Original Article

NPHS2 Mutations

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ABSTRACT

Objective. To uncover the frequency and the spectrum of *NPHS2* mutations in Egyptian children with non familial steroid-resistant nephrotic syndrome (SRNS).

Methods. Sixteen patients were screened by PCR-single-strand conformation polymorphism analysis of *NPHS2* gene followed by direct sequencing.

Results. *NPHS2* mutations were evident in four patients (25%) who were bearing four novel mutations including two frame shift mutations (R238fs and P45fs) and two missense mutations (I136L and F216Y). There were no phenotypic or histological characteristics of patients bearing *NPHS2* mutations, apart from the earlier onset of the disease, compared to those who were not bearing mutations.

Conclusion. *NPHS2* mutations are prevalent in Egyptian children with non-familial SRNS and this may in part explain the less favorable prognosis reported in these patients. **[Indian J Pediatr 2008; 75 (2) : 135-138]** *E-mail : ashbakr@mans.edu.eg*

Key words : *Egyptian children; Nephrotic syndrome; Non familial;* NPHS2 *mutations; Steroid resistant*

Steroid-resistant nephrotic syndrome (SRNS) represents a heterogeneous group of kidney disorders that often are resistant to additional immunosuppressive agents and tend to progress to end-stage renal disease (ESRD). The most prevalent histological findings associated with SRNS in children are focal segmental glomerulosclerosis (FSGS), diffuse mesangial proliferation (DMP), and infrequently minimal change nephrotic syndrome (MCNS).

In the last few years, advances in molecular genetics of familial SRNS have led to the discovery of specialized molecules endowed in podocytes as responsible for proteinuria. Podocin is one of these molecules. It is a 383-amino acid protein, that is almost exclusively expressed in glomerular podocytes. It is an integral membrane protein linking the plasma membrane and the cytoskeleton. Podocin is encoded by the gene *NPHS2* that was discovered and localized at 1q25 positional cloning in 2000.[1]

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There is now growing evidence that mutations of podocyte proteins go far behind familial cases and may occur in sporadic SRNS. Many *NPHS2* mutations have been reported in non-familial SRNS.[2-5] These studies point to the inter-ethnic differences in the spectrum of *NPHS2* mutations. Identification of *NPHS2* mutations in non-familial SRNS patients may enable clinicians to avoid unnecessary steroid or other immunosuppressive treatment in these patients and to provide prenatal diagnosis for families at high risk.[5]

In this study, mutational analysis of *NPHS2* gene was performed in non-familial Egyptian children with SRNS to determine the existence of *NPHS2* mutations in these patients.

MATERIALS AND METHODS

This cross-sectional study was performed on 16 Egyptian children with SRNS. None of them had family history of renal disease or parental consanguinity. Twenty four unrelated Egyptian children with no history of renal diseases or abnormal urinary finding, were studied as controls. Nephrotic syndrome was diagnosed in the presence of massive proteinuria (1g/m^2/day) and hypoalbuminemia (<2.5g/dL). Steroid resistance was

defined as the lack of response to daily orally administered prednisone treatment at 60mg/m^2 for 1 month followed by three pulses of methylprednisone (1g/m^2/d). Patients were recruited conductively from pediatric nephrology unit, Mansoura University Children's Hospital, Mansoura, Egypt. An informed consent was obtained from parents. This study was approved by the local ethical committee.

Detection of *NPHS2* gene mutations by PCR-SSCP and sequentional analysis

Genomic DNA was extracted from the whole blood collected from patients using convention molecular biology techniques with QIA amp DNA blood minikit for DNA extraction (QIAGen Inc Chasworthey, CA, USA). PCR was used to amplify exons 1,3,5,7 of *NPHS2* gene by using primers on the basis published in formations regarding intron-exon boundaries.[1] PCR primes are listed in Table1. The amplified products were checked on 2% agarose gel electrophoresis to show the size of each exon through using of a DNA marker.

Single stranded conformational polymorphism (SSCP) analysis was done by electrophoresis of PCR products on 10% polyacrylamide gel at 50 V overnight. Gels were visualized by silver nitrate stain.[6] Normal *NPHS2* exon products exhibit specific conformational pattern. A mutant gene displays pattern with different electrophoretic mobility or missing band. Variants seen on the SSCP gel were directly sequenced by using ABI prism 310 genetic analyzer version 2.0 (Applied Biosystem-Foster City, CA, USA).

RESULTS

The age of patients under study at the onset of the disease varied from 1 to 9 yr. Hypertension and microhematuria were reported in 5 (31.3%) and 7 (43.8%) children, respectively. None of the patients had evidence of renal impairment at the onset of the disease. Renal biopsy revealed FSGS in 13 (81.3%), MCNS in 2 (12.5%) and DMP in 1 (6.3%). However, the first patient was biopsied twice and the initial pathology was MCNS. At the time of the study 4 patients (25%) have developed ESRD on regular hemodialysis.

SSCP analysis detected abnormal electrophoretic migration in exon 1 (patient no 5), exon 3 (patient no 3) and in exon 5 (patient no 1,6,12). No abnormalities were detected in exon 7.

Direct sequencing of the abnormal PCR products showed different frame shift and missense mutations in 4 patients (25%) while the transition in exon 3 did not result in a change in the amino acid sequence. Apart from the change in exon 3, all the changes were homozygous (Table 2). None of these mutations were found in controls. Two frame shift mutations were reported. The first (713-714insG) was found in exon 5 (patients 1,6,12). Guanine base inserted at position 713-714 resulted in frame shift in codon 238 with arginine was the first affected amino acid (R238fs). The other frame shift mutation (132-133insG) was detected in exon 1 (patient 5). Guanine base was inserted at position 132-133 producing frame shift in codon 45 (P45fs) with proline was the first affected amino acid. Two missense mutations were evident. The 556A>C mutation was shown in exon 5 (patients 1,12). Adenine was mutated to cytosine at position 556 leading to the change of isoleucine to leucine (I186L). The other missense mutation (647T>A) was present also in exon 5 (patient 6); thymine was mutated to adenine at position 647 resulting in the change of phenylalanine to tyrosine (F216Y).

A silent change was noticed in exon 3 (patient 3); adenine was mutated to thymine in position 408. However, no change occurred in isoleucine in codon 136.

Table 1. Primers for DNA Sequence Analysis of *NPHS2* Exons

Exon	Forward Primer (5'-3')	Reverse Primer (5'-3')
1	GCAGCGACTCCACAGGGACT	TCAGTGGGTCTCGTGGGGAT
3	TTCTGGGAGTGATTTGAAAG	TGAAGAAATTGGCAAGTCAG
5	CATAGGAAAGGAGCCCAAGA	TTCAGGCATTGGCCATTA
7	CTAAATCAATGGCTGCACCACC	TTCCTAAAGGGGCAGTCTGG

Table 2. Types of *NPHS2* Mutations

Patient No.	Exon	Type of mutation	Nt change	AA change
1	5	Frame shift	713-714 insG	R238fs
		Missense	556A>C	I186L
5	1	Frame shift	132-133 insG	P45fs
6	5	Frame shift	713-714 insG	R238fs
		Missense	647T>A	F216Y
12	5	Frame shift	713-714 insG	R238Fs
		Missense	556A>C	I186L

Nt, nucleotide; AA, amino acid.

DISCUSSION

In the present study it has been shown that one fourth of the children with non-familial SRNS had *NPHS2* mutations. To our knowledge this is the first report that describes *NPHS2* mutations in Egyptian children with SRNS. However, *NPHS2* mutation has been identified in one consanguineous Egyptian family with autosomal recessive SRNS.[1]

Although only four exons of *NPHS2* gene were tested due to financial reasons, four novel *NPHS2* mutations (R238fs, P45fs, I186L and F216Y) have been described in the present study. The most prevalent mutation was R238fs. The relevance of the silent mutation described in patient no 3 is not clear as we did not have any functional evidence for this change. All the reported mutations, except P45fs, affected the carboxyl terminal of *NPHS2* gene. The absence of these mutations in the control DNA suggests that they are pathogenic. Moreover, podocin associates *via* its carboxyl-terminal domain with CD2AP, a cytoplasmic binding partner of nephrin, and with nephrin itself, *in vitro* and *in vivo*.[7] Huber *et al*[8] demonstrated that nephrin is a signaling molecule that stimulates mitogen-activated protein kinases and thus podocin greatly enhances nephrin-induced signaling. Collectively, podocin may serve in the structural organization of the slit diaphragm and the regulation of its filtration function *via* its carboxyl-terminal domain but not *via* its amino-terminal domain. So structural defects at the carboxyl terminal domain are usually associated with functional derangement.

The frequency as well as the type of *NPHS2* mutations in non-familial SRNS vary from one study to another. No mutations were found in Jewish SRNS children.[5] Out of 18 Arab SRNS children from unrelated families, 6 (33%) were bearing *NPHS2* mutations.[5] Özçakar *et al*[4] reported *NPHS2* mutations in only 4% of Turkish non-familial SRNS children. *NPHS2* mutations have been reported in 10-30% of non-familial SRNS cases in Western countries.[1-3]

More than 50 *NPHS2* mutations have been reported in both familial and non-familial SRNS. Reported mutations involve the whole length of the gene and determine every kind of alterations; including missense, nonsense, and deletion. Published studies report different distributions of mutations: R138Q was most frequently found in Germany and France,[2-3] while the P20L variant was observed mainly in Turkey.[4]

These variations in the frequency and the type of *NPHS2* mutations among different populations may partially explain the inter-ethnic difference in the prevalence as well as the outcome of SRNS. We have previously reported the unfavorable prognosis of our patients with SRNS-FSGS.[9]

Although the number of patients reported in this study was small with short duration of follow up, it was observed that in patients bearing *NPHS2* mutations the disease started earlier, all of them had FSGS and none of them developed ESRD. However, the other clinical characteristics were comparable in patients with *NPHS2* mutations and those without.

Age at onset of proteinuria was rather variable in different reports describing patients bearing *NPHS2* mutations in general occurring before the 10th year. A few cases with congenital or very early SRNS were also described.[3] Weber *et al*[3] report that R138Q appears to be associated with early onset (12±3 months in 15 patients); V180M and R238S are associated with late onset SRNS (129±12 months in 7 patients). Caridi *et al*[10] report that renal pathology is not suggestive of an inherited condition associated with *NPHS2* mutations. This observation is in fully agreement with the current idea on the lack of correlation between renal alterations and clinical features. Many researchers[3,4,10] observe that non-familial SRNS nephrotics bearing *NPHS2* mutations usually develop ESRD within the second decade of life 6-155 months after the onset of proteinuria. Nevertheless, all the outpatients were in the first decade of life.

CONCLUSION

In conclusion *NPHS2* mutations are prevalent in Egyptian children with non-familial SRNS and this may in part explain the less favorable prognosis reported in these patients.

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