Clinical Utility of Genetic Testing in Children and Adults with Steroid-Resistant Nephrotic Syndrome

Sheila Santín,* Gemma Bullich,* Bárbara Tazón-Vega,* Rafael García-Maset,[†] Isabel Giménez,[†] Irene Silva,*
Patricia Ruíz,* José Ballarín,[†] Roser Torra,[†] and Elisabet Ars*

Summary

Background and objectives The increasing number of podocyte-expressed genes implicated in steroid-resistant nephrotic syndrome (SRNS), the phenotypic variability, and the uncharacterized relative frequency of mutations in these genes in pediatric and adult patients with SRNS complicate their routine genetic analysis. Our aim was to compile the clinical and genetic data of eight podocyte genes analyzed in 110 cases (125 patients) with SRNS (ranging from congenital to adult onset) to provide a genetic testing approach.

Design, setting, participants, & measurements Mutation analysis was performed by sequencing the *NPHS1*, *NPHS2*, *TRPC6*, *CD2AP*, *PLCE1*, *INF2*, *WT1* (exons 8 and 9), and *ACTN4* (exons 1 to 10) genes.

Results We identified causing mutations in 34% (37/110) of SRNS patients, representing 67% (16/24) familial and 25% (21/86) sporadic cases. Mutations were detected in 100% of congenital-onset, 57% of infantile-onset, 24 and 36% of early and late childhood-onset, 25% of adolescent-onset, and 14% of adult-onset patients. The most frequently mutated gene was *NPHS1* in congenital onset and *NPHS2* in the other groups. A partial remission was observed in 7 of 26 mutation carriers treated with immunosuppressive agents and/or angiotensin-converting enzyme inhibitors. Patients with *NPHS1* mutations showed a faster progression to ESRD than patients with *NPHS2* mutations. None of these mutation carriers relapsed after kidney transplantation.

Conclusions We propose a genetic testing algorithm for SRNS based on the age at onset and the familial/sporadic status. Mutation analysis of specific podocyte-genes has a clinical value in all age groups, especially in children.

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*Molecular Biology Laboratory, Fundació Puigvert, Universitat Autònoma de Barcelona, REDinREN, Instituto de Investigación Carlos III, Barcelona, Spain; and [†]Nephrology Department, Fundació Puigvert, Universitat Autònoma de Barcelona, REDinREN, Instituto de Investigación Carlos III, Barcelona, Spain

Correspondence: Dr. Roser Torra, Nephrology Department, Fundació Puigvert, Cartagena 340-350, 08025 Barcelona, Spain. Phone: 34-93-416-97-00; Fax: 34-93-416-97-30; E-mail: rtorra@ fundacio-puigvert.es. and Dr. Elisabet Ars, Molecular Biology Laboratory, Fundació Puigvert, Cartagena 340-350, 08025 Barcelona, Spain. Phone: 34-93-416-97-00; Fax: 34-93-416-97-30; E-mail: ears@fundacio-puigvert.es

Introduction

Nephrotic syndrome (NS) is characterized by proteinuria, hypoalbuminemia, edema, and dyslipidemia. Approximately 10% of children and 40% of adults with idiopathic NS are steroid resistant (SRNS) and progress to ESRD (1–5). In these cases, renal histology typically shows focal segmental glomerulosclerosis (FSGS). The remaining patients are steroid responsive, with a favorable long-term prognosis (6). Mutations in several single genes expressed by glomerular podocytes have been identified in patients with SRNS (7).

The phenotypic spectrum caused by mutations in these genes is wider than initially expected. *NPHS1*, encoding for nephrin, was identified as the causative gene in the most common type of congenital NS (CNS), CNS of the Finnish-type (CNF) (8). Recently, *NPHS1* mutations have also been reported in childhood- (9) and adult-onset SRNS (10). *NPHS2*, encoding for podocin, was identified as the causative gene in early-onset autosomal-recessive (AR) SRNS (11). Nevertheless, *NPHS2* mutations have also been found

in CNS (12–15) and in a few cases of adult-onset FSGS (16–18). Mutations of phospholipase C-ε (*PLCE1*) have been identified in 10 to 50% of patients with NS and diffuse mesangial sclerosis (DMS) and in 12% of familial AR FSGS (19–22).Mutations in exons 8 and 9 of the Wilms tumor suppressor gene (*WT1*) were discovered in patients with syndromic SRNS (23,24) but can also cause isolated SRNS (25,26). Mutations in *LAMB2*, encoding for lamini-β2 and implicated in Pierson syndrome (27), have been found in one family with CNS (28). Finally, mutations in *TRPC6* (encoding for transient receptor potential channel 6) (29,30), *ACTN4* (α-actinin-4) (31), *CD2AP* (CD2-associated protein) (32), and lately *INF2* (33) were detected in several families with autosomal-dominant (AD) FSGS and adult-onset NS. However, some studies have reported mutations in these genes in a small number of patients with childhood-onset FSGS (34–40).

The genetic heterogeneity of SRNS, the significant phenotypic variability, and the lack of knowledge of the relative frequency of mutations in these genes in pediatric and adult patients with NS hinder the rou-

tine genetic analysis for SRNS. In fact, there are only a few studies searching for mutations in several of these genes and only in pediatric patients (14–43). The aim of this study was to compile the clinical and genetic data of eight podocyte genes analyzed in our Spanish cohort of patients with NS (ranging from congenital to adult onset) to propose an adequate genetic testing approach.

Materials and Methods
Patients
From a cohort of 204 Spanish patients with primary NS, we excluded patients who responded to steroid or immunosuppressive treatment and those developing steroid resistance at a later stage of the disease or with recurrence after kidney transplant, because we considered that an immune pathogenesis was the most likely cause of the disease in these cases. Thus, 125 patients belonging to 110 families with CNS or primary FSGS/DMS or/and SRNS were included. Diagnosis of NS and, when applicable, response to steroid treatment was determined following published criteria (1). A partial response was defined as the disappearance of edema, an increase in the serum albumin concentration, and the persistence of proteinuria below nephrotic range. Prematurely born patients presenting with a large placenta and severe proteinuria at birth or with proven renal biopsy were considered as CNF ($n = 10$). Twenty-eight patients belonging to 18 families were classified as AR SRNS, defined as families with either two or more affected children ($n = 11$) or one affected individual in consanguineous families ($n = 7$). Ten patients belonging to six families showed evidence of AD disease because multiple generations were affected. The remaining 86 patients were considered sporadic cases. The age at onset of NS, response to treatment, histology, and clinical course from first presentation to last clinical examination are listed in Table 1. We will refer to patients studied when assessing clinical data and to families studied when assessing genetic data, because siblings may follow a different clinical course but should bear the same mutations.

Mutation Analysis
Genomic DNA was isolated from peripheral blood cells using the salting-out method (44) after obtaining informed consent from affected individuals or their parents. The study was approved by the institutional review boards of each participating hospital. Mutation analysis was performed by direct sequencing of all 29 exons of NPHS1, all 8 exons of NPHS2, all 13 exons of TRPC6, all 34 exons of PLCE1, all 22 exons of INF2, exons 1 to 10 of ACTN4, and exons 8 and 9 of WT1. We only tested exons 8 and 9 of WT1 and exons 1 to 10 of ACTN4 because mutations in these genes have only been reported in these exons (26,37). Exons were amplified by PCR using intron-flanking primers, as described previously (11,19,25,29–31,33,45–47). Primer data are available on request. In addition, total RNA was extracted from peripheral blood cells with TRIzol (Invitrogen, Carlsbad, CA). Total RNA (1 µg) was reverse transcribed with the High-Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA) using random hexamers. The entire CD2AP cDNA was amplified in three overlapping fragments with primers designed with Primer

Express software (Applied Biosystems). All RT-PCR products and exons were sequenced using the Big Dye DNA Sequencing kit v1.1 (Applied Biosystems) and an ABI PRISM 3100-Avant genetic analyzer (Applied Biosystems).

Sequencing of both strands was performed for all sequence variants detected. Segregation of these changes with disease was assessed from all available family members. Unpublished missense mutations were screened in ≥200 unrelated controls of matched ethnic origin either by direct sequencing or by restriction enzyme digestion.

We first analyzed the NPHS2 gene in the entire group of patients. Afterward, we examined WT1 in those cases without clear causative mutations. The NPHS1 gene was studied in those cases without WT1 mutations. Then, patients without pathogenic mutations in the previous genes were subsequent analyzed for the ACTN4, CD2AP, and TRPC6 genes. Finally, we analyzed PLCE1 only for the familial AR cases and INF2 for the AD ones. We did not evaluate the LAMB2 gene because all our patients with DMS were explained by causative mutations in other genes. Partial data of TRPC6, NPHS1, and NPHS2 mutation analysis have been previously published (10,34,48).

Classification of Amino Acid Substitutions
To evaluate the pathogenicity of amino acid substitutions, we developed an in silico scoring system (10,34,48), as described previously for other genes (49–51). We classified patients with two NPHS1 mutations as (1) patients with two severe mutations and (2) patients with at least one mild mutation, as suggested elsewhere (9,10). In addition, we classified patients with two NPHS2 mutations as (1) patients with two pathogenic mutations and (2) patients with one pathogenic mutation plus the p.R229Q variant, as previously reported (52,53). Additional information about the classification of amino acid substitutions is provided in Supplementary Table 1.

Statistical Analyses
Data are expressed as mean ± SD or median and range. Comparisons between continuous values were made using the Mann–Whitney U test (SPSS Software), and $P < 0.05$ was considered significant.

Results
Podocyte Gene Mutation Frequencies
Mutation analysis of NPHS1, NPHS2, WT1, TRPC6, CD2AP, ACTN4, PLCE1 (only for AR familial FSGS), and INF2 (only for AD familial FSGS) showed disease-causing mutations in 34% (37 of 110) of all SRNS cases (Table 2). Recessive pathogenic mutations in NPHS1 were found in 13.5% (15 of 110) of cases. Mutations in NPHS2 were detected in 12% (13 of 110) of patients. Dominant mutations in WT1 and TRPC6 were found in 4.5 and 3% of cases, respectively. No pathogenic mutations in ACTN4 and CD2AP genes were identified in our cohort of patients. Three cases were carriers of a single NPHS1 ($n = 1$) or NPHS2 ($n = 2$) pathogenic mutation, which cannot be considered causative by itself. In AR FSGS families, no PLCE1 mutation was found, whereas one INF2 mutation was identified in one AD family (one of six).

Table 1. Clinical data according to mutation status for 125 patients from 110 families with NS

	Age at Onset of NS (Mean ± SD [years], Median, Range [years])	Response to CR (No. of Patients with NR/NG/NA)	Response to IS with/without ACEI (No. of Patients with NR/PR/NG/NA)	Histology (No. of Patients with CNF/DMS/MCNS/FSGS/NP)	ESRD (No. of patients [%])	Age at ESRD (Mean ± SD [years], Median, Range [years])	Progression Time to ESRD (Mean ± SD [years], Median, Range [years])	Kidney Transplant (No. of patients [%])
Total	17.5 ± 16.5; n = 125, 14.0 (0.0 to 56.0)	78/32/15	50/7/36/32	3/3/2/108/9	69 (55)	27.3 ± 19.1; n = 69, 24.0 (0.2 to 61.0)	5.3 ± 5.5; n = 69, 3.0 (0.0 to 26.0)	53 (76)
Congenital onset (0 to 3 months)	0.1 ± 0.1; n = 15, 0.1 (0.0 to 0.2)	4/11/0	1/0/13/1	3/3/0/2/7	7 (47)	1.4 ± 1.2; n = 7, 1.0 (0.2 to 4.0)	1.4 ± 1.2; n = 7, 1.0 (0.2 to 3.9)	5 (71)
Infantile onset (4 to 12 months)	0.8 ± 0.2; n = 8, 0.8 (0.3 to 1.0)	7/0/1	4/2/0/2	0/0/0/7/1	5 (63)	5.6 ± 0.9; n = 5, 5.0 (5.0 to 7.0)	4.8 ± 0.9; n = 5, 4.3 (4.2 to 6.4)	3 (60)
Early childhood onset (13 months to 5 years)	3.4 ± 1.3; n = 24, 3.3 (1.2 to 5.7)	22/1/1	15/1/1/7	0/0/1/22/1	9 (37)	8.3 ± 5.2; n = 9, 6.5 (3.0 to 17.0)	4.5 ± 5.0; n = 9, 1.9 (1.0 to 13.0)	9 (100)
Late childhood onset (6 to 12 years)	8.1 ± 1.9; n = 13, 7.5 (6.0 to 12.0)	13/0/0	12/1/0/0	0/0/0/13/0	4 (31)	10.0 ± 3.4; n = 4, 8.5 (8.0 to 15.0)	2.3 ± 1.9; n = 4, 1.5 (1.0 to 5.0)	2 (50)
Adolescent onset (13 to 17 years)	15.6 ± 1.3; n = 9, 16.0 (13.0 to 17.0)	6/2/1	5/0/2/2	0/0/0/9/0	7 (78)	22.7 ± 4.4; n = 7, 24.0 (17.0 to 29.0)	7.1 ± 4.9; n = 7, 8.0 (2.0 to 13.0)	6 (86)
Adult onset (>18 years)	33.9 ± 10.6; n = 56, 33.0 (18.0 to 56.0)	26/18/12	12/4/20/20	0/0/1/55/0	37 (66)	41.1 ± 13.0; n = 37, 40.0 (21.0 to 61.0)	6.4 ± 6.4; n = 37, 3.5 (0.0 to 26.0)	26 (70)
Patients with 2 NPHS1 mutations	2.6 ± 6.8; n = 16, 0.1 (0.0 to 27.0)	5/10/1	3/1/12/0	3/1/0/6/6	6 (35)	1.5 ± 1.3; n = 6, 1.5 (1.3; n = 6)	1.5 ± 1.3; n = 6	4 (67)
two severe mutations	0.1 ± 0.1; n = 12, 0.0 (0.0 to 0.1)	2/10/0	0/0/12/0	3/1/0/2/6	6 (50)	1.5 ± 1.3; n = 6, 1.0 (0.2 to 4.0)	1.5 ± 1.3; n = 6, 1.0 (0.2 to 3.9)	4 (67)
at least one mild mutation	10.3 ± 11.5; n = 4, 6.5 (1.0 to 27.0)	3/0/1	3/1/0/0	0/0/0/4/0	0 (0)	–	–	–
Patients with 2 NPHS2 mutations	11.8 ± 11.6; n = 19, 8.0 (0.02 to 39.0)	19/0/0	13/3/0/3	0/0/1/16/2	13 (68)	19.9 ± 14.5, n = 13, 15.0 (5.0 to 50.0)	6.5 ± 3.8, n = 13, 5.0 (1.0 to 13.0)	10 (77)
two pathogenic mutations	2.8 ± 2.6; n = 10, 2.3 (0.02 to 8.0)	10/0/0	6/2/0/2	0/0/1/7/2	6 (60)	7.6 ± 2.5, n = 6, 7.0 (5.0 to 12.0)	4.2 ± 2.7, n = 6, 3.7 (1.0 to 8.0)	4 (67)
one pathogenic mutation + p.R229Q	21.8 ± 9.1; n = 9	9/0/0	7/1/0/1	0/0/0/9/0	7 (78)	30.4 ± 11.8, n = 71	8.4 ± 3.7, n = 7	6 (86)

Table 1. (Continued)

	Age at Onset of NS (Mean ± SD [years], Median, Range [years])	Response to CR (No. of Patients with NR/NG/NA)	Response to IS with/without ACEI (No. of Patients with NR/PR/NG/NA)	Histology (No. of Patients with CNF/DMS/MCNS/FSGS/NP)	ESRD (No. of patients [%])	Age at ESRD (Mean ± SD [years], Median, Range [years])	Progression Time to ESRD (Mean ± SD [years], Median, Range [years])	Kidney Transplant (No. of patients [%])
Patients with WT1 mutations	19.0 (10 0.0–39.0) 1.3 ± 1.2; n = 5	3/2/0	3/2/0/0	0/2/0/3/0	2 (40)	26.0 (15.0 to 50.0) 0.3 ± 0.2, n = 2	9.0 (4.0 to 13.0) 0.2 ± 0.2, n = 2	1 (50)
Patients with TRPC6 mutations	1.0 (0.2 to 2.8) 24.4 ± 16.9; n = 4	3/1/0	0/1/1/2	0/0/0/3/1	1 (25)	3.0 (1.0 to 5.0) 39.0, n = 1	2.4 (0.7 to 4.0) 14.0, n = 1	1 (100)
Patients with INF2 mutations	25.0 (7.2 to 41.0) 32.0 ± 16.6; n = 5 31.0 (16.0 to 50.0)	0/5/0	0/0/5/0	0/0/0/5/1	4 (80)	36.5 ± 17.3, n = 4 33.5 (21.0 to 58.0)	4.5 ± 4.0, n = 4 4.5 (1.0 to 8.0)	4 (100)

CR, corticoids; IS, immunosuppressants; MCNS, minimal change nephrotic syndrome; NA, not available; NG, not given; NP, not performed; NR, no response; PR, partial response.

Table 2. Frequency of pathogenic mutations in NPHS1, NPHS2, WT1, TRPC6, INF2, ACTN4, and CD2AP in 110 unrelated cases with SRNS

Pathogenic Mutations	Total	Congenital Onset (0 to 3 months)	Infantile Onset (4 to 12 months)	Early Childhood Onset (13 months to 5 years)	Late Childhood Onset (6 to 12 years)	Adolescent Onset (13 to 18 years)	Adult Onset (>18 years)	Familial SRNS	Sporadic SRNS
No. of patients (cases)	125 (110)	15 (15)	8 (7)	24 (21)	13 (11)	9 (8)	56 (48)	35 (24)	90 (86)
NPHS1, No. of cases (%)	15 (13.5)	12 (80)	1 (14)	0 (0)	1 (9)	0 (0)	1 (2)	6 (25)	9 (11)
NPHS2, No. of cases (%)	13 (12)	1 (7)	2 (29)	3 (14)	2 (18)	1 (12.5)	4 (8)	8 (34)	5 (6)
WT1, No. of cases (%)	5 (4.5)	2 (13)	1 (14)	2 (10)	0 (0)	0 (0)	0 (0)	0 (0)	5 (6)
TRPC6, No. of cases (%)	3 (3)	0 (0)	0 (0)	0 (0)	1 (9)	0 (0)	2 (4)	1 (4)	2 (2)
ACTN4, No. of cases (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
CD2AP, No. of cases (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
PLCE1 No. of cases (%)	0 (0)	0 (0)	0 (0)	0 (0)	ND	ND	ND	0 (0)	ND
INF2 No. of cases (%)	1 (1)	ND	ND	ND	ND	1 (12.5)	0 (0)	1 (4)	ND
No. of cases with mutations (%)	37 (34)	15 (100)	4 (57)	5 (24)	4 (36)	2 (25)	7 (14)	16 (67)	21 (25)
No. of cases without mutations (%)	73 (66)	0 (0)	3 (43)	16 (76)	7 (64)	6 (75)	41 (86)	8 (33)	65 (75)

When two members of a single family presented an age of onset for the disease that fell between two different categories, we included both in the category of the patient presenting the earlier age of onset for NS. To calculate the percentage of mutations, we used the number of cases. ND, not determined.

The frequencies and distribution of mutations in podocyte genes differed depending on the age at onset (Table 2; Figure 1A). In congenital onset (onset from 0 to 3 months), mutations were detected in 100% of cases: *NPHS1,* 80% (12 of 15); *NPHS2,* 7% (1 of 15); *WT1,* 13% (2 of 15). In infantile onset (from 4 to 12 months), mutations were found in 57% of patients: *NPHS1,* 14% (1 of 7); *NPHS2,* 29% (2 of 7); *WT1,* 14% (1 of 7). However, we only explained 24% of the early childhood-onset cases (from 13 months to 5 years) caused by mutations in the *NPHS2* (14%, 3 of 21) and *WT1* (10%, 2 of 21) genes and 36% of the late childhood-onset patients (from 4 to 12 years) caused by mutations in the *NPHS1* (9%, 1 of 11), *NPHS2* (18%, 2 of 11), and *TRPC6* (9%, 1 of 11) genes. Finally, we identified pathogenic mutations for 25% (1 *NPHS2;* 1 *INF2*) of adolescent patients (from 13 to 17 years) and for 14% (7 of 48) of adult patients (>18 years): *NPHS1,* 2%; *NPHS2,* 8%; *TRPC6,* 4%.

The mutation detection rate for familial cases was 67% (16 of 24), but only 25% (21 of 86) for sporadic ones (Table 2). In familial cases, the most common mutated gene was *NPHS2* (34%), whereas *NPHS1* (11%) was the most common for nonfamilial cases. If we divide familial cases be-tween AR and AD SRNS, we identified pathogenic muta-tions in 78% (14 of 18) and 33% (2 of 6) of cases, respectively. The frequencies of mutations according to the age at onset were also different between familial and spo-radic cases (Figure 1, B and C). In familial cases, we ex-plained 100% of congenital-onset (*NPHS1* 83%, 5 of 6; *NPHS2* 17%, 1 of 6), 66% of infantile-onset (*NPHS1* 33%, 1 of 3; *NPHS2* 33%, 1 of 3), 40% of early childhood-onset (*NPHS2,* 2 of 5), 100% of late childhood-onset (*NPHS2,* 1 of 1), 100% of adolescent-onset (*NPHS2,* 1 of 2, *INF2* 1 of 2), and 43% of adult-onset cases (*NPHS2* 29%, 2 of 7; *TRPC6* 14%, 1 of 7). In sporadic cases, we also detected pathogenic mutations in 100% of congenital cases (*NPHS1* 78%, 7 of 9; *WT1* 22%, 2 of 9) and in 50% of infantile-onset cases (*NPHS2* 25%, 1 of 4; *WT1* 25%, 1 of 4), but only in 19% of early childhood-onset (*NPHS2* 6%, 1 of 16; *WT1* 13%, 2 of 16), 30% of late childhood-onset (*NPHS1* 10%, 1 of 10; *NPHS2* 10%, 1 of 10; *TRPC6* 10%, 1 of 10), 0% of adolescent-onset (0 of 6), and 10% of adult-onset cases (*NPHS1* 2.5%, 1 of 41; *NPHS2* 5%, 2 of 41; *TRPC6* 2.5%, 1 of 41).

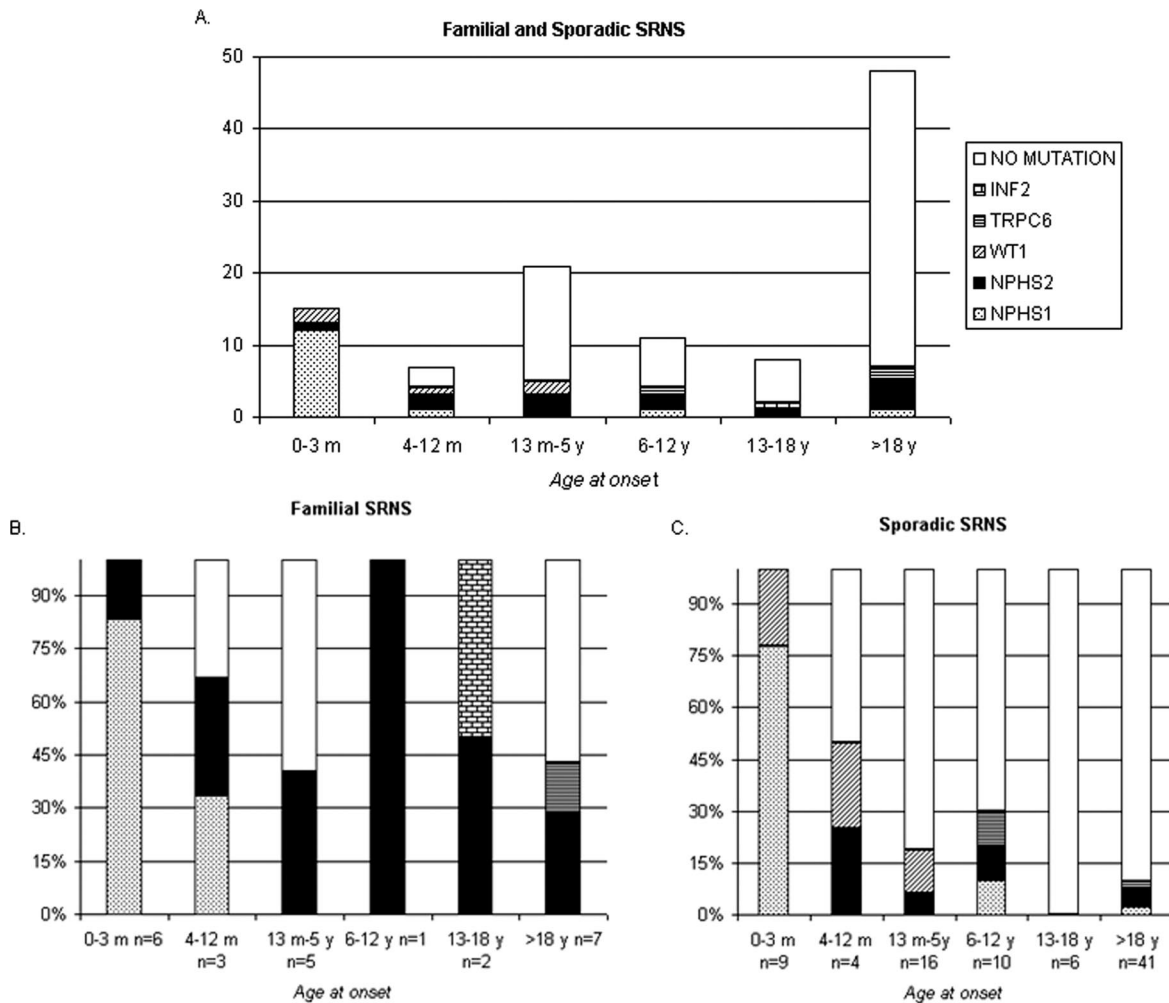A description of all of the mutations detected is given in Supplementary Table 2.

Figure 1. | Age at diagnosis of NS in correlation to detected pathogenic mutations in *NPHS1, NPHS2, WT1, TRPC6,* and *INF2* in 110 families (we did not include the *ACTN4* and *CD2AP* genes because no pathogenic mutations were identified in our cohort). (A) Familial and sporadic cases. (B) Percentage of mutations in familial cases. (C) Percentage of mutations in sporadic cases.

Genotype–Phenotype Correlations

Genotype and age at NS onset. Earlier presentation was found in patients with two severe *NPHS1* mutations, followed by patients with *WT1* mutations and two pathogenic *NPHS2* mutations. Later NS onset was found in patients with *TRPC6* and *INF2* mutations or one pathogenic *NPHS2* mutation in compound heterozygosity with the p.R229Q variant. Patients with *NPHS1* mutations showed significantly earlier onset of NS than patients with two *NPHS2* mutations (median, 0.1 [range, 0.0 to 27.0] *versus* 8.0 years [range, 0.02 to 39.0]; $P < 0.001$; Table 1). This correlation was also present among patients with two severe *NPHS1* mutations compared with patients with two pathogenic *NPHS2* mutations (median 0.0 [range, 0.0 to 0.1] *versus* 2.3 years [range 0.02 to 8.0]; $P < 0.001$). In addition, patients with at least one mild *NPHS1* mutation also manifested the disease earlier than patients with one *NPHS2* pathogenic mutation plus the p.R229Q variant (median 6.5 [range, 1 to 27.0] *versus* 19.0 years [range 10.0 to 39.0]; $P = 0.05$). In contrast, age at onset of NS was later in patients with *WT1* mutations than in patients with two severe *NPHS1* mutations ($P = 0.004$), but there was no significant difference compared with patients with two pathogenic *NPHS2* mutations. Finally, no difference was observed in the age at onset of NS between patients with *TRPC6* mutations (median, 25.0 years; range, 7.2 to 41.0 years) and patients with at least one mild *NPHS1* mutation or one *NPHS2* pathogenic mutation plus the p.R229Q variant.

Genotype and renal histology. Renal biopsy was performed in 10 of 16 patients (62.5%) belonging to 15 families with *NPHS1* mutations, and the most common histology pattern was FSGS (55%, 6 of 11; Table 1; Supplementary Table 1). For patients without FSGS, the histology findings were as follows: CNF, 3 of 11 (27%); DMS, 1 of 11 (9%). Renal biopsy was performed in 17 of 19 patients (90%) belonging to 13 families carrying *NPHS2* mutations, and the histology findings were as follows: FSGS, 16 of 17 (94%); minimal change NS, 1 of 17 (6%). For patients with *WT1* mutations, the histology findings were as follows: FSGS, 3 of 5 (60%); DMS, 2 of 5 (40%). The three patients with *TRPC6* mutations and renal biopsy performed showed FSGS. Five patients of the same family carried one pathogenic *INF2* mutation, and two of them showed FSGS, whereas the remaining three had only four glomeruli in the biopsy with complete sclerosis in one or two of them and small foci of tubular atrophy and interstitial fibrosis.

Genotype and response to treatment. One of four patients with *NPHS1* mutations treated with immunosuppressive treatment exhibited partial response to tacrolimus and angiotensin-converting enzyme inhibitors (ACEIs) (10). Data on response to immunosuppressive and ACEI treatment were available for 16 patients with *NPHS2* mutations, among them being three patients with decreased nephrotic proteinuria (Table 1; Supplementary Table 1). Three patients with *WT1* mutations received immunosuppressive therapy, and none of them experienced a sustained reduction of proteinuria. The remaining two patients with *WT1* mutations were only treated with ACEIs and they partially responded. Only one patient with a *TRPC6* mutation was treated with immunosuppressive

treatment, and she experienced a sustained reduction of proteinuria to subnephrotic levels (34). Patients with *INF2* mutations did not received steroid or immunosuppressive treatment.

Genotype and age at ESRD onset. Age at ESRD was earlier in patients with *NPHS1* mutations than in patients with *NPHS2* mutations ($P < 0.001$; Table 1). Six children with *NPHS1* severe mutations progressed to ESRD within a median time of 1.0 year after the onset of the disease, whereas the group of six children with two pathogenic *NPHS2* mutations developed ESRD after a median time of 3.7 years ($P = 0.002$). Two patients with *WT1* mutations developed ESRD at a median age of 3 years, which was significantly different from the group of two pathogenic *NPHS2* mutations. Only one patient with a *TRPC6* mutation reached ESRD in a time interval of 14 years. Patients with *INF2* mutations reached ESRD in a time interval of 4.5 years.

Discussion

The increasing knowledge of the molecular basis of NS represents a milestone in nephrology but also adds greater complexity to clinical nephrologists' decisions about when and which genetic tests should be indicated. The aim of this study was to provide a practical guideline for genetic testing in SRNS. Our study represents the largest cohort of patients with SRNS undergoing a multistep molecular evaluation of eight podocyte genes and the first to include adult-onset patients with FSGS. Our data confirm not only that mutations in different genes manifest with NS at different ages but also that different mutations in the same gene result in distinct ages of onset for NS. We show here that 34% (37/110) of SRNS patients could be explained by mutations in one of these genes, of whom 67% (16/24) were familial cases and 25% (21/86) were sporadic. We found disease-causing mutations in 100% of congenital-onset cases and in 57% of infantile-onset cases, which is higher than previously described (14,15,42,45). Interestingly, in our Spanish cohort of CNS patients, we identified more pathogenic mutations in *NPHS1* than in *NPHS2*, whereas an equal mutation rate for these two genes was reported in central European patients (15), probably because of the high frequency of the p.R138Q mutation in this region (53). The percentage of patients with mutations decreases as the age at onset increases and the distribution of mutations changes between children with congenital onset (most of them with mutations in *NPHS1*) and other ages of presentation (most of them with mutations in *NPHS2*). In childhood onset, we found a lower rate of mutations than previously published [reviewed by Benoit *et al.* (7)], but in adolescent and adult-onset patients, a higher than expected rate was observed (18,54,55).

The frequency of mutations among these genes in our Spanish cohort and the revision of the literature allowed us to propose a genetic testing approach for diagnostic purposes (Figure 2). In congenital-onset cases, it is important to analyze several podocyte genes in a multistep strategy because of the high chances of finding mutations in these cases. The first step should be to screen for *NPHS1* and, if no mutations are found, screening for *NPHS2* in familial cases and for *WT1* in the sporadic ones. If negative, in
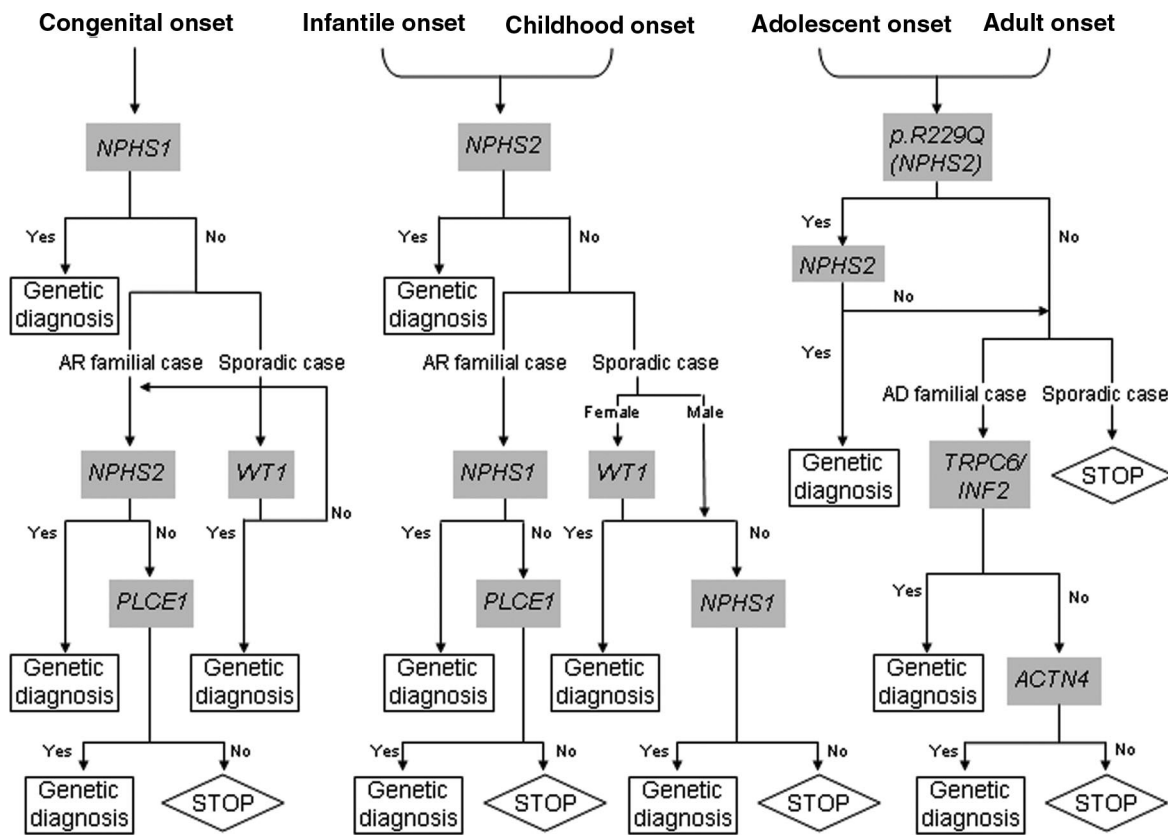
Figure 2. | Genetic approach in children and adults with SRNS.

sporadic congenital-onset cases, the next step should be to analyze the *NPHS2* gene. The last step for both sporadic and familial CNS would be *PLCE1* analysis (42). In infantile onset and in childhood onset, we propose starting with the *NPHS2* gene and, if no mutations are found, screening for *NPHS1* in familial and sporadic cases and *WT1* only in females with sporadic SRNS. Although we have not found pathogenic mutations in *PLCE1* in early-onset SRNS, *PLCE1* analysis would be indicated in those cases with familial DMS/FSGS (20,22). Finally, in adolescent- and adult-onset cases, we suggest screening for p.R229Q in the *NPHS2* gene and, if positive, to analyze the whole gene. We recommend further *TRPC6* and *INF2* testing only for AD late-onset familial FSGS and, if negative, to analyze *ACTN4* (37). Mutations in the *TRPC6* gene have been found in 1 of 41 cases of sporadic adult-onset SRNS, being a rare cause of adult-onset FSGS. It is worth noting the incomplete penetrance of *TRPC6* mutations, limiting its diagnostic value (30,34,56). Because *CD2AP* pathogenicity has been clearly shown only in one patient (38) and we have not found any mutation in our Spanish cohort with SRNS, we do not suggest screening for this gene. Benoit *et al.* (7) have recently proposed a similar genetic testing approach for SRNS, taking into account the renal biopsy. In our cohort of pediatric patients with SRNS, renal histology was not a key tool to indicate the most appropriate gene to analyze because most cases presented with FSGS, but when available, cases with FSGS must be tested for *NPHS1* and *NPHS2* and cases with DMS must first be tested for *WT1* followed by *PLCE1* (7). However, our study is limited

by the small sample size, especially for each age group under 18 years, and for the homogeneous geographic origin/ethnicity of the studied cohort; therefore, larger studies would help to improve the proposed genetic testing algorithm for SRNS.

A partial response was observed in 7 of 26 patients carrying podocyte gene mutations who received either immunosuppressive and ACEI treatment or only ACEI treatment. Calcineurin inhibitors induced a partial remission of proteinuria in several other children with genetic forms of NS (57). Recently, it has been shown that most patients with genetic CNS/SRNS presented lower response to cyclosporine A compared with nongenetic patients and showed rapid progression to ESRD (43). On the other hand, three patients with familial FSGS treated with early angiotensin inhibition resulted in a partial to complete remission of proteinuria (58), suggesting that some patients could benefit from ACEI treatment. Further studies would be necessary to adequately determine which treatment can be beneficial for genetic SRNS patients. Patients with *NPHS1* mutations showed a shorter progression time to ESRD than patients with two *NPHS2* mutations. In addition, none of the patients with pathogenic mutations relapsed after kidney transplantation.

In conclusion, our data indicate that two main criteria determine the appropriate genes to test: (*1*) age at onset and (*2*) familial/sporadic status. Because in our cohort of congenital-, infantile-, and childhood-onset patients with mutations, renal histology was not a determinant criterion because most presented with FSGS, we recommend start-

ing with genetic testing before renal biopsy because it is a noninvasive technique. In adults, renal biopsy is the first step because it allows ruling out other causes of NS such as membranous nephropathy. Although the analysis of multiple genes is time-consuming and expensive, the identification of pathogenic mutations can help (1) to avoid adverse effects of steroid/immunosuppressive treatment, (2) to encourage living donor kidney transplantation, (3) to perform a prognosis depending on which gene is mutated and which kind of mutations are detected, and (4) to offer the possibility of presymptomatic, prenatal, and preimplantation genetic diagnosis.

Disclosures
None.

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