 (27,38). NPHS2 KO heterozygous mice

spontaneously develop proteinuria with aging (21,39), depending on the genetic background determined by the mouse strain. Pathologically, these mice presented podocyte effacements and mild mesangial matrix expansion. On the other hand, CD2AP KO heterozygous mice present an increased susceptibility to glomerular injury by nephrotoxic antibodies and immunocomplexes (38). Which extra-renal factor is concurring to proteinuria in these cases is only a matter of debate, which recent data on circulating plasma factors for permeability seem to support. Carraro *et al*. (40) recently reported post-transplant proteinuria in patients with NPHS2 mutations associated with high P_{alb} values that is considered a rough estimate of something in the circulation that alters glomerular permeability to proteins (41,42). Although the relationship between elevated P_{alb} and proteinuria in NPHS2 remains to be determined this association could underscore two terms of a multi-factorial mechanism playing a role in proteinuria. The characterization of permeability plasma factors is now in progress and no clear conclusion can be reached until this step is completed.

CELL SORTING, INTERACTIONS AND RENAL PATHOLOGY RELATED TO NPHS2 PATIENTS

A few NPHS2 mutations have been studied on a functional bases, these studies providing a rationale for considering the molecular role of NPHS2 mutations in NS and possibly developing new therapeutic strategies (43,44). They followed the sub-cellular localization of podocin in HEK293 cells transfected with the wild construct as well as with various mutants and followed the maturation steps. They considered 13 podocin mutations that are distributed along the entire gene and concern aminoacids conserved among species; 11 mutations covered the stomatin homology region. Cell expressing wild-type podocin presented major plasma membrane localization where the protein was targeted *via* the classical cell sorting-Golgi dependent pathway. Most mutants (P118L, R138Q, D160G, R168C, R168H, R168S, V180M, 237_9delA and V260E) were retained in endoplasmic reticulum (ER) and one, R291W, was localized at late endosomes. Finally, normal maturation dynamics and plasma membrane localization was demonstrated for three mutants: P20L, G92C and R238S. Therefore, 10 out of 13 mutations generated aberrant proteins that are not properly processed and are dislocated within the cell. For R291W, it was hypothesized to have a stronger affinity with CD2AP that is involved in endocytosis and this explains the endosome localization. Three mutations at the COOH terminus, G92C, V180M and R238S, did not affect the targeting to plasma membrane but probably affected the interaction with nephrin and/or with other slit-diaphragm proteins altering in this way both conformation and stability of the functional unit. Other studies have been done by Tsukaguchi *et al*. (34) for the R229Q variant that is a common polymorphism occurring at an increased frequency in association with late onset FSGS. Those Authors used pull-down experiments with anti-podocin antibodies and found a decreased binding to nephrin in the presence of this polymorphism compared with the wild protein. It was proposed that this common variant may contribute to glomerulosclerosis in association with other mutants NPHS2 allele or acting in synergism with other factors. Overall, studies so far performed on podocin mutants have shown that defects in cell sorting process predominate over structural alterations of podocin. However, in both cases the mechanism of proteinuria is a modified interaction between podocin and nephrin due to de-localization or structural alteration of the former and this alters the stability of the slit-diaphragm assembly.

Analysis of podocin expression in patients with NPHS2 mutations offers an invaluable manner for defining anomalous dislocation *in vivo* suggesting functional anomalies. Zhang *et al*. (37) investigated six patients with different allelic combinations and described the following patterns: a) absence of podocin staining with antibodies against the C-terminus in two patients with homozygous 855_6delAA or 419delG; b) in four other patients carrying compound heterozygous mutations (R168S/467_8insT; R138Q/V180M), variants (R291W/R229Q) or single heterozygous 976_7insA the distribution of the protein was restricted to the podocyte body or along the GBM. Parallel to the above changes, the same patients presented an irregular distribution of nephrin along the GBM and within the podocyte cell body that are highly reminiscent of what was described in $NPHS2^{-/-}$ mice. All these changes are highly expressive of a profound alteration of the slit-diaphragm composition and furnish the basic rationale for proteinuria even in carriers of heterozygous mutations.

FUNCTIONAL VARIANTS AND NONSILENT POLYMORPHISMS

Three variants of unknown effect (P20L, A61V, and L172V) and 3 nonsilent polymorphisms (R229Q, E237Q, A242V) have been variably identified in different patient groups. With the enlargement of screening studies in different populations, including normal groups enrolled in Europe and in North Africa, definite conclusions on their significance and clinical impact can be drawn. Overall, 11 patients bearing a NPHS2 variant in patients with NS have been identified with an overall impact of 1.66% in sporadic cases. P20L is the most common variant detected in Europe, mainly in Italy (18), where 6 cases were identified. However, this variant requires comment since it is not universally considered a mutation. It determines an amino acid change that is conserved during evolution and has unknown functional consequences. Ruf *et al*. (20) reported the presence of two homozygous P20L carriers out of 80 normal controls, an incidence that is out of the Hardy-Weinberg equilibrium due to the absence of heterozygous carriers in the same group. Giving a theoretical incidence of P20L in the above normal population of 2.5% (4 alleles out of 160), the incidence in the patient group of 314 patients reported by the same authors should be 16 patients instead of one. On the other hand, we (and other authors) did not find any P20L change in 200 healthy controls (18,19,21). We have no explanation for this discrepancy and this point should be further investigated. Assuming P20L is a variant (a conclusion shared by two of the three relevant screening studies so far reported), the incidence of heterozygous variant was 1.7%. As occurs in carriers of heterozygous NPHS2 mutations, the age at onset was variable with a few carriers presenting proteinuria in adulthood. Only

one screening study in 64 adults with steroid resistant NS has been published so far (19), giving an incidence of single NPHS2 mutations in 5% of the group; this finding must be confirmed in larger groups of nephrotic adults. Four out of seven carriers of P20L for whom clinical details are available had good responses to drugs usually used in NS such as steroids and cyclosporin (Table 3). Finally, only 3 out of 11 had progression to ESRF suggesting that carriers of NPHS2 variants have a more favourable outcome in respect to response to drugs and evolution. The same considerations about NS as a recessive disease and the significance of heterozygous mutations are pertinent to carriers of single variants and additional studies are required to define whether they confer susceptibility to NS. An even more difficult task is to look at polymorphisms, two of which (E237Q, A242V) are very rare, the last one (R229Q) having instead a large impact. R229Q polymorphism was identified in 18 out of 566 normal chromosomes in Italy and France (18,21) that give an allelic frequency of 3.2%, and in 36 out of 837 chromosomes from patients with sporadic NS for an allele frequency of 4.3%. A recent report on R229Q in 1577 normal American people (35) reported an association of R229Q with microalbuminuria in the general population suggesting a more complex interaction of this podocin variant and proteinuria. Less severe phenotypes characterized by later onset of proteinuria and good response to drugs have been reported in simple carriers of heterozygous R229Q a fact that is fully consistent with functional studies.

NPHS2 PROMOTER

Pathology studies demonstrated that the molecular scaffold of the slit-diaphragm dissembles in animals and human beings with proteinuria (28–30) and in these cases, the protein expression of podocin is diminished in glomeruli (32,33). It is likely that a proper equilibrium among different components influences the maintenance of the barrier functional integrity. This appears to be a dynamic process in which the regulatory mechanisms of every different protein play a role. Data on podocin glomerular mRNA in FSGS and minimal change diseases are controversial. Koop *et al*. (32) reported on a significant increase in podocin mRNA in both conditions and proposed that this might result from a compensatory reaction of the damaged podocytes. On the contrary, results by Schmid *et al*. (33) pointed to a net differentiation in podocin mRNA glomerular expression between FSGS and minimal changes in a larger group of patients suggesting a different compensatory reaction between the 2 pathologies. While it is still unknown what determines podocin loss in such conditions it seems plausible that reparative capacities of the renal barrier strictly depend on their re-synthesis potential and the reparative process may eventually influence the proteinuria. Functional studies on NPHS2 promoter are warranted to define the reparative process within podocytes. Human NPHS2 promoter has been characterized in 2002 by Moller *et al*. (45) and its expression has been extensively studied in mice (46,47). Data on 4 NPHS2 promoter mutations in NS have been recently obtained in a group of 234 patients. All variants were studied on functional basis by sub-cloning in the luciferase-reporter vector and two of them were found to produce significant down-regulation on gene expression (personal observation). It seems reasonable to hypothesize that functional variants determining low expression of the protein play a role in the reparative process of glomerular barrier.

POST-TRANSPLANT OUTCOME

Post-transplant recurrence of FSGS is one of the most traumatic clinical events in clinical settings. It involves 35–45% of idiopathic patients according to different reports (48,49), often occurs in first few days from transplantation and is often sensitive to plasmapheresis and cyclophosphamide (42,50,51). While the clinical outcome of recurrent FSGS has received wide interest and has been exhaustively reviewed in different reports (48–52), the pathogenesis still remains an enigma. It was historically taken as a proof for the existence of circulating permeability plasma factor(s) that are also putative effectors of the original proteinuria in these patients. Indirect clinical evidence supports this possibility based on the frequent rapid onset after a few hours from the graft and the successful effect of techniques such as plasmapheresis that would act by removing protein factors from circulation (41). More recent reports describe a balance between inducers and inhibitors of permeability (53–55), but the research on this topic is still elusive, and until the characterization of permeability factors has been completed, any conclusion is speculative. The reason to consider post-transplant recurrence here resides on the fact that FSGS should not recur in cases with NPHS2 mutations in which case proteinuria is the consequence of the inherited defect. An original report by Bertelli *et al*. (56) described 5 patients out of 13 with NPHS2 mutations that presented post-transplant recurrence of proteinuria and in two cases the pathologic picture of FSGS was confirmed. Only 2 cases of the Italian group had homozygous or compound heterozygous mutations of NPHS2 (22% of those who received a renal graft) and in both cases the episode had mild clinical impact and favourable outcome. Instead, three patients had a single mutation of NPHS2 and paradoxically, the outcome for one of them was poor. One major point in considering the data by Bertelli (56) is related to the three carriers of single NPHS2 mutations whose significance has been discussed above. We now have the opportunity to consider post-transplant recurrence in a larger population and to compare the original data in 13 patients by Bertelli *et al*. (56) with other study groups (20,21). Collectively (and including the study by Bertelli), data are available for 78 patients with NPHS2 mutations who received a renal transplant. They include 65 carriers of homozygous or compound heterozygous NPHS2 mutations, one patient with single mutations plus R229Q, eight with a single mutation or variants and four carriers of polymorphisms. Post-transplant recurrence of proteinuria was documented in 5 of the 65 patients with homozygous or compound heterozygous mutations (7.7%), and in 5 out of 8 heterozygous carriers of mutations or variants (62.5%). Moreover, the unique patient with R229Q associated with NPHS2 mutation had an acute post-transplant rejection with loss of the graft and three of four carriers of a silent polymorphism had recurrence. Exhaustive

clinical details are available for all recurrent cases and are synthesized in Tables 2 and 3. Therefore, the results from the significant group of 78 patients partly reduces the impact of post-transplant recurrence in homozygous NPHS2 carriers from 22% of the original report to 7.7%, but at the same time reproduce the impressive result of a high recurrence rate in carriers of heterozygous mutation and/or nonsilent polymorphisms. One child with homozygous L347X mutation described by Ruf (20,57) and another with homozygous R138X described by Weber (21) received the kidney from their mother who was an obligatory healthy heterozygous carrier. The first child had recurrence of proteinuria on day seven from the graft that responded within one week to plasmapheresis. The second child presented nephrotic range proteinuria two years after the allograft. While a low number of patients with single NPHS2 mutation does not allow conclusive remarks these results strongly suggest that the rate of recurrence in patients with NPHS2 homozygous or compound heterozygous mutations is low; even grafts from obligate carriers of NPHS2 mutations, such as their parents, should be avoided because of the higher risk of recurrence of FSGS. Carriers of heterozygous NPHS2 mutation should be considered at risk of recurrence and strictly monitored in the post-graft phase. This implies that NPHS2 molecular test should be introduced in clinical practice to nephrotic patients, which will require the development of rapid and low-cost molecular strategies.

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