R229Q Polymorphism of NPHS2 Gene in Patients With Late-Onset Steroid-Resistance Nephrotic Syndrome
A Preliminary Study

Nikou Fotouhi, Mohammadreza Ardalan, Mortaza Jabbarpour Bonyadi, Reza Abdolmohammadi, Amir Kamalifar, Hamid Nasri, Behzad Einollahi

Introduction. Depending on the response to standard steroid therapy, nephrotic syndrome it is classified to steroid-sensitive and steroid-resistant nephrotic syndrome (SRNS). Mutations in several genes including NPHS2 have been implicated in SRNS. Gene R229Q polymorphism (p.R229Q) of NPHS2 is associated with adolescent- or adult-onset SRNS in European and South American populations. We investigated this polymorphism among a group of Iranian-Azeri patients with primary SRNS.

Materials and Methods. All participants had the primary late-onset form of focal segmental glomerulosclerosis (FSGS) and their clinical feature was steroid unresponsiveness. They were compared with a group of age- and sex-matched individuals without any renal disease for NPHS2 gene as controls. The R229Q polymorphism (p.R229Q) was investigated in the case and control groups.

Results. A total of 25 patients (mean age, 26.6 ± 8.0 years) with primary FSGS and 35 controls (mean age, 26.0 ± 8.7 years) were studied. Serum creatinine of patients and their 24-hour protein excretion at the time of study were 2.4 ± 1.94 mg/dL and 2830 ± 981 mg/dL, respectively. Molecular study showed no p.R229Q polymorphism, neither in patients nor in controls.

Conclusions. In this preliminary study, we showed that NPHS2 gene p.R229Q polymorphism does not present in Iranian-Azeri population with SRNS. Larger studies are needed to confirm our results and other mutated genes should also be considered in these patients.
with certain genetic backgrounds do not respond to aggressive immunosuppressive treatment.\textsuperscript{2,5} Since the discovery of the first podocyte gene, \textit{NPHS1}, and its mutations in Finnish type NS in 1998, mutations in 7 other genes, including \textit{NPHS2}, \textit{NPHS3/PLCE1}, \textit{WT1}, \textit{CD2AP}, \textit{ACTN4}, \textit{TRPC6}, and \textit{INF2} have been implicated in SRNS.\textsuperscript{6} The \textit{NPHS1}, \textit{NPHS2}, \textit{WT1}, and \textit{LAMB2} are recessive mutations and are associated with earlier onset and more severe disease. With the exception of \textit{WT1}, all mutations in childhood-onset SRNS are recessive.\textsuperscript{7,8} Mutations in the \textit{NPHS2} encoding podocin mainly cause an autosomal recessive childhood SRNS, but it has also been identified as a cause of late-onset FSGS.\textsuperscript{9-11} Genetic testing for all podocyte genes is expensive, and the key challenge is identifying which patients are most likely to benefit from genetic study. A family history of NS and early onset of the disease are strong markers of genetic disease mutations and are found in 67\% of familial cases (versus 25\% of sporadic cases) and 100\% of patients with congenital SRNS.\textsuperscript{6} The precise testing approach depends on the age of onset. In congenital form, the first gene to be screened should be \textit{NPHS1} and in childhood, it should be \textit{NPHS2}. Compound heterozygosity for the R229Q polymorphism (p.R229Q) of \textit{NPHS2} is associated with adolescent- or adult-onset SRNS, mostly in patients of European or South American origin.\textsuperscript{12} It should be considered that not only podocytes cytoskeleton but also other genetic backgrounds such as expression of multidrug resistant gene-1 (\textit{MDR1}) on lymphocytes could predispose to steroid unresponsiveness in NS.\textsuperscript{13} In patients with late-onset SRNS, screening for p.R229Q is indicated, followed by complete \textit{NPHS2} analysis and looking for a second pathogenic mutation if the variant is present. If this investigation is negative, no future \textit{NPHS2} analysis is indicated. In patients with familial SRNS multistep screening of \textit{INF2}, \textit{TRPC6}, and \textit{ACTN4} are indicated, but no further investigations are recommended in sporadic cases.\textsuperscript{6}

The objective of this study was to investigate the significance of p.R229Q polymorphism in \textit{NPHS2} among a group of Iranian-Azeri patients with primary SRNS.

**MATERIALS AND METHODS**

**Patients**

All patients in our cohort had late adolescent- or adult-onset of the SRNS. They had the pathologic feature of FSGS and clinical feature of steroid unresponsiveness. All individuals had a primary FSGS without any obvious secondary causes. Obesity and reflux associated FSGS were ruled out by clinical history. None of patients had a history of nephrectomy, kidney operation or any history of renal parenchyma damage. Human immunodeficiency virus-associated FSGS was ruled out by serology tests. We also studied an age- and sex-matched group of individuals without any renal disease as controls. All participants were of Azeri Turk origin from northwestern Iran. All patients were informed about the study and written informed consent was obtained. This project was approved by the ethics committee of Tabriz University of Medical Sciences.

**Molecular Analysis of Podocin (R229Q)**

Genomic DNA was extracted from peripheral leukocytes of whole-blood samples using standard laboratory protocols.\textsuperscript{14} Noted region of \textit{NPHS2} gene was amplified by polymerase chain reaction (PCR) using appropriate primers described elsewhere (Table 1).\textsuperscript{9} The “hot-start” PCR was performed with 100 ng of genomic DNA, 10 mM of dNTP, 1.5 mM of magnesium, 10 pmol of each primer, and 1U of Taq in a 25-μL reaction for 35 cycles. After PCR, presence of R229Q polymorphism was distinguished by restriction digestion testing of PCR amplicon. ClaI (Bsu15I) digestion of exon 5 PCR product (293 bp) normally produces 2 fragments of 204 bp and 89 bp for wild-type allele, while the polymorphic allele remains uncut. The results were evaluated after electrophoresis in 15\% acrylamide gel and were visualized by silver staining.

**RESULTS**

A total of 25 patients (15 men and 10 women; mean age, 26.6 ± 8.0 years) with primary FSGS and 35 controls (17 men and 13 women; mean age 26 ± 8.7 years) were studied. Serum creatinine levels of patients and their mean 24-hour protein excretion at the time of genetic analysis were 2.4 ± 1.94 mg/dL.

**Table 1. Primer Sequences Used in Polymerase Chain Reaction Amplification**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>RQ-1 F</td>
<td>5’-CATAGGAAAGGAGCCAAAGA-3’</td>
</tr>
<tr>
<td>RQ-1 R</td>
<td>5’-TTCAGCATATTGGCCATTA-3’</td>
</tr>
</tbody>
</table>
and $2830 \pm 981$ mg/dL, respectively. Demographic and clinical characteristics and identified genotypes of the patients are shown in Table 2. Two patients in our cohort had a family history of SRNS and their siblings were also involved. Molecular study of the 50 alleles of referred patients showed no R229Q polymorphism (Figure). Furthermore, the p.R229Q was not detected in the control group either.

**DISCUSSION**

In this preliminary study, we showed that *NPHS2* gene R229Q polymorphism does not present in a sample of Iranian-Azeri population with SRNS, but the major limitation of our study was the small sample size, and larger studies and study of other mutated genes should also be considered in this population to reach more strong results. Wide spectrum of p.R229Q frequency has been reported in different populations. This polymorphism is prevalence among Czech (12%), Spanish (3.1%), French (4.5%), Brazilian (3.1%), and Italian (3.2%) populations. The highest frequency of R229Q
, after Czech population, has been reported in Chileans AND ArgentineanS (7.3%).12 The R229Q polymorphism has a lower frequency among Africans, African-Americans, and Asians (zero to 1.5%).2,6,15 Our result in an Iranian Azeri cohort was in agreement with another report from Iran that suggests that NPHS2 mutations in exons 5 and 7 are not seen in Iranian children with SRNS.19

The p.R229Q is a substitution (G>A) in exon 5 of the NPHS2 gene. The R229Q allele is disease-causing rather than a benign polymorphism in European, North American Caucasian, and South American populations.12 Therefore, screening for the p.R229Q is suggested in FSGS patients.12 Tsukaguchi and coworkers analyzed NPHS2 gene in 30 FSGS families with adolescent or adult onset. In 6 of these families, affected subjects were compound heterozygous for R229Q amino acid substitution.21 The pathologic function of p.R229Q is a decreased nephrin binding efficiency to podocin.21 Tryggvason and colleagues proposed that p.R229Q, which is present in around 4% of European populations, is associated with an increased risk of microalbuminuria.22 Pereira and colleagues found that p.R229Q was associated with a 2.77-fold increased risk of microalbuminuria.23

Another investigation has proposed that the p.R229Q variant may not cause disease by itself, but may increase the susceptibility to renal diseases in compound status with the presence of other pathogenic mutations that facilitate the phenotypic result of the R229Q.10,17,18-21,22 Autosomal dominant FSGS characterized by adolescent-onset or adult-onset proteinuria progresses slowly to ESRD in the third and fourth decades of life. Recently identified INF2 gene seems to be a major gene responsible for autosomal dominant FSGS, followed by mutations in TRPC6 and ACTN4.24 The INF2 is a member of the formin family of actin regulating proteins that accelerate the actin polymerization. Screening for INF2 mutations, at least in exons 2 to 4, should be strongly considered in patients with an autosomal dominant familial history of FSGS, even before ACTN4 and TRPC6.25-27 Alfa-actinin-4 mutation is an actin-filament crosslinking protein.24 The TRPC6 gene mutation deregulates the cytoplasmatic calcium concentration, because calcium influx happens through TRPC6 protein.28

CONCLUSIONS
The R229Q allele is considered as a disease-causing, rather than a benign polymorphism, in European, North American Caucasian, and South American populations. Therefore, screening for the p.R229Q is suggested in adolescent- and adult-onset SRNS, but its pathogenic role is not clear in Iranian-Azeri population, indicating future studies with consideration of other mutated genes in this population.

CONFLICT OF INTEREST
None declared.

REFERENCES


Correspondence to:
Mohamadreza Ardalan, MD
Drug Applied Research Center and Chronic Kidney Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
Tel: +98 914 116 5818
Fax: +98 411 336 6579
E-mail: ardalan34@yahoo.com

Received November 2012
Revised March 2013
Accepted April 2013