Pediatr Nephrol (2007) 22:2031–2040
DOI 10.1007/s00467-007-0595-y

ORIGINAL ARTICLE

NPHS2 (podicin) mutations in Turkish children with idiopathic nephrotic syndrome

Afig Berdeli · Sevgi Mir · Onder Yavascan · Erkin Serdaroglu · Mustafa Bak · Nejat Aksu · Ayse Oner · Ali Anarat · Osman Donmez · Nurhan Yildiz · Lale Sever · Yilmaz Tabel · Ruhan Dusunsel · Ferah Sonmez · Nilgun Cakar

Received: 4 April 2007 / Revised: 10 July 2007 / Accepted: 13 July 2007 / Published online: 25 September 2007

Abstract The podocin (*NPHS2*) gene encodes podocin protein, which has an important role in glomerular ultrafiltration and controlling slit membrane permeability. The detection of an *NPHS2* mutation affects the treatment plan for children with nephritic syndrome (NS). The frequency and spectrum of podocin mutations in the Turkish population have remained largely unknown. The aim of this study was to screen for podocin mutations in Turkish patients with steroid-resistant NS (SRNS) and to compare it with other published series. There were 295

A. Berdeli (✉)
Department of Molecular Genetics,
Ege University Faculty of Medicine,
Izmir, Turkey
e-mail: afig.berdeli@ege.edu.tr

S. Mir · O. Yavascan
Pediatric Nephrology,
Ege University Faculty of Medicine,
Izmir, Turkey

E. Serdaroglu · M. Bak
Pediatric Nephrology,
Behcet Uz Children's Hospital,
Izmir, Turkey

N. Aksu
Pediatric Nephrology,
Tepecik Teaching and Research Hospital,
Izmir, Turkey

A. Oner
Pediatric Nephrology,
Sami Ulus Children's Hospital,
Ankara, Turkey

A. Anarat
Faculty of Medicine, Pediatric Nephrology,
Cukurova University,
Adana, Turkey

O. Donmez
Pediatric Nephrology, Faculty of Medicine,
Uludag University,
Bursa, Turkey

N. Yildiz
Pediatric Nephrology,
Goztepe Teaching and Research Hospital,
Izmir, Turkey

L. Sever
Pediatric Nephrology,
Cerrahpasa Faculty of Medicine,
Istanbul University,
Istanbul, Turkey

Y. Tabel
Pediatric Nephrology,
Inonu University Faculty of Medicine,
Malatya, Turkey

R. Dusunsel
Pediatric Nephrology,
Erciyes University Faculty of Medicine,
Kayseri, Turkey

F. Sonmez
Pediatric Nephrology,
Aydin Adnan Menderes University Faculty of Medicine,
Aydin, Turkey

N. Cakar
Pediatric Nephrology,
Ankara Diskapi Education and Research Hospital,
Ankara, Turkey

children with SRNS, originating from Turkey, included in this study. Forty-one patients (13.8%) had familial NS and 254 patients (86.2%) had sporadic NS. Mutation analysis was performed in all eight exons of the *NPHS2* gene with the direct DNA sequencing method. There were 53 different pathogenetic *NPHS2* mutations detected, including 37 novel mutations. The mutation detection rate was 24.7% for all patients, 29.2% for familial, and 24% for sporadic SRNS. The most common mutated exon was exon 5 (52 allele). The presence of mutations in exon 4 was found to increase the risk of end-stage renal disease (ESRD). Among patients with mutations, the rates of renal failure and/or ESRD (26%) were significantly higher than in those without mutations (12.6%). The mean time of progression to renal failure and ESRD in patients with mutations (1.8±2.5 years) was significantly shorter than in patients without mutations (3.7±4.0 years). Additionally, in patients with heterozygote mutations, fewer cases (13.6%) progressed to renal failure and/or ESRD than in with patients who had homozygote/compound heterozygote mutations (31.3%). In conclusion, podocin mutations are responsible for some of both familial and sporadic SRNS cases in Turkey. The mutations in this gene should be searched for in every child after presentation with the first episode of NS.

Keywords Podocin mutation · Nephrotic syndrome · Children · Focal segmental glomerulosclerosis (FSGS)

Introduction

Nephrotic syndrome (NS) is an uncommon disorder in childhood and is characterized by edema, massive proteinuria, hypoalbuminemia, and hyperlipidemia. Clinically, NS has been divided into two categories on the basis of the response to steroid therapy: steroid-sensitive nephrotic syndrome (SSNS) and steroid-resistant nephrotic syndrome (SRNS). In SRNS, more than 75% of the patients exhibit the histology of focal segmental glomerulosclerosis (FSGS) in kidney biopsies and a considerable number of patients progress to end-stage renal disease (ESRD) [1–4]. Nephrin (NPHS1), α-actinin 4 (ACT4), and podocin (NPHS2) are proteins that play an important role in glomerular slit diaphragm homeostasis. The encoding genes, if mutated, are known to cause NS [5–8]. Podocin is a lipid raft-associated protein at the filtration slit, which is exclusively expressed in the glomerular podocytes at the foot processes [9–11]. The *NPHS2* gene (OMIM number 604766) is located at chromosome 1q25–q31. It was first mapped by linkage analysis in families with autosomal recessive SRNS [3]. Then, Boute et al. [8] reported the first *NPHS2* mutation in SRNS. Caridi et al. [12–16], Ruf et al. [17], and Weber et al. [18] got down to the details of the *NPHS2* mutations, both in familial and sporadic cases of SRNS. Their studies are the most comprehensive so far.

The ethnic heterogeneity of this disease exposes the identification of *NPHS2* mutations in patients from different countries. However, there is a limited number of studies on *NPHS2* mutations from Turkey in the literature [12, 19, 20]. The aim of our study is to define *NPHS2* mutations and genotype/phenotype correlations in Turkish children with SRNS and compare our results with those of other published series.

Material and methods

Definitions

Nephrotic syndrome was defined by edema, massive proteinuria (>40 mg/m^2 per hour or a protein/creatinine ratio >2.0 mg/mg), hypoalbuminemia (<2.5 g/dl), and hyperlipidemia. Remission was defined as a urinary protein excretion below 4 mg/m^2 per hour or a protein/creatinine ratio below 0.2 mg/mg for three consecutive days. Steroid resistance was accepted as no achievement of remission in spite of treatment with prednisolone, 2 mg/kg per day for 4 weeks. If steroid resistance was seen, patients were also treated with cyclosporine A (CsA) (3–5 mg/kg per day for least 6 months) and, thereafter, if required, with cyclophosphamide (CP) (2.5–3.0 mg/kg per day for 10–12 weeks). A diagnosis of FSGS was made using the criteria recently described by D'Agati et al. [21]. Renal failure was defined as a glomerular filtration rate (GFR) below 80 ml/min per 1.73 m^2 body surface area, and ESRD was defined as a GFR below 10 ml/min per 1.73 m^2 or the necessity for any renal replacement therapy.

Patients

Two hundred and ninety-five children (170 boys and 125 girls) diagnosed with SRNS in 12 different pediatric nephrology departments in our country were included in the study. Geographical distribution of the centers was: seven in western Turkey, three in central Turkey, one in southern Turkey and one in the eastern part of Turkey. The medical records of each patient were reviewed for clinical features, laboratory values, and treatment regimens. In addition, patients with the *NPHS2* mutation were grouped according to genetic analysis results: group A, familial NS; group B1, sporadic NS with two (homozygote or compound heterozygote) mutations; group B2, sporadic NS with a single (heterozygote) mutation. Furthermore, the main clinical features in patients with heterozygote mutations and compound heterozygote/homozygote mutations in

NPHS2 were also compared. For genetic analysis, written informed consent was obtained from the parents of each patient, and the Ege University Medical School Ethics Committee approved the study.

Podocin mutation analysis

Genomic DNA from patients and healthy controls was extracted from peripheral blood leukocytes using QIAmp DNA Blood Mini Kits 50 (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Mutational analysis was performed by the direct sequencing of both strands of all eight exons of the *NPHS2* gene. Primers for the eight exons of *NPHS2* and sequencing were as described previously by Boute et al. [8] and Karle et al. [22]. Polymerase chain reaction (PCR) conditions were as follows: amplification was carried out on a GeneAmp PCR System 9700 (PE Applied Biosystems, Foster City, CA, USA) in a 50 μl reaction mixture in 0.2 ml thin-wall PCR strip tubes (Axygen Scientific, CA, USA) containing 1 μl genomic DNA solution, GeneAmp Gold Buffer (15 mmol/l Tris-HCl, pH 8.0, 50 mmol/l KCl; PE Applied Biosystems), 1.5 mmol $MgCl_2$, 50 μmol/l each of the dGTP, dATP, dTTP and dCTP (Promega, Madison, WI, USA), 5 pmol each of forward and reverse primers, and 1.0 U AmpliTaq Gold polymerase (PE Applied Biosystems). The PCR products were applied to the electrophoretic analysis with a 2% agarose gel and visualized under UV illumination. The positive PCR products were purified by PCR purification columns (Genomics Millipore, Bedford, MA, USA). The purified PCR product was used for cycle sequencing procedure. Direct sequencing was carried out with ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kits v. 3.2 (Applied Biosystems) in the automated sequencer ABI 3100.

Statistical analysis

All analyses were performed with SPSS for Windows version 11.0.0 (SPSS, USA). Differences in categorical variables between groups were tested with Fisher's exact and chi-square tests. Differences in continuous variables between two groups were tested by the non-parametric Mann–Whitney U test. $P<0.05$ was set as the level of statistical significance.

Results

Patients and mutations

The study population consisted of 295 SRNS patients. Their mean age at onset of proteinuria was 3.87±3.08 years (median 2.72 years), and their median follow-up time was 3.89 years. Forty-one patients (13.8%) had familial NS and 254 patients (86.2%) had sporadic NS. Parental consanguinity was found in 49 patients (16.6%). Of the 295 SRNS patients, 73 (24.7%) showed *NPHS2* mutation. The epidemiology, clinical data, treatment, and progression data of patients with or without *NPHS2* mutation are presented in Table 1. Furthermore, the data of patients with heterozygote mutations and compound heterozygote/homozygote mutations in *NPHS2* are presented in Table 2. *NPHS2* mutations, clinical and treatment and disease progression of patients with mutations in *NPHS2* are shown in Table 3. In familial NS, 12 (29.2%) of the 41 patients had an *NPHS2* mutation. Eight patients had homozygote and four patients had double homozygote *NPHS2* mutations (P20L and R168H in three patients, R3G and D267N in one patient). R168H, P20L and K289X were the most common mutations in familial NS patients. Among patients with sporadic NS, mutational screening gave positive findings in 61 (24%) patients. Homozygote, compound heterozygote, and heterozygote *NPHS2* mutations were found in 29 (47.6%), 12 (19.6%) and 20 (32.8%) patients in the sporadic NS group, respectively. R229Q and P20L were the most common mutations in the sporadic NS patients. The mutation frequency was not different between the familial and sporadic NS groups ($P>0.05$). We found 53 different mutations and functional variants identified from the *NPHS2* gene, as shown in Table 4. The most common mutated exons were exon 5 (52 allele), exon 1 (28 allele), and exon 3 (17 allele). We did not find any mutation in exon 6. G34G and A318A were the most common polymorphisms in patients with mutations. Among 53 different *NPHS2* mutations, 37 were novel mutations (Table 4, bold characters in Table 3). None of the novel mutations was found in at least 50 control chromosomes. Patients with novel A213T, 503delG, H228D, and V218G mutations progressed to ESRD. The age at onset of proteinuria in patients with novel M115T and 377insT mutations was under 1 year. All NPHS2 mutations are also presented in Table 4. The overall clinical features of our series, including age at onset, progression to ESRD and histological presentation, were compared with those of sporadic/familial SRNS reported in previous studies of patients of Central European, North African and Middle Eastern origins (Table 5).

Renal histology and *NPHS2* mutations

A kidney biopsy was performed in 202 (68.4%) patients. Among patients with homozygote/compound heterozygote mutations, the histological findings were as follows: FSGS in 36 (70.5%), diffuse mesangial proliferation (DMP) in four, minimal change nephrotic syndrome (MCNS) in four, membranoproliferative glomerulonephritis (MPGN) in

Table 1 Epidemiology, clinical data, treatment response, and disease progression of patients with mutation and no mutation in *NPHS2*

Parameter	No mutation (n=222)	With mutation (n=73)
Age at onset of proteinuria (years)*	4.2±0.5	3.6±3.08
Gender (male/female)*	121/101	49/24
Familial nephrotic syndrome (%)*	29 (13)	12 (16.4)
Consanguinity (%)*	33 (14.8)	16 (22)
Proteinuria (mg/m^2 per hour)	149±72	197±125
Serum albumin (g/dl)	1.9±0.8	2±0.5
Creatinine (mg/dl)	0.84±0.94	0.67±0.34
Hematuria (%)*	8 (3.6)	24 (32.8)
Hypertension (%)*	13 (5.8)	13 (17.8)
FSGS histology*	45/129	52/72
Renal failure and ESRD (%)*	28 (12.6)	19 (26)
Age at renal failure (years)	8.3±4.7	6.2±2.1
Mean time of progression to renal failure (years)	2.6±2.9	1.8±2.5
Age at ESRD (years)	10.2±5.0	11.5±2.3
Mean time of progression to ESRD (years)*	6.8±3.2	3.7±4.0

* P<0.05

three, and, IgM deposition with mesangial proliferation (IgMN) in three patients (the diagnosis failed in one patient due to insufficient biopsy material). Among patients with heterozygote mutations, the histological findings were as follows: FSGS in 16 (72.7%), MCNS in four, IgMN in one, and MPGN in one. Among patients without mutation, the most frequent histological findings were: FSGS in 45 (34.8%), MCNS in 27 (20.9%), IgMN in 16 (12.4%), and DMP in nine (6.9%) (no biopsy was performed in 93 patients). The frequency of FSGS in patients with mutations was significantly higher than in those without mutations (P< 0.05) (Table 1).

NPHS2 mutations and disease progression

The mean age at onset of NS was significantly higher in patients without mutations than in patients with mutations (P<0.05). The frequency of familial NS, consanguinity, hematuria, and hypertension in patients with mutations was significantly higher than in patients without mutations (P< 0.05) (Table 1, 2). Among patients with mutations, the rate of renal failure and/or ESRD (19/73, 26%) was significantly higher than among patients without mutations (28/222, 12.6%), (P<0.05). The mean time of progression to renal failure and ESRD in patients with mutations (1.8±2.5 years) was significantly shorter than in patients without mutations (3.7±4.0 years) (P<0.05). Additionally, in patients with heterozygote mutations, fewer (13.6%) progressed to renal failure and/or ESRD than did those with homozygote/ compound heterozygote mutations (31.3%) (Table 2, P<0.05). The mean time of progression to ESRD in patients with compound heterozygote/homozygote (5.1± 2.7 years) was significantly shorter than in patients with heterozygote mutations (8.3±3.4 years) (P<0.05) (Table 2).

P118L, R138X, R168H, R138Q, IVS7+5G>A, A212T, insT460–467, P20L, 503delG, H228D, S211A, and V218G mutations are associated with progression to ESRD. Dealing with mutations in exon 4 of the *NPHS2* gene, we observed ESRD in five of nine patients (66.6%). The presence of mutations in exon 4, regardless of other risk factors such as age at onset and duration of disease, was found to increase the risk of renal failure and ESRD by 4.3 times and 4.4 times, respectively (95% CI 1.2–15.2 and 1.2–15.8). The mutations in other exons of *NPHS2* did not correlate with disease progression. Additionally, the clinical courses regarding the age at onset and the progression of illness in the Turkish patients were consistent with those reported previously for SRNS patients (Table 5).

Table 2 Epidemiology, clinical data, treatment response and disease progression of patients with heterozygote mutations and compound heterozygote/homozygote mutations in *NPHS2*

Parameter	Heterozygote mutation (n=22)	Compound heterozygote/ homozygote mutation (n=51)
Age at onset of proteinuria (years)	3.7±0.6	3.8±0.5
Gender (male/female)	16/6	33/18
Familial nephrotic syndrome (%)*	2 (9)	10 (19.6)
Consanguinity (%)	5 (22.7)	11 (21.5)
Proteinuria (mg/m^2 per hour)	188±120	216±134
Serum albumin (g/dl)	2.1±0.7	1.9±0.4
Creatinine (mg/dl)	0.51±0.17	0.81±0.50
Hematuria (%)	8 (36.3)	16 (31.3)
Hypertension (%)*	5 (22.7)	8 (15.6)
FSGS histology	16/22	36/50
Renal failure and ESRD (%)*	3 (13.6)	16 (31.3%)
Age at renal failure (years)	6.0±0.7	6.6±3.5
Mean time of progression to renal failure (years)	2.8±3.1	2.3±2.6
Age at ESRD (years)	13.2±1.3	9.4±3.7
Mean time of progression to ESRD (years)*	8.3±3.4	5.1±2.7

*P<0.05

Table 3 *NPHS2* mutations, clinical data, treatment and disease progression of patients with mutations in *NPHS2*

Group	Initials	Gender	Fam/ Spo	Mutation (H = homozygote h = heterozygote)	Age at onset (years)	Steroid therapy	CP/CsA therapy	Biopsy	Renal failure (years after onset)	ESRD (years after onset)
A	KO[a]	M	Fam	P20L(H)-R168H(H)	0.5	SR	CP (no) CsA (no)	FSGS	Yes (8.9)	Yes (10.3)
A	AO[a]	F	Fam	P20L(H)-R168H(H)	0.7	SR	CP (par) CsA (par)	FSGS	Yes (0.5)	No
A	YEY	M	Fam	P20L(H)-R168H(H)	0.3	SR	ND	FSGS	No	No
A	AI	F	Fam	R3G(H)-D267N(H)	?	SR	ND	FSGS	No	No
A	EK	F	Fam	G140fsX180del(H)- R229Q(h)	12.2	SR	CP (no) CsA (no)	FSGS	No	No
A	AK	M	Fam	P118L(H)	0.5	SR	ND	FSGS	No	No
A	AEK	M	Fam	A301del(H)	2.6	SR	ND	IgMN	No	No
A	AC	F	Fam	E30Q(h)	7.5	SR	ND	MCNS	No	No
A	TG	F	Fam	P118L(H)-L344L(h)	3	SR	CP (no) CsA (no)	FSGS	Yes (5.4)	Yes (7.5)
A	SNA	F	Fam	R26M(h)-I192V(h)	4.0	SR	ND	FSGS	No	No
A	MD[a]	M	Fam	K289X(H)-C124W(h)	13	SR	ND	MCNS	No	No
A	SND[a]	F	Fam	K289X(h)	?	SR	ND	FSGS	Yes (?)	Yes (?)
B1	CB	F	Spo	R138X (H)- G140fsX180(h)	2.5	SR	CP (no) CsA (no)	FSGS	Yes (0.8)	Yes (1.3)
B1	HG	M	Spo	insT460-467(H)-E237Q(h)	1.3	SR	ND	FSGS	No	No
B1	BS	F	Spo	Y131Y(H)-V268L(h)	?	SR	ND	FSGS	No	No
B1	FS	M	Spo	R138X(H)	7.4	SR	CP (no)	FSGS	Yes (0.1)	Yes (4.5)
B1	DC	M	Spo	insT460–467(H)	0.7	SR	ND	MPGN	Yes (5.3)	Yes (7.5)
B1	MD	M	Spo	insT460–467(H)	0.5	SR	ND	FSGS	No	No
B1	EU	M	Spo	A213T(H)	3.2	SR	ND	FSGS	Yes (2.8)	Yes (5.8)
B1	UK	M	Spo	A213T(H)	1.1	LSR	ND	FSGS	No	No
B1	VK	M	Spo	IVS7+5G>A (H)	13.0	SR	CsA (no)	FSGS	Yes (2.5)	Yes (3.5)
B1	OI	F	Spo	R168H(H)	1.4	SR	CsA (no)	FSGS	Yes (3.0)	Yes (5.1)
B1	EK	F	Spo	P20L(H)	2.9	SR	ND	MPGN	Yes (2.1)	No
B1	NA	F	Spo	A301del(H)	5.5	SR	CP (no)	FSGS	Yes (0.1)	No
B1	CY	F	Spo	P118L(H)	1.9	SR	CP (no)	FSGS	No	No
B1	YSY	M	Spo	M115T(H)	0.9	LSR	ND	FSGS	No	No
B1	MY	M	Spo	Q39fsX60(H)	?	SR	ND	FSGS	No	No
B1	KA	F	Spo	V268fsX283(H)	5.0	SR	ND	FSGS	No	No
B1	MHO	F	Spo	R18T(H)-R229Q(h)	9.4	SR	ND	DMP	No	No
B1	SB	M	Spo	R138Q(H)	2.5	SR	ND	MCNS	No	No
B1	HE	M	Spo	503delG(h)-H228D(h) 382–384delGTA	8.6	SR	CP (no)	FSGS	Yes (0.1)	Yes (2.7)
B1	EO	M	Spo	S211A(h)-V218G(h)	1.8	SR	ND	FSGS	Yes (3.4)	Yes (5.2)
B1	RE	M	Spo	K28M(h)-M115T(h)	?	SR	ND	FSGS	No	No
B1	ES	M	Spo	I192V(h)-H228D(h) R229Q(h)	4.3	SR	ND	FSGS	No	No
B1	CK	F	Spo	T221S(h)-V370G(h)	2.9	SR	ND	FSGS	No	No
B1	BK	M	Spo	377insT(h)-I314I(h) R229Q(h)	0.8	SR	CP (no)	MCNS	No	No
B1	UC	M	Spo	R229Q(H)	?	SR	ND	DMP	No	No
B1	IN	M	Spo	R229Q(H)	8.1	SR	ND	FSGS	No	No
B1	HS	M	Spo	L159L(h)-R229L(h)	8.3	SR	ND	FSGS	No	No
B1	MS	M	Spo	382–384delGTA(H)	1.4	LSR	ND	FSGS	No	No
B1	MU	M	Spo	R322Q(H)	2	SR	ND	–	No	No
B1	BO	F	Spo	R229Q(H)	10.6	SR	CP (no) CsA (no)	FSGS	Yes (1..5)	Yes(3.5)
B1	IY	M	Spo	V188M(H)	7.2	SR	ND	FSGS	No	No
B1	YK	M	Spo	P20L(H)	1.5	SR	ND	DMP	No	No
B1	AD	F	Spo	V180M(h)-R286L(h) E30K(h)	1.6	LSR	ND	IgMN	No	No
B1	IG	M	Spo	R229Q(H)	1.2	SR	ND	FSGS	No	No
B1	BG	F	Spo	R229Q(H)	4	SR	ND	MPGN	Yes (8.2)	Yes (10.7)

Table 3 (continued)

Group	Initials	Gender	Fam/ Spo	Mutation (H = homozygote h = heterozygote)	Age at onset (years)	Steroid therapy	CP/CsA therapy	Biopsy	Renal failure (years after onset)	ESRD (years after onset)
B1	ENK	F	Spo	P118L(H)	2.9	SR	ND	DMP	No	No
B1	HS	M	Spo	L159L(h)-R229Q(h)	8.3	SR	CP (no) CsA (no)	FSGS	Yes (0.6)	Yes (5.8)
B1	RK	M	Spo	I192V(h)	4.3	SR	ND	FSGS	No	No
B1	IB	M	Spo	R138Q(h)-R229Q(h)	9.5	SR	CP (no)	FSGS	No	No
B1	BK	M	Spo	377ins**T**(h)-R229Q(h)	1.5	LSR	ND	FSGS	No	No
B1	MV	F	Spo	H276L(H)	4.2	SR	ND	IgMN	No	No
B2	ST	M	Spo	R138Q(h)	1.5	SR	ND	FSGS	Yes (5.0)	Yes (10.8)
B2	MB	M	Spo	R138Q(h)	2.9	SR	ND	FSGS	No	No
B2	MK	F	Spo	V290M(h)	8.3	SR	ND	FSGS	No	No
B2	MB	M	Spo	L169P(h)	1.0	SR	CP (no)	FSGS	Yes (5.1)	Yes (10.3)
B2	MS	M	Spo	382–384delGTA (h)	1.4	LSR	CP (par)	FSGS	No	No
B2	AOA	M	Spo	IVS7+5G>**A**(h)	2.5	SR	ND	FSGS	No	No
B2	KT	M	Spo	W122L(h)	3.5	LSR	CP (no) CsA (par)	MCNS	No	No
B2	HO	M	Spo	P20L(h)	7.9	LSR	ND	FSGS	No	No
B2	GS	M	Spo	L203P(h)	2.8	SR	ND	FSGS	No	No
B2	EU	M	Spo	P20L(h)	0.3	SR	ND	FSGS	No	No
B2	HE	F	Spo	R229Q(h)	2.8	SR	ND	IgMN	No	No
B2	OKH	M	Spo	P20L(h)	3.8	SR	ND	FSGS	No	No
B2	TO	F	Spo	P20L(h)	?	SR	ND	MPGN	No	No
B2	SK	M	Spo	R229Q(h)	2.3	SR	ND	MCNS	No	No
B2	KK	M	Spo	T53T(h)	4.3	SR	ND	FSGS	No	No
B2	ECO	M	Spo	Q39L(h)	2.5	SR	ND	FSGS	No	No
B2	KC	M	Spo	P89T(h)	2.4	SR	ND	MCNS	No	No
B2	TS	F	Spo	P20L(h)	7.7	SR	ND	FSGS	No	No
B2	CK	M	Spo	P20L(h)	2.5	SR	ND	FSGS	No	No
B2	KC	M	Spo	V180M(h)	0.3	SR	ND	MCNS	No	No

Bold characters denote novel mutations (*group A* familial nephrotic syndrome, *group B1* sporadic nephrotic syndrome with homozygote or compound heterozygote podocin mutation, *group B2* sporadic nephrotic syndrome with heterozygote podocin mutation, *Fam* familial, *Spo* sporadic, *SR* steroid resistant, *LSR* late steroid non-responder, *CP* cyclophosphamide, *CsA* cyclosporin A, *no* no response, *par* partial response, *ND* not done, *FSGS* focal segmental glomerulosclerosis, *MCNS* minimal change nephrotic syndrome, *IgMN* IgM deposition with mesangial proliferation, *MPGN* membranoproliferative glomerulonephritis, *DMP* diffuse mesangial proliferation, *ESRD* end-stage renal disease)

[a] Patients KO and AO and MD and SND are siblings

Discussion

This study is the largest evaluation of *NPHS2* gene mutations in SRNS patients in Turkey. Based on our results, the incidence of podocin mutations in familial SRNS cases in our country (29.2%) was found to be lower than in European and American children (~40%) but higher than that found in Japanese and Korean children (0%) [14, 17, 18, 23]. According to our results, the incidence of podocin mutations in sporadic SRNS cases in Turkey (24%) was similar to that among European and American children (10–30%) but higher than that found in Chinese (4%), Japanese, and Korean children (0%), [14, 16–18, 23, 24]. Furthermore, Caridi et al. [16] have demonstrated a mutation detection rate of 45–55% in families with recessive traits and 8–20% in sporadic NS. Our results are important because the frequency and spectrum of podocin mutations in Turkey are largely unknown.

Clinical developments have suggested that podocin plays an essential role both in the functional component of the slit diaphragm and in the maintenance of the glomerular permeability barrier [16, 25, 26]. Overall clinical features of our series, including age at onset, progression to ESRD, and histological presentation, were compatible with those of sporadic/familial SRNS reported in previous studies with patients of Central European, North African, Asian, and Middle Eastern origins (Table 5). Different *NPHS2* mutations have been found in Italian, French, German and, Israeli-Arab children [13, 14, 17, 18, 22, 27]. However, it has been reported that there is a lack of contribution of podocin mutations in Israeli-Jewish, Chinese, and Japanese children [23, 24, 28]. Our results are in sharp contrast with

Table 4 Mutations and functional variants identified from the *NPHS2* gene

Exon	Nucleotide change	Effect on protein	Status (patient number)	Reference
1	7A>G	R3G	Hom(1)	This study
1	52A>T	R18T	Hom(1)	This study
1	77G>T	R26M	Het(1)	This study
1	83A>T	K28M	Het(1)	This study
1	88G>C	E30Q	Het(1)	This study
1	88G>A	E30K	Het(1)	This study
1	115insT	Q39fsX60	Hom(1)	This study
1	116A>T	Q39L	Het(1)	This study
1	265C>A	P89T	Het(1)	This study
2	344T>C	M115T	Hom(1) Het(1)	This study
2	353C>T	P118L	Hom(5)	18
2	372C>G	C124W	Het(1)	This study
2	365G>T	W122L	Het(1)	This study
2	377insT	K126fsX132	Het(2)	This study
3	382–384delGTA	c.128delA	Hom(2), Het(1)	This study
3	412C>T	R138X	Hom(2)	8,13,27
3	413G>A	R138Q	Hom(1), Het(3)	8,13,22,29,36
3	419delG	G140fsX180	Hom(1), Het(1)	8,13,22
4	503delG	R168fsX180	Het(1)	This study
4	503G>A	R168H	Hom(4)	18
4	506T>C	L169P	Het(1)	13
5	538G>A	V180M	Het(2), Hom(1)	8,13,22
5	574A>G	I192V	Het(3)	This study
5	609T>C	L203P	Het(1)	This study
5	631T>G	S211A	Het(1)	This study
5	637G>A	A213T	Hom(2)	This study
5	653T>G	V218G	Het(1)	This study
5	665C>G	T221S	Het(1)	This study
5	682C>G	H228D	Het(2)	This study
5	686G>T	R229L	Het(1)	This study
5	insT460–467	V165X	Hom(3)	32
7	799G>A	D267N	Hom(1)	This study
7	802G>C	V268L	Het(1)	This study
7	827A>T	H276L	Hom(1)	This study
7	856A>C	R286L	Het(1)	This study
7	865A>T	K289X	Hom(1), Het(1)	This study
7	868G>A	V290M	Het(1)	22
8	802–803InsA	V268fsX283	Hom(1)	This study
8	901–903delGCT	A301del	Hom(2)	This study
8	965G>A	R322Q	Hom(1)	This study
8	1109T>G	V370G	Het(1)	This study
7	IVS7+5G>A	Splice side	Hom(1), Het(1)	This study
Variants of unknown significance				
1	59C>T	P20L	Hom(5), Het(6)	8, 14
5	709G>C	E237Q	Het(1)	18
Non-silent polymorphisims in the coding sequence				
5	686G>A	R229Q	Hom(5), Het(10)	22,29
Silent polymorphisims in the coding sequence				
1	102G>A	G34G	Hom(250), Het (13)	22,26
1	159C>G	T53T	Het(1)	This study
2	288C>T	S96S	Hom(3)	26
3	393T>C	Y131Y	Hom(1)	This study
4	477G>C	L159L	Het(2)	This study
8	930G>A	E310E	Het(1)	This study
8	954C>T	A318A	Hom(41), Het(6)	31
8	1038A>G	L346L	Het (1)	31

those of Asian studies, suggesting that genetic factors of FSGS differ among Asian patients and that yet unidentified genes are involved in the pathogenesis of SRNS in Asian patients [23, 24, 28].

Most of the studies so far published have referred to European populations and have reported that R138Q was most frequently found in Germany and France [17, 18], while the P20L variant was observed mainly in Italy [14] and Turkey [16]. Essentially, P20L has also been reported frequently in Europe [8, 12, 13, 15]. Also, the R168H mutation has been reported in Israeli-Arab children [27]. In our study, R168H and P20L were the most common mutations in familial NS patients.

The age at onset of proteinuria is rather variable in different reports [12, 16, 17, 18]. Weber et al. [18] reported that R138Q could be associated with early onset as well as V180M, R229Q and R238S could be associated with late onset NS. In our study, P20L and R168H were found to be associated with early onset. Seven of 14 cases with an age at onset within the first year of life showed P20L and R168H mutations. Also, R138Q mutation was determined only in four patients with sporadic NS. In three of them, the first clinical symptoms had been observed before the age of 3 years, and one progressed to ESRD. The fourth patient had compound heterozygote mutations (R138Q and R229Q), the first occurring at 9.5 years.

It has been pointed out that sporadic NS patients with a *NPHS2* mutation progressed to ESRD after 73 months from the onset of proteinuria (range 6–155 months), while familial cases reached ESRD after a follow-up of 76 months (range 18–162 months) [14, 16, 17]. Similarly, in our study, patients with a mutation progressed to ESRD after 70 months from the onset of proteinuria (range 32–130 months). In addition, the presence of a mutation in exon 4 indicated an increased risk of renal failure or ESRD in our NPHS2 population.

The diagnosis of diseases related to podocin mutations is difficult because of the heterogeneous clinical and pathological spectrum; therefore, a molecular diagnosis based on sequencing is required. In patients with the sporadic form of SRNS, identification of *NPHS2* mutations is of impor-

Table 5 Summary of clinical phenotypes of SRNS patients and *NPHS2* mutations (*MCNS* minimal change nephrotic syndrome, *FSGS* focal segmental glomerulosclerosis, *DMP* diffuse mesangial proliferation, *ND* no data, *SRNS* steroid-resistant nephrotic syndrome, *ESRD* end stage renal disease)

Ethnicity	Europeans (Africans, Middle East descendent)						Asians	
Geographic origin	France and North Africa		Central Europe, India, Italy		Turkey		Japan and Korea	Japan
No. of patients	147 (81 families)[a]	172 sporadic	169 (148 families)	120 sporadic	41 (32 families)	254 sporadic	31 (15 families)	36 sporadic
Mean age at month of onset proteinuria (range)	57.6	102	48[b]	71 (1–216)	51.7 (6–156)	45.8 (4–160)	44.4 (6–132)	46.8 (2–171)
Histology								
Total no	106	155	47[c]	120	41	161	31	36
Diagnosis (%)								
FSGS	64	67	68	69	73	71	81	86
MCNS	22	27	17	9	8	8	6	6
DMP	14	6	ND	12	6	8	ND	8
ESRD								
ESRD/total no. of patients	ND	ND	28/47[c]	58/120	3/12[d]	13/73[d]	28/31	36/36
Percentage of ESRD	ND	ND	60	48	25[d]	17.8[d]	60	100
Age at ESRD (years, range)	ND	ND	10.0	ND			6.1 (0.6–1.6)	4.6 (0–15)
Frequency of *NPHS2* mutations (%)	38[e]	10.5	26[e]	23[f]	29.2	24	0	0
References	[18]	[18]	[17]	[14]	This study	This study	[23]	[28]

[a] Defined as SRNS in families with two or more affected children or at least one affected in a consanguineous family
[b] CNS patients were excluded from calculations
[c] The proportions of patients who developed ESRD for the total biopsy-proven cases are given
[d] Frequency was calculated based on the number of patients with mutations
[e] Frequency was calculated based on the number in the family
[f] R229Q variants were not included because their carrier status (homozygote or heterozygote) were unknown

tance, not only for therapeutic considerations but also for genetic counseling. However, there is no clear genotype/phenotype correlation in children with *NPHS2* mutations [15, 17, 24–28]. The rate of familial NS, hematuria and hypertension, the mean age at diagnosis, the presence of consanguinity, and the FSGS histology in patients with mutations were found to be significantly higher than in patients without mutations ($P<0.05$). Therefore, we propose that mutational analysis of *NPHS2* should be performed in children immediately after they have presented with their first episode of NS. This information may have important implications for the clinical and therapeutic approach to patients with a nephrotic syndrome that is unresponsive to steroids, because, theoretically, ineffective and potentially harmful immunosuppression could be avoided in carriers of the mutations. Also, Caridi et al. [13], Ruf et al. [17] and Frishberg et al. [27] have suggested that children with a first episode of NS should be tested for a podocin mutation before therapy, to avoid an unnecessary steroid course in those with *NPHS2* mutations. Therefore, further studies with more patients are required to search the genotype/phenotype relationships for homozygote or compound heterozygote mutations in *NPHS2* and responses to other forms of immune suppressive treatments, such as CsA, tacrolimus, cyclophosphamide, methylprednisolone pulse therapy, or mycophenolate mofetil. For the significance of single heterozygote sequence variants, functional studies must be performed.

A small number of studies have described polymorphism in untranslated regions, including intronic positions. The significance of certain mutations is yet unknown, especially in patients affected by compound heterozygote mutations with the R229Q variant [18, 29]. In this study, the R229Q mutation was determined in one patient with familial NS and in 13 patients with sporadic NS. Five of the 13 patients with sporadic NS had a homozygote R229Q mutation, and one of them progressed to ESRD. Other reported homozygote polymorphisms are A242V, A318A, S96S, L346L, A44E, G34G, and A297A [13, 18, 22, 26, 28–36]. In our study, we found the following variants: G34G in 263 patients, A318A in 47 patients, S96S in three patients, L314L in one patient, and Y131Y in one patient. Further

studies are necessary to determine the allele and genotype frequencies across different populations.

Our study has contributed to the descriptions of novel podocin mutations. Thirty-seven new mutations (Table 4 and bold characters in Table 3) have been described in our study. Age at onset of proteinuria in patients with M115T and 377insT novel mutations was under 1 year. We think that this syndrome will be more understandable with the increase in identification of other causative genes in different populations.

In conclusion, podocin mutations are responsible for some of both familial and sporadic SRNS cases in Turkey. The mutations in this gene should be searched for in every child after presentation with the first episode of NS, thus avoiding an unnecessary second trial of standard steroid therapy with SRNS. Further genetic studies in families with NPHS2-negative SRNS are warranted for the identification of other causative genes.

References

1. Arbeitsgemeinschaft fur Pädiatrische Nephrologie (1988) Short versus standard prednisone therapy for initial treatment of idiopathic nephrotic syndrome in children. Lancet 1:380–383
2. International Study of Kidney Disease in Children (1981) Primary nephrotic syndrome in children: clinical significance of histopathologic variants of minimal change and of diffuse mesangial hypercellularity. A report of the International Study of Kidney Disease in Children. Kidney Int 20:765–771
3. Fuchshuber A, Jean G, Gribouval O, Gubler MC, Broyer M, Beckmann JS, Niaudet P, Antignac C (1995) Mapping a gene (SRN1) to chromosome 1q25-q31 in idiopathic nephrotic syndrome confirms a distinct entity of autosomal recessive nephrosis. Hum Mol Genet 4:2155–2158
4. Fuchshuber A, Gribouval O, Ronner V, Kroiss S, Karle S, Brandis M, Hildebrandt F (2001) Clinical and genetic evaluation of familial steroid-responsive nephrotic syndrome in childhood. J Am Soc Nephrol 12:374–378
5. Kestila M, Lenkkeri U, Mannikko M, Lamerdin J, McCready P, Putaala H, Ruotsalainen V, Morita T, Nissinen M, Herva R, Kashtan CE, Peltonen L, Holmberg C, Olsen A, Tryggvason K (1998) Positionally cloned gene for a novel glomerular protein—nephrin—is mutated in congenital nephrotic syndrome. Mol Cell 4:575–582
6. Winn MP, Conlon PJ, Lynn KL, Howell DN, Slotterbeck BD, Smith AH, Graham FL, Bembe M, Quarles LD, Pericak-Vance MA, Vance JM (1999) Linkage of a gene causing familial focal segmental glomerulosclerosis to chromosome 11 and further evidence of genetic heterogeneity. Genomics 58:113–120
7. Kaplan JM, Kim SH, North KN, Rennke H, Correia LA, Tong HQ, Mathis BJ, Rodriguez-Perez JC, Allen PG, Beggs AH, Pollak MR (2000) Mutations in ACTN4, encoding α-actinin-4, cause familial focal segmental glomerulosclerosis. Nat Genet 24:251–256
8. Boute N, Gribouval O, Roselli S, Benessy F, Lee H, Fuchshuber A, Dahan K, Gubler MC, Niaudet P, Antignac C (2000) NPHS2, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome. Nat Genet 24:349–354
9. Roselli S, Gribouval O, Boute N, Sich M, Benessy F, Attie T, Gubler MC, Antignac C (2001) Podocin localizes in the kidney to the slit diaphragm area. Am J Pathol 160:131–139
10. Huber TB, Simons M, Hartleben B, Sernetz L, Schmidts M, Gundlech E, Saleem MA, Walz G, Benzing T (2003) Molecular basis of the functional podocin-nephrin complex: mutations in the NPHS2 gene disrupt nephrin targeting to the lipid raft microdomains. Hum Mol Genet 12:3397–3405
11. Schwarz K, Simons M, Reiser J, Saleem MA, Faul C, Kriz W, Shaw AS, Holzman LB, Mundel P (2001) Podocin, a raft-associated component of the glomerular slit diaphragm, interacts with CD2AP and nephrin. J Clin Invest 108:1621–1629
12. Caridi G, Berdeli A, Dagnino M, DiLuca M, Mir S, Cura A, Ravazzolo R, Ghiggeri GM (2004) Infantile steroid-resistant nephrotic syndrome associated with double homozygote mutations of podocin. Am J Kidney Dis 43:727–732
13. Caridi G, Bertelli R, Carrea A, Di Duca M, Catarsi P, Artero M, Carraro M, Zennaro C, Candiano G, Musante L, Seri M, Ginevri F, Perfumo F, Ghiggeri GM (2001) Prevalence, genetics, and clinical features of patients carrying podocin mutations in steroid-resistant nonfamilial focal segmental glomerulosclerosis. J Am Soc Nephrol 12:2742–2746
14. Caridi G, Bertelli R, Di Duca M, Dagnino M, Emma F, Onetti Muda A, Scolari F, Miglietti N, Mazzucco G, Murer L, Carrea A, Massella L, Rizzoni G, Perfumo F, Ghiggeri GM (2003) Broadening the spectrum of diseases related to podocin mutations. J Am Soc Nephrol 14:1278–1286
15. Caridi G, Bertelli R, Scolari F, Sanna-Cherchi S, Di Duca M, Ghiggeri GM (2003) Podocin mutations in sporadic focal-segmental glomerulosclerosis occurring in adulthood. Kidney Int 64:365
16. Caridi G, Perfumo F, Ghiggeri GM (2005) NPHS2 (podocin) mutations in nephrotic syndrome. Clinical spectrum and fine mechanisms. Pediatr Res 57:54–61
17. Ruf RG, Lichtenberger A, Karle SM, Haas JP, Anacleto FE, Schultheiss M, Zalewski I, Imm A, Ruf EM, Mucha B, Bagga A, Neuhaus T, Fuchshuber A, Bakkaloglu A, Hildebrant F (2004) Patients with mutations in NPHS2 (podocin) do not respond to standard steroid treatment of nephrotic syndrome. J Am Soc Nephrol 15:722–732
18. Weber S, Gribouval O, Esquivel EL, Moriniere V, Tete MJ, Legendre C, Niaudet P, Antignac C (2004) NPHS2 mutation analysis shows genetic heterogeneity of steroid-resistant nephrotic syndrome and low post-transplant recurrence. Kidney Int 66:571–579
19. Ozer EA, Aksu N, Erdogan H, Yavascan O, Kara O, Gribouval O, Gubler MC, Antignac C (2004) A novel NPHS2 gene mutation in Turkish children with familial steroid-resistant nephrotic syndrome. Nephrology 9:310–312
20. Ozcakar ZB, Cengiz FB, Cakar N, Uncu N, Kara N, Acar B, Yuksel S, Ekim M, Tekin M, Yalcinkaya F (2006) Analysis of NPHS2 mutations in Turkish steroid-resistant nephrotic syndrome patients. Pediatr Nephrol 21:1093–1096
21. D'Agati VD, Fogo AB, Bruijn JA, Jennette JC (2004) Pathologic classification of focal segmental glomerulosclerosis: a working proposal. Am J Kidney Dis 43:368–382
22. Karle S, Uetz B, Ronner V, Glaeser L, Hildebrandt F, Fuchshuber A, Members of the APN Study Group (2002) Novel mutations in NPHS2 are detected in familial and sporadic steroid-resistant nephrotic syndrome. J Am Soc Nephrol 13:388–393
23. Kitamura A, Tsukaguchi H, Iijima K, Araki J, Hattori M, Ikeda M, Honda M, Nozu K, Nakazato H, Yoshikawa N, Kagami S, Muramatsu M, Choi Y, Cheong HI, Doi T (2006) Genetics and clinical features of 15 Asian families with steroid-resistant nephrotic syndrome. Nephrol Dial Transplant 21:3133–3138

24. Yu Z, Ding J, Huang J, Yao Y, Xiao H, Zhang J, Liu J, Yang J (2005) Mutations in NPHS2 in sporadic steroid-resistant nephrotic syndrome in Chinese children. Nephrol Dial Transplant 20:902–908
25. Lenkkeri U, Mannikko M, McCready P, Lamerdin J, Gribouval O, Niaudet PM, Antignac CK, Kashtan CE, Homberg C, Olsen A, Kestila M, Tryggvason K (1999) Structure of the gene for congenital nephrotic syndrome of the Finnish type (NPHS1) and characterization of mutations. Am J Hum Genet 64:51–61
26. Dusel JA, Burdon KP, Hicks PJ, Hawkins GA, Bowden DW, Freedman BI (2005) Identification of podocin (NPHS2) gene mutations in African Americans with nondiabetic end-stage renal disease. Kidney Int 68:256–262
27. Frishberg Y, Rinat C, Megged O, Shapira E, Feinstein S, Raas-Rothschild A (2002) Mutations in NPHS2 encoding podocin are a prevalent cause of steroid-resistant nephrotic syndrome among Israeli-Arab children. J Am Soc Nephrol 3:400–405
28. Maruyama K, Iijima K, Ikeda M, Kitamura A, Tsukaguchi H, Yoshiya K, Hoshii S, Wada N, Uemura O, Satomura K, Honda M, Yoshikawa N (2003) NPHS2 mutations in sporadic steroid-resistant nephrotic syndrome in Japanese children. Pediatr Nephrol 18:412–416
29. Tsukaguchi H, Sudhakar A, Le TC, Nguyen T, Yao J, Schwimmer JA, Schachter AD, Poch E, Abreu PF, Appel GB, Pereira AB, Kalluri R, Pollak MR (2002) NPHS2 mutations in late-onset focal segmental glomerulosclerosis: R229Q is a common disease associated allele. J Clin Invest 110:1659–1666
30. Franceschini N, North KE, Kopp JB, McKenzie L, Winkler C (2006) NPHS2 gene, nephrotic syndrome and focal segmental glomerulosclerosis: a HuGE review. Genet Med 8:63–75
31. Wu MC, Wu JY, Lee CC, Tsai CH, Tsai FJ (2001) A novel polymorphism (c288C>T) of the NPHS2 gene identified in a Taiwan Chinese family. Hum Mutat 17:81–82
32. Schultheiss M, Ruf RG, Mucha BE, Wiggins R, Fuchshuber A, Lichtenberger A, Hildebrandt F (2004) No evidence for genotype/phenotype correlation in NPHS1 and NPHS2 mutations. Pediatr Nephrol 19:1340–1348
33. Komatsuda A, Wakui H, Maki N, Kigawa A, Goto H, Ohtani H, Hamai K, Oyama Y, Makoto H, Sawada K, Imai H (2003) Analysis of mutations in alpha-actinin 4 and podocin genes of patients with chronic renal failure due to sporadic focal segmental glomerulosclerosis. Ren Fail 25:87–93
34. Roselli S, Moutkine I, Gribouval O, Benmerah A, Antignac C (2004) Plasma membrane targeting of podocin through the classical exocytic pathway: effect of NPHS2 mutations. Traffic 5:1–8
35. Niaudet P (2004) Podocin and nephrotic syndrome: implications for the clinician. J Am Soc Nephrol 15:832–834
36. Koziell A, Grech V, Hussain S, Lee G, Lenkkeri U, Tryggvason K, Scambler P (2002) Genotype/phenotype correlations of NPHS1 and NPHS2 mutations in nephrotic syndrome advocate a functional interrelationship in glomerular filtration. Hum Mol Genet 11:379–388