

NPHS2 gene mutation, atopy, and gender as risk factors for steroid-resistant nephrotic syndrome in Indonesians

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Abstract

Background Steroid-resistant nephrotic syndrome (SRNS) often develops into end stage renal disease. Previous studies have reported that *NPHS2* gene mutation, gender, and atopic history are risk factors associated with SRNS. Interethnic, sociocultural, and environmental differences have also been suggested to affect these mutations.

Objective To analyze possible risk factors for SRNS, including *NPHS2* gene mutations (412C→T and 419delG), gender and atopic history, in Indonesian subjects with SRNS.

Methods A case-control study with 153 subjects, consisting of 88 SRNS patients and 65 control subjects, was undertaken in 10 Indonesian teaching centre hospitals from September 2006 to December 2007. Analysis of the *NPHS2* gene mutation in 412 C→T was performed by amplification refractory mutation system-polymerase chain reaction (ARMS-PCR), while that for the *NPHS2* gene mutation in 419delG was performed by restriction fragment length polymorphism (RFLP). Data was analyzed by multiple logistic regression.

Results In our Indonesian subjects, the significant risk factors for SRNS were male gender (OR=2.21; CI 95%:1.07-4.56, P=0.036), *NPHS2* 412C→T gene mutation (OR=18.07; CI 95%:6.76-48.31, P<0.001), and *NPHS2* 419delG gene mutation (OR=4.55; CI 95%:1.66-12.47, P=0.003). However, atopic history was not a significant risk factor for SRNS (OR=1.807; CI 95%:0.642-5.086, P=0.262).

Conclusion *NPHS2* 412C→T and 419delG gene mutations, as well as male gender are risk factors for SRNS in Indonesian subjects. Atopic history was not significantly associated with SRNS in our subjects. [Paediatr Indones. 2011;51:272-6].

Keywords: Steroid-resistant nephrotic syndrome, risk factor, *NPHS2* gene mutation

In recent years, molecular genetic studies have been well-developed, including studies for genes involved in the pathogenesis of nephrotic syndrome. Of particular interest is the gene encoding a protein that maintains the diaphragm slit of podocytes.^{1,2} Gene mutation analysis is important with regards to phenotype and predicting the severity of clinical manifestations.³ The *NPHS2* gene encodes the podocin protein and is located on chromosome 1q25-31. Mutation of this gene has been associated with poor steroid response in treatment of nephrotic syndrome (NS). Previous studies have shown that SRNS patients with an *NPHS2* gene mutation had more severe clinical manifestations compared to SRNS patients without the *NPHS2* gene mutation.^{2,3,4} *NPHS2* gene mutations have been found in 20-30% of SRNS patients, mostly present in the third exon of the gene.⁵ Other studies of SRNS patients also showed mutations located on the third exon, namely, a nonsense mutation at 412C→T (R138X)³ and a deletion at 419delG.⁶

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Diversity in the *NPHS2* gene mutation pattern is thought to be due to variations in ethnicity and environment.^{3,5,7} Since there was no studies on *NPHS2* gene mutations in the pediatric Indonesian population, we focused on the third exon of *NPHS2* as a basis for study in Indonesian children with SRNS. Since many factors may play a role in the occurrence of SRNS, we included gender and history of atopy as possible risk factors of SRNS in this study. Our aim was to determine if *NPHS2* gene mutations, gender and history of atopy are risk factors for SRNS in Indonesian children.

Methods

A case-control study was conducted from September 2006 to December 2007. We included case subjects with primary SRNS aged between 1-14 years old. Control subjects were healthy children with no kidney diseases or congenital abnormalities. Case and control subjects visited the pediatric nephrology division of 10 educational hospitals in Indonesia, in the cities of Bandung, Jakarta, Yogyakarta, Semarang, Surabaya, Denpasar, Medan, Palembang, Makassar, and Menado, and were included by consecutive admission. DNA and gene mutation analyses were done in the Medical Faculty Health Research Unit at Padjadjaran University, Bandung. Approval for this study was obtained from the Ethics Committee

of the Padjadjaran University Medical School /Hasan Sadikin General Hospital, Bandung.

NS was diagnosed according to the International Study of Kidney Disease in Children (ISKDC) criteria, i.e. edema, severe proteinuria, hypoalbuminemia (< 2.5 g/dL), while SRNS was defined as NS patients not achieving remission after single drug prednisone therapy using the full dose for the first four weeks.⁸ NS diagnoses were made by pediatric nephrologists or pediatricians supervised by pediatric nephrologists.

Data analyses were performed using SPSS version 12.0. Multiple logistic regression analysis was used to determine possible risk factors for SRNS, including gene mutations *NPHS2* 412 C→T and 419delG, gender, and familial atopic history.

To detect the *NPHS2* 412 C→T mutation in exon 3, we used ARMS-PCR.⁹ Primer design is shown in **Figure 1**. Subjects' DNA specimens were placed in duplicate tubes, one tube containing the wild type and reverse primers and the other tube containing the mutated and reverse primers. Since *NPHS2* is an autosomal recessive gene, amplification should occur in only one tube.

Tubes contained a mixture of 100 ng DNA, 10 pg primers, 2.5 uL dNTP mix (200 uM), 2.5 uL 10x PCR buffer, and 0.5 unit Taq polymerase in a total vol of 25 uL. Amplification was performed as follows: denaturation at 95°C for two minutes, denaturation at 94°C for 10 seconds, annealing at 54°C for 10 seconds, and extension at 72°C for 10 seconds for 38 cycles.

Wild type (C) primer: 5'-3' GGTTGTACAAGAGTAATGGAAAGAGTAATTATATTAC
Mutated (T) primer: 5'-3' GGTTGTACAAGAGTAATGGAAAGAGTAATTATATTAT
Reverse primer: 5'-3' TGAAGAAATTGGCAAGTCAG

Figure 1. Wild type sequence, mutated sequence and reverse primers for ARMS-PCR.

T
GGTTGTACAAGAGTAATGGAAAGAGTAATTATATTACGACTGGGACATCCGCTT
CCTGGAAGAGCCAAAGGCCCTGGTAAAAAAACACTCTTTTTTTTCTAAACACCTC
TCTCCTGACTTGCCAATTTCTTCA

Figure 2. Predicted PCR product, 131 base pairs.

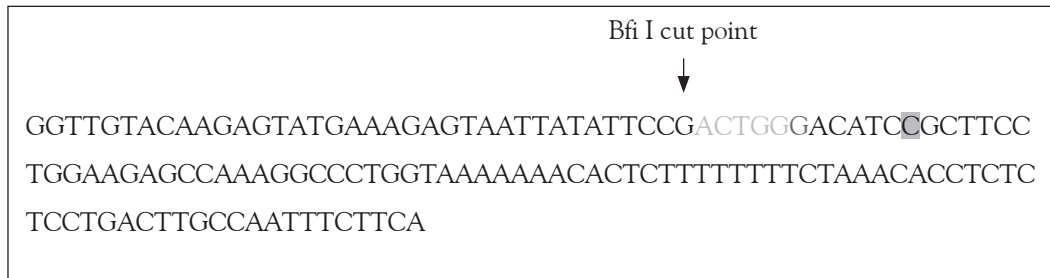


Figure 3. Bfi I recognition sequence and cut point in the wild type, *NPHS2* gene. The 419delG mutation is noted in red.

Gel electrophoresis (2% agarose) was used visualize the PCR products. Gels were photographed while under ultraviolet translumination. The predicted, 131 bp PCR product is shown in **Figure 2**.

The *NPHS2* 419delG allele was detected by RFLP analysis.¹⁰ The 131 bp-PCR products from the ARMS-PCR analysis were subjected to Bfi I restriction endonuclease. The wild type sequence (with the G present) was cut into two fragments of 39 bp and 92 bp. The mutated sequence (G deleted) remained undigested by Bfi I. Restriction analysis products were observed by gel electrophoresis as described above. The Bfi I recognition sequence located in the wild type *NPHS2* gene (131 bp-PCR product) is shown in **Figure 3**.

Table 1. Characteristics of subjects

Variable	SNRS group N = 88	Control group N = 65
Gender, n (%)		
Female	20 (22.7)	26 (40.0)
Male	68 (77.3)	39 (60.0)
History of atopy, n (%)		
Positive	25 (28.4)	8 (12.3)
Negative	63 (71.6)	57 (87.7)
412C→T mutation, n (%)		
Positive	58 (65.9)	9 (13.8)
Negative	30 (34.1)	56 (86.2)
419delG mutation, n (%)		
Positive	69 (78.4)	41 (63.1)
Negative	19 (21.6)	24 (36.9)

Table 2. The variables as risk factors for SRNS

Variable	OR	95% CI	P value
Gender	2.21	1.07-4.56	0.036
412C→T mutation	18.07	6.76-48.31	< 0.001
419delG mutation	4.55	1.66-12.47	0.003
History of atopy	1.81	0.64-5.09	0.262

Note: Accuracy 74.51%

Results

A total of 153 subjects, consisting of 88 SRNS cases and 65 control subjects were enrolled during the study period. The majority were boys in SRNS group (77.3%) and in the control group (60.0%). History of atopy was more common in the SRNS group (28.4%) than in the control group (12.3%). In addition, the *NPHS2* gene mutations were more common in the SRNS group than in the control group. (**Table 1**)

Multivariate analyses of the possible risk factors for SRNS are described in **Table 2**. Of these risk factors, male gender and the *NPHS2* 412C→T and 419delG gene mutations were significantly associated with the occurrence of SRNS ($P < 0.05$). However, the history of atopy was not significantly associated with SRNS ($P > 0.05$).

Discussion

SRNS is a common cause of chronic kidney disease in children. According to the literature, mutation in the *NPHS2* gene encoding for podocin may lead to SRNS.^{5,6,7} A case control study was performed in children presenting with SRNS in 10 Indonesian teaching hospitals from September 2006 to December 2007.

Our study results were similar to those from both Arabic³ and Caucasian children.^{4,11} The strong relationship between the occurrence of SRNS and *NPHS2* gene mutation, especially in exon 3: 412 C→T, was shown by Frishberg *et al.* in Israeli-Arab children,³ and by Caridi *et al.* in Caucasian children in Italy.¹¹ Similarly, we found that subjects with the *NPHS2* 412 C→T gene mutation had 18.0 times

higher risk for SRNS than those without this gene mutation. The *NPHS2* 412 C→T gene mutation is a nonsense mutation. The change of cytosine to thymine at base sequence 412 results in the loss of the amino acid arginine at sequence 138 (R138X).¹² This amino acid loss produces a truncated form of podocin. It is thought that the truncated podocin gets trapped in the endoplasmic reticulum, so that it loses its capability to bind nephrin in the lipid raft.^{2,13} The truncated podocin will stimulate antibody formation against the terminal part of the protein.^{14,15}

We also considered the effect of a *NPHS2* gene mutation in another location. Using the ARMS-PCR method, the exon 3: 412C→T was the only mutation able to be detected. Caridi *et al.* used a sequencing method to detect all possible *NPHS2* gene mutation in exon 3 of SRNS patients with focal segmental glomerulosclerosis (FSGS).^{4,11} They suggested that another (*NPHS2*) gene mutation in exon 3: 419delG was associated with SRNS in Italian children with SRNS¹¹ and in a large sample size of European children.⁴ By RFLP analysis, we also found this mutation to be associated with SRNS in our subjects. Those with the *NPHS2* 419delG gene mutation had a 4.5 times higher risk for SRNS than those without this mutation. This phenomenon may be explained by the nature of frameshift mutations, in which the deletion of guanine at base sequence 419 results in formation of a different podocin protein.¹¹

In comparing the effects of the *NPHS2* gene mutations at 412C→T and 419delG on the occurrence of SRNS, we observed that the 412C→T mutation was associated with a 4 times greater possibility of SRNS occurrence than the 419delG mutation. However, in theory, a frameshift mutation at 419delG should have stronger effect on the occurrence of SRNS compared to that of a nonsense mutation at 412C→T.¹⁶ One explanation for this result is that our assays detected mutations at only these specific sites, 412 and 419. We cannot exclude the possibility that mutations in other *NPHS2* gene locations exist, affecting the occurrence of SRNS.

We found the male gender to be a risk factor for SRNS, with 77.3% males and 22.7% females in the SRNS group. Similarly, an ISKDC study reported that of pediatric patients with FSGS, 69.4% were male and 30.6% were female.³ Furthermore, Caridi *et al.* observed that in Caucasian children in Italy

with sporadic SRNS, the male to female ratio of patients with an *NPHS2* gene mutation was 7:2.¹¹ It is difficult to differentiate between early FSGS and minimal change nephrotic syndrome (MCNS), as FSGS is considered to be a continuation of MCNS.¹⁷ The relationship between male gender and nephrotic syndrome remains unclear. It has been established that pathogenesis of nephrotic syndrome involves T cell dysfunction.¹⁸ Abnormal T cell clones are predominantly located in the thymus and thymus disease is more common in boys than girls. Another theory is that SRNS with gene mutation is influenced by hormones. Estrogen may prevent the glomerulosclerosis process by decreasing urinary sex-dependent low molecular weight proteins.¹⁹ The younger age of onset in sporadic SRNS, regardless of its relationship to abnormal T cell clones in the thymus, is similar to the occurrence in MCNS patients. Further research is needed.

The *NPHS2* 412C→T gene mutation leads to formation of a truncated protein. The *NPHS2* 419delG gene mutation results in formation of a protein with different amino acids than that encoded by the normal gene. However, functional changes of these mutated proteins need clarification. It is well-known that phenotype as a result from the presence of a specific gene or combination of genes, circumstances, and duration, as well as the interactions among these factors.²⁰ This theory may explain the phenotypic differences in our study results and those of previous studies. Previous studies have shown differences in type and frequency of *NPHS2* gene mutations based on racial makeup.^{3,5,21} According to multivariable analysis on **Table 2**, we can see the role of gender, 412C→T mutation, and 419delG mutation to SRNS. The value of accuracy in this role was 74.51%. It means there were other variables that affect to SRNS besides those variables.

In conclusion, the *NPHS2* 412C→T and 419delG gene mutations, as well as male gender are risk factors for SRNS. History of atopy was not a risk factor for SRNS. Further studies are needed to determine other possible associated *NPHS2* gene mutations by sequencing, as well as biopsy to establish the type of histopathological abnormalities in patients with SRNS.

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