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MDR1 Polymorphisms and Idiopathic Nephrotic Syndrome in Slovak Children: Preliminary Results

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEFG 1 Martina Cizmarikova
ABCDEFG 2 Ludmila Podracka
ADEF 3 Lucia Klimcakova
ACDEF 3 Viera Habalova
BD 4 Andrej Boor
ADFG 1 Jan Mojzis
ACDEFG 1 Ladislav Mirossay

1 Department of Pharmacology, P.J. Safarik University, Faculty of Medicine, Kosice, Slovak Republic
2 Department of Paediatrics and Adolescent Medicine, P.J. Safarik University, Faculty of Medicine, Kosice, Slovak Republic
3 Department of Medical Biology, P.J. Safarik University, Faculty of Medicine, Kosice, Slovak Republic
4 Department of Pathology, P.J. Safarik University, Faculty of Medicine, Kosice, Slovak Republic

Corresponding Author: Martina Cizmarikova, e-mail: martina.cizmarikova@upjs.sk
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Background: The role of the multidrug resistance-1 (*MDR1 or ABCB1*) gene polymorphisms 1236T>C, 2677T>G, and 3435T>C was studied in relation to susceptibility, demographics, and pathological characteristics, as well as their role in the therapeutic response (TR) to prednisone treatment in children with idiopathic nephrotic syndrome (INS).

Material/Methods: The polymorphisms were analyzed using the polymerase chain reaction-restriction fragment length polymorphism method in 46 children with INS and in 100 healthy controls. Different genetic models (codominant, dominant, recessive, and overdominant) were used for testing of associations between polymorphisms and phenotypes.

Results: Statistical analysis showed a significantly increased chance of TR in children carrying 3435TC genotype (OR=5.13, 95% CI=1.18–22.25; overdominant model). Moreover, INS patients under 6 years of age had significantly decreased frequencies of *MDR1* 1236CC (7.7% *vs.* 35%, p=0.029) or 2677GG (3.8% *vs.* 30.0%, p=0.033) genotypes. We also observed that patients with minimal change in disease and patients under 6 years of age at the onset of INS were initial responders more frequently when compared with children with focal segmental glomerulosclerosis and patients ≥6 years old at the onset (p=0.0001, p=0.027, respectively).

Conclusions: These data suggest that prednisone TR may be influenced by histology, age at the onset of INS, and *MDR1* 3435T>C polymorphism. The *MDR1* 1236T>C and 2677T>G polymorphisms were significantly associated with age at onset. Larger multicenter studies and studies across other ethnic groups are needed to elucidate the contradictory implications of *MDR1* polymorphisms with INS in children.

MeSH Keywords: Genes, MDR • P-Glycoprotein • Polymerase Chain Reaction

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Background

Nephrotic syndrome (NS) is the most common glomerular pathological condition encountered in children. In contrast to NS in adulthood, when NS is often secondary to another disorder such as diabetes mellitus, cancer, and chronic inflammatory diseases [1,2], NS in childhood (>12 months age) is largely primary or idiopathic (INS) [3]. The exact reason for this is still not understood. Generally, INS patients present diverse pathological features (e.g., minimal change disease-MCD, focal segmental glomerulosclerosis-FSGS, and mesangial proliferative glomerulonephritis-MesPGN). Variable response to immunosuppressive therapy is observed when glucocorticoids (GC) are recommended as the first-line therapy due to the immune-inflammatory characteristics of the disease. Therapeutic response (TR) to initial steroid therapy has been reported to be 60–95% of children, depending on the geographic area [4,5]. Despite the high initial response rate, relapses occur in 60–90% of the initial responders and some patients remain steroid-dependent. Clinical experience has demonstrated that patients with a poor response to steroids have an unfavorable prognosis and often develop end-stage renal failure [6]. Therefore, it is essential to identify potential factors that contribute to immunosuppressive therapy failure in order to optimize and individualize the treatment of INS.

Changes in the levels and/or function of P-glycoprotein (P-gp) are one of the possible mechanisms of drug resistance that have been published in the literature [7]. P-gp is the product of the *MDR1 (*alias *ABCB1)* gene and is an active transmembrane efflux pump for a variety of toxins and many drugs. GC (including prednisone) are also transported by P-gp and may induce P-gp expression [8]. P-gp is found in many organs and tissues (e.g., luminal cells of the lower digestive system, liver, proximal renal tubules, endothelial cells in the blood–brain barrier, placenta, and hematopoietic cells), and the tissue distribution suggests that P-gp plays an important role in excreting potentially toxic or unnecessary xenobiotics and metabolites from the body and the cells, and has significant pharmacokinetic and pharmacodynamic implications for P-gp substrates [9]. P-gp may also actively participate in the chronic inflammation in autoimmune diseases; it is up-regulated by cytokines, probably participates in releasing certain inflammatory mediators, and is probably involved in the cell activation/death pathways [10,11].

Recent experimental and clinical studies have shown that gene expression and the activity of P-gp may be affected by *MDR1* gene polymorphisms. Three single-nucleotide polymorphisms (SNPs) seem to have the most important clinical relevance: 1236T>C (rs1128503, MAF/minor allele frequency: T=0.422, silent SNP); 2677T>G/A (rs2032582, MAF: T=0.340, Ser893Ala/Thr); and 3435T>C (rs1045642, MAF: T=0.397, silent SNP). The silent 3435T>C polymorphism may have some effect on DNA structure, RNA stability, and P-gp function, or it is in linkage disequilibrium with other functional *MDR1* polymorphisms [12–14]. In most studies, the T allele is associated with decreased P-gp function. The non-synonymous SNP (2677T>G/A) has also been found to be associated with altered expression, activity and the substrate specificity of P-gp [15,16]. It is speculated that patients who carry the T/A are more resistant to drugs [17]. The silent 1236T>C polymorphism may affect translation regulation, RNA stability, or other molecular mechanisms. It may also have an indirect effect, being linked to a causal variant. In a recent study, it was shown that TT genotype minimizes P-gp activity in a substrate-dependent manner [18].

Only a few studies in different ethnic populations have evaluated the distribution of the *MDR1* polymorphisms (1236T>C, 2677T>G/A, and 3435T>C) in children with NS and controls to investigate their usefulness as markers of responsiveness of the disease to steroids, and they have shown inconsistent results [19–22].

On the basis of published data, our study focused on determining the relevance of the *MDR1* (1236T>C, 2677T>G, and 3435T>C) polymorphisms and haplotypes to childhood INS susceptibility, selected demographic and clinical characteristics of INS, and TR to prednisone-based therapy in Slovak children.

Material and Methods

Study population and data collection

The study was performed in 2 groups of participants. The patient group comprised 46 children with a diagnosis of INS treated and/or monitored in the Pediatric Nephrology Centre at the Department of Pediatrics and Adolescent Medicine of the Faculty of Medicine of P. J. Safarik University in Kosice, Slovakia. The control group comprised 100 healthy children without any known chronic disease. Blood samples from both groups were collected during routine control biochemical tests or routine preventive pediatric examination at the same department from January 2010 to January 2014.

Participation was voluntary and could be cancelled by any individual at any time during the study (according to the Helsinki II declaration). The local ethics committee approved the research protocol for this study and all volunteers and/or their parents signed the study informed consent form.

Data evaluation was based on analysis of retrospective charts. All INS patients were followed up for at least 2 years. The inclusion criteria for the patient group were a fulfilled definition of

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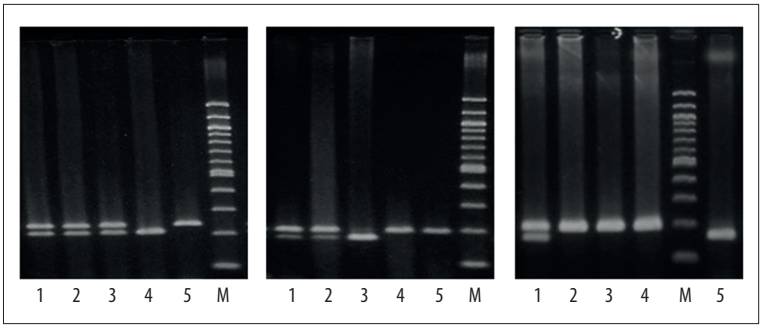

Figure 1. Electrophoretic patterns for *MDR1* polymorphisms evaluated by PCR-RFLP-based assay, M (Marker, 100 bp ladder): (**A**) 1236T>C: TT genotype (lane 5), TC genotype (lane 1, 2, 3), CC genotype (lane 4); (**B**) 2677T>G: TT genotype (lane 3), TG genotype (lane 1, 2), GG genotype (lane 4, 5); (**C**) 3435T>C: TT genotype (lane 2, 3, 4), TC genotype (lane 1), CC genotype (lane 5).

INS (massive proteinuria of ≥40 mg/h/m^2 and hypo-albuminemia of ≤2.5 g/dL), absence of secondary cause of nephrotic syndrome, and age 1–18 years. All cases were Slovaks from different regions of Eastern Slovakia.

All patients were treated with the standard initial steroid therapy, consisting of daily dosage of prednisone of 60 mg/m^2/day for 4 weeks followed by 40 mg/m^2/day on alternate days for an additional 4 weeks. Relapses were treated with daily prednisone dose of 60 mg/m^2/day until urine protein test results were negative or trace for 3 consecutive days, followed by 40 mg/m^2/day on alternate days for 4 weeks, and finally by various steps of tapering-off on alternate days.

The following definitions were considered for classification of response pattern to steroid therapy [3,4]:

1. Initial steroid response (RE-responders): attainment of complete remission (CR) within the initial 4 weeks of GC therapy. CR was defined as urinary protein excretion <4 mg/h/m^2; nil or trace by dipstick on spot sample for 3 consecutive days.
2. Initial steroid resistance (NRE-non-responders): failure to achieve complete remission following at least 4 weeks of corticosteroid therapy at a dose 60 mg/m^2/day.
3. Steroid dependence: occurrence of 2 consecutive relapses during steroid therapy or within 14 days of its cessation.
4. Frequent relapse: 2 or more relapses within the first 6 months of initial response, or 4 or more relapses in any 12-month period.

All steroid-resistant patients were successfully screened for NPHS1 and WT1 mutations, with negative results. Renal biopsy was performed in all patients except 1 (because of a hypoplastic kidney).

DNA extraction and genotyping

Genomic DNA was extracted from peripheral venous blood by using Wizard® Genomic DNA Purification Kit (Promega Corporation, USA). The SNPs in *MDR1* were analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay using the following primer sequences and corresponding restriction enzymes; (1) 1236T>C, 5'-TTACCCATCTCGAAAAGAAGTTAAGGT-3' – forward, 5'-TGCCCACTCTGCACCTTCATGTTC-3' – reverse (*HaeIII*); (2) 2677T>G, 5'-TTACCCAGAATATAGCAAATCTTGG-3' – forward, 5'-CATATTTAGTTTGACTCACCTTCTCAG-3' – reverse (*Hpy188I*); (3) 3435T>C, 5'-TGTTTTCAGCTGCTTGATGG-3' – forward, R: 5'-AAGGCATGTATGTTGGCCTC-3' – reverse (*BfuCI*). Due to the low population frequency of the more recently described A allele of 2677T>G/A polymorphism [23,24], this variant was not genotyped in the present study. The same PCR reaction pattern was used for each SNP. The PCR reaction mixture contained approximately 200 ng of genomic DNA, 1×PCR Buffer with 1.5 mM $MgCl_2$ (Solis BioDyne, Estonia), 200 µM deoxynucleotide triphosphate mix (Jena Bioscience, Germany), 0.4 µM of each primer (Sigma-Aldrich, Germany), and 1.0 U HOT FIREPol® DNA Polymerase (Solis BioDyne, Estonia). PCR-grade water was added, bringing the final volume to 25 µl. The amplification consisted of an initial polymerase activation step for 15 min at 95°C and initial denaturation step for 30 s at 95°C followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 30 s. Terminal elongation was performed at 72°C for 3 min. The PCR products were digested at 37°C overnight using 10 units of appropriate restriction endonuclease (New England BioLabs, UK). The restriction fragments obtained were separated by electrophoresis on a 3% agarose gel for 90 min at 140 V and analyzed after staining with GelRed (Biotium, USA) under ultraviolet light. The electrophoretic pattern for each SNP was: (1) 1236T>C, TT: 234 bp, TC: 234 bp, 207 bp, CC: 207 bp; (2) 2677T>G, TT: 173 bp, TG: 198 bp, 173 bp, GG: 198 bp; and (3) 3435T>C, TT: 197 bp, TC: 197 bp, 158 bp, CC: 158 bp (Figure 1).

Statistical analysis

GraphPad Prism 5 software (GraphPad Software, Inc., USA) was used for most of statistical analyses. A p value of <0.05 was taken as statistically significant. Chi-square or Fisher's exact tests were performed to compare contingency tables. The Hardy-Weinberg equilibrium (HWE) assumption was assessed for the tested group by comparing the observed numbers of each genotype with those expected under the HWE for the estimated allele frequency. Codominant, dominant, recessive, and overdominant genetic models were used to

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Table 1. Clinical characteristics of patients with idiopathic nephrotic syndrome.

Variable		RE N=33 (%)	NRE N=13 (%)	OR (95% CI)	*p* value
Age:	<6 years	22 (66.7)	4 (30.8)	1.00 (Ref.)	
	≥6 years	11 (33.3)	9 (69.2)	4.5 (1.13–17.94)	0.027*
Sex:	Males	24 (72.7)	8 (61.5)	1.00 (Ref.)	
	Females	9 (27.3)	5 (38.5)	1.67 (0.43–6.46)	0.493
Histology:	MCD	29 (87.9)	3 (23.1)	1.00 (Ref.)	FSGS *vs.* MCD
	FSGS	2 (6.1)	9 (69.2)	43.50 (6.25–302.6)	<0.0001*
	MesPGN	1 (3.0)	1 (7.7)		
Origin:	Town	14 (42.4)	9 (69.2)	1.00 (Ref.)	
	Village	19 (57.6)	4 (30.8)	0.33 (0.08–1.28)	0.102

RE – responders; NRE – non-responders; OR – odds ratio; CI – confidence interval; MCD – minimal change disease; FSGS – focal segmental glomerulosclerosis; MesPGN – mesangial proliferative glomerulonephritis; Ref. – reference; * a significant association.

analyze the association between a polymorphism and phenotype. The most commonly used model, the codominant model, is presented for all SNPs. The best-fitting genetic model was selected on the basis of Akaike's information criterion. Results from the model chosen were then discussed. Odds ratios (OR) and the corresponding 95% confidence intervals (95% CI) were used for calculating the relative associations. The SNPStats program was used to examine haplotype frequency estimates [25].

Results

Clinical characteristics of the participants

Of the 46 patients enrolled in the study, 32 (69.57%) were males and 14 (30.43%) were females (male: female ratio: 2.29:1). The mean age at onset of INS was 6.42±4.72 years. Twenty-six children were <6 years old at the onset of disease. Among the total of 46 children with INS, 33 were initial RE (71.74%) and 13 were initial NRE (28.26%). From initial RE, 27 participants (81.82%) were frequent relapsers and/or steroid-dependent INS patients.

MCD was found in 32/46 (69.57%) children, FSGS in 11/46 (23.91%), and MesPGN in 2/46 (4.35%, 1 male and 1 female) of the remaining children.

Clinical and pathological characteristics of RE and NRE are shown in Table 1. We observed significant association between histological pattern and age at onset of INS with the initial steroid response. Patients with MCD were initial RE more frequently when compared with children with FSGS ($p \leq 0.0001$). Moreover, a significantly higher frequency of RE was observed in patients younger than 6 years of age at onset of INS ($p=0.027$).

The control group comprised 100 healthy children (54 males and 46 females) with a mean age of 7.89±6.31. There were no significant differences in distributions of age and sex between controls and INS patients.

Genotype and allelic distribution of *MDR1* polymorphisms

All obtained blood samples were successfully genotyped for *MDR1* SNPs (3435T>C, 2677T>G, and 1236T>C). Overall genotype and allele frequencies for the tested *MDR1* polymorphisms in patients and control subjects are shown in Table 2. The frequency of *MDR1* 1236T and 3435T alleles in Slovak control participants was higher when compared with 1236C and 3435C alleles. For the 2677T>G polymorphism, the G allele was more frequent than the T allele in the control group.

There was no significant difference in the distribution of genotypes and alleles in all 3 SNPs between the patient group and the control group. The test for HWE showed no significant deviation from the Hardy-Weinberg equilibrium either in the total patient sample (3435T>C: $p=0.9872$; 2677T>G: $p=0.1958$; and 1236T>C: $p=0.6675$) or the control sample (3435T>C: $p=0.9335$; 2677T>G: $p=0.7651$; and 1236T>C: $p=0.3428$).

Patient characteristics according to *MDR1* polymorphisms

Distributions of genotypes and alleles of *MDR1* gene polymorphisms among INS patients with respect to age at onset are shown in Table 3. INS patients less than 6 years old had decreased frequencies of *MDR1* 1236CC (7.7% *vs.* 35%, $p=0.029$)

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Table 2. Distributions of genotypes and alleles of *MDR1* gene polymorphisms among control subjects and INS patients.

Genotype	Controls N=100 (%)	INS patients N=46 (%)	OR (95% CI)	*p* value
1236T>C				
TT	31 (31.0)	11 (23.9)	1.00 (Ref.)[a]	
TC	41 (41.0)	26 (56.5)	1.79 (0.77–4.16)	0.176
CC	28 (28.0)	9 (19.6)	0.91 (0.33–2.51)	0.849
T allele	103 (51.5)	48 (52.2)	1.00 (Ref.)	
C allele	97 (48.5)	44 (47.8)	0.97 (0.59–1.60)	0.915
2677T>G				
TT	23 (23.0)	10 (21.7)	1.00 (Ref.)[a]	
TG	49 (49.0)	29 (63.0)	1.36 (0.57–3.26)	0.488
GG	28 (28.0)	7 (15.2)	0.58 (0.19–1.75)	0.327
T allele	95 (47.5)	49 (53.3)	1.00 (Ref.)	
G allele	105 (52.5)	43 (46.7)	0.79 (0.48–1.30)	0.360
3435T>C				
TT	32 (32.0)	15 (32.6)	1.00 (Ref.)[a]	
TC	52 (52.0)	23 (50.0)	0.94 (0.43–2.07)	0.885
CC	16 (16.0)	8 (17.4)	1.07 (0.37–3.04)	0.904
T allele	116 (58.0)	53 (57.6)	1.00 (Ref.)	
C allele	84 (42.0)	39 (42.4)	1.02 (0.62–1.68)	0.950

INS – idiopathic nephrotic syndrome; OR – odds ratio; CI – confidence interval; Ref. – reference; a codominant model.

and *MDR1* 2677GG (3.8% *vs.* 30%, *p*=0.041) genotypes. The risk of early onset of INS was significantly lower among patients carrying 1236CC than patients carrying 1236TT+TC (OR=0.15; 95% CI=0.03-0.86; recessive model). Similarly, patients carrying 2677GG genotypes had lower risk of developing INS at a younger age than patients carrying TG+TT genotype (OR=0.09; 95% CI=0.01-0.86; recessive model). The difference in allele frequencies between INS participants <6 years old and ≥6 years old was not statistically significant.

There was no significant association between 3435T>C polymorphism and age of onset under any tested genetic models. No other significant differences were found across clinical and pathological characteristics (sex, histopathology, number of relapses, and origin) differentiated according to genotypes and alleles of *MDR1* polymorphisms (data not shown).

MDR1 gene variants and response to glucocorticoids

The association between *MDR1* polymorphisms and therapy response to GC was assessed in all patients (n=46). A statistically significant association was found between *MDR1* 3435T>C polymorphism and successful initial steroid treatment of INS. Statistical analysis showed a significantly increased chance of initial treatment response to GC in children carrying TC genotype (OR=5.13; 95% CI=1.18–22.25; overdominant model) (Table 4). In contrast, we did not observe any significant differences in the distribution of the *MDR1* 1236T>C and 2677T>G genotypes or alleles between steroid responders and non-responders. No statistically significant associations were found between non-frequent relapsers and frequent relapsers and/or steroid-dependent INS patients and *MDR1* genotypes or alleles (data not shown).

Haplotype analysis

Haplotype analysis of the 3 *MDR1* polymorphisms (1236T>C, 2677T>G, and 3435T>C) revealed 7 major haplotypes. TTT haplotype was the most frequent haplotype in the patients and control group. There were no significant differences in the distribution of these haplotypes between INS patients and the controls (Table 5). Moreover, we did not observe any significant difference in the distribution of these haplotypes between initial steroid responders

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Table 3. Distributions of genotypes and alleles of *MDR1* gene polymorphisms among INS patients with respect to age at the onset.

Genotype	Age at onset ≥6 years N=20 (%)	Age at onset <6 years N=26 (%)	OR (95% CI)	*p* value
1236T>C				
TT	5 (25.0)	6 (23.1)	1.00 (Ref.)[a]	
TC	8 (40.0)	18 (69.2)	1.88 (0.44–8.00)	0.465
CC	7 (35.0)	2 (7.7)	0.24 (0.03–1.71)	0.197
TT+TC	13 (65.0)	24 (92.3)	1.00 (Ref.)[b]	
CC	7 (35.0)	2 (7.7)	0.15 (0.03–0.86)	0.029*
TT+CC	12 (60.0)	8 (30.8)	1.00 (Ref.)[c]	
TC	8 (40.0)	18 (69.2)	3.38 (0.99–11.46)	0.047*
T allele	18 (45.0)	30 (57.7)	1.00 (Ref.)	
C allele	22 (55.0)	22 (42.3)	0.60 (0.26–1.38)	0.227
2677T>G				
TT	5 (25.0)	5 (19.2)	1.00 (Ref.)[a]	
TG	9 (45.0)	20 (76.9)	2.22 (0.51–9.65)	0.281
GG	6 (30.0)	1 (3.8)	0.17 (0.01–1.94)	0.129
TT+TG	14 (70.0)	25 (96.2)	1.00 (Ref.)[b]	
GG	6 (30.0)	1 (3.8)	0.09 (0.01–0.86)	0.033*
TT+GG	11 (55.0)	6 (23.1)	1.00 (Ref.)[c]	
TG	9 (45.0)	20 (76.9)	4.07 (1.15–14.49)	0.026*
T allele	19 (47.5)	30 (57.7)	1.00 (Ref.)	
G allele	21 (52.5)	22 (42.3)	0.66 (0.29–1.52)	0.331
3435T>C				
TT	7 (35.0)	8 (30.8)	1.00 (Ref.)[a]	
TC	7 (35.0)	16 (61.5)	2.00 (0.52–7.70)	0.311
CC	6 (30.0)	2 (7.7)	0.29 (0.04–1.94)	0.379
T allele	21 (52.5)	32 (61.5)	1.00 (Ref.)	
C allele	19 (47.5)	20 (38.5)	0.69 (0.30–1.59)	0.385

INS – idiopathic nephrotic syndrome; OR – odds ratio; CI – confidence interval; Ref. – reference; [a] codominant model; [b] recessive model; [c] overdominant model; * a significant association.

and non-responders (Table 5). Haplotype variations did not influence age at onset of INS or renal pathology (data not shown).

Discussion

Genomic medicine, which is the use of information from genomes and their derivatives to guide medical decision making, is a key component of personalized medicine, which is currently a rapidly advancing field of health care [26].

In the present study, we investigated whether 3 known SNPs in the *MDR1* gene (1236T>C, 2677T>G, and 3435T>C) influence INS susceptibility, selected demographics, and pathological characteristics of INS, as well as response to oral prednisone in children from Eastern Slovakia. To our knowledge, this is

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Table 4. Distributions of genotypes and alleles of *MDR1* gene polymorphisms among initial steroid responders and steroid non-responders.

Genotype	NRE N=13 (%)	RE N=33 (%)	OR (95% CI)	*p* value
1236T>C				
TT	5 (38.5)	6 (18.2)	1.00 (Ref.)[a]	
TC	6 (46.2)	20 (60.6)	2.78 (0.62–12.42)	0.244
CC	2 (15.4)	7 (21.2)	2.92 (0.41–20.91)	0.374
T allele	16 (61.5)	32 (48.5)	1.00 (Ref.)	
C allele	10 (38.5)	34 (51.5)	1.70 (0.67–4.29)	0.259
2677T>G				
TT	5 (15.2)	5 (38.5)	1.00 (Ref.)[a]	
TG	23 (69.7)	6 (46.2)	0.26 (0.06–1.21)	0.109
GG	5 (15.2)	2 (15.4)	0.40 (0.05–3.13)	0.622
T allele	33 (50.0)	16 (61.5)	1.00 (Ref.)	
G allele	33 (50.0)	10 (38.5)	0.63 (0.25–1.58)	0.318
3435T>C				
TT	7 (53.9)	8 (24.2)	1.00 (Ref.)[a]	
TC	3 (23.1)	20 (60.6)	5.83 (1.20–28.38)	0.030*
CC	3 (23.1)	5 (15.2)	1.46 (0.25–8.43)	1.000
TT+CC	10 (76.9)	13 (39.4)	1.00 (Ref.)[b]	
TC	3 (23.1)	20 (60.6)	5.13 (1.18–22.25)	0.022*
T allele	17 (65.4)	36 (54.6)	1.00 (Ref.)	
C allele	9 (34.6)	30 (45.5)	1.57 (0.61–4.04)	0.344

INS – idiopathic nephrotic syndrome; RE – responders; NRE – non-responders; OR – odds ratio; CI – confidence interval; Ref. – reference; a codominant model; b overdominant model; * a significant association.

the first study evaluating *MDR1* polymorphisms in childhood INS in a Slovak population.

Although the mechanisms of pathogenesis of INS are not clear, the justification for selecting the study of the *MDR1* gene in INS was supported by evidence of P-gp distribution and expression in many tissues with barrier function, such as intestinal tissue, liver cells, and proximal tubular epithelial cells (impact on efflux of exogenous and endogenous toxins), as well as in the membranes of leukocytes, which play an important role in the pathogenesis of immune-inflammatory diseases. Wasilewska et al. found significantly higher P-gp expression on CD3-positive lymphocytes in patients with INS than in controls [27]. Moreover, association between *MDR1* SNPs and inflammation has been reported [28,29].

In the present study, the *MDR1* genotype and allele distribution of all 3 tested SNPs were not significantly different between the patient and the control group. There have been only 4 studies conducted to evaluate *MDR1* SNPs in children with INS. Similar to our results, other researchers found no association between *MDR1* SNPs and INS susceptibility; however, in a Polish study, all INS patients were RE [19,20]. On the other hand, our results are in contrast to studies carried out in different populations, where significant differences in genotype or allele frequencies of *MDR1* 2677T>G/A and 3435T>C SNPs were observed between INS patients and control subjects. In an Indian population, people with 3435TT genotype and 2677TT+AA genotype appeared to be at increased risk of INS [21]. Similar results were observed in an Egyptian population, where *MDR1* 2677GT, GA, TT+AA genotypes or T allele,

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Table 5. Haplotype frequency distribution of *MDR1* gene polymorphisms among control subjects and INS patients and with respect to therapeutic response.

Haplotype	Controls N=100	INS patients N=46	*p* value	RE N=33	NRE N=13	*p* value
TTT	40.3%	47.5%	0.240	43.5%	57.5%	0.235
CGC	34.1%	37.7%	0.502	40.5%	30.6%	0.367
CGT	12.8%	7.9%	0.177	7.9%	7.9%	0.951
TTC	5.6%	3.6%	0.433	3.4%	4.1%	0.827
TGT	3.3%	0.0%	0.310	0.0%	0.0%	–
TGC	2.3%	1.1%	0.429	1.6%	0.0%	0.434
CTT	1.6%	2.2%	0.685	3.1%	0.0%	0.709

INS – idiopathic nephrotic syndrome; RE – responders; NRE – non-responders.

MDR1 3435TT genotype, and T allele genotype frequencies were significantly increased in the INS group [22].

Recently, *MDR1* genotyping has attracted research attention to the possibility of personalized treatment through identification of RE and NRE to a certain class of pharmacotherapy. Selection of tested SNPs in INS patients in this analysis was also based on knowledge of the importance of P-gp in pharmacokinetics or pharmacodynamics of the most commonly used GC in the treatment of the INS, including prednisone [8]. Furthermore, the importance of changes in P-gp expression in relation to TR has been confirmed in INS patients. Significantly lower expression of *MDR1* and P-gp activity in T lymphocytes was detected in steroid- and cyclosporine-sensitive patients compared with resistant patients [30]. In our study, a significantly increased chance of TR to GC was shown in children carrying the MDR1 3435CT genotype. No significant differences in the distribution of the *MDR1* 1236T>C and 2677T>G genotypes or alleles between steroid RE and NRE were confirmed. In contrast, Choi et al. found significantly higher frequencies of the *MDR1* 1236CC genotype and C allele in the initial steroid RE than in NRE [20]. A study from India revealed a significant association between steroid TR in INS children and 2677T>G/A polymorphism [21]; a higher frequency of TT+AA genotypes was detectable in NRE in comparison to RE. Similarly, in an Egyptian study, steroid NRE had significantly higher frequencies of *MDR*1 2677GT, GA, and TT+AA genotypes than responsive INS patients [22]. In a Polish study evaluating only steroid RE, a strong association was observed between all tested *MDR1* polymorphisms and time to initial steroid response [19]. The frequencies of 1236TT, 2677TT, and 3435TT genotypes were higher in late RE (time to remission: ≥7 days) than in early RE (time to remission: <7 days). There is currently no clear understanding of exactly how a heterozygous state in 3435T>C SNP confers GC sensitivity. However, some researchers proposed that heterozygous carriers of certain *MDR1* variants are less responsive to infection [31].

The tested *MDR1* polymorphisms are in strong linkage disequilibrium, and haplotype analysis was also evaluated. We identified TTT and CGC haplotypes as the most prevalent. Similar observations have been reported in other populations [19,20]. Our results revealed no significant differences in the distribution of *MDR1* haplotypes between INS patients and controls. Moreover, we did not observe any significant difference in the distribution of these haplotypes between initial steroid RE and NRE. Haplotype variations did not influence age at onset of INS or renal pathology. Similarly, Jafar et al. did not find a significant correlation between *MDR1* haplotypes and TR [21]. However, the haplotype CGC was significantly more common in patients with age at the onset ≥6 years than among patients with an age at the onset <6 years. In contrast, the frequency of the *MDR1* TGC haplotype was significantly higher in the initial steroid NRE than in RE in 2 studies [20,22]. In a Polish study, the TTT haplotype was found to be significantly associated with late oral steroid response [19].

We also tested the influence of *MDR1* SNPs on age at the onset of INS. Interestingly, *MDR1* 1236T>C and *MDR1* 2677T>G SNPs were significantly associated with age at onset. Our preliminary data indicate that genotypes 1236CC or 2677GG could delay early onset of INS but they did not influence the susceptibility to INS. We suppose that these polymorphisms might play a role in pathogenesis of NS in association with some other currently unknown predisposing factor(s). Similarly, Youssef et al. revealed significantly higher frequencies of 2677GG genotype and 3435CC genotype in patients ≥6 years old at the onset of INS [22]. This is in contrast with previous observations showing that age at onset of INS was not influenced by any *MDR1* genotype or allele variations in Polish and Korean children

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[19,20]. In another study, age at onset of INS was correlated only with *MDR1* 3435T>C SNP [21].

There are a few issues that should be discussed regarding the inconsistent results of studies evaluating P-gp and *MDR1* polymorphisms in INS patients. First, studies were done in patients with different ethnicities. It should be noted that P-gp expression and influence of *MDR1* SNPs may vary depending on ethnicity and environmental factors [32]. Second, the homogeneity of the tested group is uncertain because of the unknown pathophysiology of the diseases and because of several histological patterns included in the diagnosis of INS. Also, other factors might have influenced the results, such as drug and food interactions [33]. Finally, P-gp has functions other than those directly related to transport activity, and the impact of genetic polymorphisms on these activities may be more pronounced. All steroid-resistant patients were screened for NPHS1 and WT1 mutations, with negative results, but there are also other gene mutations associated with steroid resistance [34].

In this study, histological pattern and age at onset of INS were significantly associated with the initial steroid response. Patients with MCD were initial RE more frequently when compared with children with FSGS, which is in accordance with the data that has been published earlier [31–33]. Furthermore, a significantly higher frequency of RE was observed in patients younger than 6 years of age at the onset of INS. The higher frequency of MCD in patients younger than 6 years of age could be responsible for better TR.

The main limitation of our study was the relatively small sample size, which was because it was a single-center study and there generally are low numbers of children with INS in small populations. Therefore, we cannot exclude the possibility of bias and our preliminary results must be interpreted with caution. Moreover, because of the retrospective patient data collection from medical records and missing information, the evaluation of other parameters describing the clinical course and TR (such as early or late initial response to steroids) could not be made.

Conclusions

Our findings suggest that prednisone therapeutic response may be influenced by histology, age at onset of INS, and by *MDR1* 3435T>C polymorphism. The *MDR1* 1236T>C and 2677T>G polymorphisms were significantly associated with age at onset. Larger multicenter studies and studies across other ethnic groups are needed to elucidate the contradictory implications of *MDR1* polymorphisms in children with INS.

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Conflict of interest

The authors declare no conflict of interest in relation to the article.

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