Short Communication

Novel Genetic Polymorphisms in *NPHS2* Gene Associated with Resistance to Steroid Therapy in Iraqi Children with Nephrotic Syndrome

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Abstract

Childhood nephrotic syndrome is a group of associated symptoms which occur due to damage to the kidneys, characterized by excess protein leakage in the urine, low levels of plasma albumin, and swelling (edema). Congenital nephrotic syndrome has been caused by mutations in *NPHS2* gene. This research looks at *NPHS2* SNPs that are hypothesized to affect steroid treatment susceptibility in Iraqi children with idiopathic nephrotic syndrome. The sample size was 75 newly diagnosed cases of nephrotic syndrome in children for this study. Mutation analysis was performed using Sanger sequencing based on specific primer sets for all exons of the *NPHS2* gene. In this study, the presence of heterozygous and homozygous mutations in the *NPHS2* gene of the SRNS group compared to SSNS and control groups shows significant association between steroid resistance and mutation of *NPHS2* gene. The genetic polymorphisms identified would be important molecular markers for early intervention allowing detection of carriers, prenatal diagnosis, and providing genetic counseling to at risk couples.

Keywords: Genetic polymorphism, idiopathic nephrotic syndrome, *NPHS2* gene

Introduction

Nephrotic syndrome in children is an abnormal condition characterized by damaging the kidneys which increases protein excretion in urine, resulting in reduced plasma albumin levels, and swelling. The disorder may be confined to the kidney alone, occur as part of a malformation syndrome, or complicate systemic disorders like in diabetes. Despite having such heterogeneity, the main disadvantage stems from losing normal selective filtration attributes of the glomerular filtration barrier (GFB).[1]

Mutations of the novel renal glomerular genes *NPHS1* and *NPHS2* encoding nephrin and podocin cause two types of severe nephrotic syndrome presenting in early life, Finnish type congenital nephrotic syndrome (CNF) and a form of autosomal recessive familial focal segmental glomerulosclerosis (SRN1), respectively.

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Mutations in the *NPHS2* gene have been known to cause congenital nephrotic syndrome. As a condition, it is a disorder of the kidneys that develop in early childhood and almost always results in complete kidney failure by preschool age. Single nucleotide polymorphisms in this gene appear to be the primary functional variant responsible for congenital nephrotic syndrome.[2]

Most of these polymorphisms disrupt the construction (amino acid) sequences of podocin and lead to decreased or absent production of (functional) protein that disrupts normal formation of protective structures called slit

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diaphragms. Excluding this typical structure results in leakage of fluid containing various particles through the kidneys leading to excretory abnormalities (kidney disease).[3]

This research looks into important single nucleotide polymorphisms, or SNPs, within the NPHS2 gene that might impact the ability to resist steroid treatment in Nebuchadnezzar's children suffering from idiopathic nephrotic syndrome.

Materials and Methods

Subjects

Based on the results of the steroid therapy, all 75 cases (100%) of newly diagnosed nephrotic syndrome children (NDNS 52M:23F) were classified into two broad categories. There were 35 males and 15 females in the SSNS making total to 75, which corresponds to 67%, leading us to 15 females in the group. Twenty-five and eight make 17. Out of this, counting thus created a balance of clan that led to forming strips differentiating these two groups.[4]

DNA extraction

Total extraction of genomic DNA [Figure 1] was performed according to Promega company (USA) protocol.

Polymerase chain reaction amplification of *NPHS2* gene

Polymerase chain reaction (PCR) was carried out in a total volume of 50 µL. The promoter region and eight exons of *NPHS2* gene were amplified using nine sets of primers designed by Primer-BLAST in NCBI as well as using conventional thermal cycler device programed depending on the amplification program illustrated in Table 1. The products of amplification process were analyzed on agarose gel (2%) using DNA ladder as a marker.[5]

Sanger method sequencing

The sequencing of the amplification products of the promoter region and eight exons of *NPHS2* gene was achieved by Macrogen Company/United States of America. Then, the sequences of these products were compared with the reference data in the NCBI's GeneBank for the *NPHS2* gene, using the software (Bioedit).

Statistical analysis

The least significant difference method (LSD) test of ANOVA was used to compare the means significantly.[6]

Ethical approval

This study was conducted according to the ethical standards and guidelines of Al-Nisour University under the approval number 203 on May 22, 2024. All personal data were anonymized to ensure confidentiality and privacy.

Results

The entire exons of *NPHS2* gene were analyzed through Sanger sequencing, as demonstrated in Figure 2. The genotype and allele frequency of four polymorphisms between patients and controls are illustrated in Table 2.

The results revealed a novel SNP (503 G > A) in exon 4. In the control cohort, 93% exhibited GG genotype and 6.7%

Figure 1: Gel electrophoresis of genomic DNA on agarose gel (0.7%) for 1 h at 5v/cm2. (A): Genomic DNA extracted from blood sample of NS patients. (B): Genomic DNA extracted from blood sample of Control

Table 1: Polymerase chain reaction amplification program

Steps	Temperature (C)	Time	No. of cycles
Initial denaturation	94	2 min.	1
Denaturation	94	30 s	35
Annealing	58	30 s	
Extension	72	60 s	
Final extension	72	5 min.	1

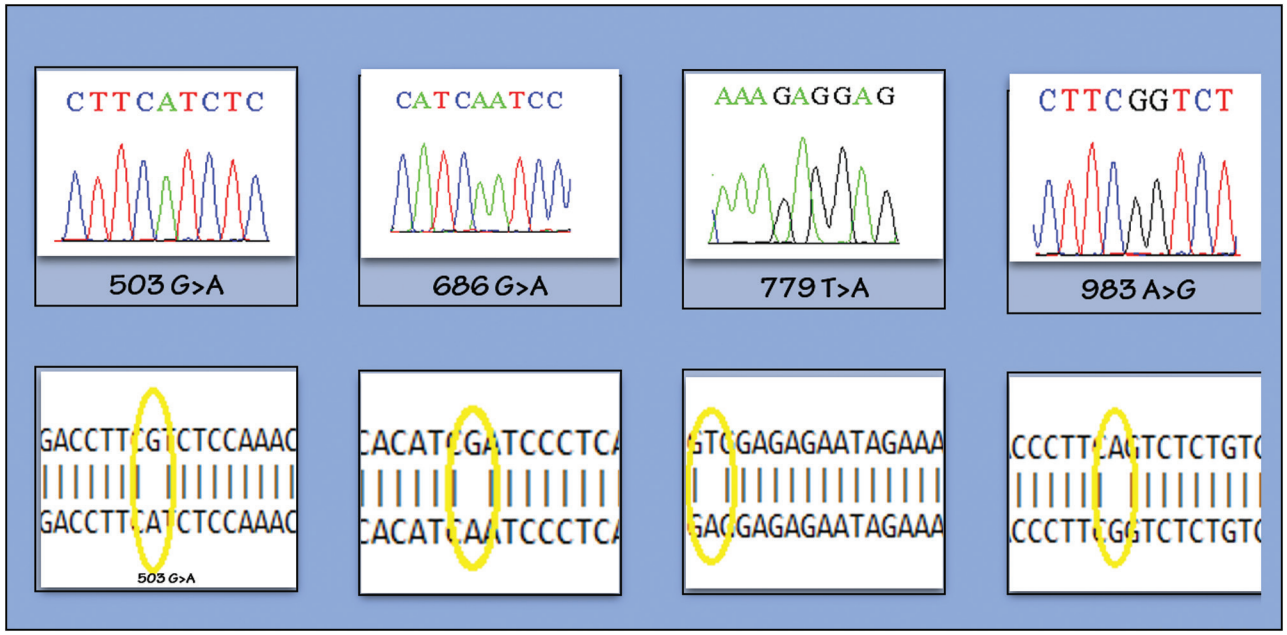

Figure 2: Sanger sequencing peaks and sequence alignments of 4 novel SNPs identified from the discovery of *NPHS2* gene in NS patients and healthy controls

Table 2: Novel and reported SNPs identified from the discovery of *NPHS2* gene in nephrotic syndrome patients and healthy individuals

NPHS2 mutations	A.A. change	Genotype and Allele frequency	Controls		Treated groups						P value	Mutation status
					SSNS		SRNS		Total			
			No. 30	%	No. 50	%	No. 25	%	No. 75	%		
503 G > A (Exon 4)	R168H	G/G	28	93.3	14	28	2	8	16	21.3	0.0001**	Novel
		G/A	2	6.7	34	68	18	72	52	69.3		
		A/A	0	0	2	4	5	20	7	9.4		
		G	0.966		0.62		0.44		0.56			
		A	0.033		0.38		0.56		0.44			
P value			0.0001**		0.0024**		0.0001**		0.009**		–	
686 G > A (Exon 5)	R229Q	G/G	29	96.7	4	8	0	0	4	5.3	0.0001**	Reported
		G/A	1	3.3	36	72	13	52	44	58.7		
		A/A	0	0	10	20	12	48	27	36		
		G	0.983		0.44		0.26		0.346			
		A	0.016		0.56		0.74		0.653			
P value			0.0001**		0.0001**		0.0001**		0.0001**		–	
779 T > A (Exon 6)	V260E	T/T	26	86.7	22	44	1	4	23	30.6	0.0001**	Novel
		T/A	4	13.3	26	52	15	60	41	54.7		
		A/A	0	0	2	4	9	36	11	14.7		
		T	0.933		0.7		0.34		0.58			
		A	0.066		0.3		0.66		0.42			
P value			0.0001**		0.0001**		0.0001**		0.0006**		–	
983 A > G (Exon 8)	Q328R	A/A	21	70	17	34	3	12	20	26.6	0.0001**	Novel
		A/G	9	30	32	64	16	64	48	64		
		G/G	0	0	1	2	6	24	7	9.4		
		A	0.85		0.66		0.44		0.586			
		G	0.15		0.34		0.56		0.413			
P value			0.0001**		0.0046**		0.0001**		0.0001**		–	

SSNS: Steroid sensitive nephrotic syndrome group; SRNS: Steroid resistance nephrotic syndrome group; A.A. change: Amino acid change;
** ($P \leq 0.01$)

GA genotype, while the SSNS group presented with 28% GG, 68% GA, and 4% AA genotypes. In the SRNS group, 8% had GG, 72% had GA, and 20% had AA genotype. Comparison of the distribution of genotype and allele frequency testing (Hardy–Weinberg equilibrium) for this SNP in SSNS and SRNS groups versus control demonstrated a marked difference ($P = 0.0001$) which is depicted in Table 2.

In exon 5 evaluation, a previously reported SNP (686 G > A) has been observed in NS Iraqi children. For the healthy controls, it is noted that 96.7% had GG genotype and 3.3% had GA genotype. On the other hand, SSNS group demonstrated that 8% had GG genotype, 72% exhibited GA while 20% presented AA genotype; in the case of SRNS group, no one had GG genotype, 52% were GA and 48% were AA. The distribution of this SNP's genotypes and alleles within the NS population showed participants deviated from Hardy-Weinberg Equilibrium ($P = 0.0001$) as shown in Table 2.

Exon 6 analysis revealed a novel SNP (779 T > A) found in NS Iraqi children. Among healthy individuals, 86.7% carried TT genotype with TA heterozygotes at 13.3%. Contrastingly, SSNS participants comprised 44%-TT/52%-TA/4%-AA while in SRNS group 4%-TT, 60%-TA, and 36%-AA The distribution of these two populations' genotype and allele frequencies relative to Hardy-Weinberg equilibrium showed significant SNPs within individual SSNS and SRNS groups which was noteworthy ($P = 0.0001$).

Throughout the analysis of exon 8, a SNP (983 A > G) has been identified in NS diagnosed Iraqi children. Among healthy controls, genotype distribution was 70% AA and 30% AG. Surprisingly, for the SSNS subgroup, there were 34% AA, 64% AG with 2% GG; while in the SRNS group, there were 12% AA, 64% AG, and 24% GG. Comparison of genotype and allele frequency distributions (Hardy–Weinberg equilibrium) for this SNP within SSNS and SRNS discordant families contrasted significantly to control subjects ($P = 0.0001$), as shown in Table 2.

Discussion

The *NPHS2* gene encodes podocin, which is partly a protein of the foot processes of podocytes. Podocytes make up part of the filtration barrier of kidney glomeruli together with glomerular capillary endothelial cells, the glomerular basement membrane and the slits themselves. Slit diaphragms are described as filtration barriers because they selectively retain molecules from the blood such as proteins while permitting other molecules like sugars or salts to be excreted through urine.[7]

Podocin likely role is assisting other proteins required for a fully functional slit diaphragm of the podocyte to reach the surface of the cell. In addition to this, the protein participates in podocyte signaling which aids in cellular adaptation to changes induced during filtration.[8]

Due to the replacement of guanine with adenine [Figure 2] in exon 4 (503 G > A) of *NPHS2* gene for participants included in our research, some changes were triggered on the amino acid sequence due to the wild type allele and an arginine "R" was replaced by Histidine "H" at R168H. Transversion mutation that happens within this area results on missense mutation that leads to the modification of arginine (an essential amino-acid with a positively electrically charged guanidino group) into histidine (an α-amino acid which is polar and bears a positively charged imidazole functional group) is shown in Table 2.

Due to the change of guanine into adenine (illustration 2) in exon 5 (686 G > A) of *NPHS2* gene, patients suffered from substitution of arginine (symbol Arg or R) by Glutamine (Gln or Q) at position R229Q. There occurs transversion mutation that leads to missense mutation and change of arginine into Glutamine which is neutral polar amino acid as indicated in Table 2.

Due to change of thymine into adenine (illustration 2) in exon 6 (779 T > A) *NPHS2* gene mutations, where present, there was substitution of valine (Val or V) with Glutamic acid at V260E. There occurs transversion mutation on this case of missense mutation where valine, nonpolar aliphatic amino acid with an α-carboxylic group and side chain i-propyl group is converted to glutamic acid, the α-amino acid classified as acidic and polar as indicated in Table 2.

As a consequence of the alteration adenine for guanine [Figure 2] in exon 8 (983 A > G), patients having mutations on NPHS2 were found having Q328R formerly Gln changed to Arg (244). It is transversion mutation that causes missense mutation from Q to R substitution as indicated in Table 2.

A number of pathological mutations have been found which may change both gene expression and the corresponding protein sequence. Therefore, the severity of the disease is determined by the replacement of an amino acid on intramolecular trafficking on podocin as well as on certain functional regions of podocin.[9,10]

As for this work, with respect to SRNS and SSNS and control groups, the presence of heterozygous and homozygous polymorphisms *NPHS2* genes in SRNS group raised noticeable association between steroid resistance occurrence and mutation of *NPHS2* gene. The finding of NPHS2 polymorphism is clinically important because in such groups the genetic defect identification has enhanced understanding for pathogenesis in termed steroid resistant nephrotic syndrome reinforcing the role toward podocyte damage leading to protein leakage associated with SRNS and/or FSGS.[11-14]

Conclusion

Our study showed that *NPHS2* gene mutational analysis should be performed as part of the initial workup. The genetic polymorphisms found in this study will serve as important molecular markers enabling early intervention in pediatric treatment, carrier detection, prenatal diagnosis, and genetic counseling for at-risk couples. Other studies focusing on excluding modifications of proteins and their relation to certain diseases are necessary to gain insight into the functional importance of those proteins.

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Conflicts of interest

There are no conflicts of interest.

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