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Research Article

Association of the rs1128503 and rs1045642 polymorphisms in the *MDR-1* gene with steroid responsiveness in Iraqi children with idiopathic nephrotic syndrome

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ABSTRACT

Steroid-resistant nephrotic syndrome (SRNS) is a leading cause of end-stage renal disease in children, with an increasing number of cases. Polymorphisms in the *MDR-1* gene were reported to contribute to SRNS development, but with varying results among different ethnicities. Thus, we investigated the association of the *MDR-1* rs1128503 (C1236T) and rs1045642 (C3435T) polymorphisms with steroid responsiveness in Iraqi children with idiopathic nephrotic syndrome (INS). This case-control study was conducted at the Babylon Hospital for Maternity and Pediatrics. Children with SRNS (n=32) and steroid-sensitive nephrotic syndrome (SSNS; n=32) were genotyped via the polymerase chain reaction-restriction fragment length polymorphism. The genotypes were subjected to association testing and haplotype analysis. The C1236T TT genotype was associated with a higher risk of developing SRNS compared to the CC and TC genotypes (odds ratio [OR]=10.33, 95% confidence interval [95% CI]=1.208-88.362; *p*-value=0.026; recessive model). The combination of the two TT genotypes of C1236T and C3435T variants was significantly more frequent (*p*-value=0.029) in SRNS (88.9%) than in SSNS (11.1%). The haplotype analysis showed no association between the C1236T and C3435T haplotypes and steroid responsiveness, but the TC haplotype was associated with an age at onset of ≥8 years (*p*-value=0.0028). In conclusion, this study revealed that children who have the *MRD-1* C1236T TT genotype alone or combined with the C3435T TT genotype may be at increased risk of developing SRNS and in need of other therapeutic strategies. Additional research is required to identify other genetic contributions to steroid responsiveness and further understand their pharmacogenetics in INS.

Keywords:
Single nucleotide polymorphism, Steroid-resistant nephrotic syndrome, Steroid-sensitive nephrotic syndrome, Iraq

1. INTRODUCTION

Nephrotic syndrome (NS) is a common glomerulopathic condition in childhood and is a serious contributor to morbidity and mortality, inflicting a significant burden on healthcare institutions. It has been defined as the episodic development of excessive proteinuria, hypoalbuminemia, and edema owing to defective filtration through the glomerular barrier[1]. In children, NS is predominantly idiopathic (INS) in etiology with diverse histopathological presentations[1-4]. The management of children with INS typically involves initial treatment with steroid regimens, which has both therapeutic and prognostic implications. Children who respond poorly to the initial steroid trial (often with stepwise increments of doses and periods) are identified as having steroid-resistant nephrotic syndrome (SRNS), which is more progressive in nature and associated with a greater risk of concurrent complications as compared to steroid-sensitive nephrotic syndrome (SSNS)[5-6]. Moreover, SRNS is one of the leading causes of end-stage renal disease and chronic renal failure among children, with an ongoing escalation in the number of cases worldwide

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as well as in Iraq[7-10]. Thus, identifying potential contributing factors to SRNS is critical to improving the management of INS patients. The multi-drug resistance-1 gene (*MDR-1*) encodes the permeability glycoprotein (P-gp), which is a trans-membrane efflux transporter for a broad range of xenobiotics. Prednisolone is a P-gp substrate as well as a possible inducer of protein expression[11]. Alterations in P-gp expression and activity have been proposed as one of the potential mediators for therapy resistance[12-13]. Moreover, P-gp may likely be an active participant in the chronic inflammatory reactions that underlie the autoimmune conditions through interaction with the cellular activation/death pathways or involvement in the pro-inflammatory mediators' release[14-15].

Investigations detected numerous *MDR-1* single nucleotide polymorphisms (SNPs) that affect P-gp expression and activity and notably change the pharmacokinetics and pharmacodynamics of medications, with consequent ramifications on treatment outcomes[16-17]. The rs1128503 **(**C1236T) and rs1045642 **(**C3435T) SNPs were related to a variation in P-gp function and expression, and the haplotypes from these polymorphisms were linked to significant alterations in the pharmacokinetics of digoxin[17]. In children with NS, the C1236T variant was reported to increase the risk of SRNS development[18-19]. Despite being synonymous SNPs, the chances of steroid resistance were significantly higher in patients who have homozygous mutant (HM) genotypes (TT) for C3435T and C1236T SNPs compared to those with heterozygous or wild genotypes (CT or CC) because of the strong linkage disequilibrium (LD) shown among several *MDR-1* synonymous and non-synonymous variants[20]. However, several studies produced variable findings in different ethnic groups of pediatric INS patients[21]. In Iraq, no prior study was conducted to determine the relevance of *MDR-1* genetic variants to the responsiveness of INS children to steroid therapy. Thus, this study aimed to investigate the association of the *MDR-1* C1236T and C3435T genotypes and haplotypes with the risk of steroid resistance development in Iraqi children with INS.

2. MATERIALS AND METHODS

2.1. Study design

This case-control study was conducted at Babylon Hospital for Maternity and Pediatrics from March to June 2022.

2.2. Participants

The study inclusion criteria included patients aged 1-16 years who were already diagnosed with SSNS or SRNS in the pediatric nephrology clinic. Patients acquiring successful remission (<1+ proteinuria on early morning urine dipsticks) after 4 weeks of daily prednisolone [2 mg/kg/d (maximum 60 mg/d)] were identified as having SSNS. SRNS was identified as failing to acquire successful remission (≥1+ proteinuria on early morning urine dipsticks) within the first 8 weeks of a trial of daily prednisolone [2 mg/kg/d (maximum 60 mg/d)]. Varying tapering regimens followed the steroid trial, such as a 4-week tapering of alternate-day prednisolone or a 6-week tapering of alternate-day prednisolone [1.5 mg/kg/d (maximum 50 mg/d)].

The exclusion criteria included patients with <1 or >16 years of age, a family history of NS, gross hematuria, an active or recurrent urinary tract infection, positivity to autoantibodies, low serum complement C3 levels (to exclude autoimmune diseases), positivity to viral antibodies to HIV, HBV, and HCV (to exclude viral infections), and diabetes history. The patients were approached during their routine follow-up visit to the clinic and were recruited consecutively after their consent and satisfaction with the study's inclusion and exclusion criteria.

2.3. Sample size estimation

The sample size was estimated using the formula described in Sharma et al.[22]. The values for the control-to-case ratio, desired power, and level of confidence were 1, 0.84 (for 80% power), and 1.96 (for 95% confidence interval), respectively. The formula yielded a sample size of 32 in each group when the estimated proportions of exposure to the genotype that predisposes to resistance (*MDR-1* C1236T TT genotype) were 70% in SRNS and 35% in SSNS based on the previous studies' findings[18-19]. Thus, we included 32 participants in the SRNS group and 32 patients in the SSNS group.

2.4. Data collection

At enrollment, the clinical and demographic data of the participants were collected 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 (described in Table 1). The estimated glomerular filtration rate (eGFR) was calculated using the updated Schwartz equation[23]. The blood samples were obtained from the patient's routine blood tests and followed up at the blood collection area in the hematology laboratory. Venous blood was collected in a sterile ethylene di-amine tetra-acetic acid-containing tube and stored at -80°C until the extraction of DNA.

2.5. Genotyping

Genomic DNA was extracted from the peripheral leukocytes of whole blood samples. The extraction

Table 1. Characteristics of the study participants.

Characteristics	The study participants (n=64)		*p*-value
	SSNS (n=32)	SRNS (n=32)	
Age at enrollment [years; median (IQR)]	5.8 (3.40)	8.3 (6.8)	0.162*
Gender [male; frequency (%)]	20.0 (62.50)	21.0 (65.6)	0.794
Age at onset of disease [years; median (IQR)]	4.0 (2.00)	3.0 (5.1)	0.142*
Weight [Kg; median (IQR)]	21.0 (10.60)	25.0 (23.3)	0.209*
Height (cm; mean±SD)	111.81 ± 17.14	117.75 ± 26.32	0.290†
Serum albumin [gm/L; median (IQR)]	38.2 (11.00)	34.0 (20.3)	0.049*
Serum creatinine [µmol/L; median (IQR)]	54.0 (22.80)	64.5 (31.5)	0.010*
Blood urea [mmol/L; median (IQR)]	2.8 (1.45)	4.2 (3.0)	0.002*
eGFR (mL/min/1.73 m2; mean±SD)	72.89 ± 14.71	63.64 ± 14.1	0.013†
Serum total cholesterol [mmol/L; median (IQR)]	4.1 (2.20)	5.5 (4.2)	0.052*
Presence of hypertension (frequency, %)			
Systolic blood pressure >95 percentile	4.0 (12.50)	5.0 (15.6)	0.500‡
Diastolic blood pressure >95 percentile	3.0 (9.40)	7.0 (21.9)	0.168
Pathology upon biopsy (frequency, %)			
Focal segmental glomerular sclerosis	-	1.0 (3.1)	NA
Membranoproliferative glomerulonephritis	-	1.0 (3.1)	NA
Minimal change disease	-	3.0 (9.4)	NA
No biopsy	32.0 (100.00)	27.0 (84.4)	NA
Immunosuppressive regimen (frequency, %)			
Prednisolone	32.0 (100.00)	4.0 (12.5)	NA
Prednisolone and cyclosporine	0	18.0 (56.3)	NA
Prednisolone and tacrolimus	0	3.0 (9.4)	NA
Prednisolone and chlorambucil	0	1.0 (3.1)	NA
Prednisolone and mycophenolate mofetil	0	6.0 (18.7)	NA
Concomitant medications (frequency, %)			
ACEI	2.0 (6.30)	8.0 (25.0)	0.039
Statin	2.0 (6.30)	6.0 (18.8)	0.257‡
Diuretic	5.0 (15.60)	15.0 (46.9)	0.007

*Significance value for the Mann-Whitney U test. † Significance value for Independent samples t-test. ‡Significance value for Fisher's exact test. Statistically significant p-values are in bold. SSNS: steroid-sensitive nephrotic syndrome; SRNS: steroid-resistant nephrotic syndrome; ACEI: angiotensin-converting enzyme inhibitor; SD: standard deviation; IQR: interquartile range.

protocol included chemically salting out the cellular proteins and debris, with subsequent separation of the soluble DNA molecules by binding to silica under high salt conditions[24]. The isolated DNA was further evaluated by electrophoresis of the samples (5 µL) on a 1% (w/v) agarose gel. The genotypic analysis was performed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. The same PCR pattern was used for each SNP. The PCR mixture contained approximately 2 µL of genomic DNA, 0.5 µL of $MgCl_2$ (Syntol, Russia), 1 µL (10 pmol/µL) of each primer (Macrogen, South Korea; Table 2), and 8 µL of PCR-master mix (Syntol, Russia). PCR-grade water was added, bringing the final volume to 20 µL. The amplification consisted of an initial polymerase activation step for 5 min at 95°C and an initial denaturation step for 30 s at 95°C, followed by 34 cycles of denaturation at 95°C for 30 s, annealing at 63°C for 30 s, and extension at 72°C for 30 s. Terminal elongation was performed at 72°C for 5 min. The PCR products were digested at appropriate temperatures (37°C for Hae III and 65°C for BstMB I) overnight using appropriate volumes (0.5 µL of Hae III and 0.25 µL of BstMB I) of restriction endonucleases (Sibenzymes, Russia). The restriction fragments were stained with loading dye (Promega, USA), separated by electrophoresis on a 2% agarose gel for 90 minutes at 100 V, analyzed under ultraviolet light, and an image was captured using photography software to visualize the bands (Figure 1).

2.6. Statistical analysis

The participants' characteristics were presented in frequencies and percentages for categorical variables, which were analyzed using the chi-square or Fisher's exact test. Analysis of the continuous variables' normality was performed via the Shapiro-Wilk test. Normally distributed data were reported as mean and standard deviation and were compared using the unpaired t-test. The non-normal data were described as the median and interquartile range and were compared using the Mann-Whitney U test. A correlation analysis was also performed to take inter-variable correlations between the groups into consideration. The chi-square analysis was used to test whether the distribution of the genotypes deviated from Hardy-Weinberg equilibrium (HWE) and to compare the distribution of *MDR-1* C1234T and C3435T genotypes and alleles between the SRNS and SSNS groups. The analysis included various genetic models (dominant, co-dominant, over-dominant, and

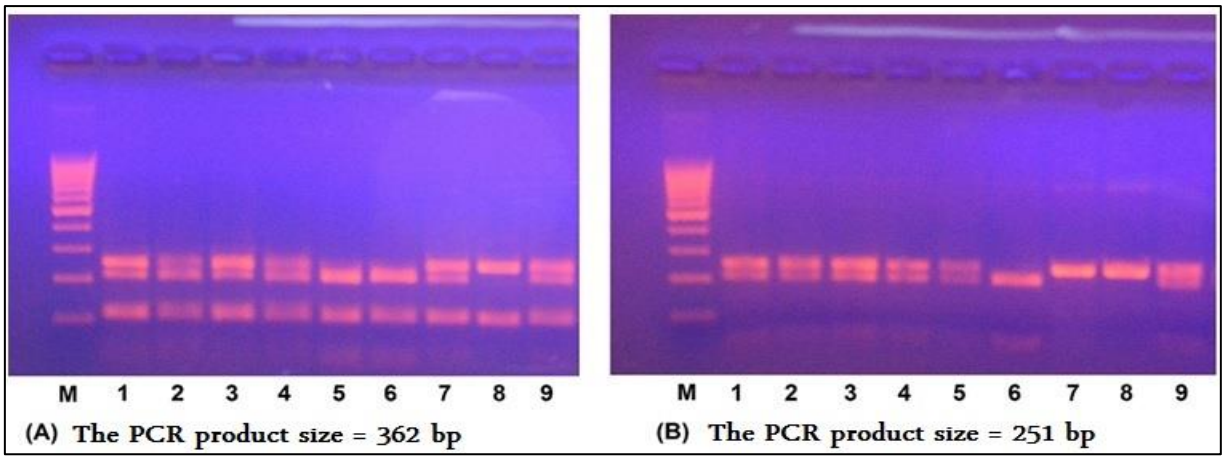

Figure 1. Electrophoretic patterns for MDR-1 polymorphisms evaluated by PCR-RFLP-based assay, M (marker, 100-bp ladder): (A) 1236C>T, TT: 253 bp, 109 bp (lane 8), TC: 253 bp, 218 bp, 109 bp (lanes 1, 2, 3, 4, 7, and 9), CC: 218 bp, 109 bp (lanes 5 and 6); (B) 3435C>T, TT: 251 bp (lanes 7 and 8), TC: 251 bp, 208 bp (lanes 1, 2, 3, 4, 5, and 9), CC: 208 bp (lane 6).

recessive). All statistical analyses were executed by the Statistical Package for Social Sciences (SPSS) statistics software (version 22). The online platform (SHEsis; http://analysis.bio-x.cn/) was used to conduct LD and haplotype analysis[25]. A *p*-value of less than 0.05 (two-tailed) was considered statistically significant.

3. RESULTS

3.1. Demographical and clinical characteristics of the study participants

The male-to-female ratio was 1.67:1 in the SSNS group, while the ratio was 1.91:1 in the SRNS group. However, the gender, age at enrollment, height, weight, age at onset, and blood pressure were not significantly different between the SSNS and SRNS groups (*p*-value >0.05). Serum albumin and eGFR were significantly lower in SRNS [median (IQR): 34 (20.3), and mean±SD: 63.64±14.1, respectively] than in SSNS [38.2 (11) and 72.89±14.71; Table 1]. The proportion of female participants who had an age at onset of less than 5 years was greater than that of the male patients (87% versus 61%, *p*-value=0.029). A positive correlation (*p*-value<0.05) was noted between age at onset and serum creatinine and between age at onset and serum albumin (Spearman's rho coefficient=0.273 and 0.289, respectively; Table 3). While serum albumin was negatively correlated with serum total cholesterol (Spearman's rho coefficient= -0.571, *p*-value<0.001).

3.2. The C1236T and C3435T SNPs of the *MDR1* gene and steroid responsiveness

The genotype distribution of the C1236T SNP was significantly different between SRNS and SSNS (*p*-value =0.018; Table 4). The TT genotype of the C136T SNP was associated with a higher risk of developing SRNS compared to the CC and TC genotypes [odds ratio (OR; 95% CI)=10.33 (1.208-88.362); *p*-value=0.026; recessive model]. The TC genotype was associated with a 0.35-fold lower risk of resistance compared to the homozygous genotypes (OR=0.354, 95% CI=0.127-0.983, *p*-value= 0.044; over-dominant model). The C3435T TT genotype frequency was higher in SRNS than in SSNS (66.7% vs. 33.3%), but not statistically significant (*p*-value=0.095; recessive model). The C3435T TC genotype frequency was significantly lower in the SRNS (37.1%) than in the SSNS group (62.9%), with an OR for developing SRNS of 0.311 (95% CI: 0.111-0.869, *p*-value=0.024; over-dominant model). The analysis also revealed that the combination of two TT genotypes of C1326T and C3435T SNPs was significantly more common in SRNS than in SSNS (88.9% vs. 11.1%, *p*-value=0.029). The C1236T and C3435T allelic distributions were not significantly different among children of both groups (C1236T *p*-value=0.283 and C3435T *p*-value=0.594). The genotypes of the C1236T and C3435T SNPs did not significantly deviate from HWE among the studied patients (C1236T *p*-value=0.472, C3435T *p*-value=0.828).

Table 2. The sequence, length, and melting temperature of the primers used in the study.

The amplified variants (rsID)	The primers	5' to 3' sequence	The primer length (bp)	The melting temperature (°C)
MDR1 C1236T	Forward	CAGGGTCTAGCTCGCATGG	19	59.93
(rs1128503)	Reverse	GTTCACTTCAGTTACCCATCTCG	23	59.07
MDR1 C3435T	Forward	ACAGGAAGTGTGGCCAGATG	20	59.96
(rs1045642)	Reverse	TGCCTATGGAGACAACAGCC	20	59.75

rsID: reference number for the studied genetic variants, MDR1: multi-drug resistant 1 gene, bp: base pair.

Table 3. Association and correlation analyses among various demographic and clinical variables of children with idiopathic nephrotic syndrome.

Association analysis				
Categorical variables		**Age at onset [frequency (%)]**		***p*-value**
		<5 years old	**≥5 years old**	
Gender	Male (n=41)	25 (61)	16 (39)	0.029
	Female (n=23)	20 (87)	3 (13)	
Correlation analysis				
Correlation variables		**Spearman's rho coefficient**		***p*-value**
Age at onset and serum albumin		0.289		0.021
Age at onset and serum creatinine		0.273		0.029
Body mass index and serum creatinine		0.278		0.026
Body mass index and serum total cholesterol		0.289		0.021
Serum albumin and serum total cholesterol		-0.571		<0.001
Serum urea and age at enrollment		0.289		0.021
Serum creatinine and diastolic blood pressure		0.290		0.02
Serum creatinine and age at enrollment		0.487		<0.001
Systolic blood pressure and age at enrollment		0.343		0.006

The negative coefficient value indicates an opposite correlation.

Table 4. The distribution of the MDR1 C1236T and C3435T variants' genotypes and alleles in children with idiopathic nephrotic syndrome.

The studied variants	**The genotypes**	**Steroid responsiveness [Frequency (%)]**		**Odds ratio (95% confidence interval)**	**P**
		SS (n=32)	**SR (n=32)**		
***MDR-1* C1236T (rs1128503)**	CC (n=19)	9 (47.4)	10 (52.6)	-	0.018*
	TC (n=36)	22 (61.1)	14 (38.9)	-	0.018*
	TT (n=9)	1 (11.1)	8 (88.9)	-	0.018*
	CC (n=19)	9 (47.4)	10 (52.6)	Reference^{CD}	
	TC (n=36)	22 (61.1)	14 (38.9)	0.573 (0.186-1.76)	0.328
	TT (n=9)	1 (11.1)	8 (88.9)	7.2 (0.747-69.381)	0.098†
	CC (n=19)	9 (47.4)	10 (52.6)	Reference^{D}	0.784
	TT + TC (n=45)	23 (51.1)	22 (48.9)	0.861 (0.294-2.519)	0.784
	TT + CC (n=28)	10 (35.7)	18 (64.3)	Reference^{OD}	0.044
	TC (n=36)	22 (61.1)	14 (38.9)	0.354 (0.127-0.983)	0.044
	CC + TC (n=55)	31 (56.4)	24 (43.6)	Reference^{R}	0.026†
	TT (n=9)	1 (11.1)	8 (88.9)	10.33 (1.208-88.362)	0.026†
	C-allele (n=74)	40 (54.1)	34 (45.9)	Reference	0.283
	T-allele (n=54)	24 (44.4)	30 (55.6)	1.471 (0.727-2.976)	0.283
***MDR-1* C3435T (rs1045642)**	CC (n=11)	4 (36.4)	7 (63.6)	-	0.077
	TC (n=35)	22 (62.9)	13 (37.1)	-	0.077
	TT (n=18)	6 (33.3)	12 (66.7)	-	0.077
	CC (n=11)	4 (36.4)	7 (63.6)	Reference^{CD}	0.169†
	TC (n=35)	22 (62.9)	13 (37.1)	0.338 (0.083-1.379)	0.169†
	TT (n=18)	6 (33.3)	12 (66.7)	1.143 (0.237-5.501)	0.868†
	CC (n=11)	4 (36.4)	7 (63.6)	Reference^{D}	0.320
	TT + TC (n=53)	28 (52.8)	25 (47.2)	0.51 (0.133-1.952)	0.320
	TT + CC (n=29)	10 (34.5)	19 (65.5)	Reference^{OD}	0.024
	TC (n=35)	22 (62.9)	13 (37.1)	0.311 (0.111-0.869)	0.024
	CC + TC (n=46)	26 (56.5)	20 (43.5)	Reference^{R}	0.095
	TT (n=18)	6 (33.3)	12 (66.7)	2.6 (0.831-8.132)	0.095
	C-allele (n=57)	30 (52.6)	27 (47.4)	Reference	0.594
	T-allele (n=71)	34 (47.9)	37 (52.1)	1.209 (0.602-2.43)	0.594
Synergism of *MDR-1* C136T and C3435T variants[a]	C1236T [0] + C3435T [0] (n=46)	26 (56.5)	20 (43.5)	-	0.029*
	C1236T [0] + C3435T [1] (n=9)	5 (55.6)	4 (44.4)	-	0.029*
	C1236T [1] + C3435T [1] (n=9)	1 (11.1)	8 (88.9)	-	0.029*

*Significance value for the likelihood ratio, †Significance value for Fisher's Exact Test; Statistically significant p-values are in bold; MDR1: multi-drug resistant-1 gene; SS: steroid-sensitive; SR: steroid-resistant. CD: co-dominance; D: dominance; OD: over-dominance; R: recessive genetic model. [a] zero in square brackets represents a homozygous wild or heterozygous genotype, while 1 represents a homozygous mutant genotype.

3.3. The Linkage disequilibrium and haplotype analysis of the *MDR1* C1236T and C3435T SNPs

The SHEsis online tool was used to construct the LD blocks among the C1236T and C3435T haplotypes in the combined SRNS-SSNS group. The values of the LD and correlation coefficients for C1236T and C3435T SNPs are D'=0.787 and r^2=0.363, respectively (Figure 2). The haplotype analysis did not reveal a statistically significant difference in the frequency distribution of the C1236T and C3435T haplotypes between SRNS and SSNS. However, the haplotype analysis with respect to the age at onset of NS highlighted that the children with the TC haplotype were eight times more likely to develop NS after eight years old than before eight years old (OR =8.034, 95%CI=1.675-38.54, *p*-value=0.0028; Table 5).

4. DISCUSSION

This research established a potential pharmacogenetic-based predictor of treatment outcome by identifying the participants' genotypes for the *MDR-1* C1236T and C3435T SNPs and studying the effect of these variants (individually and mutually) on patients' responsiveness to prednisolone therapy. The availability of early markers for steroid resistance may help guide adjustments in treatment dosage and duration, as well as prompt earlier initiation of alternative regimens with potentially more effective immunosuppression that would provide a significant benefit to the management of patients with NS. To our knowledge, no study has previously been conducted to investigate the association of the C1236T and C3435T SNPs in the MDR1 gene with steroid responsiveness in Iraqi children with INS.

This study revealed a significant association between steroid responsiveness and the genotypes of the C1236T SNP in Iraqi children with INS. A similar significant association was noted in studies in Taiwan, South Korea, Finland, and Egypt[18-19,26-27]. Han et al. conducted a systematic review and meta-analysis on several studies, which included European and Asian populations, and indicated a significantly higher risk of SRNS associated with the C1236T TT genotype compared to the CC and TC genotypes[21]. Nevertheless, other studies from Asia (India and Bangladesh), as well as Europe (Slovakia, Poland, and Turkey), found that the C1236T SNP genotypes were not associated with steroid resistance in INS patients[20,28-32]. Regarding the C3435T SNP, the genotypes were not significantly different between SRNS and SSNS. Consistent findings were observed in Indian, Taiwanese, South Korean, and Turkish populations[18,20,26,31]. However, other studies from Poland, Slovakia, Bangladesh, and

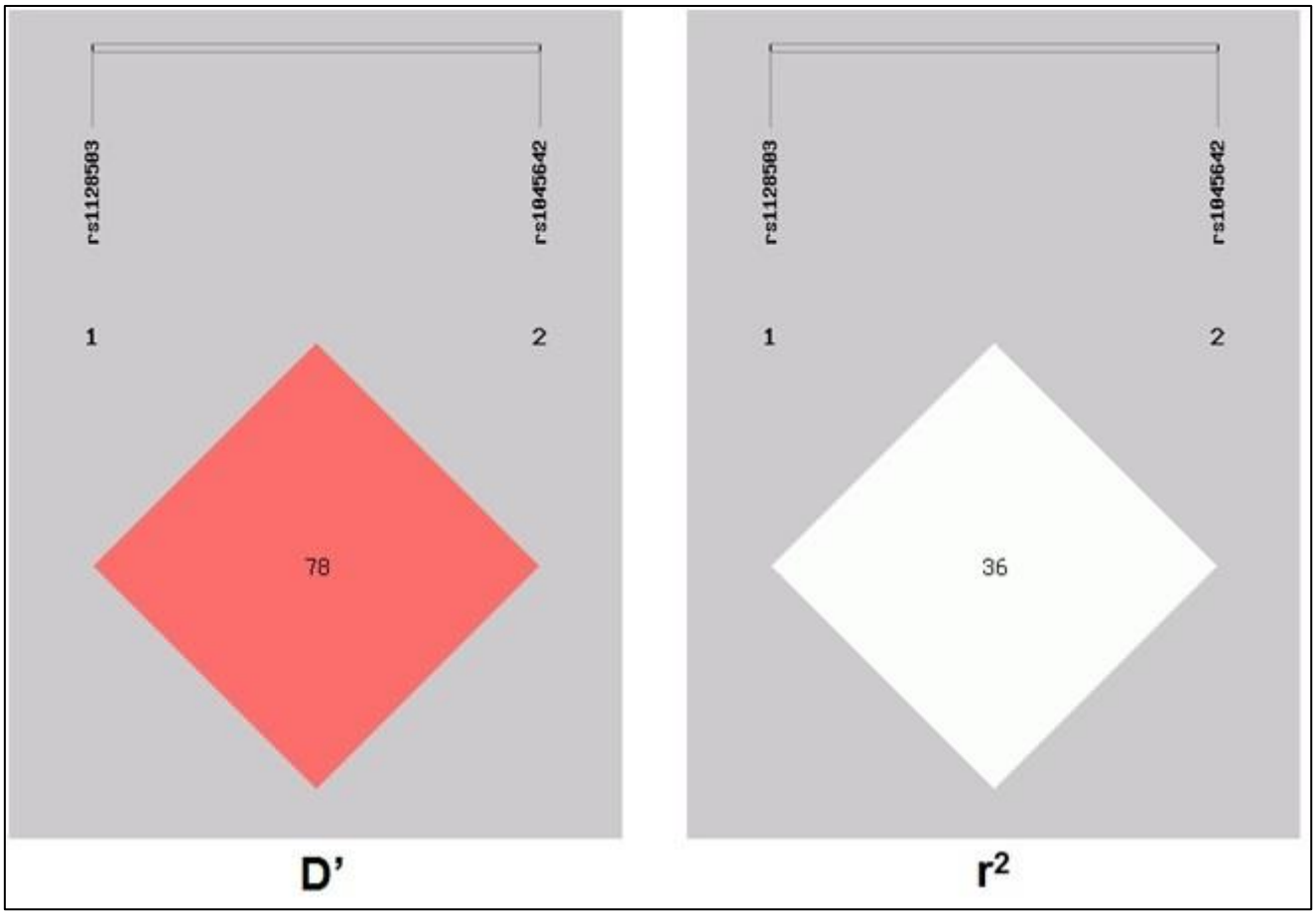

Figure 2. The values of D' and r^2 computed from the haplotype analysis of C1236T and C3435T single nucleotide polymorphisms of the MDR-1 gene in the studied groups.

Table 5. Haplotype analysis of the MDR-1 C1236T (rs1128503) and C3435T (rs1045642) SNPs with respect to steroid responsiveness and age at onset of the disease in children with idiopathic nephrotic syndrome.

Haplotype analysis with respect to steroid responsiveness						
Haplotypes	**The frequency distribution (%)**		χ^2	**Odds ratio**	**95% confidence interval (95%CI)**	***p*-value**
	SSNS (n=32)	**SRNS (n=32)**				
TT	22.54 (35.2)	26.75 (41.5)	0.534	1.305	0.639-2.666	0.4650
TC	1.46 (2.3)	3.43 (5.4)	0.832	2.436	0.339-17.479	0.3620
CT	11.46 (17.9)	10.43 (16.3)	0.058	0.893	0.356-2.244	0.8100
CC	28.54 (44.6)	23.57 (36.8)	0.802	0.724	0.357-1.469	0.3700
Haplotype analysis with respect to age at onset of nephrotic syndrome						
Haplotypes	**Age at onset<8 years (n=56)**	**Age at onset≥8 years (n=8)**	χ^2	**Odds ratio**	**95% CI**	***p*-value**
TT	43.23 (38.6)	3.5 (21.9)	1.689	0.445	0.128-1.547	0.1940
TC	3.77 (3.4)	3.5 (21.9)	8.948	8.034	1.675-38.54	0.0028
CT	19.77 (17.7)	4.5 (28.1)	0.999	1.825	0.554-6.019	0.3180
CC	45.23 (40.4)	4.5 (28.1)	0.885	0.578	0.182-1.831	0.3470

SSNS: steroid-sensitive nephrotic syndrome; SRNS: steroid-resistant nephrotic syndrome; χ^2: Chi-square; Statistically significant p-values are in bold.

Egypt found a significant variation in the C3435T genotypes between SRNS and SSNS[19,28,30,32]. The diverse genetic composition among various ethnicities might justify the variability in findings that warrant further investigations, particularly among populations with a high degree of heterogeneity in their ethnic origins. The C1236T SNP is a synonymous (silent) variant that does not alter the amino acid sequence in the protein. However, strong LD has been shown between the C1236T SNP and other variants with the ability to change *MDR1* gene expression and P-gp function[28,33]. Furthermore, the mechanisms regulating the translation of P-gp or the structure of its mRNA could be influenced by the *MDR1* C1236T SNP. A decline in the intracellular uptake of substrates has been linked to the TT genotype of the C1236T SNP[34].

Interestingly, this study also demonstrated a significantly lower risk of steroid resistance in patients with the TC genotype compared to the homozygous genotypes (CC+TT) for the C3435T polymorphism (C3435T TC: OR=0.354, 95% CI=0.127-0.983, *p*-value=0.044). Cizmarikova et al. found a significant association between steroid sensitivity and the C3435T TC genotype in Slovakian patients (OR=5.13, 95%CI=1.18-22.25, *p*-value =0.022)[30]. Similar to C1236T, C3435T is also a silent SNP, but it may affect the protein product by other mechanisms. The C3435T variant may influence the co-translational folding timing and consequently the protein-substrate and protein-inhibitor interactions[35], or destabilize the RNA structure, resulting in a modified P-gp mRNA concentration[36].

The combined genotypes of two SNPs of the *MDR1* gene were also analyzed to test whether a potential synergism of certain genotypes from both *MDR-1* SNPs determines the patients' steroid responsiveness. Patients with a combination of two HM genotypes for both the C1236T and C3435T SNPs were encountered with a significantly higher frequency in the SRNS group compared to the SSNS group (*p*-value<0.05). Jafar et al. detected a similar finding in Indian patients[20]. This finding is in line with the strong LD described by this study between the C1236T and C3435T SNPs. Strong LD was also discovered between the C3435T SNP and other *MDR-1* polymorphisms with functional impact in Asian descendants[37]. The haplotype analysis of the *MDR1* C1236T and C3435T mutations specified no statistically significant difference among patients with SSNS compared to those with SRNS. Similarly, Slovakian and Indian studies disclosed a lack of significant associations between haplotypes and steroid resistance[20,30]. Inconsistent results were presented in several studies with participants of varying ethnicities[21,28,38]. This study also examined the association of the genotypes with the age at onset of NS. No significant variation was noted between the variants' genotypes and age at onset. This was consistent with the findings of two studies[26,32]. In contrast, Jafar et al. reported a significant association between the C3435T polymorphism and age at onset[20]. Furthermore, the haplotype analysis in this study detected an association between the TC haplotype from *MDR-1* C1236T and C3435T SNPs and an age at onset of eight years or older. There was an inherent variability among the findings of different studies, which could be attributed to several considerations. In addition to ethnic variations among reporting studies, a variety of medications and foods might interact with P-gp and affect its expression and activity[39]. P-gp was reported to possess additional molecular activities not related to its carrier function, which could be affected by genetic variants in a more pronounced manner[40]. Moreover, the presence of homogeneously comparable groups of nephrotic patients cannot be ascertained because the disease pathology is not clearly established yet and the histo-pathological diagnoses are multifarious. Thus, the heterogeneity of the groups under testing cannot be excluded.

In summary, this study discerned an association between steroid resistance and the *MDR1* C1236T variant in Iraqi pediatric patients with INS. Children with

the TT genotype of the *MDR1* C1236T SNP alone or in combination with the TT genotype of the *MDR1* C3435T SNP tend to be resistant to prednisolone therapy, and management with other therapeutic strategies may be necessary.

This study had several limitations. The present work did not involve a screening of patients for genetic podocyte mutations such as those in the *NPHS1* and *WT1* genes known to cause SRNS. However, nephrotic patients with a family history were not included. Further categorization of patients' responsiveness, such as early or late sensitivity, was not possible because detailed remission information could not be retrospectively retrieved from patients' medical profiles. This study enrolled a small number of patients from a single center, which necessitates a cautious interpretation of the findings. Despite these drawbacks, this is the first attempt in Iraqi nephrotic children to provide a linkage between steroid responsiveness and *MDR1* polymorphisms, with promising preliminary findings. Further validation of the results requires subsequent studies to be conducted with a larger sample, a cohort design, and multiple centers.

5. CONCLUSION

The findings of this study suggested that the *MRD-1* C1236T SNP could increase the risk of developing resistance to prednisolone therapy in Iraqi patients with childhood INS. When children exhibit the synergistic combination of HM genotypes of *MRD-1* C1236T and C3435T SNPs, the risk of SRNS development may be elevated. Moreover, the TC haplotype of the C1236T and C3435T polymorphisms was associated with a later onset (≥8 years old) of NS. More research is needed to identify additional genetic contributors to the therapeutic response to steroids, which would allow for a better understanding of their pharmacogenetics in NS patients.

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Conflict of interest
None to declare.

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Ethics approval
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 legal caretakers) before their enrollment in the study. This work was compliant with the criteria published in the Declaration of Helsinki and its subsequent amendments.

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Author contribution
AMAA undertook literature review, data collection and analysis, and manuscript drafting. The literature review, data analysis, study design, and manuscript draft's revisions were supervised by DJK. The planning of patients' recruitment and supervision of data collection were conducted by AHAA. The finalized draft of the manuscript was approved by all authors.

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