

Association Between *NPHS1* and *NPHS2* Gene Variants Nephrotic Syndrome in Children

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Introduction. Nephrin and podocin proteins, encoded by *NPHS1* and *NPHS2* genes, are essential for the integrity of the glomerular filter. The present study was aimed to investigate whether *NPHS1* rs437168 and *NPHS2* rs61747728 genetic variants are involved in the susceptibility to nephrotic syndrome (NS).

Materials and Methods. This case-control study was performed on 108 children with NS and 97 healthy children. Genomic DNA was extracted from whole blood using the salting-out method. Polymorphism of the *NPHS1* rs437168 and *NPHS2* rs61747728 were detected by amplification refractory mutation system- and tetra primers amplification refractory mutation system-polymerase chain reaction, respectively.

Results. The results showed that the *NPHS1* rs437168 GA as well as GA+AA genotypes increased the risk of NS in comparison with GG genotype (odds ratio, 4.76, 95% confidence interval, 2.31 to 9.80; $P < .001$ and odds ratio, 4.57; 95% confidence interval, 2.31 to 9.04; $P < .001$, respectively). The A allele was associated with increased risk of NS (odds ratio, 3.53; 95% confidence interval, 1.94 to 6.42; $P < .001$) in comparison to the G allele. No association was observed between *NPHS2* rs61747728 polymorphism and NS. **Conclusions.** Our findings indicate that *NPHS1* rs437168, but not *NPHS2* rs61747728 variant, is associated with NS.

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INTRODUCTION

Nephrotic syndrome (NS), one of the most common diagnoses made in pediatric nephrology, is characterized by heavily proteinuria, hyperlipidemia, hypoalbuminemia, and peripheral edema.¹ In children, NS can be classified into steroid-sensitive NS and steroid-resistant NS (SRNS), based on the response to standard steroid therapy.^{2,3} In contrast to adults, most children with NS have idiopathic nephrotic syndrome. The most common causes of idiopathic NS in children are minimal change NS (MCNS) and focal segmental glomerulosclerosis.³

The *NPHS1* gene is located on chromosome

19q13. The transmembrane protein encoded by this gene, nephrin, is crucial for integrity of slit diaphragm.⁴ Mutation in the *NPHS1* gene is responsible for the congenital NS of the Finnish type, which can cause lethal proteinuria at birth and lack of the slit diaphragm.^{5,6} Meanwhile, the non-Finnish NS patients have different mutations, and more than 60 mutations in the *NPHS1* genes have been described.⁷⁻⁹ The *NPHS2* gene mapped on chromosome 1q25.2 encodes podocin, which is an important protein expressed by the visceral glomerular epithelial cells (podocytes).¹⁰ Podocin with several other proteins acts as scaffolding in the structural organization of the glomerular slit

membrane, which regulates the filtration function of the kidney.¹¹ Several studies have shown that mutations in the *NPHS2* gene occur in 20% to 30% of the sporadic cases of SRNS.¹²⁻¹⁵

In the present study we aimed to investigate the possible association between nephrotic syndrome and *NPHS1* rs437168 and *NPHS2* rs61747728 gene polymorphisms in a sample of Iranian children.

MATERIALS AND METHODS

This case-control study was done on 108 children admitted for NS at Ali-Ebneh Abitaleb Hospital, in Zahedan, Iran. The control group consisted of 97 healthy children. The same pediatric nephrologist examined all of the participants. The characteristic of participants are summarized in Table 1. The Ethics Committee of Zahedan University of Medical Sciences approved the study protocol, and written informed consent was obtained from the parents.

One milliliter of venous blood was drawn from each participant and genomic DNA was extracted

from peripheral blood as described previously.¹⁶ The samples were stored at -80°C until the analysis. Evaluation of *NPHS1* rs437168 polymorphism was done using amplification refractory mutation system-polymerase chain reaction (PCR). The primers used are shown in Table 2. Two tubes were used to determine the variant. Tube 1 contained command primers and A allele primer, and tube 2 contained command primers and G allele primer. The product size for control and allele were 699 bp and 351 bp, respectively. The PCR cycling condition were 5 minutes at 95°C, followed by 30 cycles of 30 seconds at 95°C, 30 seconds at 55°C, and 30 seconds at 72°C, with a final step at 72°C for 10 minutes, to allow for complete extension of all PCR fragments. The PCR products were analyzed by electrophoresis on a 2% agarose gel containing 0.5 µg/mL of ethidium bromide and visualized by transillumination with ultraviolet light. A photograph was then taken (Figure 1).

To detect *NPHS2* rs61747728 variant, we performed tetra primers amplification refractory mutation system-PCR, which is a simple rapid method for identifying single-nucleotide polymorphism.^{17,18} The primers are shown in Table 2. The PCR cycling condition were 5 minutes at 95°C, followed by 30 cycles of 30 seconds at

Table 1. Demographic and Biochemical Characteristics of Children With and Without Nephrotic Syndrome

Parameter	Study Groups	
	Nephrotic Syndrome (n = 108)	Control (n = 97)
Sex		
Male	59	49
Female	49	48
Mean age, y	5.09 ± 2.21	6.39 ± 5.49
Serum measurements		
Mean albumin, mg/dL	2.38 ± 0.49	...
Mean triglyceride, mg/dL	286.75 ± 130.18	...
Mean cholesterol, mg/dL	358.42 ± 123.22	...
Mean total protein, g/dL	4.97 ± 3.69	...

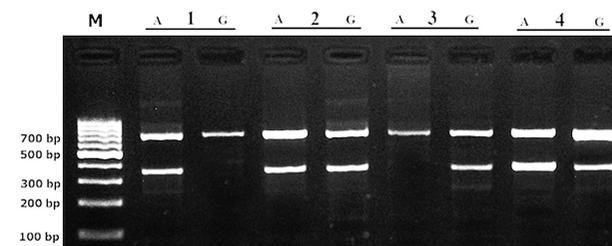


Figure 1. Polymerase chain reaction product of the rs437168 polymorphism in the *NPHS1* gene. M indicates DNA marker; lane 1, rs437168 AA; lanes 2 and 4, AG; and lane 3, GG.

Table 2. Primers Used for Detection of *NPHS1* rs437168 and *NPHS2* rs61747728 Gene Polymorphisms

Primers	5'→3' Sequence	Product Size
<i>NPHS1</i> rs437168	FO: CCATCTGTTCTCCATCCAC	
	RO: CCTAGACACGGAAGCTGCTC	699-bp
	R (G allele): TCTGTGGACATAGTCTGCACTTTC	351-bp
	R (A allele): TCTGTGGACATAGTCTGCACTTTT	351-bp
<i>NPHS2</i> rs61747728	FO: AATAAAGGGTAGGCCAACTCCATTTTTTC	469-bp
	RO: TAACTAGCTATGAGCTCCCAAAGGGATG	
	FI (A allele): ACTATGAAGCGTCTCCTAGCACAGCA	214-bp
	RI (G allele): CCTCTCTAGAAGAATTTTCAGTGAGGGCTC	310-bp

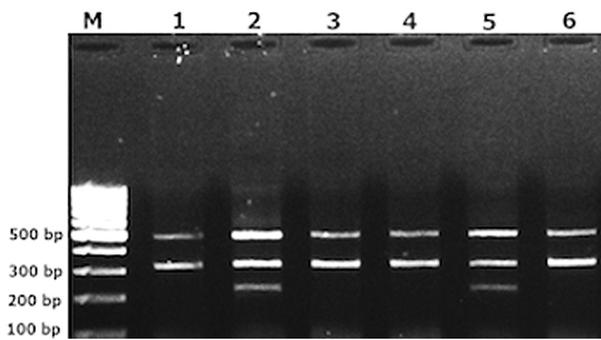


Figure 2. Polymerase chain reaction product of the rs61747728 polymorphism in the *NPHS2* gene. M indicates DNA marker; lanes 1, 3, 4 and 6, rs61747728 GG; and lanes 2 and 5, rs61747728 AG.

95°C, 27 seconds at 60°C, and 27 seconds at 72°C, with a final step at 72°C for 10 minutes to allow for complete extension of all PCR fragments. The PCR products were analyzed by electrophoresis on a 2% agarose gel containing 0.5 µg/mL of ethidium bromide and visualized by transillumination with ultraviolet light (Figure 2).

RESULTS

As shown in Table 3, the *NPHS1* rs437168 GA as well as GA+AA genotypes increased the risk of NS in comparison with GG genotype (odds

ratio [OR], 4.76; 95% confidence interval [CI], 2.31 to 9.80; *P* < .001 and OR, 4.57, 95% CI, 2.31 to 9.04; *P* < .001, respectively). The A allele was associated with increased risk of NS (OR, 3.53; 95% CI, 1.94 to 6.42; *P* < .001) in comparison to G allele. The genotype distribution of rs437168 polymorphism of *NPHS1* was in Hardy Weinberg equilibrium in the NS and control groups (chi-square = 0.44, *P* = .51 and chi-square = 3.23, *P* = .07, respectively).

The genotype distribution of *NPHS2* rs61747728 polymorphism is shown in Table 4. Neither the overall chi-square comparison of the NS and control groups (chi-square = 2.37, *P* = .12) nor the logistic regression analysis (GA versus GG; OR, 3.29; 95% CI, 0.67 to 16.25; *P* = .18) indicated any association between *NPHS2* rs61747728 polymorphism and NS. The results showed that rs61747728 A was not associated with NS in comparison with G allele (OR, 2.93; 95% CI, 0.60 to 14.30, *P* = .19). The genotype distribution of *NPHS2* rs437168 polymorphism was in Hardy Weinberg equilibrium in the NS and control groups (chi-square = 0.12, *P* = .73 and chi-square = 0.01, *P* = .92, respectively).

DISCUSSION

In the present study, we investigated the

Table 3. Genotype and Allele Frequency of *NPHS1* rs437168 Gene in Children With and Without Nephrotic Syndrome

<i>NPHS1</i> rs437168	Study Groups		Odds ratio (95% Confidence Interval)	<i>P</i>
	Nephrotic Syndrome (n = 108)	Healthy Controls (n = 97)		
Genotype				
GG	61 (56.5)	83 (85.5)	1.00	...
GA	42 (38.9)	12 (12.4)	4.76 (2.31 to 9.80)	< .001
AA	5 (4.6)	2 (2.1)	3.40 (0.64 to 18.13)	.24
GA+AA	47 (43.5)	14 (14.5)	4.57 (2.31 to 9.04)	< .001
Allele				
G	164 (75.9)	178 (91.7)	1.00	...
A	52 (24.1)	16 (8.3)	3.53 (1.94 to 6.42)	< .001

Table 4. Genotype and Allele Frequency of *NPHS2* rs61747728 Gene in Children With and Without Nephrotic Syndrome

<i>NPHS2</i> rs61747728	Study Groups		Odds ratio (95% Confidence Interval)	<i>P</i>
	Nephrotic Syndrome (n = 108)	Healthy Controls (n = 97)		
Genotype				
GG	101 (93.5)	95 (97.9)	1.00	...
GA	7 (6.5)	2 (2.1)	3.29 (0.67 to 16.25)	.18
AA	0	0
Allele				
G	229 (97.0)	192 (99.0)	1.00	...
A	7 (3.0)	2 (1.0)	2.93 (0.60 to 14.30)	.19

possible association between *NPHS1* rs437168 and *NPHS2* rs61747728 (R229Q) variants and sporadic NS in childhood. The results showed that *NPHS1* rs437168, but not *NPHS2* rs61747728 polymorphism, increased the risk of NS in our population. The R229Q in heterozygous state was identified in 7 children with NS (6.5%) and in 2 controls (2.1%). The homozygous mutant carrier was not found in the participants. It has been reported that mutations in the *NPHS2* gene cause autosomal recessive NS and have been associated with proteinuria in several populations.¹⁹ The polymorphism rs61747728 (R229Q) is one of the most commonly reported podocin polymorphisms and has frequently been found with somewhat increased frequency in patients with SRNS and focal segmental glomerulosclerosis compared to healthy individuals.²⁰⁻²² The arginine (R) residue at protein position 229 is highly conserved across species, and the arginine-to-glutamine substitution R229Q (p.229 Arg>Glu, corresponding to the nucleotide substitution g.686 G>A) has been reported to alter functional properties of podocin in vitro and possibly in vivo.^{20,23}

It has been reported that R229Q variant is not associated with focal segmental glomerulosclerosis in the United States population of African descent. However, this variant was shown to be associated with an estimated increased risk of 20% to 40% for focal segmental glomerulosclerosis in European populations.²² Otukesh and colleagues²⁴ found no mutation in exons 5 and 7 of *NPHS2* in 20 children with SRNS referred to Ali Asghar Children's Hospital, in Tehran, Iran. Ameli and coworkers²⁵ found *NPHS2* gene mutation (c.503 G>A) in an Iranian family of SRNS with 3 affected children. Fotouhi and colleagues²⁶ found no polymorphism in R229Q variant in a sample of Iranian-Azeri population with SRNS. Abid and colleagues²⁷ found R229Q mutation in the *NPHS2* gene in 2 of 36 children with childhood-onset NS.

Lo and coworkers²⁸ found an association between *NPHS1* rs437168 polymorphism and membranous glomerulonephritis. They found that GG genotype increased the risk of membranous glomerulonephritis. Basiratnia and colleagues²⁹ investigated the prevalence of *NPHS2* mutation in 49 cases of SRNS and 50 cases of steroid-sensitive NS in south-west of Iran. They sequenced the entire 8 exons of the *NPHS2* gene and found that

mutation of the *NPHS2* is frequent in Iranian population. They did not find any mutation at rs61747728 (R229Q) position.

In the present study, we found that rs437168 GA genotype increased the risk of NS in our population. Mao and coworkers³⁰ found no association between 2289 G>A (rs437168) polymorphism of *NPHS1* and sporadic NS. Narita and colleagues³¹ investigated the possible association between *NPHS1* polymorphisms and immunoglobulin A nephropathy (IgAN). They found no association between 2289 G>A variant and IgAN. Nonetheless, their findings suggested that the *NPHS1* G349A polymorphism might be associated with heavy proteinuria and a decline in kidney function in patients with IgAN. Lahdenkari and colleagues⁷ sequenced 29 exons in the *NPHS1* gene in 25 MCNS patients. They observed no homozygous mutations in MCNS patients. They found *NPHS1* 2289 G>A polymorphism in 1 child with MCNS and 2 of 25 healthy children.

Nephrin is a signaling-adhesion protein that is supposed to play an important role in modulating kidney function.³² It is a transmembrane protein of the immunoglobulin family of cell-adhesion molecules and is specifically expressed at the slit diaphragm, where its extracellular moiety forms homodimers and heterodimerizes with nephrin1/nephrin2. Nephrin molecules have been shown to be straightforwardly involved in establishing the porous, macromolecule-retaining molecular sieve of the slit diaphragm.³³ A mutation in the *NPHS1* gene, which leads to a lack of the slit diaphragm, is responsible for the congenital NS of the Finnish type, a disease that can cause lethal proteinuria at birth. Non-Finnish patients with NS, however, have different mutations; in fact, more than 60 mutations in the *NPHS1* genes have been described.^{7-9,15}

CONCLUSIONS

In the present study, we found an association between *NPHS1* rs437168 polymorphism and NS. Our findings did not support any association between *NPHS2* rs61747728 variant and NS. Further studies with larger sample sizes are needed to validate our findings.

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CONFLICT OF INTEREST

None declared.

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