Egypt. J. Chem. **Vol. 65,** No. SI:13B pp. 959 - 968 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Association between NPHS1 and NPHS2 genes polymorphism and Risk of the primary nephrotic syndrome among Egyptian Populations

Mohammed F. Al-azzawy[1], Mohammed Al-Haggar[2], Afaf M. ElSaid [2], Omali Y. El-khawaga[1]

1 Biochemistry Division, Chemistry Department, Faculty of Science, Mansoura University, Mansoura, 35516, Egypt.

2 Genetic unit, department of Pediatrics, Faculty of Medicine, Mansoura University, Mansoura , 35516, Egypt.

Abstract

Background: One of the most frequent kidney disorders in underdeveloped nations is a pediatric nephrotic syndrome (PNS), which has significant morbidity due to cardiovascular consequences such as thrombosis, increased infection risk, and malnutrition. NS has been linked to the NPHS1 and NPHS2 genes, which have been identified as the causal genes. The goal of this study was to see if the NPHS1 rs437168 and NPHS2 rs3829795 genetic variations play a role in nephrotic syndrome susceptibility (NS). **Methods**: From October 2021 to April 2022, case-control research was done in the Pediatric Nephrology Unit of Mansoura University Children's Hospital. The study included 100 children with NS who were separated into two groups: the patient group, which included 71 children with SRNS, 29 children with SSNS, and the control group, which included 100 healthy matched volunteers. Peripheral blood was used to obtain genomic DNA. ARMS-PCR was used to genotype single-nucleotide polymorphisms. **Results:** Molecular study showed NPHS1 rs437168, as well as NPHS2 rs3829795, GA, AA, genotype, dominant model. GA genotype was found in 14 SDNS cases from 29 children representing (48.3%) while SRNS cases show 42 from 71children representing (59.2%) showing a significant frequency with risk of SRNS (OR=1.806, CI (1.017-3.204), P=0.044). Moreover, AA genotype was found only in 1(3.4%) case of SDNS and 13 cases (18.3%) of SRNS showing a significant increase in the risk of SRNS (OR = 3.981, CI (1.343-11.799, P= 0.013). The A allele showed a significantly higher frequency associated with the risk of SRNS (OR= 1.687, CI. (1.146 – 2.485), P =0.008). While rs3829795 genotypes and alleles were not significantly associated with risk of SRNS where GA as well as AA genotype p>0.05. Additionally, A highly statically significant decrease in serum albumin levels between NS patients as well as SRNS and SDNS compared to healthy control cases. While A highly substantial increase in total cholesterol and triglycerides levels between NS patients (SRNS) and (SDNS) compared to the healthy control group. On the other hand, no substantial differences were found among SDNS and SRNS regarding albumin, total cholesterol, or triglyceride level. ***Conclusion***: Our results showed that the NPHS1 rs437168 polymorphism, as well as the NPHS2 rs3829795, may be associated with the development of NS in Egyptian primary nephrotic and a significant association between the NPHS1 rs437168 and the development of SRNS While no significant association between the development of SRNS and NPHS2 rs3829795 gene polymorphism.

***Keywords*:** NPHS1 gene; NPHS2 gene; nephrotic syndrome; single nucleotide polymorphism

Introduction

In developing countries, pediatric nephrotic syndrome (PNS) is among the most common kidney diseases [1]. Proteinuria, edema, hypoalbuminemia, and hyperlipidemia are the four representative hallmarks of this syndrome. Proteinuria of more than 3.5 g per day, hyperlipidemia, lipid Uria, and hypercoagulability are some of the renal and extrarenal characteristics of this clinical condition. The annual prevalence of NS in children ranges from 2 to 7 per 100,000 [2].

With a prevalence ratio of 16 occurrences per 100 000, it is one of the most common causes of pediatric kidney problems in Africa [3]. The incidence of the disease peaks between the ages of 2 and 5. Males are impacted more than females, with a 2:1 ratio. NS is 15 times more common in youngsters than it is in adults [4]. The statistical work site at Cairo University for Children's Hospital and Al-Munira Children's Hospital (2015) reported that approximately 934 cases were admitted to the hospital complaining of kidney problems. After receiving different mood states from kidney treatments, about 176 cases (18.8%) of them were diagnosed with NS [5]. NS causes 243 deaths in Egyptian children each year. Egypt is the world's second-worst country in this regard, trailing only Japan, which has 447 deaths; the United States is close behind with 153 deaths [4].

Because of cardiovascular problems such as thrombosis, increased infection risk, and starvation, NS has a significant morbidity rate. Furthermore, if NS perseveres and doesn't respond to therapy, it causes progressive scarring of the glomeruli, a condition known as glomerulosclerosis, as well as secondary tubular-

*Corresponding author e-mail: elkhawaga70s@mans.edu.eg .

Receive Date: 11 August 2022, Revise Date: 19 August 2022, Accept Date: 31 August 2022

DOI: 10.21608/EJCHEM.2022.155705.6725

interstitial compartment fibrosis. Glomerulosclerosis leads to renal dysfunction and end-stage kidney diseases, which necessitates hemodialysis or kidney transplant to survive. Glomerular disorders that lead to secondary glomerulosclerosis are a serious health concern around the world, accounting for about 85 percent of all ESRD cases [6]. PNS could be classified as steroid-dependent NS (SDNS) versus steroid-resistant NS (SRNS) according to the therapeutic efficacy [7]. Most youngsters experiencing primary nephrotic symptoms responded to corticosteroid medication within 4 weeks. Patients who respond to steroids have a long-term desirable impact and a minimal risk of developing chronic kidney disease [8]. Around 20% of youngsters having idiopathic nephrotic syndrome do not improve with steroid treatment. When a patient does not respond to prednisolone at a dosage of 2 mg/kg/day (60 mg/m2/day) over four weeks, they are classified as steroid resistance [9]. The quick revelation of genes that contribute to SRNS has assisted in the knowledge of glomerular filtration's molecular mechanics. Mutations involving genes producing podocyte-associated proteins have indeed been found in about 30% of SRNS incidences in youngsters [10], [11]. Because it is most likely a kidney-specific disease, confirmation of a genetic mutation usually means a lower chance of recurrence after transplantation [11]. The NPHS1 gene is found on the 19q13 chromosome. The formation of the zipper-like slit diaphragm (SD) is dependent on nephrin, a 136 kD transmembrane protein encoded by this gene with a substantial extracellular region with eight immunoglobulin-like domains [12], [13]. NPHS1 gene is considered the most commonest cited gene associated with congenital nephrotic syndrome and also SRNS [14].

Initially, *Kestilä et al* identified NPHS1 as the causative gene for the Finnish form of congenital nephrotic syndrome [14]. Over 250 mutations within the NPHS1 gene have already been implicated in a variety of kidney disorders [15].

Mutations in the NPHS1 gene cause CNF, which can result in deadly proteinuria and the absence of a slit diaphragm during birthing. Non-Finnish NS individuals, on the other hand, carry different mutations [16]. On chromosome 1q25-31, the NPHS2 gene was discovered [17]. This gene is 25 kb long and has a 1149 bp code region as well as eight exons [18], [19]. NPHS2 has expressed podocin, a 42 KD transmembrane proteins protein of 383 amino acids [20]. Podocin protein is synthesized in slit podocytes inside the diaphragm of the kidney and plays a significant role in filtration of glomerular wall [21]. About 30-40% of familial cases and 10-30% of sporadic cases are associated with mutations in the NPHS2 gene. In the Leiden Open Variation Database, more than 110 NPHS2 pathogenic variants have been recorded. They are found all over the gene including all type of mutations such as missense, nonsense, frame shift, and splicing mutations. A missense mutation seems to be the most common form of mutation, accounting for 42 % among all mutations [22]. The goal of this study is to see if there's a linkage between the NPHS1 rs437168 and NPHS2 rs3829795 polymorphisms and the risk of developing primary nephrotic syndrome in Egyptian children.

2. Materials and methods

2.1 Sample preparation

All included participants will be subjected to Clinical examination and management by the same clinician. Also, collecting their demographic data. The samples were collected from October 2021 to April 2022 according to the ethical standards of the Institutional Research Board (IRB), Faculty of Medicine, Mansoura University. Each participant signed his informed consent form. The study excluded any malignant cells, chronic infectious disorders (including the hepatitis B and C viruses), lupus nephritis, or drug-induced membranous glomerulonephritis (MGN).

2.2 Blood sampling

Five millilitres of blood were collected by vein puncture from all participants. Each collected blood sample was either dispensed into EDTA-tubes for molecular studies or allowed to collect for collection of serum after centrifugation for biochemical parameters measurement.

2.3 DNA extraction and genotyping

Genomic DNA will extract from peripheral blood cells according to (Thermo Fisher) [23] and using a DNA purification capture column kit supplied by (Thermo Scientific GeneJET Genomic DNA Purification Kit #K0721). The purified DNA was used immediately in PCR application. Evaluation of NPHS1 rs437168 and NPHS2 rs3829795 polymorphisms was done using amplification refractory mutation system-polymerase chain reaction (PCR). Table 1 lists the primers used.

2.4 Primer-PCR program

The optimization of amplification was performed under the conditions listed in Table 2. The PCR products were electrophoresed on 2.5%, agarose gel that was stained with ethidium bromide and visualized under UV light.

ARMS-PCR was used for the detection of NPHS1 rs437168 according to the method Hashemi et al. [16] and NPHS2 rs3829795, according to the method of Chamgordani et al. [24]. Briefly, two tubes were used to determine each variant for every subject. Each tube contained 8 µl of external primers (4 µl of forwarding control (FO) and 4µl of reverse control (RO)), 4 µl of DNA, 4 µl of A allele primer for tube 1, and 4 µl of G allele primer for tube 2 mixed with 16 µl of master mix (COSMO PCR RED Master Mix (W10203001), willow fort). In an Eppendorf Gradients Thermal cycle, PCR was performed.

2.5 biochemical parameters

2.5.1 Total cholesterol levels (T.C)

total cholesterol in serum was measured by quantitative method using BioMed- Cholesterol- LS (#CHO104090), Egypt [25]. The cholesterol is measured in the presence of phenol and Pero oxides after enzymatic hydrolysis and oxidation. Hydrogen peroxides and -4-amino antipyrine are used to produce quinonimine that absorbs strongly at 500 nm. The concentration of cholesterol has a direct relationship with the increases in absorbency. The concentration of T.C in the blood was measured in milligrams per deciliter mg/dl of blood.

2.5.2 triglyceride level (T.G)

the quantitative determination of Triglycerides in serum was determined using biomed- Triglycerides L.S (#TG117090), Egypt [25]. In the presence of lipoprotein lipase (LPL), triglycerides are converted to free fatty acids (R-COOH) and Glycerol which generates glycerol-3-phosphate that converts to hydrogen peroxide (H_2O_2) and dihydroxy acetone phosphate in the presence of glycerol-3-posphate oxidase. Finally, peroxidase converts H_2O_2, 4-aminophenazone, and p-chlorophenol to a red color. Triglyceride concentration was proportional to the absorbance of the color and expressed as mg/dl.

Table 1: Primer pairs used for screening of NPHS1 rs437168 and NPHS2 rs3829795 mutation by ARMS-PCR.

Mutation	Primer sequence	Size (bp)
NPHS1 rs437168 G>A	Common F (FO): CCATCTGTTCCTCCATCCAC	699
	Common R (RO): CCTAGACACGGAAGCTGCTC	
	R (G allele): TCTGTGGACATAGTCTGCACTTTC	351
	R (A allele): TCTGTGGACATAGTCTGCACTTTT	351
NPHS2 rs3829795 G>A	Common F (FO): CATCAACATCAGGCATAAGCAT	292
	Common R(RO): ACAAAAGGTCATCGAATTAGGGT	
	R (G allele): CCTTTCTCTCCTCCCTCCG	201
	R(A allele): CCTTTCTCTCCTCCCTCCA	201

Table 2: Optimization of PCR conditions for of NPHS1 rs437168 and NPHS2 rs3829795 mutation by ARMS-PCR.

VARIANT NAME	Cycle name	Temperature °C	Time	Number of Cycles
NPHS1 rs437168	Initial denaturation	95	5 min	1
	Denaturation	95	30 sec	30
	Annealing	55	30 sec	
	Extension	72	30 sec	
	Final extension	72	10 min	1
	Soak	4	∞	1
NPHS2 rs3829795	Initial denaturation	94	5 min	1
	Denaturation	94	40 sec	30
	Annealing	59	40 sec	
	Extension	72	50 sec	
	Final extension	72	10 min	1
	Soak	4	∞	1

2.5.3 serum albumin

Albumin was measured by using a modified Bromocresol green colorimetric method(#ALB100250) [26]. The Brom cresol green (BCG) dye binds to albumin (ALB), resulting in a rise in the blue-green color that is proportionate to the amount of albumin present in the sample. The detection of albumin is helpful in the diagnosis of hepatic and renal diseases. Albumin causes a rise in absorbance at 546 nm, which is measured spectrophotometrically and represented as mg/dl.

2.6 Statistical analysis

Statistical programmed for Social Science (SPSS) Version 25.0 was used to revise, encode, and tabulate the obtained data. Pearson's Chi-square test was used to examine the genotype distributions of mutations, as well as the frequency of heterozygous and homozygous for each variant, between patients and controls. It was considered significant if the probability (P) score was less than 0.05[27], [28].

1. **Results**

The current study represents a clinical trial including about 100 nephrotic syndrome and 100 control volunteers from Mansoura University Hospital between 2021- 2022.The present results show descriptive data of all studied quantitative parameters in control subjects compared to cases of nephrotic syndrome.

3.1 Correlation between clinical parameters and nephrotic syndrome in children

100 cases with NS including 69% males and 31% females. The median age of nephrotic syndrome patients was (11.4±3.8) years and the median age of control cases was (10.6±4.3) years (Table 3). All cases were divided into two groups based on treatment response: steroid-dependent nephrotic syndrome (SDNS) (29%) and steroid resistant nephrotic syndrome (SRNS) (71 %). As seen in Table 4, there was a statistically significant decline in serum albumin levels between patients (2.8±0.6) as well as SRNS (2.81±0.59) and SDNS (2.81±0.71) compared to control (4.5±0.4). A highly significant increase in total cholesterol level between patients [410(133-784)] as well as SRNS (417(133-784)) and SDNS (402(232-769)) compared to control (158 (98-230)). Also, a highly significant increase in triglycerides levels too observed between patients (195(34-608)) as well as seen in SRNS (195(100-608)) and SDNS (196(34-436)) compared to control (78(50-102)). On the other hand, no significant differences were found between SDNS and SRNS regarding albumin, total cholesterol, or triglyceride level.

3.2 Distribution of NPHS1 rs437168 gene polymorphism in Controls compared to nephrotic cases:

Genotyping study was performed on 100 patients with nephrotic syndrome and 100 healthy controls. Both in NS and control groups, the genotype distribution of the rs437168 of NPHS1 was in the Hardy Weinberg equilibrium. Table 5 shows a very statically significant difference from controls regarding NPHS1 rs437168 polymorphic genotype. The GA heterozygous genotype was identified in 56 children with NS (56%) higher than in controls (38%). The AA homozygous genotype was observed in 14 (14%) of NS children and 4 (4%) of healthy controls. while the GG homozygous genotype was lower in the patient (30%) than in the control (58%). GA genotype (Odds ratio [OR], 1.92; Confidence interval [CI], 95% (1.323 to 2.786); Probability (P=0.001)) as well as GA+AA genotypes (OR, 2.072, [CI] 95% (1.447 to 2.967); (P<.001)) increased chance of NS In comparing to th GG genotype (wild type). In compared to the G allele (wild type), the A allele which represents 84(42%) of NS and 46(23%) of healthy volunteers, was linked with a higher incidence of NS (OR, 1.737; [CI] 95 %, (1.33 to 2.27); (P <0.001)). Figure (1)

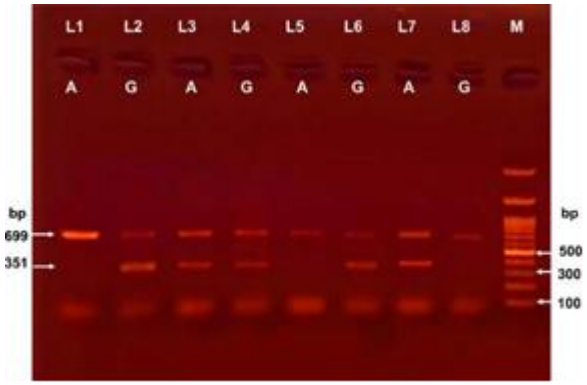

Figure (1) Individual ARMS PCR electrophoretic pattern of NPHS1rs437168, where every two lanes represent one participant. M stands for DNA marker (100 bp). The internal control is shown by the 699bp band. Depending on the primer, specific 351 bp bands represent G or A alleles. AG heterozygous is represented by lanes 3 and 4, where lane 3 represents the A allele and lane 4 represents the G allele. Lanes 1,2,5, and 6 indicate GG homozygous; the A allele is absent from lanes 1 and 5, whereas the G allele is present at 351bp on lanes 2 and 6. Lanes 7 and 8 are AA homozygous, with the A allele appearing at 351bp on lane 7 and the G allele absent from lane 8

Table 3: Comparison of age and gender among studied groups

		Control n=100	NS n=100	test of significance	P
Age (years)	mean±SD	10.6±4.3	11.4±3.8	t=1.426	0.155
Males	N (%)	65(65%)	69(69%)	X2=0.362	0.547
Females	N (%)	35(35%)	31(%31)		

Table 4: Comparison of biochemical parameters among the studied groups

		Control N=100	N S N=100			test of significance	P1	P2	P3	P4
			NS N=100	SDNS N=29	SRNS N=71					
S. albumin (g/dL)	Mean ±SD	4.5±0.4	2.8±0.6	2.81±0.71	2.81±0.59	F=24.49	<0.001	<0.001	<0.001	0.995
TC (mg/dL)	Median (range)	158 (98-230)	410 (133-784)	402 (232-769)	417 (133-784)	H=12.132	<0.001	<0.001	<0.001	0.960
TG (mg/dL)	Median (range)	78 (50-102)	195 (34-608)	196 (34-436)	195 (100-608)	H=12.761	<0.001	<0.001	<0.001	0.988

TC, total cholesterol, TG, triglyceride, SD, standard deviation, F, ANOVA coefficient; H, Kruskal Wallis coefficient. P = probability, P≤0.001= highly significant, P>0.05 = non-significant, n= number of cases.

P1, comparison between control and all NS.
P2, comparison between control and SSNS.
P3, comparison between control and SRNS.
P4, comparison between SSNS and SRNS.

Table 5: Genotype and Allele Frequency of NPHS1 rs437168 Gene in Children with and Without Nephrotic Syndrome

NPHS1 rs437168		Study Groups				
		Control (n = 100) %	NS (n = 100) %	OR	(95% Confidence Interval)	P
Genotype	GG	58 (58) %	30(30%)	Wild type		
	GA	38(38%)	56(56%)	1.92	))1.323-2.786	0.001
	AA	4(4%)	14(14%)	3.237	(1.609-6.513)	0.001
	GA+AA	42(42%)	70(70%)	2.072	(1.447-2.967)	<0.001
Allele	G	154 (77%)	116(85%)	Wild type		
	A	46(23%)	84(42%)	1.737	(1.33-2.27)	<0.001

P = probability, P≤0.001 = highly significant,

3.3 Distribution of NPHS2 rs3829795 gene polymorphism in Controls compared to nephrotic cases:

Genotyping study was performed on 100 patients with nephrotic syndrome and 100 healthy controls. In both of NS and healthy control groups, the genotype distribution of rs3829795 polymorphism of NPHS2 was in the Hardy Weinberg equilibrium

Table 6 shows a statically significant difference from controls regarding NPHS2 rs3829795 polymorphic genotype. The GA heterozygous genotype was identified in 53 children with NS (53%) higher than in controls (31%). Also, The AA homozygous genotype was higher in NS with 7(7%) children than in healthy control children with only 2 (2%). while the GG homozygous genotype was lower in patients (40%) than control (67%).GA genotype shows highly significant difference from controls (odds ratio [OR], 1.927; [CI], 95% (1.338 to 2.777); (P < 0.001)) as well as GA+AA genotypes (OR, 2.002, [CI] 95% (1.402 to 2.858); (P<0.001)) increased the risk of NS in comparison with GG genotype (wild type). The A allele which represents 67 (33.5%) of NS cases and 35(17.5%) of controls was associated with increased risk of NS (OR, 1.714; [CI] 95%, (1.285 to 2.278) ;(P 0.001)) in comparing to G allele (wild type) Figure (2).

3.4 Association of rs437168 and rs3829795 genotypes and alleles with risk of SRNS.

A total of 100 children with nephrotic syndrome were divided into 71 children with (SRNS) and 29 children with (SDNS) based on treatment response. Regarding rs437168 GA, AA, genotype, dominant model. GA genotype was found in 14 SDNS cases from 29 children representing (48.3%) while SRNS cases show 42 from 71children representing (59.2%) showing a significant frequency with the incidence of SRNS (OR=1.806, CI(1.017-3.204), P =0.044). Additionally, genotype was found only in 1(3.4%) case of SDNS and 13 cases (18.3%) of SRNS showing a significant increase in the risk of SRNS (OR = 3.981, CI (1.343-11.799, P= 0.013). The A allele showed a significantly higher frequency related to a higher incidence of SRNS (OR= 1.687, CI (1.146 – 2.485), P =0.008). While rs3829795 genotypes and alleles were not significantly associated with risk of SRNS where GA as well as AA genotype P> 0.05, Table 7.

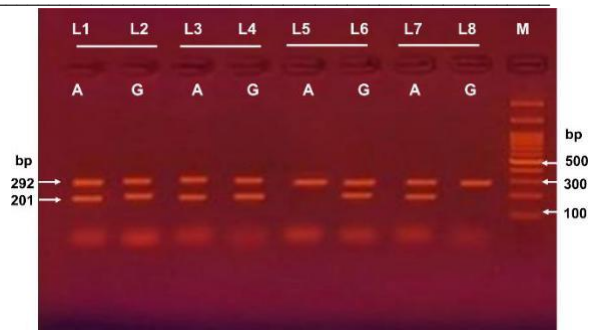

Figure (2): Individual ARMS PCR electrophoretic pattern of NPHS2 rs3829795, where each lane represents one person. M stands for DNA marker (100 BP). The internal control is shown by the 292bp band. Based on the primer, specific 201 bp bands represent G or A alleles. Lanes 1,2,3, and 4 represent AG heterozygous, with lanes 1 and 3 representing A alleles and lanes 2 and 4 representing G alleles, respectively. Lanes 5 and 6 are GG homozygous; where the A allele is absent from lane 5, the G allele shows at 201bp in lane 6. Lanes 7 and 8 are AA homozygous, with the A allele appearing at 201bp on lane 7 and the G allele absent from line 8

Table 6: Genotype and Allele Frequency of NPHS2 rs3829795 Gene in Children with and Without Nephrotic Syndrome

NPHS2 rs3829795		Study Groups: Control (n = 100)%	NS (n = 100)%	OR	(95% Confidence Interval)	P
Genotype	GG	67 (67)%	40(40%)	Wild type		
	GA	31(31%)	53(53%)	1.927	(1.338 – 2.777)	<0.001
	AA	2(2%)	7(7%)	2.964	(1.154-7.614)	.024
	GA+AA	33 (33%)	60 (60%)	2.002	(1.402 - 2.8587)	<0.001
	G	165(82.5%)	133(66.5%)	Wild type		
Allele	A	35(17.5%)	67(33.5%)	1.714	(1.285-2.278)	<0.001

P = probability, P ≤ 0.05 = significant, P ≤ 0.001 = highly significant,

Table 7: Association of rs437168 and rs3829795 genotypes and alleles with risk of SRNS

		SDNS n=29		SRNS n=71		p	OR	(95% CI)
		N	%	N	%			
rs437168	GG	14	48.3	16	22.5	Reference		
	GA	14	48.3	42	59.2	0.044	1.806	(1.017-3.204)
	AA	1	3.4	13	18.3	0.013	3.981	(1.343-11.799)
	GA+AA	15	51.7	55	77.5	0.013	2.03	(1.163-3.543)
	G	42	72.4	74	52.1	Reference		
	A	16	27.6	68	47.9	0.008	1.687	(1.146-2.485)
rs3829795	GG	14	48.3	26	36.6	Reference		

	GA	14	48.3	39	54.9	0.373	1.278	(0.745-2.192)
	AA	1	3.4	6	8.5	0.272	1.978	(0.586-6.675)
	GA+AA	15	51.7	45	63.4	0.283	1.335	(0.788-2.263)
	G	42	72.4	91	64.1	Reference		
	A	16	27.6	51	35.9	0.255	1.259	(0.847-1.874)

OR odds ratio; CI, confident interval. Logistic regression analysis was used. Dominant model=GA+AA. P = probability, p≤0.05 significant, P> 0.05 non-significant.

4. Discussion

NS is a primary disease that is part of a group of glomerular heterozygous disorders clinically characterized by peripheral edema, excessive proteinuria, hypoalbuminemia, and hypercholesterolemia [29]. Our Findings indicated that albumin levels were decreased in NS patients. while the levels of total cholesterol and triglyceride were significantly increased. These observations were in agreement with the results of Vaziri who discovered that patients experiencing nephrotic syndrome get a much greater burden of coronary heart disease and that hyperlipidemic alterations in these patients may contribute to this elevated risk[30]. The most prevalent genes reported to be mutated are currently limited to a few genes, including NPHS1 and NPHS2, particularly in people with nephrotic syndrome before or after the age of 18 years [14]. Principally, molecular detection of nephrotic syndrome is mostly based on direct evaluation of genetic alterations and indirect investigation through linkage analysis. Because of a large number of actual mutations in the NPHS1 and NPHS2 genes, sequencing and direct detection of mutations is an expensive and time-consuming operation. As a result, an alternate method for genetic diagnosis is focused on an association study employing polymorphic markers to detect carriers (heterozygote) as well as prenatal testing in families with affected children[24].

NPHS1 product, Nephrin, which is contributed to the creation of slit diaphragms and intercellular junctions of adult podocytes, has a larger role in the pathophysiology of nephrotic syndrome after childhood [31]. The current study looked at the possible link between the NPHS1 rs437168 variant and primary NS in children. The GA was found to be heterozygous in 56 children with NS (56 %) and 38 controls (38 %). In addition, the AA homozygous state was found in 14 (14%) of the children with NS and 4 (4%) of the controls. The G2289A (rs437168) genotype increases the risk of NS in our sample, as the A allele is associated with an elevated risk of NS. This is consistent with the findings of ***Hashemi et al***, who discovered a linkage between the (rs437168) GA polymorphism of NPHS1 and occasional NS [32]. Many studies reported the potential correlation between NPHS1 polymorphisms and immunoglobulin A nephropathy (IgAN) [33], [34]. Also, there had been a substantial difference in the genotype frequency distribution of the rs437168 polymorphism between membranous glomerular nephritis (MGN) patients and healthy controls, also with frequency of the G allele being significantly greater in the patient group [31]. In other diseases, according to one study, the C allele of rs437168 is substantially connected with the pathophysiology of early-onset pre-eclampsia PE and could be responsible for renal dysfunction, which is a risk factor in the development of early-onset pre-eclampsia in women of African ancestry [35]. On contrary, Mao and coworkers found no association between G2289A (rs437168) polymorphism of NPHS1 and sporadic NS but associated with heavy proteinuria [36]•

NPHS2 gene, which encodes a transmembrane protein containing 383 amino acids called Podocin, has several polymorphisms that are reported in association with NS [37]. As a result, NPHS2 gene markers could be used as biomarkers to assess an individual's risk of developing the disease. This study is the first evaluation of NPHS2 rs3829795 gene mutations in NS patients in Egyptian children. As regards, the distribution of the NPHS2 rs3829795 polymorphism genotypes GG, GA and AA and the frequency of G and A alleles in patient children and healthy samples. The patient group had an overrepresentation of the GA and AA genotypes with 53% and 7% respectively and low presentation of GG (40%) compared with healthy children with a high presentation for GG (67%) and low frequency of GA (31%) and AA (2%). Indicating that there is a statical association between NPHS2 G>A and nephrotic syndrome expressing that the G allele is the codominant allele in a healthy individual and the A allele increase the risk of nephrotic syndrome. This is following an Iranian study, they found out that the G>A allele might be employed as single-nucleotide markers in linkage analysis to reveal NPHS2 gene alterations in the disease's molecular diagnosis [38]. Steroid resistance is a powerful predictor of future end-stage renal disease risk. Even though clinical development in SRNS was already limited, the value of this increased understanding of the disease's pathogenic genesis has been substantial. It is widely acknowledged that early reliable identification of gene variants would change the therapeutic strategy, allowing for the treatment

of uncommon instances while avoiding inappropriate immunosuppression. In the last 15 years, the medical community has determined that more than 70 recessive or dominant genes can cause SRNS in humans if they are altered. Podocytes are linked to most of the encoded proteins [39].

In terms of the SRNS correlation, the polymorphism NPHS1 rs437168 G2289A was observed in 55 (77.5%) of 71 children having SRNS both in homozygous and heterozygous genotypes in this investigation. GA, as well as AA genotype, show a significant frequency with risk of SRNS. The A allele showed a significantly higher frequency associated with the risk of SRNS. In the current investigation, either SRNS or SDNS showed a significant difference regarding the polymorphism NPHS2 rs3829795 G670A. Where GA and AA genotype P >0.05, genotypes and alleles were not significantly linked with the incidence of SRNS. Otherwise, Mutations in the NPHS2 gene containing our SNP were discovered in Azerbaijani children with SRNS In a previous study carried out by the finding of Baylarov and co-workers [40].

At least one polymorphic allele of G670A appears to be involved in SRNS. To make an accurate conclusion about the involvement of G670A in the development of SRNS, researchers must look for other exon mutations in the NPHS2 gene in the same study groups. In conclusion, because of the genetic heterogeneity of NS, screening methodologies should continue to encompass several NS genes, as well as rare and newly identified genetic causes, to assure a high yield of molecular genetic diagnosis. In this study, we show that rs437168 mutation in NPHS1 and rs3829795 mutation in NPHS2 might cause NS in Egyptian children. The diagnosis of mutations that cause NS is essential for therapeutic considerations and genetic guidance. In the present study, both rs437168 and rs3829795 variants indicate a substantial difference in the polymorphism. To our best knowledge, this work provides the first genotype correlations of those variants in Egyptian children which could be employed as single-nucleotide biomarkers in genetic analysis to aid in disease diagnosis

5. Declaration of interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

6. Data Availability:

The data that supports this work is available upon reasonable request.

7. Funding:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

8. Acknowledgment

We would like to thank the Chemistry Department, Faculty of Science, Mansoura University, Egypt for providing some of the facilities required during this study. We would like to thank the Pediatric Hospital Mansoura University, Egypt for providing the facilities required during this study.

9. References:

[1] Gebrehiwot M., Kassa M., Gebrehiwot H, and Sibhat M., "Time to relapse and its predictors among children with nephrotic syndrome in comprehensive specialized hospitals, Tigray, Ethiopia, 2019," International journal of pediatrics, vol. 2020, 2020.

[2] Bayomy N. R., Alfottoh W. M. A., Eldeep S. A. A., Mersal A. M. S. I. M., Abd El-Bary H. M. A., and Abd El Gayed E. M., "Mir-142-5p as an indicator of autoimmune processes in childhood idiopathic nephrotic syndrome and as a part of MicroRNAs expression panels for its diagnosis and prediction of response to steroid treatment," *Mol.Immunol.*, vol. 141, pp. 21–32, 2022.

[3] Esezobor C. *et al.*, "Management of idiopathic childhood nephrotic syndrome in sub-Saharan Africa: Ibadan consensus statement," *Kidney Int.*, vol. 99, no. 1, pp.59–67, 2021.

[4] Meena P. and Renuga S., "Assess the knowledge on nephrotic syndrome among mother of under five children at SMCH," *Age (Omaha).*, vol. 21, no. 30, pp. 31–40, 2019.

[5] Khalil S. H., Attia A. A., Mahmoud N. F., Abdelaziz H. E., and others, "Self-concept among school-age children with nephrotic syndrome," *Egypt. Nurs. J.*, vol. 18, no. 1, p. 11, 2021.

[6] Tharaux P.-L. and Huber T. B., "How many ways can a podocyte die?," in *Seminars in nephrology*, 2012, vol. 32, no. 4, pp. 394– 404.

[7] Liu I. D., Willis N. S., Craig J. C., and Hodson E. M., "Interventions for idiopathic steroid-resistant nephrotic syndrome in children," *Cochrane Database Syst. Rev.*, vol. 2019, no. 11, 2019, doi:10.1002/14651858.CD003594.pub6.

[8] Noone D. G., Iijima K., and Parekh R.,"Idiopathic nephrotic syndrome in children," *Lancet*, vol. 392, no. 10141, pp. 61–74, 2018, doi: 10.1016/S0140-6736(18)30536-1.

[9] Yildiz N., Cicek N., Guven S., Gokce I., and Alpay H., "Clinical Course of Idiopathic

Nephrotic Syndrome in Children: predictors of steroid resistance," 2022.

[10] Sadowski C. E. *et al.*, "A single-gene cause in 29.5\% of cases of steroid-resistant nephrotic syndrome," *J. Am. Soc. Nephrol.*, vol. 26, no. 6, pp. 1279–1289, 2015.

[11] Wang F. *et al.*, "Spectrum of mutations in Chinese children with steroid-resistant nephrotic syndrome," *Pediatr. Nephrol.*, vol. 32, no. 7, pp. 1181–1192, 2017.

[12] Lenkkeri U. *et al.*, "Structure of the gene for congenital nephrotic syndrome of the finnish type (NPHS1) and characterization of mutations," *Am. J. Hum. Genet.*, vol. 64, no. 1, pp. 51–61, 1999

[13] Zhuo L., Huang L., Yang Z., Li G., and Wang L., "A comprehensive analysis of NPHS1 gene mutations in patients with sporadic focal segmental glomerulosclerosis," *BMC Med.Genet.*, vol. 20, no. 1, pp. 1–8, 2019, doi:10.1186/s12881-019-0845-4.

[14] Zhuo L., Huang L., Yang Z., Li G., and Wang L., "A comprehensive analysis of NPHS1 gene mutations in patients with sporadic focal segmental glomerulosclerosis," *BMC Med. Genet.*, vol. 20, no. 1, pp. 1–8, Jun. 2019, doi: 10.1186/s12881-019-0845-4.

[15] Park E., "Genetic Basis of Steroid Resistant Nephrotic Syndrome," *Child. Kidney Dis.*, vol. 23, no. 2, pp. 86–92, 2019, doi:10.3339/jkspn.2019.23.2.86.

[16] Hashemi M., Sadeghi-Bojd S., Rahmania K., and Eskandari-Nasab E., "Association between NPHS1 and NPHS2 gene variants nephrotic syndrome in children," *Iran. J. Kidney Dis.*, vol. 9, no. 1, pp. 25–30, 2015.

[17] Ranganathan S., "Pathology of podocytopathies causing nephrotic syndrome in children," *Front. Pediatr.*, vol. 4, no. MAR, p. 32, 2016, doi:10.3389/fped.2016.00032.

[18] McKenzie L. M. *et al.*, "NPHS2 variation in sporadic focal segmental glomerulosclerosis," *J. Am. Soc. Nephrol.*, vol. 18, no. 11, pp. 2987–2995, 2007.

[19] Chamgordani L. E., Ebrahimi N., Amirmahani F., and Vallian S., "CG/CA genotypes represent novel markers in the NPHS2 gene region associated with nephrotic syndrome," *J. Genet.*, vol. 99, no. 1, pp. 1–7, 2020, doi: 10.1007/s12041-020-1188-9.

[20] Gbadegesin R. *et al.*, "Mutational analysis of NPHS2 and WT1 in frequently relapsing and steroid-dependent nephrotic syndrome," *Pediatr. Nephrol.*, vol. 22, no. 4, pp. 509– 513, 2007, doi: 10.1007/s00467-006-0377-y.

[21] Dong L. *et al.*, "Integration of cistromic and transcriptomic analyses identifies Nphs2, Mafb, and Magi2 as Wilms' tumor 1 target genes in podocyte differentiation and maintenance," *J. Am. Soc. Nephrol.*, vol. 26, no. 9, pp. 2118–2128, 2015.

[22] Bouchireb K. *et al.*, "NPHS 2 Mutations in Steroid-Resistant Nephrotic Syndrome: A Mutation Update and the Associated Phenotypic Spectrum," *Hum. Mutat.*, vol. 35, no. 2, pp. 178–186, 2014.

[23] Thermo Fisher, "Thermo Scientific GeneJET Genomic DNA Purification Kit #K0721, #K0722," vol. 2016, no. October, pp. 6–13, 2016.

[24] Chamgordani L. E., Ebrahimi N., Amirmahani F., and Vallian S., "CG/CA genotypes represent novel markers in the NPHS2 gene region associated with nephrotic syndrome," *J. Genet.*, vol. 99, no. 1, 2020, doi: 10.1007/s12041-020-1188-9.

[25] Reagent P. and Chloride M., "Consult Instructions for Use Caution , Consult accompanying Documents In Vitro Diagnostic Medical Device Temperature Limitation Manufacturer Catalogue Number Batch Code Use by," vol. 114100, no. 1x50 ml.

[26] Point E. and Albumin B., "BioMed-Albumin," [Online]. Available: www.egy-chem.com.

[27] Zhao F., Song M., Wang Y., and Wang W.,"Genetic model," *J. Cell. Mol. Med.*, vol. 20, no. 4, p. 765, 2016, doi:10.1111/jcmm.12751.

[28] Kwok P.-Y., "Approaches to allele frequency determination," *Pharmacogenomics*, vol. 1, no. 2, pp. 231–235, 2000.

[29] Go A. S. *et al.*, "Primary nephrotic syndrome and risks of eskd, cardiovascular events, and death: The kaiser permanente nephrotic syndrome study," *J. Am. Soc. Nephrol.*, vol. 32, no. 9, pp. 2303–2314, 2021, doi:10.1681/ASN.2020111583.

[30] Vaziri N. D., "Molecular mechanisms of lipid disorders in nephrotic syndrome," *Kidney Int.*, vol. 63, no. 5, pp. 1964–1976, 2003.

[31] Lo Wan-Yu W. Y. *et al.*, "Association between genetic polymorphisms of the NPHS1 gene and membranous glomerulonephritis in the Taiwanese population," *Clin. Chim. Acta*, vol. 411, no.9–10, pp. 714–718, 2010, doi:10.1016/j.cca.2010.02.004.

[32] Hashemi M., Sadeghi-Bojd S., Rahmania K., and Eskandari-Nasab E., "Association between NPHS1 and NPHS2 gene variants nephrotic syndrome in children," *Iran. J. Kidney Dis.*, vol. 9, no. 1, p. 25, 2015.

[33] Narita I. *et al.*, "Genetic polymorphism ofNPHS1 modifies the clinical manifestations of Ig A nephropathy," *Lab. Investig.*, vol. 83, no. 8, pp. 1193–1200, 2003, doi:10.1097/01.LAB.0000080600.49276.31.

[34] Hashemi M. *et al.*, "Association betweenNPHS1 and NPHS2 gene variants

nephrotic syndrome in children," *Iran. J. Kidney Dis.*, vol. 9, no. 1, pp. 25–30, Jun. 2015, doi:10.3923/pjbs.2020.365.374.

[35] Khaliq O. P., Konoshita T., Moodley J., and Naicker T., "The association of NPHS1 andACNT4 gene polymorphisms with pre-eclampsia," *Eur. J. Obstet. Gynecol. Reprod. Biol.*, vol. 266, pp. 9–14, 2021, doi: https://doi.org/10.1016/j.ejogrb.2021.09.006.

[36] Mao J. *et al.*, "NPHS1 and NPHS2 gene mutations in chinese children with sporadic nephrotic syndrome," *Pediatr. Res.*, vol. 61, no. 1, pp. 117–122, 2007, doi:10.1203/01.pdr.0000250041.19306.3d.

[37] Joshi B. B. *et al.*, "Characterization of NPHS2 gene polymorphisms associated to steroid resistance nephrotic syndrome in Indian children," *Gene*, vol. 628, no. July, pp. 134–140, Sep. 2017, doi:10.1016/j.gene.2017.07.029.

[38] Chamgordani L. E., Ebrahimi N., Amirmahani F., and Vallian,S., "CG/CA genotypes represent novel markers in the NPHS2 gene region associated with nephrotic syndrome," *J. Genet.*, vol. 99, no. 1, pp. 1–7, 2020.

[39] Tullus K., Webb H., and Bagga A.,"Management of steroid-resistant nephrotic syndrome in children and adolescents," *Lancet Child Adolesc. Heal.*, vol. 2, no. 12, pp. 880–890, 2018, doi: 10.1016/S2352-4642(18)30283-9.

[40] Baylarov R., Senol O., Atan M., and Berdeli A., "NPHS2 gene mutations in azerbaijani children with steroid-resistant nephrotic syndrome," *Saudi J. Kidney Dis. Transpl.*, vol. 31, no. 1, pp. 144–149, 2020, doi: 10.4103/1319-2442.279934.