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# Study of genetic polymorphisms of adenosine triphosphate-binding cassette B1 (ABCB1) gene in Iraqi nephrotic syndrome patients on prednisolone therapy

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## ABSTRACT

*Introduction:* Steroids are the primary treatment for idiopathic nephrotic syndrome. Pharmacogenomic factors, including genetic and histological modifications, play a significant role in influencing the response to steroids. One such factor is the excessive synthesis of P-glycoprotein (permeability glycoprotein) and multidrug resistance-associated protein 1 (MDR-1), which may contribute to the development of steroid resistance and alterations in steroid pharmacokinetics.

*Objectives:* We examined the correlation between steroid responsiveness and the MDR-1 gene variations, specifically rs1045642 (3435T/C) and rs2032582 (2677G/T/A), in children in Iraq diagnosed with idiopathic nephrotic syndrome (INS).

*Patients and Methods:* In this cross-sectional study, one hundred Iraqi pediatric patients aged one to 16 years, all diagnosed with primary nephrotic syndrome and treated with prednisolone, were enrolled. After isolating genomic DNA, genotyping was performed using the allele-specific polymerase chain reaction (PCR) technique. A notable correlation was identified between the adenosine triphosphate-binding cassette B1 (ABCB1) gene SNPs 3435T>C and 2677G>T/A, and the likelihood of prednisolone resistance in these patients with nephrotic syndrome.

*Results:* The study included 45 cases of steroid-sensitive nephrotic syndrome (SSNS), 38 cases of steroid-dependent nephrotic syndrome (SDNS), and 17 cases of steroid-resistant nephrotic syndrome (SRNS). Our investigation revealed a significant correlation between the 3435T>C polymorphism of the ABCB1 gene and the likelihood of resistance to prednisolone in pediatric patients with nephrotic syndrome ($p = 0.02$). The genotype distribution for rs1045642 (3435T>C SNP) was 14 TT, 68 TC, and 18 CC. For rs2032582 (2677G/T/A SNP), the genotype distribution was 18 GG, 16 GT, 24 TT, 23 TA, and 19 AA. Additionally, the 2677G>T/A polymorphism was significantly associated with the onset of prednisolone-resistant nephrotic syndrome ($P = 0.043$).

*Conclusion:* This study concluded that children with the MDR-1 3435T/C and 2677G/T/A polymorphisms may be more vulnerable to SRNS and, therefore, may require alternative therapeutic approaches.

Original Article

*Implication for health policy/practice/research/medical education:*
In our cross-sectional study, we investigated the association between steroid resistance and ABCB1 (adenosine triphosphate-binding cassette B1) gene polymorphisms (3435T/C and 2677G/T/A) in one hundred Iraqi pediatric patients aged one to 16 years with primary nephrotic syndrome, all of whom were treated with prednisolone. Our findings revealed that both polymorphisms were significantly associated with increased steroid resistance. Furthermore, the results suggest that children with these genetic variations may require alternative treatments for steroid-resistant nephrotic syndrome.

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## Introduction

Nephrotic syndrome is a prevalent glomerular disease in pediatric populations, significantly affecting morbidity and mortality while placing a substantial strain on healthcare systems. The condition is characterized by the episodic onset of significant proteinuria, low-albumin levels, and edema, resulting from impaired filtration across the glomerular barrier. In children, this syndrome is primarily classified as idiopathic nephrotic syndrome (INS), which presents with diverse histological features (1).

Approximately 90% of nephrotic syndrome cases in childhood are attributed to this condition, which has an incidence rate of two to seven per 100 000 children. In Europe and the United States, the annual prevalence among children under the age of 16 years is approximately 1–3 per 100 000. The male-to-female ratio among affected children is 2:1, with a higher prevalence in males. However, during adolescence, the condition affects both genders equally (2).

Steroid therapy is the principal treatment for nephrotic syndrome. According to the international study of kidney disease in children, prednisolone is the recommended initial treatment for INS. Nephrotic syndrome is primarily classified into two categories; steroid-sensitive (SSNS) and steroid-resistant (SRNS), based on the patient's response to steroid treatment (3). However, relapse occurs frequently in 60%–90% of initial responders, despite the high initial response rates. Although some patients experience relapses, they remain dependent on steroids. To optimize the therapy for INS, it is essential to identify the factors that influence an individual's response to immunosuppressive treatment. This is particularly important, as patients who are steroid-dependent or steroid-resistant are at a higher risk of developing irreversible renal failure and typically have a poor prognosis (4).

The multidrug resistance gene 1 (MDR-1) encodes the permeability glycoprotein transmembrane export transporter, which is responsible for the export of a diverse range of xenobiotics. Prednisolone is classified as a P-glycoprotein substrate and has the potential to increase protein expression. Variations in P-glycoprotein (permeability glycoprotein) expression and activity have been suggested as potential factors contributing to therapeutic resistance (3,5). Furthermore, P-glycoprotein may actively release pro-inflammatory mediators or interact with cellular activation and death pathways, thereby contributing to the persistent inflammatory responses that are fundamental to autoimmune disorders. Various single nucleotide polymorphisms (SNPs) in MDR-1 have been identified, which may significantly affect the pharmacokinetics and pharmacodynamics of medications. As a result, the activity and expression of P-glycoprotein can impact treatment outcomes (1,6). The MDR1 gene is believed to encompass over 50 genetic variants. The predominant genetic polymorphisms in the coding region of MDR1 include C1236T (rs1128503), G2677T/A (rs2032582), and C3435T (rs1045642). However, the influence of genetic variation on P-glycoprotein expression is not yet fully understood and may vary depending on tissue type, clinical state, and ethnicity (6). Extensive research has been conducted on the SNP (rs1045642) 3435C/T in exon 26, which has hypothesized that it may be associated with other polymorphisms or impact RNA stability and structure (4,7).

Numerous investigations have examined the correlation between glucocorticoid responsiveness and P-glycoprotein polymorphisms, yielding contradictory results (2). Prior studies indicate that individuals with nephrotic syndrome who possess a homozygous mutation in 2677G>T exhibit an increased vulnerability to steroid resistance. The adenosine triphosphate-binding cassette B1 (ABCB1) gene is characterized by two key variations: 2677G>T (Ala893Ser/Thr, rs2032582) and 3435C>T (Ile1145Ile, rs1045642), which may increase the propensity for developing SRNS in various combinations. Additionally, numerous genetic polymorphisms exist in the ABCB1 gene, with some showing significant linkage disequilibrium (8).

However, the results of numerous investigations have been inconsistent across a diverse range of ethnic groups of pediatric INS patients. Moreover, the significance of MDR-1 genetic variations in the response of children with INS to steroid treatment in Iraq has not been extensively studied.

## Objectives

Our study aims to ascertain the genotype and allele frequencies of the most prevalent ABCB1 gene variations (3435T/C and 2677G/T/A) among children with SSNS, steroid-dependent nephrotic syndrome (SDNS), and SRNS. Additionally, we will evaluate the effects of these SNPs on the responsiveness to steroid therapy in Iraqi children, thereby facilitating the prediction of their response to treatment.

## Patients and Methods

### *Study design*

This cross-sectional observational study was conducted at Kerbala Teaching Hospital for Children from August 2023 to March 2024. The inclusion criteria for the study comprised participants aged one to 16 years who had previously been diagnosed with SRNS, SDNS, or SSNS at the pediatric nephrology clinic. Patients diagnosed with SSNS demonstrated successful recovery, indicated by <1+ protein urine on early morning urine dipsticks,

following a four-week regimen of daily prednisolone at a dosage of 2 mg/kg/d, with a maximum of 60 mg/d. In the initial eight weeks of the trial involving daily prednisolone (2 mg/kg/d [maximum 60 mg/d]), the SRNS was determined to be unsuccessful in attaining sustained recovery (≥1+ protein urine on the first morning urine dipsticks). Steroid dependence (two consecutive relapses during corticosteroid therapy or within 14 days after cessation of therapy). In the steroid trial, various tapering regimens were employed, include a four-week reduction of alternate-day prednisone or a six-week discontinuation of alternate-day prednisolone (1.5 mg/kg/d [maximum 50 mg/d]).

Patients who presented with secondary nephrotic syndrome or were older than 16 years were excluded from the study. These individuals were addressed during their routine follow-up consultations at the clinic. After obtaining informed consent and confirming their compliance with the study's inclusion and exclusion criteria, they were recruited sequentially.

All participants underwent a general and local medical examination, urinalysis, and assessments of serum levels of creatinine, urea, albumin, and total cholesterol. Additionally, a comprehensive medical history was taken for each subject.

### *Collection of blood samples*

Following an overnight fast, 5 ml of venous blood was drawn and divided into two tubes. Three milliliters were placed in a plain tube, allowed to clot for 30 minutes, centrifuged, and the resulting serum was stored at −20 °C for the assessment of total cholesterol, albumin, urea, and creatinine levels using the Roche Diagnostics Cobas c111 autoanalyzer chemistry system from Germany. The remaining 2 ml of blood were collected in a tube containing ethylenediaminetetraacetic acid (EDTA), and genomic DNA was subsequently isolated from the peripheral whole blood.

### *Urine sampling*

Participants were required to submit 24-hour urine samples in sterilized containers, which were subsequently employed to ascertain the protein level of the urine specimen.

### *Genotyping*

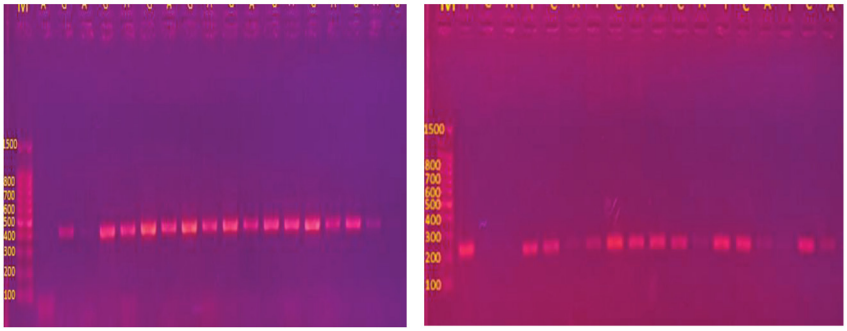
The complete blood sample was processed to extract genomic DNA from peripheral leukocytes. Soluble DNA molecules were isolated by binding to agarose under elevated salt conditions, following the chemical salt extraction of cellular proteins and debris. To further evaluate the extracted DNA, 5 µL of the sample was electrophoresed on a 1.5% (w/v) agarose gel. Our research indicates that allele-specific amplification via polymerase chain reaction (PCR) is commonly conducted for identifying single-nucleotide polymorphisms. According to the manufacturer (AddBio/Korea, AddPrep Genomic DNA Extraction Kit), the conventional protocol involves isolating genomic DNA from whole blood, which is suitable for PCR compatibility. The primers conducted in our study were designed based on the research introduced by Hasan et al (Table 1) (9). The PCR amplification protocol consisted of an initial denaturation step at 95 °C for 5 minutes, followed by 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 60 °C for 45 seconds, and extension at 72 °C for 30 seconds. The amplification was concluded with a final extension at 72 °C for five minutes. The resulting PCR products were then subjected to DNA gel electrophoresis as follows: a 1.5% agarose gel (1.5 g/100 mL 1X TBE buffer) was prepared, and 3 µL of loading buffer along with 5 µL of the sample were added. Electrophoresis was performed at 100 V for 35 minutes. After electrophoresis, Red Safe was added to re-stain the gel. DNA bands were visualized using an ultraviolet (UV) transilluminator, and their molecular weights were determined using a 100–1500 bp DNA ladder as shown in Figure 1.

### *Statistical analysis*

The Statistical Package for the Social Sciences (SPSS version 26) was used to conduct the statistical analysis. Descriptive statistics for numerical data were reported as the mean and standard deviation (Mean ± STD). Non-numerical data were expressed as percentages and counts.

**Table 1.** The length and sequence of the primers employed in the investigation

| Primers | Allele-specific | Primer sequence (5'→3') | Product size (bp) |
|---|---|---|---|
| Primers sequences of BCB1 3435T>C (rs1045642) | Reverse allele A | 5-GGGTGGTGTCACAGGAAGAGATT-3 | 400 |
| | Reverse allele G | 5-GGGTGGTGTCACAGGAAGAGATC-3 | |
| | Forward Common | 5-TAAGGCTGACAAAGGTGGAGCC-3 | |
| Primers sequences of ABCB1 2677G>T/A (rs2032582) | Forward allele T | 5-TGAAAGATAAGAAAGAACTAGAAGGTT 3 | 222 |
| | Forward allele G | 5-TGAAAGATAAGAAAGAACTAGAAGGTG 3 | |
| | Forward allele A | 5-TGAAAGATAAGAAAGAACTAGAAGGTA 3 | |
| | Common Reverse | 5-AGTCCAAGAACTGGCTTTGC-3 | |



(A) The PCR product size = 400 bp (B) The PCR product size = 222 bp

**Figure 1.** A) PCR results illustrate the amplification of a partial portion of the ABCB1 gene for the identification of SNP rs1045642. The PCR product measures 400 base pairs. The gel concentration was 1.5%, and the DNA dye utilized was Red Safe (Intron, Korea). Volume: 90, Duration: 45 minutes. B) PCR results resulting from the amplification of a partial portion of the ABCB1 gene for the identification of SNP rs2032582.The PCR product measures 222 base pairs. The gel concentration was 1.5%, and the DNA dye utilized was Red Safe (Intron, Korea). Volume: 90, Duration: 45 minutes.

The Shapiro-Wilk test was applied to assess the normality of the data distribution. For numerical data that were not normally distributed, nonparametric tests, including the Kruskal-Wallis test, was used to analyze variables such as age, body mass index (BMI) percentile, albumin, cholesterol, urine protein, creatinine, and blood urea. The chi-square test was conducted to analyze non-numerical data, including variables such as gender, family history, and obesity status. Statistical significance was indicated by *P* values less than 0.05 in the logistic regression analysis.

## Results

### *The study of patient's demographic and biochemical characteristics*

The study included 100 children with nephrotic syndrome, aged one to 16 years. Among the children diagnosed with nephrotic syndrome, 45 were identified as having SSNS, 38 cases were identified as having SDNS, while 17 individuals were categorized had SRNS. The SDNS group was significantly older than both the SSNS and SRNS groups, with the average age of the groups showing a statistically significant difference. No substantial differences were observed in the distribution of obesity status, mean BMI values, BMI percentiles, or family history among the groups. However, the gender distribution across the categories was markedly distinct, with male children exhibiting a higher propensity for nephrosis compared to female children (Table 2).

The biochemical profiles of individuals diagnosed with SSNS, SDNS, and SRNS are outlined in Table 3. Serum albumin levels showed a significant difference among the groups, with SRNS patients having the lowest levels compared to those with SSNS and SDNS. Low-serum albumin levels were associated with more severe nephrotic syndrome and increased proteinuria. In patients with SRNS, the decrease in albumin reflects more severe protein loss and potentially greater kidney injury. Additionally, significant differences were observed in serum cholesterol

**Table 2.** Demographic attributes of pediatric patients diagnosed with nephrotic syndrome

| Variables | | Steroid responsiveness | | | *P* value |
|---|---|---|---|---|---|
| | | SSNS (n=45) | SDNS (n=38) | SRNS (n=17) | |
| Age (y), mean ± SD | | 7.47 ± 3.86 | 9.47 ± 2.93[a] | 8.12 ± 2.6 | 0.024[b] |
| BMI percentile, mean ± SD | | 54.83 ± 36.22 | 63.56 ± 41.93 | 71.43 ± 40.22 | 0.275[b] |
| BMI (kg/m$^2$), mean ± SD | | 17.33 ± 3.65 | 18.87 ± 5.06 | 18.6 ± 4.63 | 0.348[b] |
| Obesity status, No. (%) | Underweight | 3 (6.7%) | 5 (13.2%) | 1 (5.9%) | 0.399[c] |
| | Healthy weight | 31 (68.9%) | 18 (47.4%) | 8 (47.1%) | |
| | Overweight | 5 (11.1%) | 5 (13.2%) | 4 (23.5%) | |
| | Obesity | 5 (11.1%) | 8 (21.1%) | 2 (11.8%) | |
| | Severe obesity | 1 (2.2%) | 2 (5.3%) | 2 (11.8%) | |
| Gender, No. (%) | Male | 31 (68.9%) | 25 (65.8%) | 6 (35.3%) | 0.043[c] |
| | Female | 14 (31.1%) | 13 (34.2%) | 11 (64.7%) | |
| Family history, No. (%) | No | 38 (84.4%) | 32 (84.2%) | 14 (82.4%) | 0.979[c] |
| | Yes | 7 (15.6%) | 6 (15.8%) | 3 (17.6%) | |

SSNS, Steroid-sensitive nephrotic syndrome; SDNS, Steroid-dependent nephrotic syndrome; SRNS, Steroid-resistant nephrotic syndrome; BMI, Body mass index. [a] Significant ($P<0.05$) compared to SSNS. [b] Kruskal Wallis test. [c] Chi-square test.

**Table 3.** The serum biochemical measurements of pediatric patients with nephrotic syndrome

| Variables | Steroid responsiveness | | | *P* value |
|---|---|---|---|---|
| | SSNS (n=45) | SDNS (n=38) | SRNS (n=17) | |
| Albumin (g/dL) | 3.54 ± 1.06 | 3.14 ± 1.23 | 2.66 ± 0.94[a] | 0.034 |
| Cholesterol (mg/dL) | 228.42 ± 117.9 | 261.83 ± 138.76 | 370.19 ± 158.21[ab] | 0.008 |
| Urine protein (mg/dL) | 76.89 ± 215.34 | 184.21 ± 306.8[a] | 255.88 ± 370.05[a] | 0.003 |
| Creatinine (mg/dL) | 0.36 ± 0.15 | 0.38 ± 0.21 | 1.02 ± 1.9 | 0.196 |
| Urea (mg/dL) | 22.63 ± 7.57 | 29.9 ± 23.61 | 34.74 ± 28.28 | 0.400 |

SSNS, Steroid-sensitive nephrotic syndrome; SDNS, Steroid-dependent nephrotic syndrome; SRNS, Steroid-resistant nephrotic syndrome; BMI, Body mass index. [a] Significant effect ($P < 0.05$) compared to the SSNS group. [b] Significant effect ($P < 0.05$) compared to SDNS group. The laboratory data was analysed using Kruskal Wallis test (Data presents as mean ± SD).

levels among the groups. Consistent with the more severe form of nephrotic syndrome characterized by proteinuria and dyslipidemia, SRNS patients exhibited the highest serum cholesterol levels.

The difference in urinary protein levels between the groups was statistically significant, with the most severe proteinuria observed in SRNS patients, as indicated by their elevated urinary protein levels. However, serum creatinine and blood urea levels did not show significant differences across the groups.

### *Molecular analysis*

The genetic variations of 3435T/C in the ABCB1 gene, as they relate to the response to prednisolone in individuals with INS, are presented in Table 4. The genotype frequency distribution of steroid-sensitive, steroid-dependent, and SRNS patients, in relation to the ABCB1 C3435T genotypes and their response to prednisolone, showed significant differences. The TC genotype was more common in the SDNS group, suggesting a potential association with steroid dependence as shown in Figure 2.

### *The association between the 2677 G/T/A SNP and steroid responsiveness*

The genotypes of G2677T/A in the ABCB1 gene of INS patients were examined in relation to their response to prednisolone treatment (Table 5). The distribution of the ABCB1 G2677T/A genotypes and their responses to prednisolone therapy showed significant differences among the genotype frequency distributions of SSNS, SDNS, and SRNS patients. The GT genotype was found to be associated with SDNS, suggesting a potential link between this genotype and steroid dependence.

Compared to SSNS and SDNS patients, the SRNS group exhibited a relatively lower prevalence of GG and GT genotypes. In contrast, the TT+AA genotypes were more prevalent in the SRNS group as shown in Figure 3. A statistically significant association was observed between different genotypes and steroid resistance. Each genotype appears to be linked to a specific form of steroid responsiveness, highlighting the potential influence of genetic variants on treatment outcomes in nephrotic syndrome.

## Discussion

Steroid response serves as a crucial prognostic marker in INS. Adverse outcomes, including the development of end-stage kidney disease, are associated with the diagnosis of steroid resistance. This condition affects approximately 10–20% of children with INS, who exhibit an inadequate response to corticosteroids (2).

A notable difference in the age distributions ($P = 0.024$) was observed among the demographic parameters of the study samples; steroid sensitive (7.47 ± 3.86 years), steroid dependence (9.47 ± 2.93 years), and steroid resistance (8.12 ± 2.6 years). These results, however, are not consistent with those reported by Roy et al (10). Furthermore, the male-to-female ratio indicates that male children are more susceptible to nephrotic syndrome than female children, as suggested by previous studies (8,11,12). Urine protein and total cholesterol levels in the serum of steroid resistance patients were statistically

**Table 4.** Genotype frequency of the 3435 T/C polymorphism in patients with SSNS, SDNS, and SRNS

| Variables | | Alleles of rs1045642 (3435 T/C) | | | *P* value |
|---|---|---|---|---|---|
| | | TT (n=14) | TC (n=68) | CC (n=18) | |
| Steroid responsiveness | SSNS | 1 (7.1%) | 14 (20.6%) | 2 (11.1%) | 0.02 |
| | SDNS | 2 (14.3%) | 31 (45.6%) | 5 (27.8%) | |
| | SRNS | 11 (78.6%) | 23 (33.8%) | 11 (61.1%) | |

SSNS, Steroid-sensitive nephrotic syndrome; SDNS, Steroid-dependent nephrotic syndrome; SRNS, Steroid-resistant nephrotic syndrome; BMI, Body mass index. Data were analyzed by using the chi-square test.

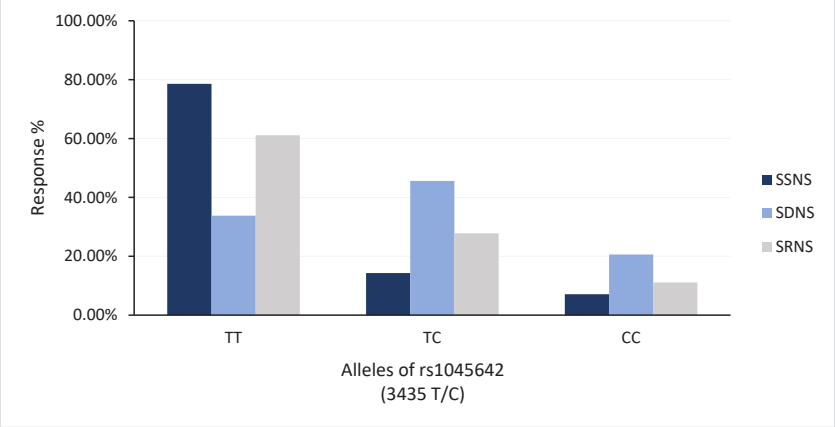



**Figure 2.** The incidence distribution of the ABCB1 3435T/C polymorphism genotype among patients with childhood nephrotic syndrome.

significantly higher than those in steroid sensitive and steroid dependence-INS children. Meanwhile, serum albumin levels in steroid resistance patients experienced a statistically significant decline. These results are consistent with the findings of Parvin et al (8). The alteration of P-glycoprotein, an active transmembrane efflux pump for various toxins and medications, including prednisone, is a potential cause of drug resistance. It has been shown that, P-glycoprotein is responsible for transporting these substances and may also regulate its own expression (13). Furthermore, it is likely that P-glycoprotein plays a role in releasing specific inflammatory mediators and may be implicated in the chronic inflammation associated with autoimmune diseases. The C3435T (rs1045642, a silent SNP) and 2677G/T/A polymorphisms in the ABCB1 gene may influence P-glycoprotein function and gene expression (14, 15). In our study, the genotype distributions of the two examined SNPs in the ABCB1 gene showed significant differences among the SS, steroid dependence, and steroid resistance patient cohorts. The frequency distribution of the ABCB1 3435T/C and 2677G/T/A mutations demonstrated notable differences between the steroid sensitive, steroid dependence, and steroid resistance patient cohorts ($P=0.02$ and $P=0.043$, respectively). Numerous studies have shown that the expression and function of P-glycoprotein are influenced

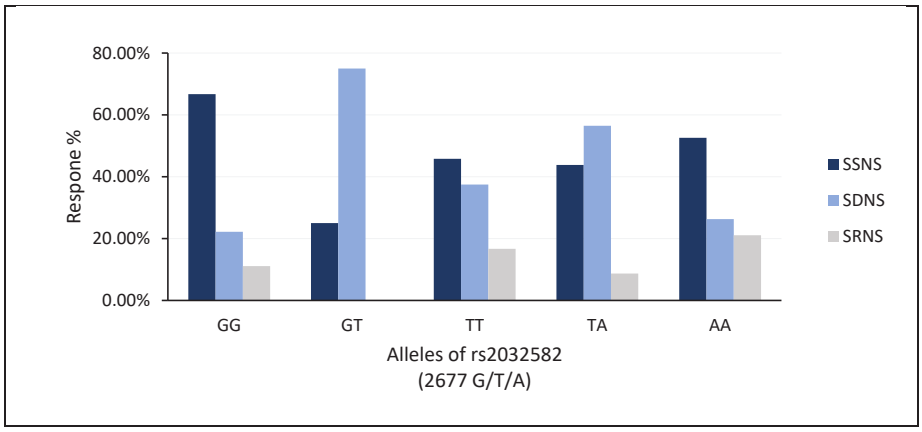



**Figure 3.** The genotype frequency distribution of the ABCB1 2677G>T/A polymorphism in cases of childhood nephrotic syndrome.

**Table 5.** Genotype frequency of 2677 G/T/A polymorphism among the SSNS, SDNS and SRNS patients

| Variables | | Alleles of rs2032582 (2677 G/T/A) | | | | | *P* value |
|---|---|---|---|---|---|---|---|
| | | GG (n=18) | GT (n=16) | TT (n=24) | TA (n=23) | AA (n=19) | |
| Steroid responsiveness | SSNS | 12 (66.7%) | 4 (25%) | 11 (45.8%) | 8 (43.8%) | 10 (52.6%) | 0.043 |
| | SDNS | 4 (22.2%) | 12 (75%) | 9 (37.5%) | 13 (56.5%) | 5 (26.3%) | |
| | SRNS | 2 (11.1%) | 0 (0%) | 4 (16.7%) | 2 (8.7%) | 4 (21.1%) | |

SSNS, Steroid-sensitive nephrotic syndrome; SDNS, Steroid-dependent nephrotic syndrome; SRNS, Steroid-resistant nephrotic syndrome; BMI, Body mass index. Data were analyzed by using the chi-square test.

by specific single nucleotide polymorphisms in the ABCB1 gene locus across different ethnic groups. Among these, the 3435C>T/C SNP has been the primary focus of most research (16). The 3435T/C polymorphism may alter the function of P-glycoproteins, RNA stability, and DNA structure. Studies found an association of 3435C > T polymorphism with SRNS in South Indian children (17) and Finnish patients (18). However, another survey conducted by Wasilewska et al (19) has demonstrated a robust correlation with nephrotic syndrome. The results presented in this study are based on our research, while studies conducted on a variety of ethnic groups, including the North Indian (20), Slovakian (21), Egyptian (7), Chinese (22), and the Indian population (23), were found to be inconsistent. However, the genotype distributions of steroid resistance and steroid sensitive patients were found to be significantly distinct (2).

Our data indicated that the frequencies of ABCB1 G2677T/A genotypes showed significant differences ($P = 0.043$) among the SSNS, SDNS, and SRNS groups, with a correlation to an elevated risk of developing SRNS in patients with nephrotic syndrome. A notable association between steroid resistance and nephrotic syndrome was identified in Indian children with the G2677T/A variant, while no such association was found with ABCB1 C1236T and ABCB1 C3435T (20). The amino acid mutation Ala899Ser/Thr arises from two prevalent SNPs, rs2032582. This modification may improve the cell's drug resistance (24,25). This characteristic may be attributed to the replacement of alanine at position 2677 with serine or threonine, which transforms a lipophilic residue into a hydrophilic one. The substitution of alanine, a neutral amino acid, with serine or threonine may affect the geometric precision of the interaction site and alter the secondary structure (25). This likely resulted in enhanced effectiveness of the modified protein, improving its capacity to remove steroids or their active metabolites, thereby promoting steroid resistance (21).

Studies by Mohammed et al and Suvanto et al (4,18) have determined that all three ABCB1 SNPs were associated with treatment choices. Patients requiring immunosuppressive drugs had a higher prevalence of the T allele and TT genotype than those receiving glucocorticoids. This suggests that T and TT are associated with a more complex form of the disease.

## Conclusion

This study indicates that the MDR-1 3435T/C and 2677G/T/A single nucleotide polymorphisms may increase the likelihood of developing resistance to prednisolone treatment in Iraqi children with INS.

## Limitations of the study

The investigation had several limitations. Firstly, the current study did not screen for genetic podocyte alterations, particularly in the NPHS1 and WT1 genes associated with SRNS. Additionally, the inclusion of a limited group of patients from a single center necessitates careful interpretation of the study's outcomes.

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## Authors' contribution

**Conceptualization:** Rawan Azad Mohammed, Ahmed Salih Sahib.
**Data curation:** Rawan Azad Mohammed
**Investigation:** Rawan Azad Mohammed, Qahtan Mohammed Ali.
**Methodology:** Ahmed Salih Sahib, Rawan Azad Mohammed.
**Supervision:** Ahmed Salih Sahib, Qahtan Mohammed Ali.
**Writing–original draft:** Ahmed Salih Sahib, Rawan Azad Mohammed
**Writing–review & editing:** Ahmed Salih Sahib, Rawan Azad Mohammed.

## Conflicts of interest

All authors declare that they have no conflicts of interest

## Ethical issues

This study was conducted in accordance with the principles outlined in the Declaration of Helsinki. The research was conducted at Kerbala Teaching Hospital for Children after receiving approval from the scientific and ethical committee of the University of Kerbala College of Pharmacy. Prior to initiating the study, informed consent was obtained from each participant or their relatives/parents. Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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