

NPHS2 Mutations in Children With Steroid-Resistant Nephrotic Syndrome

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Introduction. Congenital nephrotic syndrome may be caused by mutations in *NPHS1* and *NPHS2*, which encode nephrin and podocin, respectively. Since the identification of the *NPHS2* gene, various investigators have demonstrated that its mutation is an important cause of steroid-resistant nephrotic syndrome. We aimed to evaluate frequency and spectrum of podocin mutations in the Iranian children with steroid-resistant nephrotic syndrome.

Materials and Methods. We examined 20 children with steroid-resistant nephrotic syndrome referred to Ali Asghar Children's Hospital, in Tehran, Iran. Mutations in the 5th and 7th exons of *NPHS2* were assessed. The mutational analysis of *NPHS2* was performed by DNA sequencing.

Results. The mean age at the onset of proteinuria was 6.4 ± 3.6 years. None of the children had mutations in the exons 5 or 7.

Conclusions. Our study suggests that *NPHS2* mutations in exons 5 and 7 are not seen in our children. Therefore, we cannot recommend *NPHS2* (exons 5 and 7) mutation for screening in Iranian children with steroid-resistant nephrotic syndrome. Other exons of podocin or other podocyte proteins in Iranian children may play a role in pathogenesis of steroid-resistant nephrotic syndrome.

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INTRODUCTION

Idiopathic nephrotic syndrome (NS) has an annual incidence ranging from 2 to 7 new cases in children less than 16 years of age in the United States, corresponding to a cumulative prevalence of 15.7 per 10 children.¹ Most patients with idiopathic NS respond to steroid therapy and have a favorable long-term outcome, although a number of them may experience steroid-dependent relapses. Ten percent of children with idiopathic NS, however, fail to respond to glucocorticoids. These patients are at risk of end-stage renal disease and account for more than 10% of children who progress to kidney failure.² In many patients with the steroid-resistant

form, circulating agents produced by immune cells increase the glomerular basement membrane permeability to proteins. In other patients, genetic factors may be involved. A growing number of genes have been identified that, when defective, lead to inherited forms of idiopathic NS. Podocin-mediated focal segmental glomerulosclerosis seems to be the most common inherited form of this set of diseases.

Podocin is one of the podocyte proteins which is produced by encoding the recessive gene of *NPHS2* located in 1q25. Congenital NS may be caused by mutations in one of the two genes, *NPHS1* and *NPHS2*, which encode the proteins nephrin and

Sequences of Primers Used for Polymerase Chain Reactions

NPHS2 Exon	Forward Primer	Reverse Primer
Exon 5	AGGATTACCACAGGATTAAGTTGTGCA	TAGCTATGAGCTCCCAAAGGGATGG
Exon 7	AGTCTGTGTGAAAGCTTTGG	CCTTCCTAAAGGGCAGTCT

podocin, respectively. These components are thought to play an essential role in the normal function of the glomerular filtration barrier.³ Although recessive *NPHS2* mutations initially were reported to cause familial steroid-resistant NS in children aged between 3 months and 5 years with kidney failure,⁴ recent studies have shown that they are associated with a broader clinical spectrum regarding age of onset in steroid-resistant nephrotic syndrome, late steroid resistance, and relapse.^{5,6} In Iran, knowledge on mutations of podocin in children with steroid-resistant NS (SRNS) is lacking. Thus, we decided to evaluate the presence of a probable mutation in most-common exons of podocin gene which are involved in SRNS.

MATERIALS AND METHODS

This study was a prospective assessment of 20 children with SRNS referred to Ali-Asghar Children's Hospital, in Tehran, Iran. Nephrotic syndrome was defined as proteinuria (> 40 mg/m²/h), hypoalbuminemia (< 2.5 g/dL), hyperlipidemia (serum total cholesterol > 250 mg/dL), and edema. Familial and sporadic NS types were also determined by familial history. Steroid-resistant NS was defined as the failure to respond to 6- to 8-week daily administration of prednisone, 2 mg/kg, from the onset of the disease. In patients who responded to steroids, relapses during the protocol were managed by restarting corticosteroids. Relapse was defined as the relapse of proteinuria in nephritic range in association with edema. Patients with prednisolone-resistant relapse were included as "late nonresponders." All the eligible children with SRNS were selected consecutively.

This study was approved by the Ethics Committee of the Iran University of Medical Sciences and Health Services and consent of the children's parents was taken. On first admission, baseline demographics were determined. Hypertension was recorded, as defined by systolic and/or diastolic blood pressure above the 95 percentile for age and sex. Microscopic hematuria was defined as 1+ hematuria or more by dipstick, or 5 erythrocytes or more per high-power

field. Additionally, pathologic examination results of the renal specimens were evaluated.

Genomic DNA was isolated from peripheral blood leukocytes. Mutation analysis was performed in the 5th and 7th exons of the *NPHS2* gene with the direct DNA sequencing method. These two exons of *NPHS2* were amplified from genomic DNA by polymerase chain reaction assay and directly sequenced. All polymerase chain reaction primers were designed from intronic sequences (Table). Polymerase chain reaction products were reacted with Big Dye Terminator Mix (Applied Biosystems, Foster City, California, USA) using an M13 universal primer. Sequences were determined using ABI software. Sequences were analyzed using Mutation Surveyor software (Soft Genetics, State College, Pennsylvania, USA) and by inspection by eye.

RESULTS

Twenty children suffering from SRNS (10 girls and 10 boys) in Ali-Asghar Children's Hospital, in Tehran, were assessed. Their mean age at the onset of proteinuria was 6.4 ± 3.6 years (range, 1 to 12 years). Five patients (25.0%) had familial NS and 15 (75.0%) had sporadic NS. The histological findings were as follows: focal segmental glomerulosclerosis in 7 (35.0%), diffuse mesangial proliferation in 7 (35%), and minimal change NS in 6 (30.0%). Immunoglobulin M deposition with mesangial proliferation was seen in 1 patient. Another patient had both immunoglobulin G and M deposition. Eighteen children (90.0%) had negative findings on fluorescent antibody technique studies. Seven patients (35.0%) ended up with end-stage renal disease. Finally, in the 20 children with SRNS, no mutations were found in the 5th and 7th exons of the *NPHS2* gene.

DISCUSSION

Podocin, a 24-KDa protein with 383 aminoacids, localizes the slit diaphragm and interacts directly with nephrin and CD2-associated protein.⁷ Nephrin and podocin are key components of the slit diaphragm of the glomerular epithelial cell and

play an essential role in the normal function of the glomerular filtration barrier. Podocin defects alter slit diaphragm permeability and can also alter the processing and localization of nephrin.³

More than 50 putative diseases causing mutation of podocin gene have been identified. Diseases linked genetically to these mutations were seen in 50% of families with SRNS and autosomal recessive transmissions. In sporadic steroid-resistant cases, these mutations are responsible for 10% to 30% of diseases, reportedly by large studies.⁷⁻¹¹ Patients with podocin mutations have a large variability in the severity of their disease suggesting that other features both genetic and nongenetic are also important in modulating this disease. On the other hand, there may be a rough correlation between the type of mutation and severity of phenotype. Patients with frameshift or nonsense in both alleles appear to have a more aggressive course. In contrast, patients with other variant alleles, particularly R229Q, tend to have later-onset disease. Typically, focal segmental glomerulosclerosis is the most common pathology associated with these cases, although a spectrum of glomerular lesions, including mesangial proliferation and minimal change lesion, may also be seen.^{12,13}

It has been clearly shown that children with podocin-mutant disease do not respond to steroids. On the other hand, there is no clear difference in clinical and pathological phenotype between podocin-mediated disease and idiopathic NS. Given the adverse effects of prolonged steroid treatment, particularly in children, patients can undergo genetic testing for *NPHS2* mutations soon after disease presentation to help assess the risk that steroid therapy will fail.

Berdeli and colleagues assessed 295 children with SRNS originating from Turkey. Mutation analysis was performed in all 8 exons of the *NPHS2* gene with the direct DNA sequencing method. The mutation detection rate was 24.7% for all cases, 29.2% for familial and 24% for sporadic SRNS cases. They showed that the most common mutated was exon 5 and the presence of mutations in exon 4 was found to increase the risk of end-stage renal disease.¹⁴ Maruyama and coworkers isolated genomic DNA from 36 Japanese children with chronic renal insufficiency caused by SRNS or heavy proteinuria, and analyzed all 8 exons and exon-intron boundaries of *NPHS2* using the

polymerase chain reaction and direct sequencing. They concluded that *NPHS2* gene mutations are not a major cause of chronic renal insufficiency caused by sporadic SRNS or heavy proteinuria in Japanese children.¹⁵

In one of the most recent studies by Chernin and colleagues on 18 African-American children in 2008, after evaluating all 8 exons of *NPHS2*, the researchers declared that in African-American children with SRNS, the frequency of *NPHS2* mutations is much lower than in large cohorts of children with SRNS in the general population.¹⁶ On the other hand, Bakr and associates suggested that *NPHS2* mutations are prevalent in Egyptian children with nonfamilial SRNS, and this may in part explain the less favorable prognosis reported in these patients.¹⁷

Another study by Cho and colleagues showed that the incidence of *NPHS2* mutations seemed to be very rare in Korean children. They suggested that genetic diagnosis of *WT1* mutations should be recommended for children with SRNS, especially in cases involving a female phenotype or in males with genital anomalies.¹⁸ Different reports of the frequency of *NPHS2* mutations in different populations and countries show that the ethnicity may play an important role. However, the number of evaluated exons and the sample size are among the most important sources of these discrepancies.

Our study suggests that *NPHS2* mutations in exons 5 and 7 are not seen in our children. Therefore, we cannot recommend *NPHS2* (exons 5 and 7) mutation screening in Iranian children with SRNS. Although this study was the first among Iranian children, low sample size of children with steroid-resistant nephrotic syndrome and evaluating few numbers of exons in comparison with other large studies are our limitations in this study, and additional studies with larger sample size and considering more number of exons are needed to confirm these findings.

CONCLUSIONS

Knowledge of mutation rate of *NPHS2* in different populations of patients with SRNS facilitates the physician in planning a suitable genetic screening strategy for the patients. The *NPHS2* mutations in exons 5 and 7 were not seen in our children. Therefore, we cannot recommend *NPHS2* (exons 5 and 7) mutation for screening in Iranian children

with nephritic syndrome. However, due to our limitations, more studies are needed. Other exons of podocin or other podocyte proteins in Iranian children may play a role in pathogenesis of SRNS.

CONFLICT OF INTEREST

None declared.

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