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NPHS1 and NPHS2 Gene Mutations in Chinese Children With Sporadic Nephrotic Syndrome

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ABSTRACT: Recent discoveries indicate that the molecules in glomerular podocytes and slit diaphragms may play an important role in the development of proteinuria and nephrotic syndrome. Mutational analyses of *NPHS1* and *NPHS2* were performed to verify this hypothesis in sporadic nephrotic syndrome (NS) patients. Clinical characteristics and DNA samples were collected from 38 Chinese children with sporadic steroid-sensitive NS, 22 with steroid-resistant NS and 30 controls. Direct sequencing was performed after PCR amplification of all 29 and 8 exons of the *NPHS1* and *NPHS2* genes, respectively. In *NPHS1*, 4 patients had heterozygous missense mutations leading to amino acid substitutions (R800C, Q453R). Furthermore, 3 known single nucleotide polymorphism (SNP) were found (T741T, V763V, S1105S). In *NPHS2*, 3 patients had novel heterozygous allelic variants leading to amino acid substitutions (S206I, E188D), while 1 patient was found to carry a novel nonsense mutation leading to a truncated protein product (Glu237STOP). Two known polymorphisms were also found (A318A, L346L). The results demonstrate that *NPHS1* and *NPHS2* mutations are also present in Chinese sporadic NS patients, suggesting that genetic changes of nephrin and podocin may play pathogenetic roles in some patients with sporadic steroid resistant NS. **(*Pediatr Res* 61: 117–122, 2007)**

Nephrotic syndrome (NS), defined as the association of proteinuria, hypoalbuminemia, edema and hyperlipidemia, constitutes one of the most common diagnoses made in pediatric nephrology and is emerging as a leading cause of uremia. For decades, NS has been separated into two broad categories based upon its response to standard steroid therapy: steroid-sensitive nephrotic syndrome (SSNS) and steroid-resistant nephrotic syndrome (SRNS) (1,2). About 20% of all children with sporadic NS do not respond to steroid treatment. Renal histology reveals focal segmental glomerulosclerosis (FSGS), minimal change nephrotic syndrome (MCNS) and mesangioproliferative glomerulonephritis (MsPGN) in 75%, 20% and 5% of the SRNS patients, respectively (3). The management of steroid-resistant nephrotic syndrome continues to pose a therapeutic challenge to nephrologists.

Although loss of glomerular selectivity for albumin is common to all types of NS, the related pathogenesis has been elusive despite decades of studies into its renal histology and protein biochemistry. It appears to be a heterogeneous clinical condition characterized by histologic variants and different genetic backgrounds. Many different and seemingly unrelated genomic and molecular markers correlate with the NS disease course, again supporting the molecular heterogeneic nature of the NS group of disorders. These markers can be divided into two categories: immune-related and structural. In recent years, advances in molecular genetics of familial NS have led to the discovery of several podocyte specific genes. Identification of these candidate genes for monogenic forms of SRNS indicates the importance of genetic factors in the pathogenesis of NS.

Many exciting advances have increased our understanding of genetic causes of familial SRNS recently. Mutations in the *NPHS1* gene encoding nephrin, the *NPHS2* gene encoding podocin, the *ACTN-4* gene encoding α- actinin-4, the WT1 gene coding Wilm's Tumor 1 and TRPC6 gene coding the ion-channel protein transient receptor potential cation channel 6 have been identified (4–8). However, these mutations in these genes do not account for all cases of familial FSGS, especially for nonfamilial SRNS.

Several studies have indicated that nephrin, a 136 kD transmembrane protein with a large extracellular portion with eight immunoglobulin-like domains, is crucial for the structure of slit diaphragm (SD). Mutation in the *NPHS1* gene is responsible for the congenital nephrotic syndrome of the Finnish type (CNF), which can cause lethal proteinuria at birth and lack of the SD. Meanwhile, the non-Finnish NS patients have different mutations, and more than 60 mutations in the *NPHS1* genes have been described (9–11).

Podocin is another important molecule located at the slit diaphragm. It is encoded by the *NPHS2* gene, mutation of which can cause autosomal recessive steroid-resistant nephrotic syndrome. Podocin is expressed in the podocyte foot

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Abbreviations: CNF, congenital nephrotic syndrome of the Finnish type; **ESRD,** end-stage renal disease; **FSGS,** focal segmental glomerulosclerosis; **IgAN,** IgA nephropathy; **MCNS,** minimal change nephrotic syndrome; **MsPGN,** mesangial proliferative glomerulonephritis; **NS,** nephrotic syndrome; **SD,** slit diaphragm; **SRNS,** steroid-resistant nephrotic syndrome; **SSNS,** steroid-sensitive nephrotic syndrome

Table 1. *General features and clinical details in 60 Chinese children with sporadic nephrotic syndrome*

	n	Mean age at onset	Male/female	Hypertension	Renal function	Renal histology	Follow-up duration (year)
SSNS	38	8.2	22/16	Normal 35, Abnormal 3	Normal 36, Transient abnormal 2	MCNS, 4; FSGS, 1; IgAN, 2	4.5
SRNS	22	9.3	12/10	Normal 15, Abnormal 7	Normal 16, Abnormal 6	MCNS, 6; FSGS, 5; IgAN, 2; MsPGN, 1	3.7

process cell membrane at the insertion site of the SD as shown by immunoelectron microscopy, which proposes that podocin might be important for maintaining the integrity of the SD. Since the identification of the *NPHS2*, different groups have demonstrated that mutations in the *NPHS2* gene represent a frequent cause of SRNS, occurring in 20–30% of the sporadic cases of SRNS (12–14).

The role of nephrin and podocin in children with sporadic nephrotic syndrome is not clearly known till now. Some studies reported of NPHS1 gene mutations in patients with MCNS (*9,15*). Also, altered expression of nephrin and podocin has been reported in MCNS and FSGS patients. We also identified recently that the expression of nephrin and CD2-associated protein (CD2AP) were significantly reduced at protein level in nephrotic patients with MCNS and IgA nephropathy (IgAN), though no significant reduction in podocin expression was found compared with the controls. This prompted us to investigate whether mutations in the *NPHS1* and *NPHS2* genes lie behind Chinese patients with sporadic nephrotic syndrome. Specifically, we performed mutational analysis for all 29 and 8 exons of *NPHS1* and *NPHS2*, respectively, among 38 patients with SSNS, 22 patients with SRNS and 30 controls. We identified 8 SRNS patients carried missense and nonsense mutations in the *NPHS1* or *NPHS2* genes. Therefore, mutational analysis of *NPHS1* and *NPHS2* among patients with sporadic nephrotic syndrome might be helpful for predicting their response to standard steroid treatment during the early stage of onset, and will also be valuable for forecasting their prognosis after onset.

MATERIALS AND METHODS

Patients and controls. The study was approved by The Ethic Committee of Children's Hospital of Medical College, Zhejiang University. Informed consent was obtained from all the parents of the patients and controls, or from patients and controls themselves, depending on their perceptive capability. The study group consisted of 60 children who had been diagnosed as sporadic nephrotic syndrome in Children's Hospital of Medical College, Zhejiang University from 2001 to 2004. Clinical data including the medication, number of relapse and laboratory findings were also recorded. A family history was taken and the absence of the nephrotic syndrome in the first-degree relatives was required. Controls included 30 children with no personal or family history of a kidney disease. Both the study subjects and the control subjects are of Mongolian origin. Exclusion criteria were low C_3-complement; post-infectious glomerulonephritis; and systemic diseases such as lupus erythematodes, diabetes, amyloidosis, vasculitis, Schönlein-Henoch purpura nephritis, metabolic or toxic nephritis, hepatitis B, or hereditary glomerular diseases.

Definitions and treatment. Sporadic nephrotic syndrome was defined as heavy proteinuria (urine protein exceeding 40 mg/m^2/h), and hypoalbuminemia (serum albumin ≤25 g/L) without familial history of proteinuria. All patients received a standard treatment with daily corticosteroid (prednisone) at a dose of 2 mg/kg/d (maximum 60 mg/d) for 4 wk and were then switched to alternate day therapy. The patients who respond to initial treatment after 4 wk of the daily steroid treatment as complete resolution of proteinuria and edema were defined as steroid-sensitive. If no improvement was observed, the patients were defined as SRNS. Patients with late steroid resistance were excluded in this study.

Hypertension was defined as blood pressure higher than 95th percentile for age according to data from Task Force Report on High Blood Pressure in Children and Adolescents. Hematuria was defined as a dipstick reading of 1+ or more for blood or as more than 3 red blood cells per high-power field in urine sediment. Estimated GFR (GFR) was calculated by the Schwartz formula: GFR = k × height (cm)/plasma creatinine (mg/dL), where k is 0.45 for infants (≤18 mo of age), 0.55 for older children and adolescent girls, and 0.7 for adolescent boys over 13 y of age. Decreased kidney function was defined as GFR ≤90 mL/min/1.73m (2), CRF as GFR ≤60 mL/min/1.73m (2), and end-stage renal disease (ESRD) as GFR ≤15 mL/min/ 1.73m (2).

DNA samples. Peripheral blood samples were collected from 38 Chinese sporadic SSNS patients, 22 SRNS patients and 30 controls in Hangzhou area of Zhejiang province of P. R. China. DNA preparation Kit (Takara, Japan) was used to purify DNA from a 150-μL EDTA-treated blood samples.

PCR conditions. Genomic DNA (50 ng) was subjected to 35–40 cycles of PCR amplification in a volume of 50 μL consisting of 2 μL 5 pmol/L sense primer, 2 μL 5 pmol/L antisense primer, 1.5–3.5 mM $MgCl_2$, 100 mM dNTPs, and 2.5 U *Taq* polymerase (Perkin-Elmer Applied Biosystems, Foster City, CA). DNA was denatured at 95°C for 10 min, followed by 35–40 cycles of denaturing for 1 min at 95°C, annealing for 45 s at 55–68°C, extension for 45 s at 72°C, and final extension of 7 min at 72°C in a thermocycler (GeneAmp PCR system 2400, Perkin-Elmer, CA).

Detection of genomic mutation of NPHS1 and NPHS2 The primers were designed on the basis of previously published information regarding intron-exon boundaries (4,5). The PCR products were purified with the QIA Quick PCR Purification Kit (Qiagen, Hilden, Germany). The purified products were subjected to cycle-sequenced with Big-Dye terminators (Applied Biosystems, Foster City, CA, USA). The cycle sequence product was analyzed with an automated sequencer (ABI Prism 310 Genetic Analyzer, Applied Biosystems).

All mutations were confirmed by sequencing the complementary strand. Known single nucleotide polymorphisms within the primer sequences were avoided (http://genome.ucsc.edu/), because single nucleotide variants within the primer sequence can suppress amplification of one of the two alleles of the amplified product. To rule out polymorphisms, 30 healthy control individuals (age and ethnic background similar to the patients) were checked for novel mutations by direct sequencing.

Statistical analysis. χ^2 test were used to analyze the difference of genotypic and allelic frequencies between NS patients and controls by SPSS software. $p < 0.05$ was viewed as statistically significant.

RESULTS

Clinical data of the patients with SSNS and SRNS. The clinical data are listed in Table 1. In 22 SRNS patients, renal insufficiency was seen in 6 patients. By renal biopsy, 6

Table 2. *Amino acid substitutions and polymorphisms of the NPHS1 gene in 38 SSNS patients, 22 SRNS and 30 controls subjects*

Exon/ intron	Nucleotide change	Effect on protein	No. of changes per Alleles		Comment
			NS	Control	
Intron	7906G→A	Unknown	4/60	1/30	Homozygous
Intron	7906G→A	Unknown	5/60	2/30	Heterozygous
Intron	7892C→T	Unknown	2/60	1/30	Heterozygous
Exon11	1358A→G	Q453R	1/60	0/30	Heterozygous
Exon17	2223C→T	T741T	9/60	4/30	Heterozygous
Exon17	2289C→T	V763V	6/60	2/30	Homozygous
Exon17	2289C→T	V763V	10/60	3/30	Heterozygous
Exon18	2398C→T	R800C	3/60	0/30	Heterozygous
Exon26	3315G→A	S1105S	2/60	2/30	Homozygous

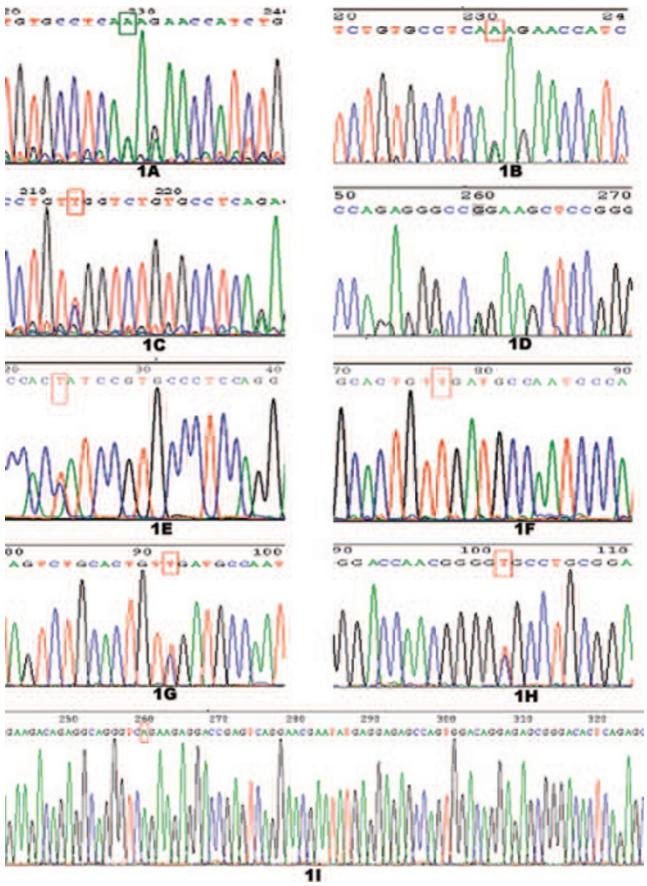

Figure 1. *NPHS1* mutation: (*A*) intron, 7906G→A, homozygous; (*B*) intron, 7906G→A, heterozygous; (*C*) intron, 7892 C→T, heterozygous; (*D*) exon11, 1358A→G'Q453R'heterozygous; (*E*) exon17, 2223C→T'T741T'heterozygous; (*F*) exon17, 2289 C→T'V763V, homozygous; (*G*) exon17, 2289 C→T'V763V, heterozygous; (*H*) exon18, 2398C→T, R800C, heterozygous; (*I*) Exon26, 3315 G→A'S1105S'homozygous.

patients showed evidence of MCNS, 5 of FSGS, 2 of IgAN and 1 of mesangial proliferative glomerulonephritis (MsPGN). The mean follow-up duration in 22 SRNS was 3.7 y.

Allelic variants of NPHS1. None of the patients had the Fin-major mutation in the exon 2 or the Fin-minor mutation in the exon 26. A total of 9 sequence variants were observed in 4 exons and 3 introns, as summarized in Table 2 and listed from Fig. 1*A–I*. To examine the significance of these mutations, the same exons were also sequenced from 30 control subjects, and the results were compared with the published data and database (dbSNP, SNPper).

Three of 9 nucleotide changes (T741T heterozygous, V763V heterozygous and homozygous, S1105S homozygous) as known polymorphic variants (SNP) (Table 2 and Fig. 1*E–G, I*) were found in 9, 16 and 2 patients respectively, and none of them caused an amino acid substitution. Nucleotide changes were found particularly at exon 17, but also at exon 18 and 26.

Three patients were found to have a 2398C>T nucleotide change resulting heterozygous R800C (Fig. 1*H*) transition leading to amino acid substitution of arginine by cysteine in Ig-7 domain of nephrin. The onset of proteinuria in patients with this mutation occurred, respectively, at age 8.4, 7.3 and 6.5 y (Table 3). All of them are steroid-resistant. Among them, 1 patient carries also the T741T polymorphism. However, no differences were found in symptom severity and response to drug therapy between this patient and other 2 patients with the R800C mutation.

One patient was detected as carrying a novel 1358A≥G mutation (heterozygous) in the exon 11 leading to the substitution of Q453 with arginine (Fig. 1D). He is a young boy with no history of renal disease, with poor response to the standard steroid treatment and abnormal renal dysfunction 2.7 y after onset. Moreover, a renal biopsy showed evidence of FSGS. The same mutation was not detected in his parents at all (Table 3).

Allelic variants of NPHS2. A total of 5 sequence variants were observed in 2 exons, as summarized in Table 4 and Fig. 2*A–E*. To examine the significance of these mutations, the same exons were also sequenced from 30 control subjects, and the results were compared with the published data and database (dbSNP, SNPper).

A novel homozygous nonsense mutation in *NPHS2*, E237X (709G>T in the exon 5) was detected in one patient (Table 3, Fig. 2*E*). This nucleotide change introduces a stop codon (TAG), leading to a truncated protein product. This patient is a 6.8-y-old boy with no family history of proteinuria and renal disease. He was diagnosed as having nephrotic syndrome 1.2 y ago. His condition has deteriorated even after the treatment with corticosteroid and cyclosporine A, and presently his renal function is abnormal (serum creatinine 219.6 μM). The par-

Table 3. *Clinical data of patients carrying NPHS1 and/or NPHS2 mutation*

Patient	Gender	Age (years)	Age at onset (years)	Proteinuria (g/24h)	Serum creatinine (μmol/L)	BUN (mmol/L)	C_3mg/dl	Renal biopsy	Mutation	
									NPHS1	NPHS2
1	M	11.2	8.5	3.52	112.2	8.46	90.3	FSGS	Q453R	A318A
2	F	10.3	8.4	4.26	114.5	9.68	120.5	MsPGN	R800C	L346L
3	F	8.2	7.3	3.84	105.6	6.67	114.8	ND	R800CandT741T	
4	M	8.7	6.5	7.29	98.2	5.81	91.7	MCNS	R800C	
5	M	14.8	6.8	9.23	120.4	9.53	143.6	FSGS	S1105S	S206I
6	F	10.6	8.1	2.47	96.5	6.68	135.9	FSGS	T741T	E188D
7	F	9.2	6.4	5.68	102.5	5.11	98.5	MCNS	V763V	S206I
8	M	6.8	5.6	4.10	219.6	15.43	125.6	FSGS		Glu237STOP
9	F	7.8	6.7	3.25	110.7	6.54	113.7	ND	V763V	

BUN, serum urea nitrogen; C3, complement C3; MCNS, minimal change nephrotic syndrome; FSGS, focal segmental glomerular sclerosis; MsPGN, mesangial proliferative glomerulonephritis.

Table 4. *Amino acid substitutions and polymorphisms of the NPHS2 gene in 38 SSNS patients, 22 SRNS and 30 controls subjects*

Exon/ Intron	Nucleotide change	Effect on protein	No. of changes per Alleles		Comment
			NS	Control	
Exon8	954T→C	A318A	5/60	4/30	Homozygous, known polymorphism, no amino acid change
Exon8	1038A→G	L346L	7/60	4/30	Heterozygous, known polymorphism, no amino acid change
Exon5	564G→T	E188D	1/60	0/30	Homozygous
Exon5	617G→T	S206I	2/60	0/30	Heterozygous
Exon5	709G→T	Glu237STOP	1/60	0/30	Homozygous

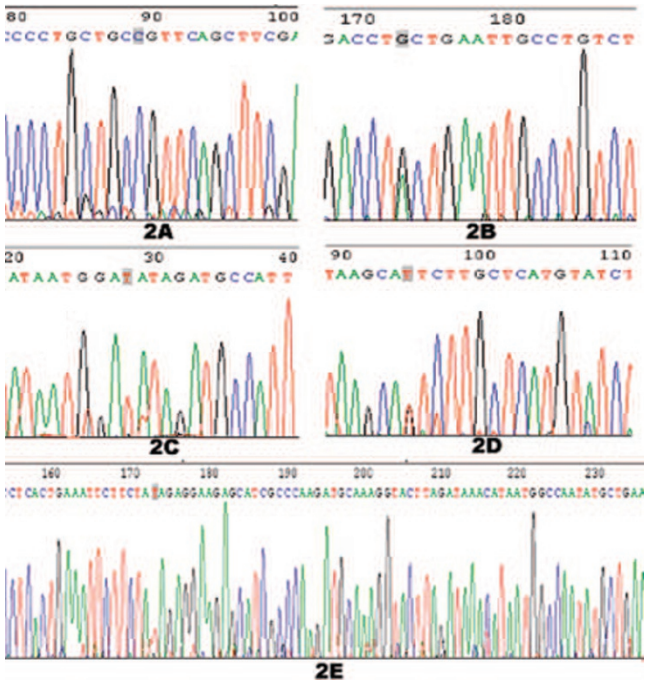

Figure 2. *NPHS2* mutation: (*A*) Exon8, 954T→C'A318A'homozygous; (*B*) Exon 8, 1038A→G'L346L'heterozygous; (*C* Exon 5, 564G→T'E188D'homozygous; (*D*) Exon 5, 617G→T'S206I, heterozygous; (*E*) Exon 5, 709G→T'Glu237STOP, homozygous.

ents of the patient do not carry the identity mutation in *NPHS2* or *NPHS1*.

Two novel missense mutations in the *NPHS2* gene, E188D (564G>T in the exon 5, homozygous, Fig. 2*C*) and S206I (617G>T in the exon 5, heterozygous, Fig. 2*D*) were detected in 3 patients. The patient carrying the 564G>T mutation is a girl without family history of renal disease. At the time of the onset she was 8.1 y old. She has received the standard steroid and cyclosporine A treatment and her renal function remains normal 2.5 y after the onset. Her mother is healthy though carrying same heterozygous mutation. This mutation was not identified in the father of the patient. One patient carrying the 617G>T mutation is a boy with no history of renal disease. At the age of 6.8 years he was diagnosed as having nephrotic syndrome. He is resistant to the standard steroid therapy and his renal function is abnormal with the serum creatinine concentration of 120.4 μM and BUN of 9.53 mM. The parents were not consanguineous. This mutation was not identified in the parents of the patient. Another patient carrying the same 617G>T mutation is a 9.2-y-old girl. Her renal function remains normal.

A single nucleotide polymorphisms of 954T≥C in the exon 8 was observed in 5 patients and 4 controls. Another polymorphism of 1038A≥G in the exon 8 was observed in 7 patients and 4 controls. Both polymorphisms do not cause amino acid substitution (A318A & L346L) (Fig. 2*A–B*).

Genotype/phenotype correlation. We found 5 patients with mutations in both *NPHS1* and *NPHS2* genes. However, all of these are known polymorphisms with no amino acid substitution (same sense mutation, Table 5). Patients 10 and 11 are homozygous for V763V in the *NPHS1* gene and heterozygous for L346L in the *NPHS2* gene; Patient 12 is heterozygous for V763V in the *NPHS1* gene and homozygous for A318A in the *NPHS2* gene. This patient is steroid resistant. Patient 13 is heterozygous for T741T in the *NPHS1* gene and homozygous for A318A in the *NPHS2* gene. Finally patient 14 is homozygous for S1105S in the *NPHS1* gene and heterozygous for L346L in the *NPHS2* gene. Four of patients (patient 10, 11, 13 and 14) are sensitive to standard steroid treatment strategy. No correlation of genotype/phenotype was found among them in *NPHS1* and *NPHS2* gene.

Genotypic and allelic frequencies. There was no significant difference in the genotypic and allelic frequencies of the 2223C>T, 2289C>T, 3315G>A polymorphisms in the *NPHS1* gene or the 954T>C, 1038A>G polymorphisms in the NPHS2 gene between patients and controls (Table 6).

Table 5. *Clinical data of 5 NS patients carrying both NPHS1 and NPHS2 mutation*

Patient	Gender	Age (years)	Age at onset (years)	Proteinuria (g/24h)	Serum creatinine (μmol/L)	BUN (mmol/L)	C_3mg/dl	SS/SR	Renal biopsy	Mutation	
										NPHS1	*NPHS2*
10	F	8.5	6.3	5.57	102.6	6.46	90.3	SS	ND	V763V (H)	L346L(h)
11	F	9.3	8.4	3.58	123.5	5.63	120.5	SS	ND	V763V (H)	L346L(h)
12	M	7.2	4.1	3.84	115.6	9.67	114.8	SR	MCNS	V763V (h)	A318A(H)
13	M	11.7	6.5	3.29	96.2	5.81	81.7	SS	ND	T741T(h)	A318A(H)
14	M	12.8	10.8	6.23	127.7	5.53	143.6	SS	ND	S1105S(H)	L346L(h)

H, homozygous; h, heterozygous; ND, not determined; MCNS, minimal change nephrotic syndrome; BUN, serum urea nitrogen; C3, complement C3; SS, steroid sensitive; SR, steroid resistant.

Table 6. *Genotypic and allelic frequencies of 5 polymorphisms in 60 patients and 30 controls*

	Patients (n = 60)	Controls (n = 30)	χ^2	p
2223C>T of NPHS1				
Genotype				
CC	51 (85%)	26 (87%)	0.000	1.000
CT	9 (15%)	4 (13%)	0.000	1.000
TT	0 (0%)	0 (0%)	—	—
Allele				
C	0.93	0.94	0.000	1.000
T	0.07	0.06	0.000	1.000
2289C>T of NPHS1				
Genotype				
CC	44 (73%)	25 (83%)	1.118	0.290
CT	10 (17%)	3 (10%)	0.281	0.596
TT	6 (10%)	2 (7%)	0.017	0.896
Allele				
C	0.82	0.88	1.315	0.251
T	0.18	0.12	1.315	0.251
3315G>A of NPHS1				
Genotype				
GG	58 (97%)	28 (94%)	0.033	0.856
GA	0 (0%)	0 (0%)	—	—
AA	2 (3%)	2 (6%)	0.033	0.856
Allele				
G	0.97	0.94	0.409	0.523
A	0.03	0.06	0.409	0.523
954T>C of NPHS2				
Genotype				
TT	55 (92%)	26 (87%)	0.139	0.709
TC	0 (0%)	2 (7%)	—	0.109
CC	5 (8%)	2 (7%)	0.000	1.000
Allele				
T	0.92	0.90	0.137	0.711
C	0.08	0.10	0.137	0.711
1038A>G of NPHS2				
Genotype				
AA	53 (88%)	26 (87%)	0.000	1.000
AG	7 (12%)	2 (7%)	0.139	0.709
GG	0 (0%)	2 (7%)	—	0.109
Allele				
A	0.94	0.90	0.508	0.476
G	0.06	0.10	0.508	0.476

DISCUSSION

In this study we investigated the possible role of the major slit diaphragm proteins, nephrin and podocin, by analyzing the structure of their coding gene in patients with sporadic nephrotic syndrome in childhood. One novel missense mutation (Q453R), 1 known missense mutation (R800C), and 3 known polymorphisms (T741T, V763V, S1105S) in the *NPHS1* gene were identified. Additionally 2 novel missense mutations (E188D, S206I), 1 novel nonsense mutation (Glu237STOP), and 2 known polymorphisms (A318A, L346L) in the *NPHS2* gene were found in 38 SSNS and 22 SRNS patients.

There are several studies reporting mutations in both *NPHS1* and *NPHS2* gene in congenital FSGS. Koziell (11) *et al.* first reported mutations both in NPHS1 & NPHS2 in 4 individuals from 3 different families with congenital FSGS. They found an overlap in the *NPHS1/NPHS2* mutation spectrum with the characterization of a unique di-genic inheritance of NPHS1 and *NPHS2* mutations, which results in a 'tri-allelic' hit and appear to modify the phenotype from CNF to one of congenital FSGS. *Caridi et al.* (16) reported one patient with heterozygous *NPHS1* mutation and a homozygous *NPHS2* mutation (R229Q). Schultheiss *et al.* (17) reported 5 patients with combined mutations in the *NPHS1* and *NPHS2* genes, but absence of evident phenotype/genotype correlation was found in their study. In present study no correlation of genotype/phenotype was found either in the *NPHS1* and *NPHS2* genes.

Some studies have showed that polymorphisms, even when not result in amino acid substitutions, might cause phenotypic variations either by affecting the structural of the mRNA or by inactivating genes through the change in gene splicing machinery to skip the mutant exons (18). This possibility remains to be investigated in our patients in the future. In 23 Chinese sporadic SRNS patients by Yu *et al.* (18), 1 case of heterozygous missense mutation and 7 polymorphisms of the NPHS2 gene in some patients and controls were reported. In addition, no significant differences in genotypic and allelic frequencies of these polymorphisms between patients and controls were found which was similar to our present study.

European and North American studies (3,16,19) of large cohorts of SRNS patients revealed a detection rate of homozygous or compound heterozygous *NPHS2* mutations of 38% and 26%, respectively, in patients with familial SRNS and of 6–19% in sporadic cases. The percentage of patients with only one *NPHS2* mutation was low, being only 2–3%. Both studies (16,19) confirm that FSGS in patients with two pathogenic *NPHS2* mutations is generally characterized by early-onset, resistance to standard steroid treatment, and a reduced risk of FSGS recurrence after kidney transplantation. Ruf *et al.* (20) reported in 26 NS patients carrying compound heterozygous mutations (double mutations) in the NPHS2 gene. Of them, 22 patients showed steroid resistance and 11 patients developed ESRD. In contrast to this, in 9 patients with single heterozygous mutation in the NPHS2 gene, 4 patients showed steroid response, and only 1 patient developed ESRD after 8 y of the onset. Caridi *et al.* (21) described an infantile variant in 2 families (3 patients) from Turkey, characterized by homozygosity of a complex haplotype, in which 2 NPHS2 mutations (P20L & R168H) are present. The patients reported developed NS within the first 6 mo of life with strict resistance to drugs and a histologic background of FSGS. The very early onset of proteinuria might mimic severe NS patients such as congenital NS of the Finnish type. These results imply that, while compound heterozygous mutations of gene(s) is associated with strictly steroid/cyclosporine resistance, patients with single mutation are more likely to respond to therapy and may have a better long-term outcome than patients with compound mutations (22). In present study, 1 patient carried compound mutations in *NPHS1* gene (T741T in exon 17, heterozygous and R800C in exon 18, heterozygous), and was steroid resistant. No differences were found in symptom severity or response to drug therapy in this patient and the 2 others with the R800C mutation. Normally most cases of monogenic diseases are heterozygous, because homozygous or compound heterozygous states might be either lethal or associated with a

more severe phenotype than in single heterozygotes (23,24). This is also the case in common multigenic disorders.

According to our results and others (25), screening the newly diagnosed patients with sporadic NS, especially with SRNS, for the presence of possible *NPHS2* mutations is evidently necessary. It might be also meaningful to screen the mutation of *NPHS1* in SRNS. Differences in clinical findings of patients with or without *NPHS1* and/or *NPHS2* mutation are not always distinct, so the genetic analysis might be valuable to separate these two entities as early as possible to avoiding unnecessary steroid therapy, because missense and nonsense mutations as these may be present in sporadic SRNS patients without a familial history of renal disease.

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