

Mutational analysis of NPHS2 and WT1 genes in Saudi children with nephrotic syndrome.

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Abstract

Background: Nephrotic syndrome is the predominant glomerular disease in childhood. Mutations in numerous genes are known to be the reason for steroid-resistant nephrotic syndrome; however, the presence of these mutations seems to be effected by race sociocultural differences and interethnic group. Mutations in *NPHS2* and *WT1* genes record for nearly 20% and 5% of all children cases with steroid-resistant nephrotic syndrome, respectively. By contrast, mutations are absent from children with either steroid-dependent nephrotic syndrome or frequently-relapse nephrotic syndrome.

Methods: Mutation analysis was accomplished by direct sequencing of the complete 8 exons of *NPHS2* and exons 8 and 9 of *WT1* in 20 patients with steroid-resistant nephrotic syndrome, 25 with steroid-dependent nephrotic syndrome, and 13 with frequently-relapse nephrotic syndrome.

Results: Three pathogenic mutations in *NPHS2* were detected within steroid-resistant nephrotic syndrome patients. One as a non-sense mutation in exon 1 that was reported previously, while the second was novel and found as missense mutation in exon 7. The third one was found in the *NPHS2* promoter region. Additionally, for the first time, one pathogenic missense mutation in exon 8 of *WT1* gene was found in one Saudi patient with steroid-resistant nephrotic syndrome. All four mutations were documented and submitted to the ClinVar database.

Conclusion: *NPHS2* and *WT1* genes mutations are risk factors for steroid-resistant nephrotic syndrome with about 15% and 5% in Saudi pediatric patients. More molecular studies are required to clarify other possibility genes that responsible for the development of steroid-resistant nephrotic syndrome in Saudi children.

Keywords: Steroid-resistant nephrotic syndrome, Steroid-dependent nephrotic syndrome, Pediatric, WT1, NPHS2, Saudi Arabia.

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Introduction

Nephrotic Syndrome (NS) is defined as the occurrence of proteinuria, hypoalbuminemia, edema, and hyperlipidemia and it represents one of the most serious diagnoses made in pediatric nephrology [1]. The appearance of NS may be

as a result of primary (idiopathic) glomerular or secondary (systemic) disease [2]. According to the response of the patients to the steroid therapy, NS has been divided into two different categories: Steroid-Sensitive Nephrotic Syndrome (SSNS) versus Steroid-Resistant Nephrotic

Syndrome (SRNS) [3]. About 70% of SSNS patients will have one or more relapses, and a significant percentage will go on to develop either Frequent-Relapsing NS (FRNS) or steroid-dependent NS course (SDNS) [2].

It is a well-known that SRNS is the familiar phenotype of the genetically forms of NS whether recessive or dominant [4]. More recent report indicates that the occurrence of single-gene causation of SRNS can be found in at least a one-third of all tested families [5]. Up to date, several causative genes related to NS have been identified by either using direct DNA sequencing approaches or next-generation sequencing technology [6-12]. From literature, *NPHS1*, *NPHS2* and *WT1* are the most analyzed genes especially in a large cohort of patients with NS [3,9,13]. The results proposed that, *NPHS1* and *WT1* mutations were essentially found in children with congenital NS, while *NPHS2* mutations might be more prevalent in children with idiopathic SRNS [3,13]. The age of onset for *NPHS2* mutation is varying but commonly before 6 years of age [14]. By contrast, adult-onset before 18 years old cases of SRNS related with *NPHS2* are also well documented, these mutations are often found to be together as a compound heterozygous state [15].

Mutations in *NPHS2* and *WT1* genes have been reported to contribute nearly to 20% and 5% of all cases of SRNS, respectively [3,9,16]. On the other hand, *NPHS2* and *WT1* gene mutations are absent from children with SSNS, even though, all SSNS patients are not clinically same group as some may be FRNS or SDNS [17]. Until now, the influence of *NPHS2* and *WT1* mutations in FRNS or SDNS patients is fully unknown [17]. It was suggested that children with heterozygous mutations in *NPHS2* gene may have middle disease course between SSNS and SRNS that displays as the FRNS/SDNS course, but this finding did not report in children with uncomplicated SSNS [18].

With the growing number of undiagnosed missense mutations, many diagnostic laboratories use *in silico* prediction tools, e.g. sequence and evolutionary conservation-based tools and protein sequence and structure-based tools, to anticipate the effect the pathogenicity of a novel mutation in relation to the evolutionary conservation of specific amino acids, as well as protein structure and function [19]. Some of these tools are used frequently by diagnostic labs to recommend clinicians of disease probability in the absence of previous indication [19,20].

The population of the Kingdom of Saudi Arabia (KSA) is categorized with high consanguinity rate (52%-56% of marriages), therefore, it is documented that the high frequency of autosomal recessive genetically mediated renal diseases is due to the high consanguinity rate [21,22]. Also, it is well recognized that NS is more common in Asian children than in Caucasian children [23]. Furthermore, congenital and infantile NS was also reported to be higher in KSA than in other countries [22,24].

Therefore, in the present study, we performed mutational analysis of the entire exons of *NPHS2* plus exons 8 and 9 of *WT1* genes in 20 idiopathic cases of SRNS, 25 with SDNS and 13 cases with FRNS.

Materials and Methods

Patients

We identified all children with a clinical diagnosis of NS aged between 1-16 years old who were being followed at different Children Hospitals in western area of KSA under parent's consent and approved protocols of the central Hospital Bioethics committee of Alhada armed forces hospital under number (H-02-T-001-PTRC-15-04-226). NS patients were entered to the hospital from January, 2012 to December 2015. NS patients was defined as proteinuria (>40 mg/m²/h), hypoalbuminemia, and generalized edema [25]. SRNS patients were defined as the failure to response to daily therapy of prednisone (2 mg/kg) for 4-6 weeks [17,25]. Congenital Nephrotic Syndrome (CNS) was defined as the presentation of NS within the first three months of life [25]. FRNS patients defined as two or more relapses during the first 6 months or more than three relapses during any 12 month period, while, SDNS defined as two successive relapses at the time of tapering period of prednisone therapy or relapse within 2 weeks of the discontinuation of steroid therapy [25]. Inclusion criteria was made up of all Saudi patients with nephrotic syndrome of children below 16 years, while, exclusion criteria was patients with NS of a known secondary cause (e.g. IgA nephropathy). Familial and sporadic NS types were also determined by family history. Renal pathologists assessed renal biopsy specimens. Characteristic features of the clinical diagnosis were documented such as: name, sex, family history, HTN, hematuria, age of onset, response to steroid therapy, histological features of kidney biopsy, and interval time of progression toward End Stage Renal Disease (ESRD).

Genomic DNA Extraction

Genomic DNA was extracted directly from blood according to instructions of genomic DNA extraction kit (Thermo Fisher; USA). Quality of extracted DNA was assessed via electrophoresis on agarose gel and concentration of DNA was estimated by UV Spectrophotometry.

Amplification of DNA via PCR

All eight exons of *NPHS2* and exons 8 and 9 of *WT1* were amplified from genomic DNA by polymerase chain reaction (PCR) and directly sequenced. All PCR primers were designed from intronic sequences. The sequences of the forward and reverse primers, PCR conditions and the sizes of PCR products are given in Table 1.

Detection of *NPHS2* Gene Mutations and Variants by Direct DNA Sequencing

Sequencing of the purified specific fragments using the same primers were employed in the PCR amplification process on both forward and reverse directions and it was

repeated to confirm reproducible results. The products were sequenced using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (ABI Applied Biosystems, Life Technologies) on a 3130 Genetic Analyzer (Applied Biosystems) and the raw sequencing results were collected using the Data collection software version 3.1 from ABI Applied Biosystems. The DNA sequences were analyzed using the Seqscape software version 2.7 from ABI Applied Biosystems for base-calling and mutation detection. The results were compared against the references DNA sequence of *NPHS2* (GenBank; NG_007535) and *WT1* (GenBank; NG_009272).

In Silico Analysis of the Nucleotide Changes Identified

To assess the possible effect of the pathogenic mutations that found in the present study, three software programs were used: (1) PolyPhen2 (Polymorphism Phenotyping 2) (<http://genetics.bwh.harvard.edu/pph2/>), which expects possible effect of the non-synonymous exonic nucleotide change in the function of the human proteins [26]. (2) Sorting Tolerant from Intolerant (SIFT) and PROVEAN program (http://provean.jcvi.org/seq_submit.php). PROVEAN is a new calculation method which works for both single nucleotide polymorphisms (SNPs) and/or indels [27]. In PROVEAN program -2.5 is consider as a default threshold, therefore, variants with a score equal to or below -2.5 are considered deleterious, while, variants with a score above -2.5 are considered neutral [27]. (3) The RWebLogo program (plotting custom sequence logos. R package version 1.0.3.) (<https://CRAN.R-project.org/package=RWebLogo>), which is a statistical method for

detecting amino acid conservation [28]. From information theory, conservation can be calculated at each amino acid position that ranges from zero to 4.3 bits at an equally position for by all the 20 amino acids at an invariant position [29]. Therefore, strongly conserved positions are expected to be more deleterious when substitute with other amino acid, whereas weakly conserved positions are anticipated to tolerate more substitutions. Additionally, we used ConSite program (<http://consite.genereg.net>) for searching of any transcription factor binding site at the position of the variant that was identified in the promoter region.

SNP Database Submission

The mutations and novel polymorphisms variants that detected in this study were documented and submitted to the ClinVar under the accession numbers SCV000265969, SCV000265972, SCV000265973 and SCV000266491.

Results

We selected 58 Saudi pediatric patients with a diagnosis of NS who visited different hospitals from the western area of KSA during the past 3 years. These selections were including 25 patients (43%) with SDNS, 13 patients (22%) with FRNS, and 20 patients (35%) with SRNS. The mean age at the onset of the NS is 4.5 years while the mean age for the total years is 7.8 years. The majority was boys (55%). 33% of the patients had a positive family history of NS.

The nomenclature for describing the sequence variations that used here was based on the reference sequence of

Table 1. Primers sequences and annealing temperature conditions used in this study

Fragment (bp)	Annealing temperature (°C)	Reverse Primer (5'→3')	Forward Primer (5'→3')	Exon	Gene
447	55	TCAGTGGGTCTCGTGGGGAT	GCAGCGACTCCACAGGGACT	1	<i>NPHS2</i>
197	55	ATTGGGTCCTTATGGAATCT	CCAACAGATGCTAGTCAGTG	2	
205	55	GTTGAAGAAATTGGCAAGTC	CATTCTTATGCCAAGGCCT	3	
181	55	TAGAGAAAGCAAAGCCATC	GAAAGGTGAAACCCAAACA	4	
547	58	TAGCTATGAGCTCCCAAAGG	ACCACAGGATTAAGTTGTGC	5	
302	55	CAGTTCTTGCTAGTTAATTTCC	ATACCACACCATCTTCAATTG	6	
191	55	CCTTCCTAAAGGGCAGTCT	AGTCTGTGTGAAAGCTTTGG	7	
959	60	GCCCCTTACAGTCACATTA	TACTGCATAGTGGTGAAGCC	8	
299	60	CACATGGCTGACTCTCTCATT	CCTTAGGCATTTGGGATCT	8	<i>WT1</i>
366	60	TAGCCACGCAC TATTCCTTC	GTGAGGCAGATGCAGACATT	9	<i>WT1</i>

Table 2. Clinical data of the individual cases with mutations identified in SRNS patients associated with *NPHS2* and *WT1* genes

	Gender	Gene Mutation	Age of onset ^a	Renal Biopsy ^b	Age at ESRD ^c	HPT ^d	Hematuria	Site of mutation	Nucleotide change	Effect on coding
1	Female	<i>NPHS2</i>	9 y	FSGS	12 y	Yes	Microscopic	Promoter region	[c.-52C>G(; c.-51G>T]	-
2	Female	<i>NPHS2</i>	5 y	FSGS	7 y	Yes	Microscopic	Exon 1	c.115C>T	p.Q39X
3	Female	<i>NPHS2</i>	6 y	FSGS	9 y	Yes	Macroscopic	Exon 7	c.812C>T	p.P271L
4	Female	<i>WT1</i>	3 m	DMS	1 y	Yes	Negative	Exon 8	c.1301G>A	p.R434H

a) y: years; m: months; b) FSGS: Focal Segmental Glomerulosclerosis; DMS: Diffuse Mesangial Sclerosis; c) ESRD: End-Stage Renal Disease; d) HPT: Hypertension

GENBANK database as NM_014625, AJ279254, and NP_055440 for *NPHS2* gene, while NM_024426.3, NP_077744.3 for *WT1* gene. A summary of results from the mutation analysis of *NPHS2* and *WT1* genes is given in Table 2. DNA sequence analysis of all 8 exons of *NPHS2* gene revealed 3 pathogenic mutations (3/20; 15%) that

were found in SRNS cases only (Figure 1). First mutation was found as homozygous mutation in one girl at 5 years old and located in exon 1 (c.115C>T; p.Q39X). Two years later, this girl had developed to ESRD. This nonsense mutation was reported previously [9]. The second pathogenic mutation (c.812C>T; p.P271L) was found in female patient aged 6 years and diagnosed as SRNS with focal segmental glomerulosclerosis (FSGS) pattern and had macroscopic hematuria (Table 2). This missense mutation is novel and found in exon 7 as a heterozygous state (Figure 1). These two mutations were documented and submitted to the ClinVar database under the accession numbers SCV000265972 and SCV000265973, respectively.

Interestingly, we found one SRNA girl at age 9 years with hypertension and microscopic hematuria having a haplotype or compound heterozygous mutation located side by side [c.-52C>G(;); c.-51G>T] in the binding site of Upstream Stimulatory Factor (USF) of the *NPHS2* promoter region (Figure 1). Her renal biopsy was demonstrated as FSGS pattern. Three years later, she had developed ESRD (Table 2). Unfortunately, we could not screen the DNA of the parents for these specific mutations to explore if these variant are found on one chromosome and thus represent as a haplotype heterozygous mutation or they are present on two separate chromosomes and thus the genotype will be a compound heterozygous mutation. This mutation was submitted to the CLinVar under the accession number SCV000266491.

By contrast to these results, no pathogenic homozygous or heterozygous mutations were found in any SDNS or FRNS cases. But, we detected known and novel Single Nucleotide Polymorphisms (SNPs) in some patients with SDNS, FRNS and SRNS. Total seven SNPs variation in *NPHS2* gene was detected (Table 3). In the promoter region, one reported SNP was found (c.-51G>T) as heterozygous state in 11 patients with SDNS or FRNS; while, as a homozygous state in 3 patients with SDNS (Table 3 and Figure 1). In exon 1, one silent homozygous mutation (c.102A>G; p.Arg34Arg) was found in 25 patients (Table 3). Exon 8 had five SNPs; c.954 C>T

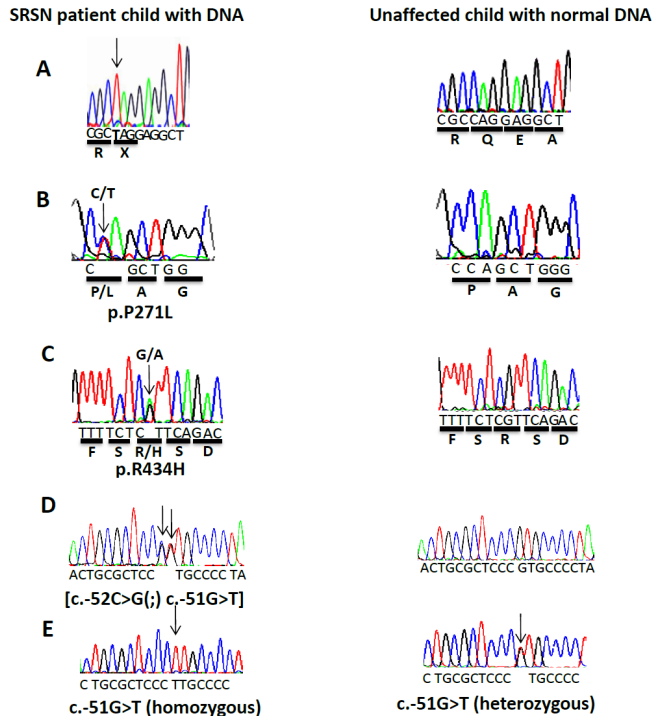


Figure 1. Detection of the *NPHS2* and *WT1* mutations by direct sequencing (A to C)

The corresponding nucleotide sequence with the respective amino acids is shown at the bottom of each chromatogram. The right column exemplifies the respective chromatograms of unaffected individuals. The left column shows the chromatograms of patients with steroid-resistant nephrotic syndrome with a homozygous nonsense mutations in *NPHS2* (A), a heterozygous mutation of p.P271L in *NPHS2* (B) and a heterozygous mutation of pR424H in *WT1* gene (C). D and E are the DNA variants in the promoter region of *NPHS2*

Table 3. *NPHS2* and *WT1* polymorphisms in 58 patients with SDNS, FRNS, and SRNS

Gene	Exon	Polymorphism	Heterozygous/ Homozygous	Patients (n=58)	Effect on coding
<i>NPHS2</i>	Promoter region	c.-51G>T	Homozygous Heterozygous	3 (5%) 11 (20%)	-
	1	c.102A>G	Homozygous	25 (43%)	p.Arg34Arg
	8	c.954C>T	Homozygous Heterozygous	12 (20.6%) 17 (29%)	p.Ala318Ala
	8	c.*157G>A	Homozygous	30 (52%)	-
	8	c.*200G>A	Homozygous Heterozygous	15 (26%) 6 (10%)	-
	8	c.*258A>G	Homozygous Heterozygous	2 (3.5%) 2 (3.5%)	-
	8	c.*428A>G	Homozygous	20 (34.5%)	-
	<i>WT1</i>	Intronic	c.1250-52G>T	Heterozygous	2 (3.5%)
Intronic		c.1250-32C>A	Heterozygous	2 (3.5%)	-

(as homozygous state in 12 patients and heterozygous state in 17 patients), c.*157G>A (as homozygous state in 30 patients), c.*200G>A (as homozygous state in 15 patients and heterozygous state in 6 patients), c.*258A>G (as homozygous state in 2 patients and as heterozygous state in 2 patients), and finally c.*428A>G was found as a homozygous state in 20 patients (Table 3).

When we screened exons 8 and 9 of *WT1* gene for possible mutation, we found one female SRNS patient (1/20; 5%) identified as a congenital nephrotic syndrome carrying a heterozygous mutation in exon 8 at the position c.1301G>A (p.R434H) (Figure 1). This mutation was documented and submitted to the ClinVar database under the record SCV000265969. Her biopsy was confirmed with diffuse mesangial sclerosis (DMS). At one year old this patient progressed to ESRD.

Also, we did not detect any disease-causing mutation correlated with *WT1* gene in patients with SDNS or FRNS. While, two intronic SNPs (c.1250-32C>A) and (c.1250-52G>T) were found in *WT1* gene in heterozygous state with 2 patients (3.5%) for each SNP (Table 3).

Based on *in-silico* evaluation for the two heterozygous mutations that found in *NPHS2* and *WT1* gene by using the web-based programs SIFT/PROVEAN, both mutations of P271L in *NPHS2* and R434H in *WT1* genes found to be affected the function of the two proteins with a deleterious score of -9.46 and -4.57 for *NPHS2* and *WT1* proteins, respectively. This prediction is further supported by

PolyPhen2 program, which also predicted that the amino acid substitution is probably damaging to the proteins. These results were in agreement with the distribution of the conserved amino acids along the sequences of *NPHS2* and *WT1* proteins. The RWebLogo program was used to measure the correlation between sequence conservation and tolerance to substitutions [28]. As shown in Figure 2, the two amino acids (P in *NPHS2* and R in *WT1* proteins) are shown to be well conserved among *NPHS2* and *WT1* proteins with more than 2 bits, respectively. Therefore the mutations of p.P271L in *NPHS2* and p.R434H in *WT1* might be a deleterious within the SRNS patients.

Discussion

Although *NPHS2* gene mutations cause autosomal recessive familial SRNS, it is also possible that heterozygous mutations of *NPHS2* may be related with FRNS or SDNS [9,18]. Here, we present for the first time a mutation analysis of *NPHS2* and *WT1* in children patients lived in western region of KSA, with SRNS, SDNS and FRNS. Table 4 shows a comparison of our findings with those of other studies related to SRNS cases. The mean age of our patients at the onset of NS was 4.5 years, which is similar to that report by Guaragna et al. [30] and Cho et al. [31]. While it was 2.2 years lower in comparison with the African American children patients with SRNS [32]. We found that the male gender may be had a possibility as a risk reason for SRNS, with about 60% in SRNS group. Similar results were reports in a study by Guaragna et al. [30], Chernin et al. [32] and Dhandapani et al. [33].

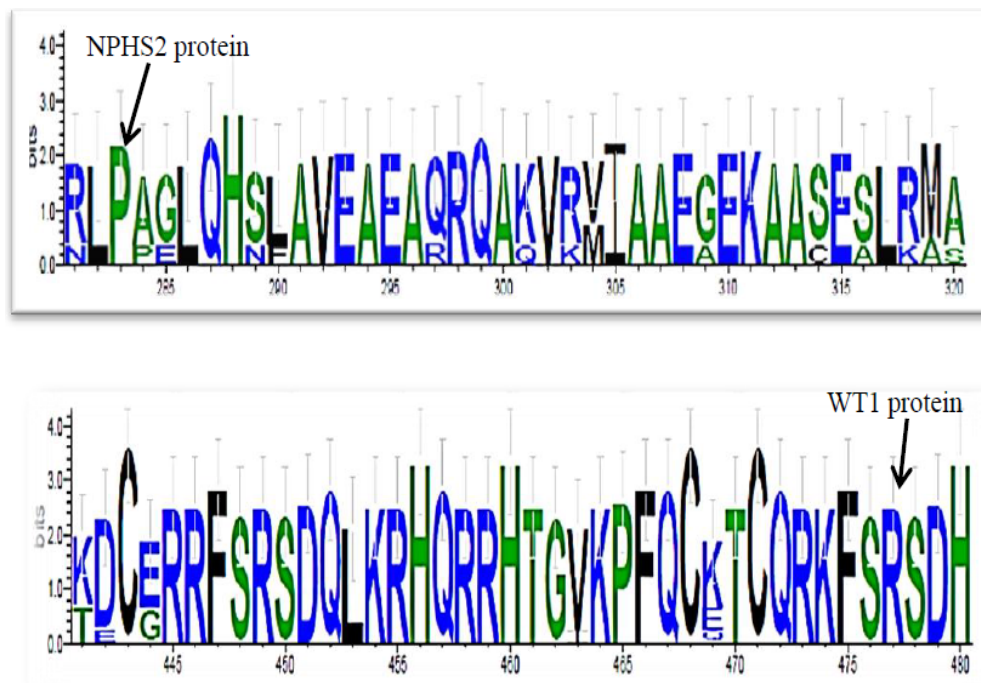


Figure 2. LOGO representation of the degree of amino acid conservation

Top figure represent the logo representation of 40 amino acids for *NPHS2* protein along with five proteins from *Homo sapiens* (NP_055440.1), *Mus musculus* (NP_569723.1), *Rattus norvegicus* (NP_570841.2), *Bos Taurus* (NP_001193036.1) and *Danio rerio* (NP_001018155.2). While, **Bottom** figure represent the logo representation of 40 amino acids along for *WT1* protein of *Homo sapiens* (NP_077744.3), *Mus musculus* (NP_659032.3), *Rattus norvegicus* (NP_113722.2), *Danio rerio* (NP_571121.1), *Sus scrofa* (NP_001001264.1), *Xenopus tropicalis* (NP_001135625.1), *Salmo salar* (NP_001167249.1), *Oryzias latipes* (NP_001098390.1) and *Gallus gallus* (NP_990547.1)

Table 4. Comparison of *NPHS2* and *WT1* mutations patterns in the 20 patients of the SRNS in this study with other studies

	This study	Al-Hamed et al. [9]	Kari et al. [34]	Dhandapani et al. [33]	Karle et al. [35]	Guaragna et al. [30]	Cho et al. [31]
Country	KSA	KSA	KSA	India	Germany	Brazil	Korea
No. patients	20	62	36	100	31	27	70
M %	60	ND	ND	62	58	55.5	44.2
F %	40	ND	ND	38	42	44.5	55.8
MN (years)	4.5	ND	ND	5.39	3.0	4.0	4.7
<i>NPHS2</i>	15%	22%	6.8%	18%	28%	15%	0%
<i>WT1</i>	5%	0%	0%	ND	ND	0%	5.7%

M: Male; F: Female; MN: Mean age at onset; ND: Not Determined

Our results revealed that 15% of children with primary SRNS had mutations in the *NPHS2*. Kari et al. [34] documented the rate of *NPHS2* gene mutation in KSA by 6.8%. Several groups from different countries reported that 15%-30% of patients with primary SRNS were caused by mutations in *NPHS2* gene [9,30,33,35]. Our results are lower than these frequencies. This could be interpreted by the small number of our patients or possibly reflects ethnic differences reducing the rate of these mutations in Saudi Arabia. It is a well-known that *NPHS2* gene mutations are more dominant in Europe and North or South America as the rate of *NPHS2* mutations reached to 28% [30,35]. While, Maruyama et al. [36] and Cho et al. [31] did not detect any mutation in *NPHS2* within 36 Japanese children or 70 Korean children with SRNS, respectively. Therefore, the variety in the *NPHS2* gene mutation frequency is believed to be due to variations in ethnicity and environment [31,36,37].

The nonsense mutation p.Q39X in *NPHS2* is the result of c.115C>T transition in exon 1. This mutation had previously been reported [9]. The loss of this amino acid produces a short form of podocin protein. It is supposed that this truncated protein gets stuck in the endoplasmic reticulum, so it loses its ability to bind nephrin in the lipid raft [9,38].

The second heterozygous mutation of *NPHS2* gene (c.812C>T; p.P271L) has not been reported in the literature until now. This mutation is located in exon 7 which is considered as a one of c-terminal domain for the *NPHS2* protein. It is well-known that the disease-causing mutations of *NPHS2* located in the C-terminal domains, which constitute the highly conserved amino acid region of homology with other stomatin protein family, are essential to podocin homo-oligomerization and interaction with other slit diaphragm proteins, especially *NPHS1* [5].

In the present study, one gene mutation was found in exon 8 of *WT1*. This mutation was found in female patient at age 3 months diagnosed as CNS case with renal biopsy of DMS (Table 2). It is a distinguished that *NPHS1* is the essential gene that has been recognized in patients presenting CNS in the first 3 months of life [9,13,25]. However, it has also been reported that CNS may be caused by mutations in several other genes, including *NPHS2*, *PLCE1*, and *WT1* [3,9,16,39]. The low rate of

mutation frequency in *WT1* gene of our study (5%) are similar to some reports those stated the *WT1* mutations may account for about 5% of patients with SRNS, and they have been recognized in patients with isolated DMS, with a clinical onset varying from a few days of life up to 2 years of age [3,16,31]. Interestingly, almost all cases were those of phenotypically female patients, and the mutations occurred mainly in exons 8 and 9, which code for zinc finger domains 2 and 3, respectively [16,31].

We believe that this is the first mutation found in *WT1* gene within Saudi children patients with SRNS. Recently, Al-Hamed, et al. [9] performed molecular genetic analysis in a cohort of 49 Saudi Arabian families that included families with CNS, infantile NS and childhood SRNS. They detected mutations in *NPHS2*, *NPHS2*, *PLCE1* and *MYOIE* genes, while no mutations were found in *WT1* gene [9]. Moreover, Kari et al. [34], retrospectively reviewed 36 children with a clinical diagnosis of SRNS and their results documented that the probably disease-causing mutations were identified in 5 children, 3 (6.8%) with *NPHS2* mutation and 2 (4.5%) had *NPHS1* mutation, while there is no mutation found in *WT1* gene [34]. Interestingly, the same position of *WT1* mutation (c.1301G>A) that found in our patient was considered as a pathogenic mutation in different study that causing the Congenital Diaphragmatic Hernia (CDH), a disorder of the development of the lung and diaphragm that related with pulmonary hypoplasia and pulmonary hypertension [40]. These results confirm the issues about *WT1* function as a network complex gene that regulates many functions in human.

Here, *NPHS2* or *WT1* mutations were not found with SDNS or FRNS patients. Our finding is different from that of Caridi et al. who stated significant heterozygous mutations in children with FRNS and SDNS [18]. Conversely, we detected 9 polymorphic variants, 7 in *NPHS2* and 2 in *WT1*, within the SDNS, FRNS, and SRNS patients. The two silent exonic mutation of exon 1 (c.102A>G) and exon 8 (c.954C>T) in *NPHS2* gene were published previously [4].

Interestingly, the SNPs [c.-51G>T] and [c.-52C>G(;)] c.-51G>T] were identified in the promoter region of *NPHS2* gene. The c.-51G>T variant was published previously, while the (c.-52C>G(;)] c.-51G>T) was novel [4]. The

Consite web-program predicted the sequences from -55 to -49 (TCCC**CG**TG) as the binding site for the upstream stimulatory factor (USF) in *Homo sapiens*. Di Duca et al. [41] studied the effect of the regulatory elements in the *NPHS2* promoter on the *NPHS2* protein function. They reported that the c.-51G>T variant as a functional polymorphism are affecting the gene expression by 80% down-regulation of the podocin protein when transfected in podocytes [41]. Therefore, the change of nucleotide from G to T in USF binding site resulted in the loss of function of the podocin protein [41]. These facts support the idea that our SRNS patient with the two variants [c.-52C>G (;) c.-51G>T] and SDNS or FRNS patients with the c.-51G>T variant might be affected through the reduction of the *NPHS2* protein level. These facts are in agreement with the published data that suggested children with heterozygous mutations in *NPHS2* gene may exhibit the FRNS/SDNS course [18].

We know that the high rate of family history with NS make it difficult to estimate pathogenicity of novel mutations without segregation and extensive family analysis. However, *in silico* tools have been used to identify pathogenicity of our novel mutations. Similar studies have been used same *in silico* tools to predict the effect of missense variants with SRNS patients [9,14,19,20,42,43].

Conclusion

We identify 15% of *NPHS2* mutations and 5% of *WT1* mutation in Saudi SRNS children patients. Interestingly, we detect for the first time a mutation in *WT1* gene amongst Saudi children populations. Also, we reported for the first time a haplotype or compound heterozygous mutations [c.-52C>G(;) c.-51G>T] in the promoter region of *NPHS2* gene. The low mutations frequencies in the two genes suggest that gene mutations of other podocyte-specific proteins could be responsible for the development of SRNS in Saudi children.

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