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NPHS1 Gene Mutations in Children with Nephrotic Syndrome in Northwest Iran

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Abstract: Idiopathic Nephrotic Syndrome (NS) is the prevalent glomerular disease in childhood. It is treated with steroid and according to its response is defined as steroid sensitive NS (SSNS) and steroid resistance NS (SRNS). Mutation in NPHS1 gene is reported in children with SRNS and few cases of SSNS. The aim of current study is to evaluate NPHS1 gene mutations in idiopathic NS (SSNS and SSRS) in Northwest Iran. In this cross-sectional analytic study 20 children from Azeri population in Iran with idiopathic NS including 10 cases with SRNS (5 male and 5 female) and 10 cases with SSNS (7 male and 3 female) were evaluated for NPHS1 gene mutations. DNA was extracted from peripheral blood and NPHS1 gene analysis was performed by PCR and direct sequencing method with the use of standard primers. Mutations in NPHS1 gene occurred in 6 cases of SSNS including 3 heterozygous and 3 homozygous mutations and in 8 cases of SRNS including 5 homozygous, one compound heterozygous and 2 heterozygous mutations. Overall 6 different mutations were detected in NPHS1 gene: one deletion, one insertion, 3 missense and one nonsense mutations. Mutations in exon 4 and 27 were only seen in SRNS patients. Mutations in NPHS1 gene could occur in both SRNS and SSNS patients; however, considering higher incidence of heterozygous mutations in SSNS, the existence of milder phenotype in these cases would be the reason for steroid response.

Key words: Idiopathic nephrotic syndrome, steroid sensitive, steroid resistance, NPHS1 gene mutation

INTRODUCTION

Idiopathic Nephrotic Syndrome (INS) which is characterized with severe proteinuria, hypoalbuminemia, edema and dyslipidemia, is the most common glomerular disease in childhood with annular incidence of 2-7 per 100000 children (Eddy and Symons, 2003; Kim *et al*., 2005). Most patients with INS 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 INS, however, fail to respond to glucocorticoids. These patients are at risk of end-stage renal disease (Tarshish *et al*., 1997).

Clinically, NS has been divided into two categories based on the response to steroid therapy: steroid-sensitive NS (SSNS) and steroid-resistant NS (SRNS) (Eddy and Symons, 2003; Kim *et al*., 2005). Epidemiologic studies are indicative of increase in SRNS incidence, although the reasons for this increase are unclear. SSNS and SRNS patients have similar clinical manifestations and there is no laboratory parameter differentiating these two. Pathologic evaluations of renal biopsies are usually used to define SSNS and SRNS patients, as minimal change nephrotic syndrome in SSNS and Focal Segmental Glomerulosclrosis (FSGS) in SRNS patients was reported (Fuchshuber *et al*., 2001; Ichikawa and Fogo, 1996).

There is an emerging picture of the role of genetics in nephrotic syndrome. To date, mutations in seven genes (NPHS1, NPHS2, CD2AP, PLCE1, ACTN4, TRPC6 and INF2) expressed by glomerular podocytes have been identified in patients with NS (Beltcheva *et al*., 2001; Boute *et al*., 2000; Kaplan *et al*., 2000; Kestila *et al*., 1998; Lahdenkari *et al*., 2004; Orloff *et al*., 2005; Winn *et al*., 2005) NPHS1 codes for the nephrin protein, an essential component of the interpodocyte-spanning slit diaphragm (Ruotsalainen *et al*., 1999). Nephrin forms a zipper-like filter structure in the center of the slit and plays an important role in cell-cell signaling in the slit diaphragm (Khoshnoodi *et al*., 2003; Patari-Sampo *et al*., 2006) Mutations in NPHS1 lead to disruption of the filtration barrier and cause massive protein loss. Most mutations in NPHS1 are reported in congenital NS (Beltcheva *et al*., 2001); however, recently NPHS1 gene mutations are reported in childhood onset SRNS (Philippe *et al*., 2008).

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Some studies reported of NPHS1 gene mutations in patients with MCNS (Lahdenkari *et al*., 2004, 2005). As SSNS patients usually show MCNS, there is possibility of existing NPHS1 gene mutations in SSNS patients like SRNS cases. In this study we aim to evaluate NPHS1 gene mutations in SSNS and SRNS children in Azeri Turkish population in northwest of Iran.

MATERIALS AND METHODS

Twenty unrelated Iranian children with Azeri Turkish origin diagnosed with idiopathic nephrotic syndrome (10 with SSNS and 10 with SRNS) by the Department of Pediatrics, Tabriz Kudakan Hospital, between 2010 and 2012 were included in this study. This study was approved by the Ethics Committee of the Tabriz University of Medical Sciences. Informed consent from patients or their parents had been obtained. Nephrotic syndrome was defined as proteinuria (>40 mg m^2 h^{-1}), hypoalbuminemia (<2.5 g dL^{-1}), hyperlipidemia (serum total cholesterol >250 mg dL^{-1}) and edema. SRNS was defined as the failure to respond to 6- to 8-week daily administration of prednisone, 2 mg kg^{-1}, from the onset of the disease.

Inclusion criteria were patients with idiopathic nephrotic syndrome diagnosed before 18 years of age, being definite steroid sensitive or resistance with pathologic examination results of the renal specimens in SRNS cases. Patients with congenital or secondary nephrotic syndrome were excluded. Patients with late steroid resistance were not included in this study. Patients' baseline findings were recorded and were matched between groups (Table 1).

DNA samples: Peripheral blood samples were collected from 10 SSNS patients and 10 SRNS patients in Tabriz area of East Azerbaijan province of Iran. Genomic DNA was isolated from peripheral blood cells using the salting-out method (Miller *et al*., 1988). All exons of NPHS1 were amplified from genomic DNA by polymerase chain reaction (PCR) and directly sequenced. The primers were designed on the basis of previously published information regarding intron-exon boundaries (Lenkkeri *et al*., 1999) (Table 2). Genomic DNA then was sent to Macrogen Company, South Korea for further sequencing. The findings were compared with normal gene sequences and mutations were recorded.

Data analysis: Statistical package for the social sciences (SPSS) version 16 was used for data analysis. Quantitative variables were presented as Mean±Standard Deviation. For comparison of differences between qualitative variables, chi-square or exact Fisher's tests were used and for comparison of quantitative variables, nonparametric Mann-Whitney U test was used. A p value less than 0.05 was considered significant.

Table 1: Patients' baseline findings in SSNS and SRNS

Characteristics	SSNS	SRNS	p-value
Gender (male)	7 (70%)	5 (50%)	0.360
Age at onset (months)	58.70±36.15	30.70±22.79	0.053
Disease duration (months)	20.90±8.30	37.90±32.38	0.120
Weight (Kg)	21.50±10.79	21.40±12.60	0.980
Height (cm)	109.20±19.48	102.20±23.28	0.470
Familial history of renal disease	1 (10%)	4 (40%)	0.300

Table 2: PCR Primer Sets for Amplifying Exons of *NPHS*1 (Lenkkeri *et al*., 1999)

Exon	5" Primer	3' Primer'	Product Size (bp)
50 UTR	GCTGACTCTGCCAGT GCCTG C	AGGGCCATCACAGGTCCCC	643
1-2	GAGAAAGCCAGA CAGACGC	AG AGCTTCCGCTGGTGGCT	491
3-4	AGCCACCAGCGG AAGCT	CTCCCTTCCCACTCCAGAGG	530
5	CAGAATCTATCTT GCGGGGAG	CATGGGGAAAATTAG GGGTCAAG	187
6-7	TCTCCCTGACTCCCC AAATTTC	CTCAGGACTGGCTCCCAGAC	544
8-9	GACAGTGGGGTCTG GGAGCCAGTCCTGAG	GAGTCATGCCCTCAGCCCC	623
10-11	CACGATGGATAGG GGTGCTG	CCTGGTCCTTCCCCCACATT	465
12-13	AACCCAGTGGGCA GGGTAGGGG	GACATGCGTGGAG GGGGCGA	668
14	CCTAGTGCCTCT CCAGCC	GAGTAGTTTAGGGTC AAGAAGG	288
15-16	CCTGATCTCCAAT CTGTCCTTG	CCACAATGGGCAAG GTTCCTTG	484
17	CACCCAGACCTGT CTGGGCC	GTCCCCACTCCC AAGGAACTC	257
18-19	GAGGCTACAGAA GGGACAATTTG	GCTGGAGGTCCAGA CCTGGG	561
20	GGATGGATGCAT AGATGATTCC	CAATCAGGGATGTG GGAATG	297
21-22	CCTGGACAGAATC TTCTGGAATT	CCTACACATCCTCTG AGGAATAC	487
23	GAGGCTGAGAAATA TTTAAAGCTTAT	GAGACCAGGAGGTT CCATTCT	188
24-26	CTCGGGGAGACCC ACCCC	CCTGATGCTAACGG CAGGGC	611
27-28	TGCCCTTCCGGGCA CAGTGG	TACAAGCAATAGGA GGTAGGC	438
29	CAGATCTTCAATGAA GACCTACA	GAGACAGAATCTCGCTCTG	747

RESULTS

Demographic and biochemical profiles: Patients' baseline findings are shown in Table 1. Two groups are matched for baseline findings; however, although not significant, SRNS cases were diagnosed at younger age had more positive familial history. SSNS cases all had minimal change disease. Pathologic evaluations showed focal segmental glomerulosclerosis (FSGS) in all SRNS cases.

SSNS patients had significantly lower serum albumin levels in comparison to SRNS patients (Table 3); other laboratory findings were not significant between groups. SSNS patients also had insignificantly lower hemoglobin levels.

HNPS1 genotypes and alleles in patients with SSNS versus patients with SRNS: Mutation information for SSNS and SRNS patients are shown in Table 4. Overall, mutations were seen in 14 patients. NPHS1 gene mutations were seen in 6 SSNS patients including 3 homozygous and 3 heterozygous genotypes. Eight SRNS patients had NPHS1 gene mutations with genotypes of homozygous in 5 cases, compound heterozygous in 1 case and heterozygous in 2 cases. The other six patients with no NPHS1 mutation would have mutation in other genes which was not evaluated. There was no significant difference between groups according to NPHS1 gene mutation rate (p = 0.61). Overall we detected 6 different mutations in NPHS1 gene including one deletion (Exon 19), one insertion (exon 24) and 3 missense mutations (exons 4,10,16), all leading to a frameshift and premature truncation of the protein. There was also a nonsense mutation in exon 27. Among these mutations, mutations in exon 4 and 27 were seen only in SRNS patients and mutation in exon 24 was seen mostly in SSNS patients. The only compound heterozygous truncating and missense mutation in exon 10 and 24, respectively, was seen in a SRNS patient (SRNS7-A).

Table 3: Laboratory findings between SSNS and SRNS patients

Parameters	SSNS	SRNS	p-value
Hemoglobin (mg dL^{-1})	12.67±2.04	10.68±2.28	0.055
24 h Urine volume (mL day^{-1})	1089.50±999.55	978.00±288.74	0.730
24 h Urine albumin (mg day^{-1})	3718.50±2497.15	4439.30±1758.63	0.460
24 h Urine creatinine (mg day^{-1})	64.29±24.32	99.69±29.41	0.360
Serum albumin (mg dL^{-1})	2.35±0.74	3.02±0.64	0.04*
Serum creatinine (mg dL^{-1})	0.60±0.03	1.55±0.63	0.15
Serum blood urea nitrogen	17.90±9.58	19.80±13.87	0.72
Serum urea	35.60±18.92	39.50±27.60	0.71
Serum Triglyceride (mg dL^{-1})	335.40±117.59	390.20±271.18	0.56
Serum cholesterol (mg dL^{-1})	344.70±68.19	322.70±161.85	0.69

*p is two tailed significant

DISCUSSION

In this study we evaluated NPHS1 gene mutations in SRNS and SSNS Azeri Turkish children and observed 6 different mutations in 14 cases (6 SSNS and 8 SRNS) including 8 homozygous, 5 heterozygous and one compound heterozygous.

Unfortunately most studies have evaluated mutations in NPHS2 gene in non congenital NS patients, especially in steroid resistance cases. NPHS1 gene mutations are usually seen in congenital NS; however, new studies have shown that patients with NPHS1 mutations present with NS, mostly during the first 3 month of life (Hinkes *et al*.; 2007; Koziell *et al*., 2002; Lenkkeri *et al*., 1999; Sako *et al*., 2005).

In our study mutations especially homozygous mutations were more prevalent in SRNS cases. Similar to our findings, Santin *et al*. (2011) reported that NPHS1 gene mutation is the most common finding in congenital and non-congenital SRNS cases.

Table 4: Mutation information for 10 children with SSNS and 10 children with SRNS

Patient ID	Exon	Nucleotide exchange	Effect on coding sequence	Mutation status
SSNS-1B	16	c.2126T>G	V709G	Heterozygous
SSNS-2B	10	c.1234G>T	G412C	Homozygous
SSNS-3B	24	c.3243_3250ins G	V1084fsX1095	Homozygous
SSNS-4B	19	c.2548del10	A850fsX87	Heterozygous
SSNS-5B	-			-
SSNS-6B	-			-
SSNS-7B	10	c.1234G>T	G412C	Homozygous
SSNS-8B	24	c.3243_3250ins G	V1084fsX1095	Heterozygous
SSNS-9B	-			-
SSNS-10B	-			-
SRNS-1A	10	c.1234G>T	G412C	Homozygous
SRNS-2A	4	c. 512T>A	I171N	Homozygous
SRNS-3A	24	c.3243_3250ins G	V1084fsX1095	Heterozygous
SRNS-4A	-			-
SRNS-5A	16	c.2126T>G	V709G	Homozygous
SRNS-6A	-			-
SRNS-7A	10	c.1234G>T	G412C	Heterozygous
	24	c.3243_3250 insG	V1084fsX1095	
SRNS-8A	19	c.2548del10	A850fsX87	Heterozygous
SRNS-9A	10	c.1234G>T	G412C	Homozygous
SRNS-10A	27	c.3478C>T	R1160X	Homozygous

It seems that NPHS1 gene mutation in congenital NS and INS has different effects on disease manifestations and the outcome. Caridi *et al.* (2009) observed that cases with NPHS1 gene mutation have good response to the treatment and these variations have no effect on the INS outcome.

Normally most cases of monogenic diseases are heterozygous, because homozygous or compound heterozygous states might be either lethal or associated with a more severe phenotype than in single heterozygotes (De Bernabe *et al.*, 2003; Weatherall, 2000). The NPHS1 gene mutations heredity is autosomal recessive; so in cases with homozygous mutations there is definite interruption in nephrin activity.

As mentioned above, homozygous mutations have more severe phenotype. It is reported that children with NPHS1 gene mutations causing disease usually have milder phenotype in comparison to severe phenotype of congenital NS. Liu *et al.* (2001) showed that nephrin is a flexible protein which is altered easily and could be trapped in endoplasmic reticulum after a missense mutation. However, the variations in heterozygous form are not adequate to make structural damage and so are manifested with milder phenotype. Likewise, Caridi *et al.* (2009) observed that children with heterozygous mutations with different variants have similar renal survival probabilities that are significantly better than those of children with homozygous mutations.

Heterozygous mutations in our study especially in Exon 16 were higher in SSNS patients that could be a clue of presumed milder phenotype. Mutations in exons 4 (c. 512T>A) and 27 (c.3478C>T) were only seen in SRNS children in our study. Considering the nonsense mutation in exon 27, it could be presumed that mutation in exon 4 of NPHS1 gene is appropriative for SRNS cases.

CONCLUSION

Results of current study demonstrate that the disease manifestations and its outcome in cases with NPHS1 gene mutations in INS is completely different with congenital NS. Mutations in NPHS1 gene could occur in both SRNS and SSNS patients; however, considering higher incidence of heterozygous mutations in SSNS, the existence of milder phenotype in these cases would be the reason for steroid response.

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