NPHS2 Gene in Steroid-resistant Nephrotic Syndrome

Prevalence, Clinical Course, and Mutational Spectrum in South-West Iranian Children

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Introduction. Mutations in podocin (*NPHS2*) gene have the key role in the pathogenesis of steroid-resistant nephrotic syndrome (SRNS) in children, but data is scarce regarding their prevalence and natural course among different all ethnic groups. This study was aimed to demonstrate the spectrum of *NPHS2* mutations in children with SRNS and to compare the clinical course of disease in patients with and without mutation.

Materials and Methods. All 8 exons of *NPHS2* were sequenced in 99 children, including 49 with SRNS and 50 with steroid-sensitive nephrotic syndrome (control group) by DNA sequencing.

Results. The prevalence rates of *NPHS2* gene mutation among children with SRNS and SSNS were 31% and 4%, respectively. The prevalence rates of mutation among familial and sporadic forms were 57% and 26%, respectively. Thirty-three percent of the children experienced recurrence of primary disease after kidney transplantation, none of whom had a mutation. The clinical response to treatment was poorer in children with mutation in comparison with patients without mutation (12% versus 32%, respectively; odds ratio, 3.29, 95% confidence interval, 0.40 to 25.64). Patients with and without mutation could not be differentiated by demographic and histological features, glomerular filtration rate at onset, hypertension, progression to end-stage renal disease, and proteinuria.

Conclusions. Mutations of *NPHS2* gene are frequent among Iranian children with SRNS. Regarding similar clinical features in patients with and without mutation and poor response to pharmacotherapy in patients with mutation, a molecular approach might be necessary for different treatment plans and prediction of prognosis.

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INTRODUCTION

Idiopathic nephrotic syndrome is the most common cause of nephrotic syndrome in children. Most children are steroid sensitive; however, 10% to 20% of patients are steroid resistant, and up to 50% develop end-stage renal disease (ESRD) after 5 years.[1,2] Focal segmental glomerulosclerosis (FSGS) is the most common cause of steroid-resistant nephrotic syndrome (SRNS) and is a heterogeneous entity characterized by a common kidney biopsy findings of segmental sclerosis.[3] It can occur as a primary disorder, secondary to different exogenous factors, or genetic mutation in podocytes.

Recent advances have uncovered new genes and signaling pathways that were linked to FSGS. They all produce structural defects in the glomerular

barrier that terminates to podocyte dysfunction. Since from the first report of the genetic form of FSGS due to podocin gene mutation, 7 other genetic defects—*CD2AP*, laminin b2, Wilms tumor-1, phospholipase C epsilon-1, α-actininin-4, *TRPC6*, and *IFN2*—have been identified.[4] Podocin gene has the key role in the pathogenesis of SRNS in children and accounts for disease in 26% of familial FSGS and 12% to 19% of sporadic forms.[5,6] Although the exact role and function of the new proteins has opened new windows to the pathogenesis of FSGS, there is not precise data regarding the prevalence, genetics, and natural course of FSGS among different ethnic groups around the world.

The aim of our study was to demonstrate the spectrum of mutations in a case-control study of children with SRNS and to compare the clinical course of disease in patients with and without *NPHS2* mutation from the south-west of Iran.

MATERIALS AND METHODS

We reviewed the clinical charts of all patients younger than 18 years old, diagnosed with primary SRNS from 1990 to 2010. The data at presentation were collected retrospectively from medical records, including age, sex, weight, height, parent consanguinity, blood pressure, creatinine clearance, 24-hour urinary protein excretion. The collected follow-up period data included response to therapy, time interval to renal insufficiency, ESRD, and transplantation. Children with poor follow-up and those who did not undergo kidney biopsy were excluded and the remaining 49 patients were included in the study. The study was approved by the local Ethics Committee. Informed consent was obtained from the participants` parents.

Steroid-sensitive nephrotic syndrome (SSNS) was defined as attainment of complete response within initial 8 weeks of corticosteroid therapy, and SRNS, as the persistence of proteinuria 8 weeks after steroid therapy. Chronic kidney disease was defined as an estimated glomerular filtration rate (GFR) less than 50 mL/min/1.73 m^2 and ESRD as a GFR less than 10 mL/min/1.73 m^2. The GFR was measured using the Schwartz formula. Posttransplant recurrence of disease was demonstrated by reappearance of proteinuria and documented by transplant kidney biopsy.

Kidney biopsy was performed in all patients with SRNS and sections were re-examined by an expert nephropathologist and classified according to the working proposal.[7] Mutation analysis of *NPHS2* gene was performed for 99 patients including 49 SRNS patients and 50 SSNS patients as controls. DNA was extracted from formalin-fixed tissue or peripheral leukocyte samples and exons and boundary region of the gene examined by direct sequencing method as previously described.[8] The polymerase chain reaction assay of exons 3, 4, and 8 was carried out as previously described.[9] For the remaining exons, the primers (Table 1) and conditions were applied at annealing temperatures of 52°C (exon 5) and 60°C (exons 1, 2, 6, and 7). Because of the high CG content of exon 1, polymerase chain reaction was performed with hot start Taq polymerase and Q solution (Qiagen, Hilden, Germany).

Data analysis was performed by the SPSS software (Statistical Package for the Social Sciences, version 15.0, SPSS Inc, Chicago, Ill, USA). The results were expressed as frequencies or mean ± standard deviation. Comparison of means and proportions was performed by the Student *t* test, chi-square test, and the Fisher exact test, respectively. The Pearson correlation coefficient was used to determine the association between subgroups. A *P* value less than .05 was considered significant.

RESULTS

A total of 49 children with SRNS (28 boys and 21 girls) were included in this study as the SRNS group. The mean age was 6.82 ± 4.24 years (range,

Table 1. List of Primers Used for Sequencing of Exons

Exon	Primers	
1	5'-GCAGCGACTCCACAGGGACT-3'	5'-TCCACCTTATCTGACGCCCC-3'
2	5'-AGAATTGGACCAACAGATGC-3'	5'-AAGTGAGAATGGGCATGGTG-3'
5	5'-AAAGGAGCCCAAGAATCAAG-3'	5'-AAATATTTCAGCATATTGGCC-3'
6	5'-GTTTAGGCATGCTC TCCTC-3'	5'-GATATGGCTATAGTACTCAGTG-3'
7	5'-GTCTGTGTGAAAGCTTTGGC-3'	5'-GCAAAGGGGAAATGTTCTCC-3'

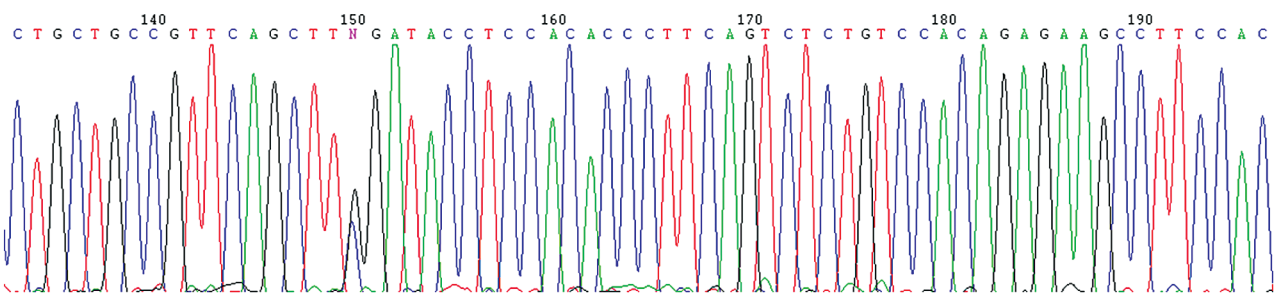
10 months to 15 years). The mean duration of follow-up was 42.0 ± 39.5 months (range, 6 to 186 months).

The prevalence of *NPHS2* gene mutation among the children with SRNS was 30.6% (15 out of 49 cases). Of the 50 children with SSNS (control group), 2 (4.0%) had a heterozygote pattern mutation on the *NPHS2* gene (Figure). Details of mutations found among the 15 SRNS patients and 2 children with SSNS are shown in Table 2.

Pathologic examination revealed that FSGS comprised the majority of cases with SRNS (43 cases, 87.7%) followed by 6 with diffuse mesangioproliferative glomerulonephritis (MPGN). The following histologic variants of FSGS were noted: not otherwise specified (NOS), 16 cases (37%); cellular, 13 cases (30%); prehilar, 11 cases (26%); and collapsing, 3 cases (7%). The histologic features of global sclerosis, segmental sclerosis, mesangial hypercellularity, tubular atrophy, and interstitial fibrosis could not differentiate between patients with and without mutation ($P = .79$, $P = .13$, $P = .52$, $P = .30$, and $P = .80$, respectively). The most common pathologic variants among patients with *NPHS2* mutation were cellular (7 cases, 47%), followed by NOS (5 cases, 33%), prehilar (2 cases, 13%), and MPGN (1 case, 7%). In contrast, NOS was more prevalent in patients without mutation (11 cases, 32%), followed by prehilar (9 cases, 26%), cellular (6 cases, 18%), MPGN (5 cases, 15%), and collapsing (3 cases, 9%). Seven patients had a family history of SRNS in their first-degree relatives, and the remainders (42 patients) were sporadic cases. The prevalence of mutation among familial and sporadic forms was 57% (4 of 7) and 26% (11 of 42), respectively.

Nine patients underwent kidney transplantation and 3 of them (33%) experienced recurrence of the primary disease shortly after engraftment (1 patient during the first week and the others during the first month posttransplantation). Only 1 of 9 transplanted children (11%) showed *NPHS2* gene mutation, and all of the children with recurrence of the primary disease did not reveal any mutation at the studied region of *NPHS2* gene.

There was no difference between patients with and without mutation regarding age, sex, GFR at onset, hypertension, progression to ESRD, and proteinuria ($P = .25$, $P = .78$, $P = .40$, $P = .43$, $P = .51$, and $P = .64$, respectively). Eight of 15 patients with *NPHS2* mutation were treated with cytotoxic drugs (cyclosporine, cyclophosphamide, or both) and none of them except 1 child (12%) had clinical response. In contrast, 25 patients without mutation received cytotoxic medications, and 8 of 25 children (32%) demonstrated partial or complete response (odds ratio, 3.29, 95% confidence interval, 0.40 to 25.64).



A mutation at the cd322 (CGA>GGA) that substituted argninine by glycine.

Table 2. Spectrum of Mutations at *NPHS2* Gene in Children With Nephrotic Syndrome from South-West of Iran

Nucleotide Change	Number of Patients	Mutant Alleles	Genotype	Clinical Characteristic
353 C>T	3	P118L	Homo	Steroid Resistant
479 A>G	1	D160G	Homo	Steroid Resistant
502 C>T	2	R168C	Het	Steroid Resistant
538 G>A	1	V180M	Homo	Steroid Resistant
555 del T	2	F185fsX186	Het	Steroid Resistant
714 G>T	4	R238S	Homo	Steroid Resistant
864 G>A	2	A288T	Het	Steroid Resistant
966 C>G	2	R322G	Het	Steroid Sensitive

DISCUSSION

Podocin is encoded by *NPHS2*, localized at chromosome 1q25 by positional cloning. This protein plays an important role in the regulation of glomerular permeability and acts as a linker between the plasma membrane and the cytoskeleton. It is believed that podocin has interaction with nephrin, α-actinin-4, and *CD2AP*.[10] Podocin mutations predominate in patients with infantile and early childhood SRNS.[8] Our important finding of this study was that mutations of podocin were frequent among south-west Iranian pediatric population with SRNS. About one-third of our study children with SRNS presented mutation in the protein coding region or interon-exon area of *NPHS2* gene. The prevalence rates of mutation among familial and sporadic forms were 57% and 26%, respectively. The allele 714 G>T and 353 C>T at the position of amino acid 118 and 238 were the most prevalent alleles in the studied region, respectively. A patient from the SSNS control group showed a new mutation (966 C>G, cd322) in heterozygote pattern that was not reported so far (Figure). Although the substitution of 1 amino acid by another one in heterozygote pattern will affect structural and chemical property of podocin, its pathophysiologic role is unclear.

There are different reports regarding the prevalence of podocin mutation in various ethnic groups. In populations of European ancestry, Ruf and colleagues[5] reported homozygote or compound heterozygote *NPHS2* mutations in 26% of families with familial FSGS. Caridi and colleagues[6] reported podocin mutation in 12% to 19% of sporadic pediatric FSGS. In contrast to previous reports, *NPHS2* mutations appear to be uncommon in Japanese pediatric patients with SRNS.[11] Chernin and associates showed that in African-American children with SRNS, the frequency of *NPHS2* mutations is much lower than in large cohorts of pediatric SRNS patients in the general population.[12] As in other genetic disorders, the different incidence rates of podocin mutation in other reports are related to different genetic backgrounds among various ethnic groups.

We demonstrated that patients with and without podocin mutation mimicked each other with respect to the severity of proteinuria, age, sex, GFR at onset, and hypertension. This is in accordance with the study of Caridi and coworkers[6] that demonstrated no genotype-phenotype correlation between children with *NPHS2* mutation and idiopathic FSGS. Although these variables cannot define the clinical course between patients with and without mutation, some studies have shown that specific podocin mutations correlate with age of onset in SRNS.[13,14] This study showed that patients with *NPHS2* mutations had less clinical response to treatment in comparison to patients without *NPHS2* mutation. Our data and most studies[5,6,15-17] on response to cytotoxic therapy in children with podocin mutation have indicated that this genetic form of nephrotic syndrome is mostly drug resistant. However, one of the children with molecular defect of *NPHS2* in the present study had complete response to cyclosporine after 6 months, and this might be attributed to the direct effect of cyclosporine on the stabilization of the podocyte actin cytoskeleton.[18] The latest guidelines recommend cyclosporine rather than alkylating agents in patients with SRNS or FSGS.[19] Regarding the high prevalence of genetic causes of SRNS, Rood and coleagues[20] suggested mutation analysis in patients with familial and sporadic SRNS in order to avoid patient exposure to prolonged treatment with corticosteroids or cyclophosphamide.

Approximately, 30% of patients with primary FSGS will develop recurrent disease in the allograft.[21,22] We demonstrated the same rate of recurrence (33%) among our patients. All of our patients with recurrence of primary disease were without *NPHS2* mutation. Only 1 of 9 transplant children exhibited NPHS2 mutation and he did not experience recurrent disease during 5-year posttransplant follow-up. Although the number of transplant cases and those with mutation were very low in the present study, this preliminary result is in line with the other reports with respect to low recurrence rate after kidney transplantation in genetic forms of FSGS.[23,24] If one considers that the defective protein is the cause of FSGS, the disease should be cured in allograft recipients, but recurrence of the primary disease has been reported in 5% to 10% of patients with podocin mutations[5,22]; therefore, some extrarenal factors are needed for reappearance of proteinuria. Several investigators have proposed potential permeability factors including cardiotrophin-like cytokine-1 and soluble urokinase receptor, responsible for

increased permeability of glomerular basement membrane, but their mechanisms of action are not well understood.[25-27] Caridi and colleagues[28] have suggested 2 different phases of recurrence with separate mechanisms. Plasma factors cause prolonged loss of podocin in the urine and its resynthesis is linked to the donor's genetic background.

Analysis of histological variants demonstrated that the majority of cases (88%) corresponded to FSGS. The NOS variant was the prevailing form as reported by other studies.[29,30] The tip variant was not found in the biopsies. Malhotra and associates[31] also identified greater response rates to steroid therapy in the tip variant cases and poor renal outcome for NOS subtype of FSGS. This study once more shows the usefulness of Columbia classification[7] for prediction of response to steroid therapy. Among 15 patients with mutation, 80% had pathologic features of either cellular (47%) or NOS (33%), while this combination was found in 50% of patients without mutation (NOS, 32% and cellular,18%). Although it seems that cellular variant is more common in patients with mutation, further studies with larger number of cases are needed to address the relationship between genotype and histological classification.

The current study had some limitations; it was not a prospective longitudinal trial and had a small sample size. Prospective longitudinal and multicentral studies are required to delineate the association between genotype and different histological variants of FSGS.

CONCLUSIONS

Our study demonstrates that mutations of *NPHS2* gene are frequent among Iranian pediatric population with SRNS. Regarding similar presenting symptoms in patients with and without mutation and poor drug response and low recurrence of primary disease after transplantation in patients with mutation, a molecular approach might be necessary for different treatment plan and prediction of prognosis.

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CONFLICT OF INTEREST

None declared.

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