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Research Article

Association of the *MDR1* variants (rs2032582 and rs2032583) with Steroid Response in Iraqi Children with Idiopathic Nephrotic Syndrome

Ali Mohammed Abd Alridha[1]*, Dheyaa Jabbar Kadhim[2], Ayad Hussein Ali Alkhazrajy[3]

[1]*Department of Clinical Pharmacy and Therapeutics, Faculty of Pharmacy, University of Kufa, Najaf, Iraq;* [2]*Department of Clinical Pharmacy, College of Pharmacy, University of Baghdad, Baghdad, Iraq;* [3]*Babylon Hospital for Maternity and pediatrics, Babylon, Iraq*

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Abstract

Background: Several studies linked the development of steroid-resistant nephrotic syndrome (SRNS) to genetic variations in the multidrug resistance 1 (*MDR1*) gene, though a disparity in findings was underlined among children with different ethnic origins. ***Objective***: This study examined the relationship between *MDR1* variants (rs2032582 and rs2032583) and the risk of developing SRNS in Iraqi patients with idiopathic nephrotic syndrome (INS). ***Methods***: This case-control study included children with steroid-sensitive INS (SSNS; n=30) and SRNS (n=30) from the Babylon Hospital for Maternity and Pediatrics. Sanger sequencing was used to determine the participants' genotypes. ***Results***: The rs2032582 genotypes and alleles were not associated with SRNS development risk. It was also found that kids who had both the wild or mutant homozygous genotypes for rs2032583 and rs2032582 variants were more likely to get SRNS [OR (95%CI):30.18 (1.55–588.5), p=0.008] than kids who had both the heterozygous genotypes for rs2032583 and either genotype of rs2032582. ***Conclusions***: Nephrotic children who have homozygous genotypes (wild or mutant) for the rs2032583 and rs2032582 variants likely resist prednisolone therapy, and an alternative therapeutic regimen may be warranted. Further investigations are needed to elucidate the potential implications of *MDR1* variants for personalizing drug therapy in INS children.

Keywords: Genetic polymorphisms, Iraq, *MDR1*, Nephrotic syndrome, Steroid resistance.

ارتباط المتباينات الجينية في جين المقاومة للأدوية المتعدد-1 (rs2032582 و rs2032583) مع الاستجابة للعلاج بالأدوية الستيرويدية لدى الأطفال العراقيين المصابين بمتلازمة التنادر الكلوي مجهولة السبب

الخلاصة

الخلفية: وجدت العديد من الدراسات أن متلازمة التنادر الكلوي المقاومة للأدوية الستيرويدية لها علاقة بالتغيرات الجينية في جين المقاومة للأدوية المتعددة 1، وهناك تباين ملحوظ في نتائج الدراسات بين الأطفال ذوي الأصول العرقية المختلفة. **الهدف**: لدراسة العلاقة بين المتباينات الجينية (rs2032582 و rs2032583) في الجين المقاوم للأدوية المتعدد 1 وخطر الاصابة بمتلازمة التنادر الكلوي المقاومة للأدوية الستيرويدية لدى الاطفال العراقيين الذين يعانون من المتلازمة مجهولة السبب. **الطرائق:** أجريت دراسة الحالات والشواهد وشملت 30 الأطفال الذين يعانون من متلازمة التنادر الكلوي المستجيبة للأدوية الستيرويدية و 30 متلازمة التنادر الكلوي المقاومة للأدوية الستيرويدية في مستشفى بابل للولادة وطب الأطفال. تم استخدام التحليل التسلسلي لسانكر لتحديد الأنماط الجينية للمشاركين. **النتائج**: لم ترتبط الأنماط الجينية والأليلات للمتباينة الجينية (rs2032582) بخطر الاصابة بالمتلازمة المقاومة للأدوية الستيرويدية. وكان الأطفال الذين لديهم مزيج من اي نمط وراثي للمتباينة (rs2032582) مع النمط الجيني المتماثل (الطبيعي أو المتغاير) للمتباينة الجينية (rs2032583) أكثر عرضة للإصابة بالمتلازمة المقاومة للأدوية الستيرويدية مقارنة بأولئك الذين لديهم مزيج من أي نمط وراثي للمتباينة (rs2032582) مع النمط الوراثي المتخالف للمتباينة (rs2032583) حيث كانت نسبة الأرجحية= 30.18 ومدى الثقة 95% (1.55 - 588.5)، ومستوى الدلالة المعنوية الاحصائية (p=0.008). **الإستنتاجات**: وجدت الدراسة أن الأطفال المصابين بمتلازمة التنادر الكلوي مجهولة السبب و الذين لديهم النمط الوراثي المتماثل (الطبيعي أو المتغاير) للمتباينة الجينية (rs2032583) في الجين المقاوم للأدوية المتعددة 1 من المرجح أن يكونوا مقاومين لعلاج البريدنيزولون، وقد يحتاجون لتداخل علاجي بديل. كما وإن اجراء المزيد من البحوث هو أمر ضروري لأي توضيح اضافي للعلاقة بين التباين الجيني و الإستجابة للخطة العلاجية لدى الأطفال المصابين بمتلازمة التنادر الكلوي مجهولة السبب.

* ***Corresponding author***: Ali M. Abd Alridha, Department of Clinical Pharmacy and Therapeutics, Faculty of Pharmacy, University of Kufa; Email: alim.abdalridha@uokufa.edu.iq

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INTRODUCTION

Nephrotic syndrome (NS) is one of the most common glomerulopathies among children, with serious health-related burdens in terms of morbidity, mortality, and financial implications. The etiology of NS during childhood has been defined in the predominant majority of cases as idiopathic (INS), and the underlying histopathological presentation is diverse [1–4]. Patients with poor responsiveness to an initial steroid trial are categorized as presenting with steroid-resistant NS (SRNS). The associated risk of disease progression and concurrent complications is greater for children with SRNS compared to those with steroid-sensitive NS (SSNS) [5–7]. Furthermore, an ongoing increase in the cases' number of SRNS has been observed among children globally and regionally (in Iraq) [2,8–12]. This is an alarming finding because SRNS represents a leading cause of childhood chronic renal failure and end-stage kidney disease. Because of this, finding out what might cause steroid resistance is important for improving the drug treatment of INS in children [2,8–12]. Studies have investigated numerous biochemical and pharmacogenetic markers for their potential association with SRNS development [13–16]. Studying single nucleotide polymorphisms (SNPs) has the potential to make patient-personalized treatments possible for a wide array of disorders, which is the main focus of pharmacogenetic investigations [17–19]. The permeability glycoprotein (P-gp), also known as the multidrug resistance 1 protein, is encoded by the *MDR1* gene. The P-gp acts as an efflux pump for several xenobiotics, including prednisolone. It has been reported that the altered expression and/or activity of P-gp were possible mediators for developing resistance to drug therapy [20,21]. Furthermore, it has been suggested that P-gp is probably actively participating in the chronic inflammation of autoimmune diseases [22]. Some SNPs in the *MDR1* gene have been linked to alterations in the expression and efflux function of P-gp [21,23]. With respect to children with INS, the rs2032582 variant has been suggested to increase the risk of developing SRNS [24]. However, variability among the findings has been noted in several investigations that included INS children of different ethnicities [25]. Considering the lack of a pharmacogenetic study and the reported ethnic-based variability, further research is necessary to investigate the role of the rs2032582 variant on the development of SRNS in Iraqi children. Furthermore, several other *MDR1* variations remain unexplored, particularly a low-frequency variation such as the rs2032583 variant. This variant was reported to influence the responsiveness of patients with major depressive disorder and Crohn's disease to drug therapy [26–28]. To our knowledge, no previous work was undertaken to investigate the prevalence of the rs2032583 SNP in the Middle Eastern population. Moreover, the clinical relevance of the rs2032583 variant was not explored in a prior study on nephrotic children. Studying the rs2032583 *MDR1* variant could provide new insights into the genetic basis that underlies the responsiveness of INS children to steroid therapy. This study aimed to determine the frequency rates of the *MDR1* variants (rs2032583 and rs2032582) and analyze the association of their genotypes, alleles, and haplotypes with the risk of developing SRNS in Iraqi children.

METHODS

Study design and setting

This case-control study was undertaken at the pediatric nephrology clinic of Babylon Hospital for Maternity and Pediatrics from March to June 2022. The study involved 60 NS children, aged 1-13 years, who had already received a diagnosis of either SSNS (n=30) or SRNS (n=30). Patients who developed successful remission (<1+ proteinuria on early morning urine dipsticks) after the first 4 weeks of daily prednisolone [2 mg/kg/d (maximum 60 mg/d)] were identified as having SSNS. After the first 4, 6, or 8 weeks of taking daily prednisolone [2 mg/kg/d (maximum 60 mg/d)], SRNS was found to not be remission-compatible (≥1+ proteinuria on early morning urine dipsticks). Varying tapering regimens followed the steroid trial, such as a 4 or 6-week tapering of alternate-day prednisolone [1.5 mg/kg/d (maximum 50 mg/d)] for SSNS patients or a 6-month (or longer) tapering of alternate-day prednisolone regimen for SRNS patients. The exclusion criteria included patients with <1 or >16 years of age, a family history of NS, gross hematuria, acute kidney injury, and an active or recurrent urinary tract infection. We also excluded patients with NS secondary to systemic diseases such as lupus nephritis (low serum complement C3 levels or positive for auto-antibodies such as ANA and anti-dsDNA antibodies), viral infections (positive for viral antibodies to HIV, HBV, and HCV), or diabetes history.

Ethical considerations

The Human Research Committee of the Babylon Directorate of Health (Approval Number: 44 on March 28, 2022) and the Research Ethics Committee of the University of Baghdad, College of Pharmacy (Approval Number: RECAUBCP17102021A on October 17, 2021) approved the study protocol. Informed consent was obtained from all study participants (their parents or caregivers) before their enrollment in the study. This work was compliant with the criteria published in the Declaration of Helsinki and its subsequent amendments.

Data collection

At enrollment, we collected the clinical and demographic data of the participants on a predetermined sheet. The gender, age, blood pressure, age at onset, height, weight, intake of concomitant medications, urinalysis, proteinuria (early morning by urine dipstick), serum urea,

creatinine, albumin, total cholesterol, steroid-response history, and immunosuppressant/drug intake were recorded. The eGFR was calculated using the updated Schwartz equation [eGFR= (0.413*height in cm)/serum creatinine in mg per dL] [29]. Serum-fasting blood glucose was also documented to exclude diabetes mellitus.

DNA extraction and participants' genotyping

For the purposes of extracting the genomic DNA, a venous blood specimen was obtained from each patient, and the specimens were collected in a sterile EDTA tube and stored at -80 °C until the time of DNA extraction. The genomic DNA was extracted by chemically salting out the proteins and debris (phospholipids and polysaccharides), which was followed by the separation of soluble DNA by binding to a silica column under high salt conditions. The extracted DNA was further evaluated using agarose gel electrophoresis [Bio-Rad Systems, USA] [30]. The *MDR1* gene fragment (the sequence included the variants rs2032583 and rs2032582) was amplified by polymerase chain reaction [Biometra, Germany] (Table 1).

Table 1: The primers and conditions of the amplification reaction of the studied variants

The primers				
Sequence (5'->3')	Length	Tm	GC%	PCR product length (bp)
Forward primer: AGTCCAAGAACTGGCTTTGCT	21	60.13	47.62	480
Reverse primer: AGTCTCATGAAGGTGAGTTTTCAGA	25	59.93	40.00	
The PCR conditions				
Step	Temperature		Time	Cycle number
Initial activation	95 °C		5 min	1
Denaturation	95 °C		30 sec	34
Annealing	63 °C		30 sec	34
Extension	72 °C		30 sec	34
Final extension	72 °C		5 min	1

Tm: melting temperature in °C, PCR: polymerase-chain reaction, bp: base-pair.

The primers were designed using NCBI Primer BLAST after retrieving the gene fragment sequence from the NCBI database. The PCR mixture contained 2 µL of genomic DNA, 1 µL of $MgCl_2$ (Syntol, Russia), 1 µL (10 pmol/µL) of each primer (Macrogen, South Korea, detailed in Table 1), and 10 µL of PCR master mix (Syntol, Russia). PCR-grade water was added, bringing the final volume to 25 µL. The presence of amplified PCR products was confirmed in a later step of agarose gel electrophoresis. The PCR products were sent to a sequencing company (Macrogen, South Korea), where Sanger sequencing (via an automated DNA sequencer) was performed to identify the participants' genotypes for the studied SNPs. The results were received by email and analyzed by BioEdit 7.1 software. The genotype of the rs2032583 variant (139th position of the 480 bp-PCR amplicon) with the homozygous A allele was determined based on the presence of a single "A" peak (illustrated by a green line), whereas the heterozygous variant genotype (A/G) was identified when both "A" and "G" peaks (illustrated by overlapping green and black lines) were present. The finding of a "G" peak (illustrated by a black line) alone was indicative of a homozygous mutant genotype (G/G; see Figure 1A). With respect to the rs2032582 variant (196th position of the 480 bp-PCR amplicon), the detection of a singular "A" peak (illustrated by a green line) indicated a homozygous A/A wild genotype, while the combined "A" and "C" peaks (illustrated by overlapping green and blue lines) indicated a heterozygous A/C carrier genotype. Finally, the presence of a single "C" peak (illustrated by a blue line) characterized the homozygous mutant genotype (C/C; see Figure 1B).

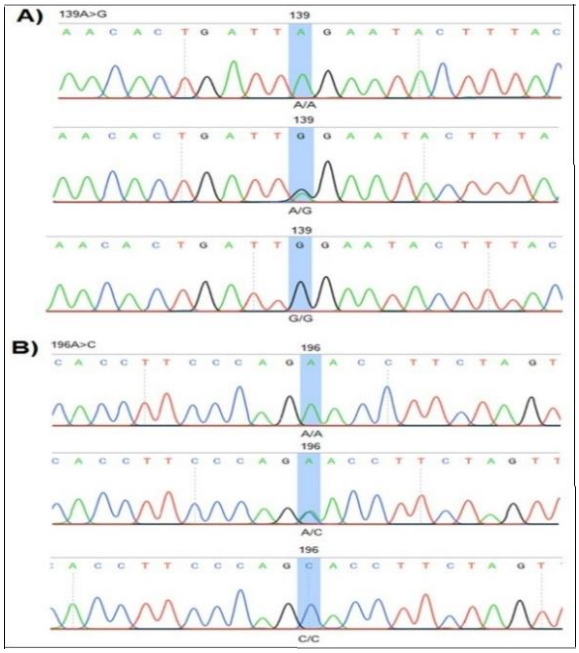

Figure 1: Sequencing genotyping of the studied *MDR1* gene variant

Statistical analysis

The statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS) statistics software (version 22). Categorical data for the demographical and clinical characteristics of the study subjects were presented as frequencies and percentages. We performed the chi-square analysis or Fisher's exact test, to check for differences among the categorical variables. The Shapiro-Wilk test was used to assess the distribution normality of the continuous data. The normally distributed variables were presented using the mean and standard deviation. The median and inter-quartile range (IQR)

were used to describe the variables with non-normal data. The normal data of both study groups were compared using the unpaired t-test. We used the Mann-Whitney U test to compare the non-normal data. Regarding the genotypic data, chi-square analysis was used to compare the distribution of genotypes and alleles between the SRNS and SSNS groups. The analysis also included a binary logistic regression for the likelihood of developing steroid resistance. The variants' genotypes were tested in four genetic models: co-dominant, dominant, over-dominant, and recessive genetic models. The online platform (SHEsis; http://analysis.bio-x.cn/) was used to conduct linkage disequilibrium (LD) and haplotype analysis [31,32]. The LD analysis was executed to test for non-random association (i.e., coinheritance) of alleles of the studied SNPs, whereas the haplotype analysis was utilized to check for the association of the SNPs' haplotypes with steroid responsiveness. A p-value of less than 0.05 (two-tailed) was considered statistically significant.

RESULTS

The patient's gender, age, height, weight, age at onset, and blood pressure were not significantly different among children in the SSNS group compared to the SRNS group (p>0.05) (Table 2).

Table 2: The demographical and clinical characteristics of the study participants

Characteristics	Total subjects (n=60)		*p*
	Steroid-sensitive (n=30)	Steroid-resistant (n=30)	
Demographical characteristics			
Age [in years; median (IQR)]	6(5,8.25)	8.5(5.38-11.25)	0.08 †
Sex [male; frequency (%)]	19(63.3)	21(70)	0.584
Age at diagnosis [in years; median (IQR)]	4(2.88-4.63)	3(1.88-7)	0.447 †
Weight [Kg; median (IQR)]	21.5(19-25.75)	26(19.63-43.5)	0.072 †
Height (cm; mean±SD)	110.53±16.92	121.33±24.93	0.055 ‡
Blood pressure (BP; frequency, %)			
Elevated Systolic BP (>95 percentile)	4(13.3)	5(16.7)	0.718
Elevated diastolic BP (>95 percentile)	3(10)	6(20)	0.472
Biopsy histological findings (frequency, %)			
Focal-segmental glomerular sclerosis	-	1(3.3)	NA
Membrano-proliferative glomerulo-nephritis	-	1(3.3)	NA
Minimal-change disease	-	3 (10)	NA
No biopsy	32(100)	25(83.3)	NA

IQR: interquartile range (Q1, Q3).

The participants in the SRNS group had significantly lower (p<0.05) serum albumin (mean±SD: 32.3±10.98) and eGFR (64.08±14.26) than those with SSNS (37.61±7.19 and 73.32±15.11, respectively). Significantly higher serum creatinine [median (Q1, Q3): 66.5 (55.75, 87.25)] and urea levels [4.2 (2.9, 6.03)] were found in the SRNS patients as compared to the SSNS [53 (46.5, 67.75) and 2.8 (2.5, 3.85), respectively; p<0.05] (Table 3).

Table 3: The biochemical characteristics and medication use of the study participants

Characteristics	Total subjects (n=60)		*p*
	Steroid-sensitive (n=30)	Steroid-resistant (n=30)	
Serum biochemical and functional measures			
Albumin level (gm/L; mean±SD)	37.61±7.19	32.3±10.98	0.031
Creatinine level [µmol/L; median (IQR)]	53(46.5-67.75)	66.5(55.75-87.25)	0.002
Urea level [mmol/L; median (IQR)]	2.8(2.5-3.85)	4.2(2.9-6.03)	0.004
Total cholesterol level [mmol/L; median (IQR)]	4.05(3.45-5.63)	4.75(3.7,8.03)	0.117
eGFR (mL/min/1.73 m^2; mean±SD)	73.32±15.11	64.08±14.26	0.018
Immunosuppressant therapy (frequency, %)			
Prednisolone	30(100)	2(6.7)	NA
Cyclosporine plus prednisolone	0	18(60)	NA
Tacrolimus plus prednisolone	0	3(10)	NA
Mycophenolate mofetil plus prednisolone	0	6(20)	NA
Chlorambucil plus prednisolone	0	1(3.3)	NA
Concomitant supplemental and adjuvant therapies (frequency, %)			
Angiotensin-converting enzyme inhibitor use	1(3.3)	8(26.7)	0.026
Statin use	2(6.7)	6(20)	0.254
Diuretic use	5(16.7)	12(40)	0.045

IQR: interquartile range (Q1, Q3).

There were also more patients who received ACEIs and diuretics in the SRNS group than in the SSNS group (8 and 12 *vs*. 1 and 5, respectively). The wild genotype of the rs2032583 variant was prevalent in the majority of the studied Iraqi children with INS (73.33%). The minor allele frequency (MAF) of the studied polymorphism rs2032582 [MAF(C)=56.67%] was higher than that of the rs2032583 variant [MAF (G)= 19.17%] (Table 4). The T allele of the rs2032582 polymorphism was not found among the study subjects. The frequency distribution of genotypes of the rs2032583 polymorphism (V1) was significantly associated with patients' steroid responsiveness (p<0.001) (Table 5). Children with the variant carrier genotype (A/G) were less likely to develop SRNS [odds ratio (OR) and 95% confidence interval (95% CI): 0.044 (0.002~0.8), p=0.008] compared to those with the wild genotype (A/A) in the co-dominant genetic model and compared to those with homozygous genotype (A/A or G/G) in the over-dominant genetic model [OR (95% CI): 0.037 (0.002~0.67), p=0.002).

Table 4: The frequency distribution of the studied variants' genotypes and alleles in children with idiopathic nephrotic syndrome

The variants' genotypes and alleles	Frequency (%) in INS cases	
	rs2032583	rs2032582
Homozygous wild genotype	A/A: 44(73.33)	A/A: 8(13.33)
Heterozygous genotype	A/G: 9(15)	A/C: 36(60)
Homozygous mutant genotype	G/G: 7(11.67)	C/C: 16(26.67)
Wild allele	A: 97(80.83)	A: 52 (43.33)
Mutant (minor) allele	G: 23(19.17)	C: 68(56.67) T: 0(0)

rs: reference single nucleotide polymorphism; INS: idiopathic nephrotic syndrome.

Table 5: The analysis of the variants' genotypes and alleles with respect to steroid response

The variants' genotypes and alleles	Frequency (%)		OR (95%CI)	*p*
	SSNS (n=30)	SRNS (n=30)		
rs2032583 (A>G)				
A/A genotype (n=44)	20(45.5)	24(54.5)	-	
A/G genotype (n=9)	9(100)	0(0)	-	<0.001†
G/G genotype (n=7)	1(14.3)	6(85.7)	-	
A/A genotype (n=44)	20(45.5)	24(54.5)	Reference CD	
A/G genotype (n=9)	9(100)	0(0)	0.044(0.002~0.8)	0.008 WH*
G/G genotype (n=7)	1(14.3)	6(85.7)	5(0.55~45.06)	0.151
A/G + G/G genotypes (n=16)	10(62.5)	6(37.5)	Reference D	0.247
A/A genotype (n=44)	20(45.5)	24(54.5)	2(0.62~6.47)	
A/A + G/G genotypes (n=51)	21(41.2)	30(58.8)	Reference OD	0.002 WH
A/G genotype (n=9)	9(100)	0(0)	0.037(0.002~0.67)	
A/A + A/G genotypes (n=53)	29(54.7)	24(45.3)	Reference R	0.076
G/G genotype (n=7)	1(14.3)	6(85.7)	7.25(0.82~64.46)	
A wild allele (n=97)	49(50.5)	48(49.5)	Reference	0.817
G mutant allele (n=23)	11(47.8)	12(52.2)	1.11(0.45~2.77)	

SSNS: steroid-sensitive nephrotic syndrome; SRNS: steroid-resistant nephrotic syndrome; OR: odds ratio; CI: confidence interval; rs: reference single nucleotide polymorphism. CD: co-dominant, D: dominant, OD: over-dominant, R: recessive, WH: Woolf-Haldane correction (WH was applied by adding 0.5 to each cell count if a zero was in at least one cell of the 2*2 table.) * *p*-value by the Fisher's exact test. † *p*-value for likelihood ratio.

In contrast, a significant association with steroid response was not found for the rs2032582 (V2) genotypes and alleles ($p>0.05$) (Table 6). Moreover, we analyzed the different combinations of genotypes from the two variants (V1 and V2) between the SRNS and SSNS groups. It was found that the risk of developing SRNS was significantly higher when V1 and V2 were both homozygous (wild or mutant) [OR (95%CI): 30.18 (1.55~588.5), $p=0.008$] compared to when V1 was heterozygous and either of V2 genotypes.

Table 6: The analysis of the variants' genotypes and alleles with respect to steroid response

The variants' genotypes and alleles	Frequency (%)		OR (95%CI)	*p*
	SSNS (n=30)	SRNS (n=30)		
rs2032582 (A>C)				
A/A genotype (n=8)	3(37.5)	5(62.5)	-	
A/C genotype (n=36)	19(52.8)	17(47.2)	-	0.735†
C/C genotype (n=16)	8(50)	8(50)	-	
A/A genotype (n=8)	3(37.5)	5(62.5)	Reference CD	
A/C genotype (n=36)	19(52.8)	17(47.2)	0.54 (0.11~2.59)	0.439
C/C genotype (n=16)	8(50)	8(50)	0.6 (0.11~3.4)	0.564
A/C + C/C genotypes (n=52)	27(51.9)	25(48.1)	Reference D	0.452
A/A genotype (n=8)	3(37.5)	5(62.5)	1.8 (0.39~8.32)	
A/A + C/C genotypes (n=24)	11(45.8)	13(54.2)	Reference OD	0.598
A/C genotype (n=36)	19(52.8)	17(47.2)	0.76 (0.27~2.13)	
A/A + A/C genotypes (n=44)	22(50)	22(50)	Reference R	1
C/C genotype (n=16)	8(50)	8(50)	1 (0.32~3.14)	
A wild allele (n=52)	25(48.1)	27(51.9)	Reference	0.713
C mutant allele (n=68)	35(51.5)	33(48.5)	0.87 (0.42~1.8)	
The genotype combinations of both rs2032583 (V1) and rs2032582 (V2) variants				
V1[HT] + V2[HT/HM] (n=9)	9(100)	0(0)	Reference	
V1[HM] + V2[HT] (n=30)	13(43.3)	17(56.7)	24.63 (1.31~461.8)	**0.012** WH*
V1[HM] + V2[HM] (n=21)	8(38.1)	13(61.9)	30.18 (1.55~588.5)	**0.008** WH*

SSNS: steroid-sensitive nephrotic syndrome; SRNS: steroid-resistant nephrotic syndrome; OR: odds ratio; CI: confidence interval; rs: reference single nucleotide polymorphism. CD: co-dominant, D: dominant, OD: over-dominant, R: recessive, WH: Woolf-Haldane correction (WH was applied by adding 0.5 to each cell count if a zero was in at least one cell of the 2*2 table.) * *p*-value by the Fisher's exact test. † p-value for likelihood ratio. Statistically significant *p*-values are in bold. [HT]: heterozygous; [HM]: homozygous wild or mutant; [HT/HM]: hetero or homozygous genotypes.

The linkage disequilibrium (LD) blocks were constructed between the haplotypes of the studied SNPs in both groups (SSNS and SRNS) using the SHEsis online platform (Figure 2).

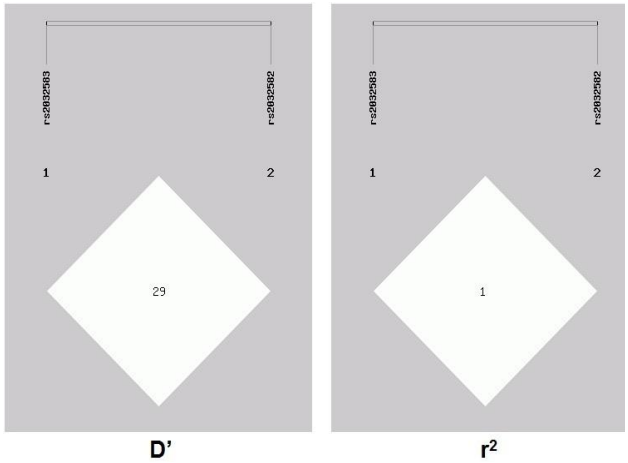

Figure 2: Linkage disequilibrium between the studied polymorphisms.

The LD among the SNPs' haplotypes was presented using the computed values of the Lewontin coefficient (D') and correlation coefficient (r^2). A weak LD was found between the rs2032583 and rs2032582 polymorphisms (D'= 0.295, r^2= 0.016). The haplotype analysis indicated the presence of four haplotypes for the rs2032583 and rs2032582 variants among the study participants (Table 7). The two most prevalent haplotypes were AC (haplotype frequency= 51.65) and AA (haplotype frequency= 45.35) in both groups. The distribution of the variants' haplotypes was not significantly different among children with SRNS compared to those with SSNS (p>0.05).

Table 7: Analysis of the variants' haplotypes and patients' steroid responsiveness

Haplotype analysis for the rs2032583 and rs2032582 variants based on steroid response					
Haplotypes	Frequency (%)		χ^2	OR (95% CI)	*p*-value
	SSNS (n=30)	SRNS (n=30)			
A A	22.35(37.3)	23(38.3)	0.015	1.047 (0.5~2.19)	0.903
A C	26.65(44.4)	25(41.7)	0.092	0.894(0.434~1.842)	0.761
G A	2.65(4.4)	4(6.7)	0.292	1.548 (0.314~7.636)	0.589
G C	8.35(13.9)	8(13.3)	0.009	0.951(0.335~2.7)	0.925

SSNS: steroid-sensitive nephrotic syndrome; SRNS: steroid-resistant nephrotic syndrome; χ^2: chi-square; OR: odds ratio; CI: confidence interval. Global χ^2 for the distribution of haplotypes between SSNS and SRNS is 0.345 while Pearson's p-value is 0.951.

DISCUSSION

Children with INS are initially treated with a prednisolone trial, the outcome of which determines the subsequent therapeutic course for patients as well as the prognosis of their condition [33,34]. For pediatric nephrology researchers and physicians, the management of SRNS is a challenging encounter. Prednisolone's therapeutic window is broad, with an established efficacy of remission induction in nephrotic children. However, the concern over ineffective treatment and inadvertent toxic events caused by steroid trials is still an ongoing therapy issue for children with SRNS [35]. Moreover, clinical practice currently lacks a diagnostic marker for identifying SRNS. This study attempted to provide information to help bridge this gap. The availability of early markers for steroid resistance may help guide adjustments in treatment dosage and duration. Furthermore, such markers can prompt earlier initiation of alternative regimens with potentially more effective immunosuppression. This can provide a significant benefit to the effectiveness and safety of the management plan for patients with NS [36]. This work established a possible pharmacogenetic-based contributor to developing steroid resistance in pediatric patients with INS. This study found that the rs2032583 SNP was significantly associated with steroid responsiveness in INS patients. Several studies reported the link between the variant rs2032583 and the therapeutic responsiveness of patients with major depression to antidepressant medications as well as the steroid-dependency of children with Crohn's disease [26–28]. Nevertheless, the pharmacogenetic role of the rs2032583 SNP was not, to our knowledge, previously studied in children with INS. Thus, a comparison to findings from similar investigations was not possible. Moreover, the rs2032583 variant was previously deposited in the NCBI dbSNP database as a low-frequency variation [global MAF(G)=0.13], with no prior reports of clinical relevance to NS. However, the results of this study emerged as new findings, uncovering the prevalence of the variant in a Middle Eastern population (specifically, in Iraq) and its relationship to steroid responsiveness in children with INS [37]. Despite being synonymous, the impact of the rs2032583 SNP on the MDR1 protein could be attributed to the LD of the variant with other SNPs capable of exerting activity- or expression-related changes in the MDR1 protein. Additionally, the rs2032583 polymorphism might affect the regulatory factors of the MDR1 protein translation or its mRNA structure [38]. Regarding the rs2032582 SNP, the MAF of the C allele was 56.67%, whereas the T allele was not found among the studied children. The T allele was not reported in the study because the report of the sequencing company did not include a T allele finding for the rs2032582 SNP. Moreover, we noted the possibility of zero-frequency occurrence for the T allele of the rs2032582 SNP that was reported on the NCBI website for several ethnicities, such as African, Latin American, and South Asian populations [39]. This study also found that the risk of developing SRNS was not associated with the rs2032582 SNP, which is a coding variant with an amino acid change. This finding was in agreement with prior studies in Slovakia and South Korea, which reported a lack of relationship between the rs2032582 SNP and steroid responsiveness in INS children [40,41]. In contrast, Parvin *et al*. suggested

the presence of a significant association between the rs2032582 variant and SRNS development in Bangladeshi children with NS [24]. The variability in findings among different populations reflects the heterogeneous genetic composition that is possibly attributed to the disparity in ethnic origins. Regionally, a local Iraqi study found that another common *MDR1* variant (rs1128503) was significantly associated with developing steroid resistance in INS children [42]. However, such a significant association was not identified between the studied rs2032582 polymorphism and SRNS development in this work. P-gp is a multi-drug transporter protein, and the genetic variations that are expected to influence a protein site might not be associated with a modifying effect. This may partially be due to the ability of the protein to tolerate a slight structure alteration by accommodating the variant residue and limiting its interference [43]. Furthermore, this study analyzed different genotype combinations from both the rs2032583 and rs2032582 variants and assessed their relationship with SRNS development. The study results indicated that children who had the combination of the homozygous genotype (mutant or wild) of the rs2032583 with either genotype of the rs2032582 polymorphism were associated with a higher risk of developing SRNS compared to those who had the combination of the heterozygous genotype of the rs2032583 with either genotype of the rs2032582 polymorphism. This finding is consistent with the study results that linked SRNS and the rs2032583 variant and suggested a lack of association between SRNS development and the rs2032582 variant. Moreover, this study demonstrated weak LD between the rs2032583 and rs2032582 polymorphisms as well as the absence of an associating haplotype with SRNS, which is in agreement with the relatively independent nature of the variants' coinheritance that was apparent in the results of this study. Children who are heterozygous for rs2032583 may be less likely to be steroid resistant than those who are either homozygous wild-type or homozygous mutants. A possible interpretation might be that children with the rs2032583 heterozygous genotype express the P-gp protein with a change in the dimensional conformation that allows entry of prednisolone substrate into the podocytes, which mitigates the underlying nephrotic changes and improves the treatment outcome in INS children. Homozygous genotype carriers may not have such an altered dimensional conformation, which hinders the entry of prednisolone into the podocytes and renders the patient resistant to steroid therapy. The association between treatment-responsive outcomes and the rs2032583 heterozygous genotype was also reported in other studies involving children with Crohn's disease [28] and patients receiving antidepressants [27]. Overall, this study discerned a link between the *MDR1* variant (rs2032583) and developing resistance to steroid therapy in children with INS. Furthermore, this work revealed a lack of relationship between the rs2032582 polymorphism and steroid responsiveness in Iraqi nephrotic children. We should conduct further investigations to evaluate additional variations in the *MDR1* gene and other genes that may have implications for steroid therapy, based on these findings. This could personalize drug therapy for children with INS by providing a worthwhile opportunity to optimize treatment effectiveness and safety, especially for medications with multiple toxic concerns, such as steroid regimens in childhood INS. It is necessary to address several limitations in this study. This study was conducted in a single center with a small number of patients who were receiving ongoing treatment. The present study did not involve screening patients for genetic podocyte mutations such as those in the nephrin (*NPHS1*) and Wilm's tumor-1 (*WT1*) genes known to cause SRNS. However, nephrotic patients with a family history and children that are less than one-year-old were not included. Considering these limitations and to validate the study findings, multicenter, large sample, and cohort-designed investigations are recommended. Also, more research needs to be done on other pharmacogenetic markers (within the *MDR1* gene or other relevant genes) to find out how to predict when children with INS will become resistant to steroids.

Conclusion

The study's findings suggested a potential clinical relevance for the *MDR1* variant (rs2032583) in patients with INS. This work established that rs2032582 is in weak LD with rs2032583 and that the former SNP has no influence on steroid responsiveness. Children who have a homozygous genotype (A/A or G/G) at the rs2032583 SNP tend to be prednisolone-resistant regardless of the genotype at the rs2032582 SNP and likely require an alternative therapeutic approach. Additional research is required to validate the study findings and explore other pharmacogenetic-related variations, which would provide essential insight for tailoring drug therapy in nephrotic children.

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Conflict of interests

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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