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NPHS2 Mutations in Steroid-Resistant Nephrotic Syndrome: A Mutation Update and the Associated Phenotypic Spectrum

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ABSTRACT: Mutations in the *NPHS2* gene encoding podocin are implicated in an autosomal-recessive form of nonsyndromic steroid-resistant nephrotic syndrome in both pediatric and adult patients. Patients with homozygous or compound heterozygous mutations commonly present with steroid-resistant nephrotic syndrome before the age of 6 years and rapidly progress to end-stage kidney disease with a very low prevalence of recurrence after renal transplantation. Here, we reviewed all the *NPHS2* mutations published between October 1999 and September 2013, and also all novel mutations identified in our personal cohort and in international genetic laboratories. We identified 25 novel pathogenic mutations in addition to the 101 already described. The mutations are distributed along the entire coding region and lead to all kinds of alterations including 53 missense, 17 nonsense, 11 small insertions, 26 small deletions, 16 splicing, two indel mutations, and one mutation in the stop codon. In addition, 43 variants were classified as variants of unknown significance, as these missense changes were exclusively described in the heterozygous state and/or considered benign by prediction software. Genotype–phenotype analyses established correlations between specific variants and age at onset, ethnicity, or clinical evolution. We created a Web database using the Leiden Open Variation Database (www.lovd.nl/NPHS2) software that will allow the inclusion of future reports.

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KEY WORDS: *NPHS2*; steroid-resistant nephrotic syndrome; podocin; FSGS

Additional Supporting Information may be found in the online version of this article.

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Introduction

Nephrotic syndrome (NS) is characterized by massive proteinuria, hypoalbuminemia, and edema. Clinically, NS has been divided into two categories based on the response to steroid therapy: steroid-sensitive NS (SSNS) and steroid-resistant NS (SRNS). The most prevalent histological finding associated with SRNS in children and adults is focal segmental glomerulosclerosis (FSGS). It is characterized by the presence in some—but not all—glomeruli of segmental areas of mesangial collapse and sclerosis. Over the last two decades, the molecular bases of SRNS have been elucidated in a subset of familial forms of SRNS, and mutations in genes encoding podocyte proteins have been described as causes of hereditary SRNS. To date, mutations in 10 genes (*NPHS1, NPHS2, PLCE1, CD2AP, ACTN4, TRPC6, INF2, MYO1E, PTPRO*, and *ARHGDIA*) have been implicated in different forms of nonsyndromic SRNS, whereas in the less frequent syndromic forms, 11 other genes have been identified (*WT1, LMX1B, LAMB2, ITGB4, SCARB2, COQ2, PDSS2, MTTL1, SMARCAL, MYH9*, and *NXF5*) [Buscher and Weber, 2012]. Among these genes, *NPHS1* and *NPHS2* encoding nephrin and podocin are the two main genes implicated. The human *NPHS2* gene (MIM #604766, GenBank accession number NM_014625.2), identified by positional cloning in 2000, spans approximately 25 kb on chromosome 1q25-q31 and contains eight exons [Boute et al., 2000]. The protein product, podocin, is almost exclusively expressed in the glomerular podocytes. Podocytes are specialized epithelial cells that form the outer surface of the glomerular filtration barrier (GFB), also composed of the glomerular basement membrane (GBM), and the fenestrated endothelium. These octopus-like cells are composed of a cell body, and several cytoplasmic expansions called foot processes, which are interconnected on the top of the GBM by a specialized adherens junction, the slit diaphragm. Podocin is a 42-kDa integral membrane protein of 383 amino acids that belongs to the stomatin protein family. This hairpin-like protein has a unique topology with a single short transmembrane domain and cytosolic N- and C-terminal domains. It also contains a single central hydrophobic domain and the prohibitin homology (PHB) domain that allows podocin binding to cholesterol in the plasma membrane [Huber et al., 2006]. Podocin was shown to accumulate in oligomeric form in lipid raft microdomains and to recruit nephrin in these specialized microdomains of the plasma membrane [Huber et al., 2003]. By also interacting with CD2AP and TRPC6, podocin represents an important link between the slit diaphragm and the podocyte cytoskeleton. Its dysfunction results in disorganization of the GFB and development of proteinuria. Volker et al. (2013) have evidenced a human podocin splice variant that lacks exon 5. This splice variant is translated into a protein that lacks part of the PHB domain and does not reach the plasma membrane in detectable amounts. Therefore, its function remains elusive.

Mutations in the *NPHS2* gene were first described in a subset of children with autosomal-recessive SRNS and FSGS [Boute et al., 2000] and thereafter in sporadic childhood cases [Caridi et al., 2001; Karle et al., 2002], and also in adult-onset SRNS [Tsukaguchi et al., 2000, 2002]. In the largest European and North American pediatric series, mutations in the *NPHS2* gene account for approximately 30%–40% of familial cases of SRNS [Karle et al., 2002; Weber et al., 2004; Berdeli et al., 2007; Hinkes et al., 2007, 2008] and 10%–30% of sporadic SRNS [Caridi et al., 2001, 2003; Karle et al., 2002; Ruf et al., 2004; Weber et al., 2004; Berdeli et al., 2007; Hinkes et al., 2008; Megremis et al., 2009; Jungraithmayr et al., 2011]. Patients with homozygous or compound heterozygous mutations commonly present with SRNS before the age of 6 years [Weber et al., 2004; Hinkes et al., 2008], and rapidly progress to end-stage kidney disease with a low prevalence of recurrence after renal transplantation [Boute et al., 2000; Weber et al., 2004]. According to the age at onset, podocin mutations were also found to be a frequent cause of congenital NS manifesting in utero or during the first 3 months of life (15%–39%) and infantile NS with onset between 4 months and 1 year of age (29%–35.2%) [Hinkes et al., 2007, 2008; Machuca et al., 2010; Santin et al., 2011]. On the opposite, most series found that *NPHS2* mutations are a rare finding in adult-onset FSGS [Caridi et al., 2003; Weber et al., 2004; Aucella et al., 2005; Monteiro et al., 2006; He et al., 2007; McKenzie et al., 2007; Tonna et al., 2008; Buscher and Weber, 2012].

Herein, we reviewed a total of 126 mutations of *NPHS2*, 25 of them being novel, and we evaluated the genotype–phenotype correlations.

Spectrum of Mutations

We searched for all *NPHS2* mutations published between October 1999 and September 2013, by using the combination of the key words "*NPHS2*," "podocin," and "NS" in PubMed. Approximately 750 patients were described in the literature, with ≥1 mutation in the *NPHS2* gene. However, there is some redundancy, some patients being included in several cohorts and thus reported several times. We also reviewed all the mutations described in our personal cohort that included all the patients screened for podocin mutations in Necker hospital between October 1999 and September 2013 and in international laboratories involved in molecular screening of hereditary glomerular diseases. Functional studies lacked for the majority of these mutations, and therefore the role of some variants described is unclear and would require experimental confirmation. Therefore, we classified as variants of unknown significance variants that were not predicted to be deleterious by the Polyphen 2 software and/or variants that were exclusively described in the heterozygous state. We entered all these mutations in the Leiden Open Variation Database (LOVD 3.0: www.lovd.nl/NPHS2).

In summary, we identified 25 novel mutations (Table 1), in addition to the 101 already described. *NPHS2* mutations were spread across the entire gene (Fig. 1, Supp. Table S1) and led to all kinds of alterations including missense, nonsense, frameshift, and splice-site mutations. The mutation nomenclature follows current guidelines.

Missense Mutations

Missense mutations represented the largest group of *NPHS2* mutations with a total of 53 different mutations described (42%) (Table 1, Supp. Table S1). The p.R138Q (c.413G>A) mutation in exon 3 was the most prevalent mutation in European series, likely due to a founder effect in Northern Europe [Niaudet, 2004]. It was found in 32% and 44%, respectively, of all affected *NPHS2* alleles in two large European series [Weber et al., 2004; Hinkes et al., 2008]. This arginine residue at position 138 is highly conserved among the stomatin-like protein family members and is crucial for podocin function [Boute et al., 2000]. The protein resulting from the substitution of glutamine for arginine is retained in the endoplasmic reticulum (ER) and loses its ability to recruit nephrin in lipid rafts [Huber et al., 2003]. ER retention has also been proven for other missense mutation proteins such as: p.P118L (c.353C>T), p.D160G (c.479A>G), p.R168S (c.502C>A), p.R168C (c.502C>T), p.R168H (c.503G>A), and p.V260E (c.779T>A) [Roselli et al., 2004].

On the other hand, some missense mutations lead to podocin mutants that are correctly targeted to the plasma membrane: p.G92C (c.274G>T), p.V180M (c.538G>A), and p.R238S (c.714G>T). Their

Table 1. Novel *NPHS2* Mutations in SRNS

Exon	Nucleotide change	Predicted amino acid change	Polyphen2 score
Missense mutations			
2	c.320T>C	p.Leu107Pro	0.707 (possibly damaging)
2	c.365G>C	p.Trp122Ser	0.920 (probably damaging)
5	c.547G>T	p.Asp183Tyr	1.000 (probably damaging)
7	c.841G>A	p.Glu281Lys	0.991 (probably damaging)
8	c.886G>A	p.Glu296Lys	0.888 (possibly damaging)
8	c.926C>T	p.Ala309Val	0.742 (possibly damaging)
Indel deletions			
4	c.522_524delinsTGT	p.Pro175Val	0.995 (probably damaging)
Variants of unknown significance			
2	c.289G>A	p.Gly97Ser	0.004 (benign)
8	c.938A>G	p.Thr315Ile	0.078 (benign)
8	c.998A>G	p.Glu333Gly	0.341 (benign)

Exon	Nucleotide change	Predicted amino acid change
Nonstop mutation		
8	c.1150T>C	p.X384GlnextX7
Nonsense mutations		
1	c.137C>A	p.Ser46X
5	c.655C>T	p.Gln219X
5	c.685C>T	p.Arg229X
7	c.809T>A	p.Leu270X
Small insertions		
1	c.138_142dup	p.Ser48TrpfsX53
7	c.824dup	p.His276AlafsX8
Small deletions		
1	c.134del	p.Pro45ArgfsX54
1	c.167del	p.Glu56GlyfsX43
1	c.249del	p.Leu84TrpfsX15
5	c.706del	p.Leu236X
8	c.989_992del	p.Leu330ProfsX17
8	c.1133_1136del	p.Lys378ThrfsX11

Exon	Nucleotide change	Predicted amino acid change	Splice-site prediction score change
Splice-site mutations			
IVS1	c.275–1G>C	Splice site	0.90–0
IVS3	c.378+5G>A	Splice site	0.91–0.31
IVS3	c.379–1G>C	Splice site	0.99–0
IVS6	c.794+5G>T (§)	Splice site	0–0.30
IVS7	c.874–2A>G	Splice site	0.89–0

Mutation numbering is based on the cDNA reference sequence (GenBank accession number NM_014625.2). A Polyphen2 score (http://genetics.bwh.harvard.edu/pph2/) is predicted to be "probably damaging" if it is greater than 0.85, "possibly damaging" between 0.85 and 0.2, and "benign" if it is less than 0.2. Splice-site prediction scores were calculated with BDGP Splice Site Prediction by Neural Network (http://www.fruitfly.org/seq_tools/splice.html). The mutation in IVS6 (§) creates a new potential donor splice site that might add three nucleotide at the end of exon 6. Calculation and interpretation of prediction software data comply with the journal standards.

deleterious effect could affect the function of the protein by directly modifying its signaling properties and/or altering its interaction with other protein at the slit diaphragm [Roselli et al., 2004].

Missense mutations were not confined to specific exons and were distributed throughout the length of the gene. Therefore, the different functional podocin domains were uniformly affected (Fig. 1). Among these 53 missense mutations, three are reported in the public whole-exome sequence databases such as EVS: p.V290M (c.868G>A) and p.V180M (c.538G>A) with a frequency of 1/13006 alleles (0.008%), and p.R138Q (c.413G>A) with a frequency of 8/13006 (0.06%), which is consistent with the higher prevalence of the latter mutation in the patients.

Nonsense Mutations

Seventeen nonsense mutations have been described of whom four were novel (p.S46X [c.137C>A], p.Q219X [c.655C>T], p.R229X [c.685C>T], and p.L270X [c.809T>A]). The other mutations previously described were: p.Q39X (c.115C>T), p.R71X (c.211C>T), p.E87X (c.259G>T), p.W122X (c.366G>A), p.Q129X (c.385C>T), p.R138X (c.412C>T), p.Y162X (c.486C>A), p.R196X (c.586C>T), p.Q215X (c.643C>T), p.E237X (c.709G>T), p.E264X (c.790G>T), p.K289X (c.865A>T), and p.R322X (c.964C>T). It has been shown that, in vitro, the p.R138X (c.412C>T) mutant protein is present at the plasma membrane but fails to recruit nephrin in lipid rafts [Huber et al., 2003]. However, a barely detectable product was found by amplification in a renal biopsy of a patient with p.R138X (c.412C>T), suggesting that RNA is subjected to mRNA decay [Boute et al., 2000].

Small Insertions and Deletions

Short genomic deletions or insertions accounted for 37 of the overall 126 mutations (29%), with 11 small insertions and 26 small deletions. All but three of these mutations are frameshifting changes. Small insertions and small deletions were located throughout the podocin gene coding sequence; however, small deletions clustered in the PHB and C-terminal domains. Only four deletions out of 26, p.Pro89ArgfsX13 (c.264_265del) and the novel ones (p.P45RfsX54 [c.134del], p.E56GfsX43 [c.167del], and p.L84WfsX15 [c.249del]) were located in the first exon and affected the N-terminal part of podocin.

Indel Mutation

The first indel mutation described in the *NPHS2* gene was recently described [Santin et al., 2011]. It is the p.L324HfsX20 (c.971_987delinsACAG). The second one was a novel indel in frame mutation found in our cohort: p.P175V (c.522_524delinsCGT).

Splice-Site Mutations

We describe five novel variants altering splice-site sequences in addition to the 11 already described in the literature: 275–1G>C, 275–2A>C, 378+1G>A, 378+5G>A, 379–1G>C, 451+2T>A, 451+3A>T, 452–2A>C, 534+1G>T, 535–1G>T, 794+5G>T, 873+5G>A, 873+2T>A, 873+1G>A, 874–1G>C, and 874–2A>G. All but four alter the obligatory consensus donor or acceptor splice sites and are considered pathogenic. Most of splice-site mutations affected introns 3, and 7 (58% of all the splice-site mutations).

Mutation in the Stop Codon

This peculiar novel mutation was recently found in our cohort, p.X384QextX7 (c.1150T>C). In this mutation, a single base substitution in the stop codon generates a longer open reading frame, resulting in a larger 390 amino acid protein. Transient overexpression of this mutant podocin in cultured podocytes led to its retention in the ER, however, with a partial plasma membrane localization (data

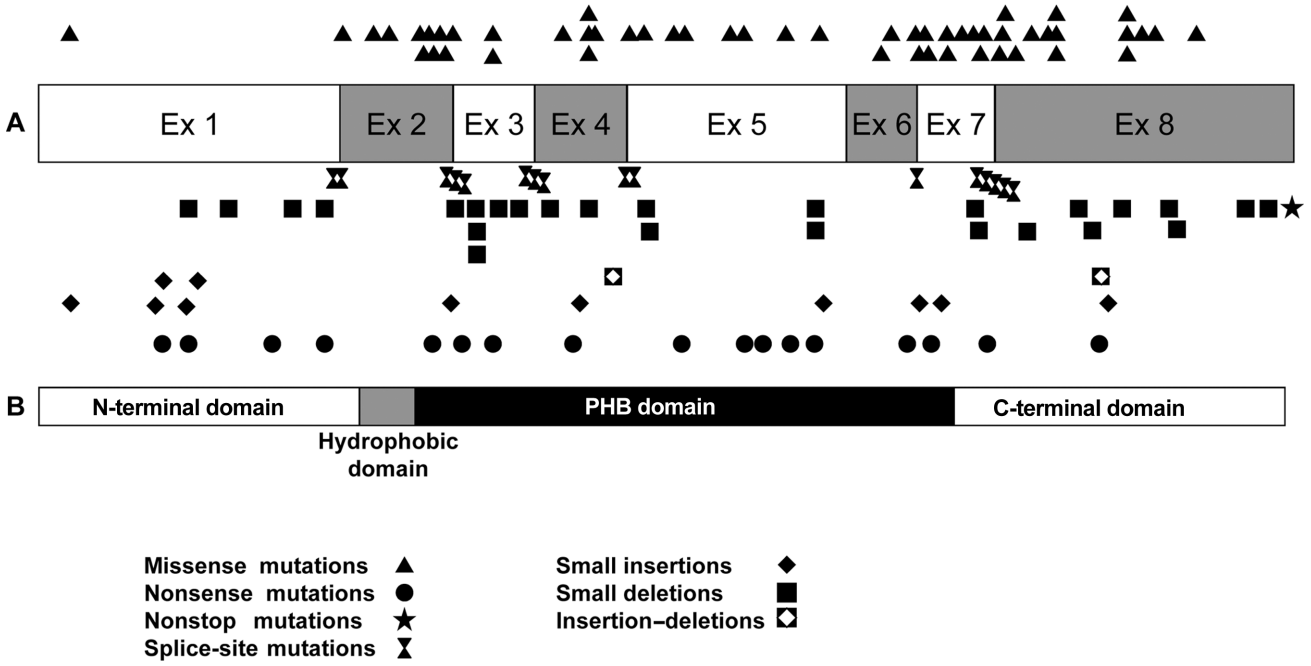

Figure 1. *NPHS2* mutations reported in the literature. **A:** Exon structure of the *NPHS2* gene with geometric shapes indicating relative positions of different types of mutations. **B:** Podocin domain positions.

not shown). Further experimental studies are needed to evaluate the functional consequences of this singular mutation.

Polymorphisms

A total of 67 *NPHS2* polymorphic variants were published (Table 2), including 22 coding SNPs, of whom eight were nonsynonymous and 14 were synonymous changes. The remaining 45 variants located in the noncoding regions with 13 intronic changes, 26 in the promoter region, and 6 in the 3′UTR.

Among the nonsynonymous SNPs, a close attention must be paid to the p.R229Q (c.686G>A) variant. This G to A nucleotide substitution at position 686 in exon 5 results in a nonconservative amino acid exchange with arginine to glutamine substitution. The biologic properties of the encoded peptide are altered. Indeed, the resulting podocin has a significantly decreased binding to nephrin in vitro [Tsukaguchi et al., 2002]. It has been demonstrated that p.R229Q (c.686G>A) in association with a pathogenic mutation on the second *NPHS2* allele leads to FSGS, especially among adults (cf. *Genotype–Phenotype Correlations*). Additionally, it has been suggested that p.R229Q (c.686G>A) allele can predispose to proteinuria in the general population [Pereira et al., 2004] and in patients with thin membrane nephropathy [Voskarides et al., 2012]. On the opposite, several other articles reported a relatively similar frequency of the p.R229Q (c.686G>A) variant in patients with SRNS and normal control subjects [Ruf et al., 2004; Weber et al., 2004; McKenzie et al., 2007], hence suggesting that this variant is not a risk factor for SRNS in the heterozygous state [Karle et al., 2002; McKenzie et al., 2007]. The estimated prevalence of the p.R229Q (c.686G>A) allele varies depending on the ethnicity: 0.03–0.13 in Europeans [Karle et al., 2002; Tsukaguchi et al., 2002; Caridi et al., 2003; Lowik et al., 2003; Pereira et al., 2004; Ruf et al., 2004; Weber et al., 2004; Aucella et al., 2005; Franceschini et al., 2006; Kottgen et al., 2008], and 0.005–0.025 in individuals from African descent [Tsukaguchi et al., 2002; Pereira et al., 2004; Dusel et al., 2005]. In conclusion, the p.R229Q (c.686G>A) is currently considered as a non-neutral polymorphism. When associated in trans to another deleterious mutation, it may lead to a wide spectrum of disease phenotypes from adult-onset FSGS to late childhood-onset SRNS with a slow progression to end-stage kidney disease (cf. *Genotype–Phenotype Correlations*). The variants p.A61V (c.182C>T) and p.A242V (c.725C>T) are nonsynonymous SNPs predominant in individuals of African American descent, with a relative frequency of 0.015 and 0.076, respectively (NHLBI Exome Sequencing Project).

Variants of Unknown Significance

Forty-three variants were classified in this category (Table 1). Mostly initially reported as pathogenic, these mutations were classified in our paper as variants of unknown significance because they were exclusively reported in the heterozygous state, or their Polyphen2 score was considered as benign.

Genotype–Phenotype Correlations

We analyzed all patients with *NPHS2* mutations in the homozygous, compound heterozygous, or heterozygous state that have been reported in the literature since the identification of the gene in 2000 until September 2013. We reviewed the available data of all these patients to find correlations between genotype and some clinical features.

Age at Disease Onset

Reports showed a significantly lower mean age at onset of SRNS in children with two identified pathogenic mutations (41.2 ± 5.9 and 45.6 ± 6 months) than in children with no identified mutation (76.8

Table 2. Published *NPHS2* Polymorphisms

Nucleotide change	Amino acid exchange	References	dbSNP name
Coding SNP			
Nonsynonymous change			
c.59C>T	p.Pro20Leu[a]	[Boute et al., 2000; Ruf et al., 2004]	rs74315344
c.101G>A	p.Gly34Glu	[Maruyama et al., 2003]	
c.182C>T	p.Ala61Val[a]	[Dusel et al., 2005]	rs201050491
c.176C>A	p.Ala44Glu	[Dusel et al., 2005]	
c.686G>A	p.Arg229Gln[b]	[Tsukaguchi et al., 2002]	rs61747728
c.709G>C	p.Glu237Gln[a]	[Caridi et al., 2005]	rs146906190
c.725C>T	p.Ala242Val	[Weber et al., 2004]	rs61747727
c.790G>C	p.Glu264Gln	[Santin et al., 2011]	rs179523615
Synonymous change			
c.87C>G	p.Ala29Ala	[Lahdenkari et al., 2005]	rs12123397
c.102G>A	p.Gly34Gly	[Karle et al., 2002]	rs1079292
c.159C>G	p.Thr53Thr	[Berdeli et al., 2007]	
c.288C>T	p.Ser96Ser	[Wu et al., 2001]	rs3738423
c.289C>T	p.Ser96Ser	[Dusel et al., 2005]	
c.393T>C	p.Tyr131Tyr	[Berdeli et al., 2007]	
c.408A>T	p.Ile136Ile	[Bakr et al., 2008]	
c.477G>C	p.Leu159Leu	[Berdeli et al., 2007]	
c.610C>T	p.Leu204Leu	[Gbadegesin et al., 2007]	rs199837664
c.891G>A	p.Ala297Ala	[Dusel et al., 2005]	rs5005771
c.930G>A	p.Glu310Glu	[Berdeli et al., 2007]	
c.951T>C	p.Ala317Ala	[Boute et al., 2000]	
c.954T>C	p.Ala318Ala	[Karle et al., 2002]	rs1410592
c.1038A>G	p.Leu346Leu	[Boute et al., 2000]	rs3818587
c.1206C>G	3′UTR	[Dusel et al., 2005]	rs1410591
c.1309A>G	3′UTR	[Dusel et al., 2005]	rs1410590
c.1352G>A	3′UTR	[Dusel et al., 2005]	rs2274623
c.1410A>G	3′UTR	[Dusel et al., 2005]	rs2274622
c.1580G>A	3′UTR	[Dusel et al., 2005]	rs1060775
c.1589G>T	3′UTR	[Dusel et al., 2005]	rs78606873
Noncoding SNP			
Intronic changes			
IVS3+9insA		[Dusel et al., 2005]	
IVS3−144C>T		[Dusel et al., 2005]	rs41267604
IVS3−46C>T		[Aucella et al., 2005]	rs12401711
IVS3−32T>G		[Dusel et al., 2005]	rs138367742
IVS3−31T>C		[Dusel et al., 2005]	rs16854341
IVS3−21C>T		[Aucella et al., 2005]	rs12401708
IVS4−97C>T		[Dusel et al., 2005]	rs112373866
IVS5+110T>A		[Dusel et al., 2005]	rs73051834
IVS7+7A>G		[Caridi et al., 2001]	rs115778946
IVS7+132A>G		[Dusel et al., 2005]	
IVS7+261C>T		[Dusel et al., 2005]	
IVS7+304T>C		[Dusel et al., 2005]	
IVS7−74G>C		[Dusel et al., 2005]	rs2274624
Promoter changes			
−2169A>T		[Dusel et al., 2005]	rs72706703
−1842C>G		[Dusel et al., 2005]	rs188059625
−1709G>A		[Fu et al., 2008]	rs75264651
−1707delCT		[Dusel et al., 2005]	
−1628G>C		[Dusel et al., 2005]	rs36057553
−1441G>A		[Dusel et al., 2005]	rs76801034
−1376G>A		[Dusel et al., 2005]	rs115824309
−1363T>C		[Dusel et al., 2005]	
−1147_1144del4		[Dusel et al., 2005]	rs72049094
−1112G>A		[Dusel et al., 2005]	
−1087A>C		[Dusel et al., 2005]	rs61378320
−1000A>T		[Fu et al., 2008]	rs2026014
−999T>A		[Dusel et al., 2005]	
−770C>T		[Dusel et al., 2005]	rs61583057
−748C>T		[Dusel et al., 2005]	rs115040496
−704G>A		[Dusel et al., 2005]	rs57596064
−670C>T		[Fu et al., 2008]	rs3829795
−537_531del7		[Dusel et al., 2005]	rs146791300
−494G>A		[Dusel et al., 2005]	rs76432654
−486_485insA		[Dusel et al., 2005]	rs150839022
−440T>C		[Dusel et al., 2005]	rs74520747
−364C>T		[Dusel et al., 2005]	
−185T>C		[Dusel et al., 2005]	rs115256710
−116C>T		[Fu et al., 2008]	rs1079291
−52C>G		[McKenzie et al., 2007]	rs78541594
−51G>T		[Yu et al., 2005]	rs12406197

Mutation numbering is based on the cDNA reference sequence (GenBank accession number NM_014625.2).
[a]Variant initially published as a mutation, then reclassified as a polymorphism.
[b]Indicates mutations with functional studies.

months, range from 0 to 16 years) [Weber et al., 2004; Berdeli et al., 2007; Hinkes et al., 2008]. Among patients carrying two pathogenic mutations, the lowest mean age at onset of 21 months was found in patients with two p.R138Q (c.413G>A) alleles, or with truncating mutations (nonsense or frameshift) in the homozygous or compound heterozygote states [Weber et al., 2004; Hinkes et al., 2008]. In congenital and infantile forms of SRNS in European children, ≥1 truncating mutation(s) or p.R138Q (c.413G>A) in the homozygous state were found in 94.1% of cases [Hinkes et al., 2007].

The p.R168H (c.503G>A) mutation was also associated with an early-onset SRNS [Berdeli et al., 2007]. A total of 21 patients with the p.R168H (c.503G>A) in the homozygous or compound heterozygous state and an available age at onset were reported. Of whom 13 patients (61%) developed SRNS before 6 months of age, six of them bearing homozygous p.R168H (c.503G>A) mutation, and six bearing the association of p.R138Q (c.413G>A) and p.R168H (c.503G>A). In contrast, the p.V180M (c.538G>A) was associated with a later onset of SRNS [Weber et al., 2004; Hinkes et al., 2008]. Indeed, in 22 patients with the p.V180M (c.538G>A) and an available age at onset, 20 were diagnosed after 8 years. Some biological data may explain these genotype–phenotype correlations [Roselli et al., 2004]. Indeed, the p.R138Q (c.413G>A) and p.R168H (c.503G>A) mutations have been demonstrated to result in retention of mutant podocin in the ER, whereas the p.V180M (c.538G>A) allows the targeting of the mutant podocin to the cell membrane. It is postulated that mutants targeted to the plasma membrane such as the p.V180M (c.538G>A) may be able to assume a partial function sufficient to maintain normal glomerular function until later in life [Roselli et al., 2004] and lead to a less severe disease.

Patients carrying the p.R229Q (c.686G>A) non-neutral polymorphism associated with a pathogenic mutation had a significantly later onset of disease [88.1 ± 12.6 months in Weber et al. (2004), 80.88 months in Hinkes et al. (2008), and represented almost all adult onset cases with *NPHS2* mutations [Tsukaguchi et al., 2002; McKenzie et al., 2007; Machuca et al., 2009]. Indeed, 49 adult patients (age >18 years) with at least one pathogenic mutation have been reported, among whom 47 (95%) had the p.R229Q (c.686G>A) variant associated with one deleterious mutation. Almost all the pathogenic mutations associated to the p.R229Q (c.686G>A) were missense mutations clustered in the C-terminal part of the protein and the most frequently associated mutation was the p.A284V (c.851C>T), especially for patients of South American [Machuca et al., 2009] and Spanish [Santin et al., 2011] descent. Whereas patients with homozygous or compound heterozygous *NPHS2* mutations progressed to end-stage kidney disease at a mean age of 8.6 ± 5.2 years, patients bearing the p.R229Q (c.686G>A) allele and one pathogenic mutation developed end-stage kidney disease significantly later at a mean age of 26.1 ± 18.9 years [Machuca et al., 2009].

Extrarenal Manifestations

To date, podocin has not been shown to be expressed at the protein level outside of the podocyte, although at the RNA level, a weak expression was detected in the adult testis, fetal heart, and fetal liver [Boute et al., 2000]. Rare cardiac and ophthalmologic abnormalities [Frishberg et al., 2006; Ozaltin et al., 2008; Sonmez et al., 2008; Machuca et al., 2010] have been described in patients with *NPHS2* mutations. The spectrum of cardiac abnormalities was essentially represented by left ventricular hypertrophy and pulmonary stenosis. For most children, these cardiac anomalies were detected at the time of diagnosis of SRNS, while they had normal blood pressure

and preserved renal function. Most patients were asymptomatic, and had homozygous *NPHS2* mutations (p.R138X [c.412C>T], p.R138Q [c.413G>A], and p.V180X [c.419delG]). However, in the two studies that addressed this issue, the prevalence was very different ranging from 89% (18/20) of the Israeli Arab children tested [Frishberg et al., 2006] to 28% [Caridi et al., 2007] in a European children series of 12 patients. Regarding the eye involvement in *NPHS2* mutations, Ozaltin et al. (2008) found at least one ocular abnormality in 4/5 patients with homozygous *NPHS2* mutations mainly exotropia ($n = 1$), anisometropic amblyopia ($n = 2$), and Mittendorf's dot ($n = 2$). These important ocular findings are surprising as it has never been described in any other series. It also should be noted that these observations have been made in patients originating from highly consanguineous populations, so we cannot exclude coinheritance of two separate genetic defects. Therefore, clinical significance and prognosis of these nonspecific extrarenal abnormalities, with an unknown prevalence, should be evaluated by further studies in larger cohorts to draw certain conclusions.

Geographical Origin

Not all ethnic groups share the same frequency of mutations in this gene. Indeed, many studies strengthen the notion that *NPHS2* mutations are not a major cause of SRNS in Israeli Jewish children or in some Asian countries with a prevalence of: 0/13 in Israeli Jewish [Frishberg et al., 2002], 0/52 in Japanese children [Maruyama et al., 2003; Furue et al., 2008], 0/70 in South Korean children [Cho et al., 2008], 0/15 mixed Japenese and Korean families, 0/20 in Iranian children [Otukesh et al., 2009], 2/145 in Pakistani children [Abid et al., 2012], and 1/25 in Indian children [Vasudevan et al., 2012]. The same trend is described in African Americans with 0/18 children with SRNS, and 0/247 late-onset FSGS [McKenzie et al., 2007; Chernin et al., 2008]. Regarding Chinese children, in addition to single patient reports [Yu et al., 2005; Mao et al., 2007; Sun et al., 2009], the reported prevalence is variable, from 1/23 to 4/22 patients [Yu et al., 2005; Mao et al., 2007], which does not allow definitive conclusions about the prevalence in China. Even though the sample size of the previous studies was relatively small, a first overview suggests that *NPHS2* mutations do not seem to be a major cause of SRNS in African-American and Asian patients, and therefore, a systematic screening for *NPHS2* mutations in these SRNS children can be questioned.

Many studies point to the interethnic differences in the spectrum of *NPHS2* mutations, and interestingly some mutations have been found with a special geographical pattern suggesting that some founder effects may exist. Indeed, the 18 patients of Israeli Arab descent reported in the literature with SRNS and *NPHS2* mutations were all homozygous for the p.R138X (c.412C>T) [Frishberg et al., 2002; Frishberg et al., 2006; Becker-Cohen et al., 2007]. The p.V260E (c.779T>A) seemed to be predominant in patients originating from the Comoros and the sultanate of Oman. In our personal cohort, we identified 12 different families bearing the p.V260E (c.779T>A) in the homozygous state, six of them originating from Comoros, five from the sultanate of Oman, and the last one from Bahrain. Noteworthily, some Omani migrated to both Bahrain and the Comoros in the 17th century, resulting in a mixed population due to geographic proximity. The p.A284V (c.851C>T) was especially observed in South American and/or Spanish patients. In fact, 35 patients were reported in the literature with the association of p.A284V (c.851C>T) and p.R229Q (c.686G>A) and an available ethnicity, of whom 26 originated from Spain, South America, or were Hispanic Americans [Chernin et al., 2008; Tonna et al., 2008; Machuca et al., 2009; Santin et al., 2011]. The p.G140DfsX41 (c.419delG) is particularly prevalent in some Mediterranean countries such as France, Italy, Turkey, and Cyprus [Caridi et al., 2001; Ozcakar et al., 2006; Voskarides et al., 2008]. It also appeared that the p.P118L (c.353C>T) mainly affected individuals of Turkish descent. Indeed, 20 patients with this particular mutation in the homozygous or compound heterozygous state have been reported. Among them, ethnicity was available in 10 patients, of whom nine were of Turkish. We also made another personal observation about patients bearing the p.V180M (c.538G>A) mutation in the homozygous state that we found in four families from North-Africa. The last correlation is the well-known high prevalence of the p.R138Q (c.413G>A) in European patients that roughly represented 30%–40% of all the mutations detected in Europe.

NPHS2 Heterozygous Carriers

Seventy-nine heterozygous mutation carriers with SRNS were reported. The clinical significance of heterozygous *NPHS2* mutations is unclear since they theoretically segregate as an autosomal-recessive trait requiring defects on both alleles to induce a pathologic effect. A possible explanation is the presence of another mutation in the regulatory sequences or noncoding regions yet to be identified. Based on this theory, we retrospectively studied five patients of our personal cohort with SRNS and a heterozygous mutation in the *NPHS2* gene using a custom array targeting *NPHS1, NPHS2, WT1*, and *INF2*, searching for a deletion of one or several exons in the second allele. Nevertheless, no rearrangement was found in the analyzed patients. However, and given the genetic heterogeneity of SRNS, other mutations in other genes may also be likely.

Clinical Relevance

SRNS is a paradigmatic disease to illustrate the major clinical consequences that can arise from genetic testing. The clinical interest of diagnosing *NPHS2* mutations in SRNS is not only the genetic counseling of families with respect to family planning but also the suitability of relatives as organ donors. Most importantly, mutated patients can be spared unnecessary hazardous immunosuppressive treatment, and have a very low risk of recurrence after kidney transplantation. Indeed, in hereditary forms of SRNS and unlike the most prevalent immune-mediated forms, the protein loss is caused by a structural abnormality of the podocyte and it seems reasonable to postulate that hereditary cases of SRNS cannot respond to steroids or other immunosuppressive drugs, and should not recur after transplantation. Although this was true in most patients with *NPHS2* mutations, there were some intriguing data such as the sensitivity to steroids found in a 38-year-old patient with compound heterozygous mutations p.[R286TfsX17];[R229Q] (c.[855_856del];[686G>A]) [He et al., 2007], or the response to immunosuppressive drugs in 19 patients. However, in all reported cases, only a partial remission of SRNS was observed (proteinuria between four and40 mg/m^2/hr and albumin >30 g/l). All the patients had been treated with at least cyclosporine or tacrolimus and/or angiotensin-converting enzyme inhibitor (ACEi). This partial response observed can be explained, as ACEi is a family of drugs that lower urinary protein excretion by reducing the glomerular filtration rate, whereas cyclosporine has been shown to have a direct effect on the actin cytoskeleton of kidney podocytes [Faul et al., 2008].

As expected, most papers found a very low recurrence rate of SRNS in patients with compound heterozygous or homozygous

mutations compared with the nongenetic forms [Karle et al., 2002; Ruf et al., 2004; Weber et al., 2004; Jungraithmayr et al., 2011]. Out of the 174 patients published in the literature with pathogenic homozygous and or compound heterozygous podocin mutations, who received a renal graft, and for which the posttransplantation outcome is available, only 16 cases (9%) of recurrent SRNS have been reported so far, a prevalence far less important than the recurrence rate found in idiopathic forms of SRNS, which is of 30%–50% [Senggutuvan et al., 1990; Crosson, 2007]. One hypothesis proposed is that the recurrence might be induced by the formation of autoantibodies against podocin. A total of five patients were tested in the literature for these antibodies and no evidence of anti-podocin antibodies in the recipient serum was detected to date [Bertelli et al., 2003; Weber et al., 2004; Becker-Cohen et al., 2007].

Animal Models

Two mouse models of *NPHS2* constitutive mutations have been generated by constitutively inactivating the *Nphs2* gene [Roselli et al., 2004] or via the targeted knock-in of the murine equivalent of the human mutation p.R138Q (c.413G>A) [Philippe et al., 2008] (*Nphs2*$^{R140Q/R140Q}$). The phenotypic consequences of podocin loss in these two models were similar. These mice manifest severe proteinuria at birth and develop massive foot process effacement, as well as lesions of mesangiolysis and mesangial sclerosis and succumb to end-stage kidney disease within the first 5 weeks of life [Roselli et al., 2004; Philippe et al., 2008]. These effects were modulated not only by genetic background, as previously shown in other murine models of FSGS [Fogo, 2003] but also, in *Nphs2* null mice, by the maternal environment in which newborn mice are nurtured [Ratelade et al., 2008]. However, the early demise of these mice precluded more intensive study of the role of podocin in the mature kidney as well as the test of novel therapeutic strategies. Therefore, a novel model was generated in which podocin is conditionally inactivated specifically in podocytes in the mature kidney using the tamoxifen-inducible *Cre-loxP* system [Mollet et al., 2009]. Conditional inactivation of podocin in the mature kidney resulted in the development of progressive renal insufficiency and NS leading to death at a median time of 11 weeks after tamoxifen injection. Rather than displaying diffuse mesangial sclerosis lesions, the mice demonstrated lesions of FSGS, hence recapitulating the most common lesions found in renal biopsies of patients bearing *NPHS2* mutations. The crucial role of podocin in maintaining GFB integrity was further confirmed in zebrafish where knockdown of podocin using morpholino led to the absence of slit diaphragms and an altered plasma filtration [Kramer-Zucker et al., 2005]. Very recently, it has also been shown that *Mec2*, encoding the *Drosophila* homolog of podocin, is required for the function of the insect nephrocyte, which is a podocyte-like cell with a filtration slit diaphragm [Zhang et al., 2013].

Conclusion

Mutations in the *NPHS2* gene are responsible for congenital, infantile, childhood, and adult-onset SRNS. Most of the 126 mutations reported to date are missense mutations, but interethnic variability is reported in the frequency and the type of mutations. The classical phenotype is an early-onset "multidrug-resistant" NS with evolution to end-stage kidney disease and a very low recurrence rate after renal transplantation. This review gives a first exhaustive overview of all the reported *NPHS2* mutations, as well as some genotype–phenotype correlations.

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