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Frequency of the C1236T, G2677T/A and C3435T *MDR1* gene polymorphisms in the Serbian population

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Abstract:
The multi-drug resistance 1 (*MDR1*) gene encodes for a P-glycoprotein (PGP), which acts as a gate-keeper against various kinds of xenobiotics. Several single nucleotide polymorphisms (SNPs) in the *MDR1* gene that may influence PGP level and function have been identified. The aim of this study was to simultaneously analyze the three most important *MDR1* SNPs, C3435T, G2677T/A and C1236T, in the Serbian population and to compare the results with those published for other ethnic groups. A group of 158 unrelated, healthy subjects was included in the present study. For determination of *MDR1* SNPs, a multiplexed mutagenically separated PCR was performed. The genotype frequency of the analyzed *MDR1* SNPs was as follows: 3435 nt – 0.19 (CC), 0.54 (CT) and 0.27 (TT); 2677 nt – 0.26 (GG), 0.52 (GT), 0.15 (TT), 0.03 (GA) and 0.064 (TA), and 1236 nt – 0.23 (CC), 0.61 (CT) and 0.16 (TT). Our results for the Serbian population could be relevant for further investigation of drugs that are substrates of PGP and for studies of interethnic diversity in *MDR1* polymorphism frequency.

Key words:
MDR1, genetic polymorphism, P-glycoprotein, C1266T, G2677T/A, C3435T

Introduction

P-glycoprotein (PGP) is a transmembrane transporter protein expressed in various organs, including the liver, kidney, intestine and blood-brain barrier. It acts as a gate-keeper against numerous xenobiotics. It plays a protective role for cells in mediating DNA damage, secretion of toxic compounds, apoptosis and the immune response [27]. The range of substrates transported by PGP is broad and includes drugs used for treatment of hypertension, allergy, infection, immunosuppressive agents, and cancer chemotherapy [24].

PGP is a product of the multi-drug resistance gene (*MDR1*) [36], which is located on the human chromosome 7p21-21.1 and extends over greater than 100 kb. Genetic variants of *MDR1* can influence inter-individual variability in the bioavailability and pharmacoki-

netics of various drugs [13]**.** The most significant correlation was found between the PGP expression level and the C3435T polymorphism in exon 26 [14]. Individuals homozygous for 3435T had significantly lower *MDR1* and PGP expression levels than homozygous C3435 carriers [14]. Two additional single nucleotide polymorphisms (SNPs), G2677T/A in exon 21 and C1236T in exon 12, were found to be in linkage disequilibrium with *MDR1* C3435T and also seem to influence PGP function [5].

So far, significant interethnic differences in allele and genotype frequencies of C3435T and other *MDR1* SNPs have been reported. Considering the well-known influence of *MDR1* on the bioavailability and pharmacokinetics of various drugs, genotyping of *MDR1* polymorphisms and determination of haplotypes may become an important tool for predicting individual susceptibility to development of drug resistance. In this investigation, the allele and genotype frequencies of the C1236T, G2677T/A and 3435T *MDR1* gene were determined in the Serbian population to obtain data relevant for this ethnic group.

Materials and Methods

Subjects

After obtaining the patient's informed consent for DNA analyses, five ml of peripheral venous blood were drawn with EDTA as an anticoagulant from 158 healthy, unrelated subjects (113 male, 45 female) during routine venepuncture. All subjects were of Slavic origin, Caucasian, from southeast Serbia, and aged 48.35 ± 9.8 years on average, with an age range from 19 to 65 years. The study was approved by the Research Ethics Committee of the Medical Faculty in Nis, University in Nis (approval no. 01-1591/10).

PCR analysis

DNA was isolated according to standard procedures. For determination of the *MDR1* SNPs (C3435T, G2677T/A, C1236T), a mutagenically separated PCR (MS-PCR) protocol was followed. This single-tube-based PCR-technique relies on allele-specific primers that differ in length by 8–10 bp for each SNP and results in PCR products of different sizes. Various single-base mismatches in the allele-specific primers introduce deliberate differences into the allelic PCR products to minimize cross-reactions between primers and PCR products in subsequent cycles (primer sequences are shown in Tab. 1). All primers were synthesized by TibMolBiol (Berlin, Germany) and were purified by HPLC. The reaction mixture contained 2 µl of DNA (pre-diluted to 1:50; approx. 4 ng of genomic DNA), 2 µmol of each dNTP (Amersham Pharmacia Biotech, Uppsala, Sweden), 1.5 mmol/l $MgCl_2$, 0.5 U of AmpliTaq Gold (Perkin Elmer Cetus, Norwalk, CT, USA), 2 µl of 5x Rapid Load Buffer, 3 µl of 10x PCR Buffer (Perkin Elmer) and 1 pmol of the primers for G2677, C3435, 3435T and 3435 common reverse, 2 pmol of C1236, 5 pmol of 2677 common reverse, 10 pmol of 2677T and 2677A, and 20 pmol of 1236T and 1236 common reverse in a reaction volume of 30 µl. PCR was performed in an Eppendorf cycler (Mastercycler, Eppendorf AG, Hamburg, Germany) as follows: after a denaturation period of 10 min at 95°C, the reaction mixture was subjected to 35 cycles of denaturation for 45 s at 95°C, annealing for 45 s at 49°C, elongation for 45 s at 72°C and a terminal extension period of 5 min at 72°C. Subsequently, the PCR products were separated for 55 min at 150 V on pre-cast 5% TBE polyacrylamide gels (Criterion, Bio-Rad Inc., Hercules, CA, USA) and visualized by staining double-stranded DNA with SybrGreen (1:10,000 dilution, Molecular Probes, Eugene, OR, USA) followed by exposure to UV light and digital imaging.

For each SNP, one or two different PCR products were amplified, depending on the genotype. The product lengths were as follows: 2677A, 175 bp; 2677T, 169bp; 2677G, 161bp; 3435T, 135 bp; 3435C, 126 bp; 1236, 114 bp; and 1236C, 105 bp [33].

Tab. 1. Primers used for multiplex *MDR 1* PCR

	Primer Sequence 5´ to 3´
Primer 2677 t	CAC TGA AAA TAA AGA AAG AAC TAG AAT GTT
Primer 2677 a	GAC AAG ATC TGA AAT AAA AGA AAG AAC TAG TAG GTA
Primer 2677 g	GAT AAG AAA GAA CTA GAG GGT G
Primer 2677 rev	GAA AAA GAT TGC TTT GAG GAA TGG
Primer 3435 c	GGT GTC ACA GGA AGA GAT C
Primer 3435 t	CAG CCG GGT ATA GTC ACA GGA AGA TAT T
Primer 3435 rev	GGC CAG AGA GGC TGC CAC AT
Primer 1236 t	CTC ACT CGT AAA GGT AGA TCT TGA AGA GT
Primer 1236 c	CCT GGT AGA TCT TGA ACG GC
Primer: 1236 rev	GCA TCA GCT GGA CTG TTG TG

Tab. 2. Allele and genotype frequencies of *MDR1* SNPs in healthy Serbian subjects

SNP	Exon	Allele frequency			Genotype frequency					
C3435T	26	C	T		CC	CT	TT			
		0.47	0.53		0.19	0.54	0.27			
G2677T/A	21	G	T	A	GG	GT	GA	TT	TA	AA
		0.53	0.43	0.04	0.26	0.52	0.03	0.15	0.04	0.00
C1236T	12	C	T		CC	CT	TT			
		0.54	0.46		0.23	0.61	0.16			

Tab. 3. Genotype frequencies of MDR1 variants observed in this study compared with those found in other populations

Position		C3435T			G2677T/A						T1236C			Reference
Population	N	CC	CT	TT	GG	GT	GA	TT	TA	AA	CC	CT	TT	
Serbian	158	0.19	0.54	0.27	0.26	0.52	0.03	0.15	0.04	0.00	0.23	0.61	0.16	This study
German	461	0.21	0.50	0.29	0.31	0.49	0.02	0.16	0.02	0.00	0.35**	0.49*	0.16	[4]
Russian	290;59	0.21	0.49	0.30	0.30	0.45	0.04	0.18	0.03	0.00	0.24	0.56	0.20	[10, 11]
Portuguese	100	0.12	0.47	0.41*	0.31	0.43	N. a.	0.26*	N. a.	N. a.		N. a.		[6]
Turkish	150;100	0.20	0.53	0.27				N. a.			0.20	0.51	0.29*	[12, 35]
Polish	204	0.22	0.51	0.27	0.39*	0.40*	0.02	0.17	0.02	0.00		N. a.		[22]
Czech	189	0.21	0.45	0.34	0.30	0.47	0.01	0.22	0.00**	0.00*	0.32	0.47*	0.21	[28]
UK (Caucasian)	190	0.24	0.48	0.28				N. a.				N. a.		[1]
Spanish	204	0.27	0.51	0.22				N. a.				N. a.		[37]
Japanese	154	0.36**	0.47	0.17*	0.19	0.32**	0.15**	0.18	0.13**	0.03	0.11**	0.47*	0.42**	[19]
Chinese	200	0.30*	0.53	0.17*	0.18	0.37**	0.11**	0.21	0.10*	0.03	0.11**	0.47*	0.42**	[39]

N. a. – not assessed. * $p < 0.05$, ** $p < 0.01$ compared with the Serbian population

Statistical analysis

Allele and genotype frequencies for *MDR1* SNPs were assessed for deviation from the Hardy-Weinberg equilibrium using the Fisher exact test. The F-statistic was used to compare the genotype frequencies of different populations for C3435T, G2677T/A, C1236T SNPs, $p < 0.05$ was considered statistically significant.

Results

We analyzed samples obtained from 158 healthy subjects to detect *MDR1* polymorphisms in positions 1236, 2677 and 3435. The allele and genotype frequencies of *MDR1* variants are given in Table 2. In healthy Serbians, the frequencies of analyzed *MDR1* SNPs were as follows: 3435 – 0.19 (CC), 0.54 (CT) and 0.27 (TT); 2677 – 0.26 (GG), 0.52 (GT), 0.15 (TT), 0.03 (GA) and 0.04 (TA), and 1236 – 0.23 (CC), 0.61 (CT) and 0.16 (TT). The observed genotype frequencies did not deviate significantly from those expected at Hardy-Weinberg equilibrium.

The genotype frequencies of *MDR1* variants observed in healthy Serbians, compared with those found in other populations, are shown in Table 3.

The relative frequencies of all possible combined C3435T and G2677T/A genotypes are described in Table 4. The wild-type sequence in both of these SNPs sites was found in 22 subjects, while the number of

heterozygotes for both SNPs was 60 (38% of investigated individuals). The homozygous presence of variant sequences in both positions was found in 13% of the subjects.

Discussion

Expression of P-glycoprotein, the product of the *MDR1* gene, is an important factor influencing the bioavailability of many cardiovascular and anticancer medications with a narrow therapeutic window. Because *MDR1* polymorphisms have an impact on the pharmacokinetic and pharmacodynamic profiles of drug substrates and directly influence the outcome and prognosis of certain diseases [26], it is clear that *MDR1* polymorphism analysis can provide important information to optimize the individualized therapeutic approach. Significant interethnic variations have been identified worldwide, but there were no data available for the Serbian population. Determination of the prevalence of functionally important SNPs in the *MDR1* in this population is of great interest, because determination of the *MDR1* will become increasingly important.

Currently, more than 100 mutations have been identified in the human *MDR1* gene [23]. A conservative, wobble polymorphism in exon 26 (C3435T) is the most commonly reported SNP of the *MDR1* gene. Although silent, it was demonstrated to affect the function of P-glycoprotein by altering its substrate specificity [18]. Additionally, it was found to be associated with reduced expression of PGP in the intestinal epithelium [36]. Studies of C3435T suggested a correlation between allele and genotype frequency and variability in response to drug treatment. This synonymous polymorphism has been associated with higher oral bioavailability of digoxin in patients homozygous for the variant T allele [36], but lower plasma concentrations after oral doses of fexofenadine [17] and nelfinavir [9]. This SNP has also been linked to better CD4+ cell recovery in HIV-infected patients who were treated with HIV protease inhibitor [9], lower frequency of resistance to anti-epileptics [31] and beneficial pharmacological effects of atorvastatin [16]. The role of C3435T in disease susceptibility has also been evaluated [7, 30], and it was linked with a risk for cancer development [15, 21, 32]. Recent findings suggested that the risk of male infertility is significantly elevated in individuals carrying at least one T allele [8].

The frequency of wild-type and variant alleles in position 3435 detected in a healthy, southeast Serbian population did not differ significantly from those reported for other Caucasian populations, except for those reported for the Portuguese [6]. The homozygosities for both wild-type and variant alleles were significantly lower than those observed in Asian populations [19, 39] but were similar to those observed in other European ethnicities [4, 22, 35].

The C3435T SNP was found to be linked to a G2677T transversion in exon 21 (Ala893Ser) and a synonymous C1236T SNP in exon 12; however, it should be noted that the linkage between wild-type-wild-type and variant-variant sequences in these positions is not complete and can occur independently [17]. Similar findings were observed for the healthy Serbian subjects in the present investigation. Almost 65% of our subjects were either wild-type or variant homozygotes or were heterozygotes simultaneously in both SNPs. The contradictory results in studies investigating drug efficacy/toxicity or disease susceptibility in correlation with a single, specific *MDR1* polymorphism suggested the need for complete haplotype analyses instead of individual analyses within

Tab. 4. Linkage of *MDR1* polymorphisms between positions 2677 and 3435

	2677GG		2677GT		2677TT		2677GA		2677AT	
	N	f	N	f	N	f	N	f	N	f
3435CC	**22**	**0.14**	3	0.019	1	0.006	4	0.025	1	0.006
3435CT	17	0.11	**60**	**0.38**	3	0.019	1	0.006	4	0.025
3435TT	2	0.012	19	0.12	**19**	**0.12**	**0**	0	2	0.012

N – number of subjects, f – relative frequency

the study population; this is expected to provide a stronger prediction of the PGP phenotype. Haplotype analysis in other healthy populations indeed suggested strong linkage disequilibrium between the C3435T SNP and many SNPs across the *MDR1* gene [20, 34].

G2677T/A was the first *MDR1* polymorphism identified [25]. Among commonly observed SNPs, it is the only polymorphism that results in an amino acid change (Ala893Ser/Thr), with three possible variants at the same gene locus. This non-synonymous polymorphism has great impacts on both the ATPase activity and the substrate specificity of PGP toward various drugs [29]. The silent C1236T polymorphism in exon 12 (Gly412Gly) has also been linked to variability in the response to various PGP substrates. It has been shown that the frequency of the mutant T allele was higher in late responders to oral prednisone than in early responders among children with steroid-responsive nephrotic syndrome [38]. The homozygous variant allele carriers presented with a decreased docetaxel clearance [3]. The G2677T/A and C1236T polymorphisms have also been linked to several diseases [2, 22].

In this study, the observed distribution of alleles in locus 2677 was similar to that in most European populations. No carriers of the homozygous 2677A allele were found, although heterozygous genotypes containing the 2677A allele were found in twelve subjects. Our results showed a highly significant difference for C1236T, compared with the Japanese and Chinese populations. In general, the A allele in exon 21 and the T allele in exon 12 are significantly more common in Asian populations than in the Serbian population or in other Caucasian populations.

Turbulent historical circumstances and a geographical location on the crossroads of Western and Eastern cultures defined the Serbian population as open to the influences of neighboring and other European and Asia Minor ethnicities. Therefore, because of the common Indo-European origin, we expected to find a similar *MDR1* polymorphism distribution in study population as has been observed in other European and Asian populations.

In conclusion, our study established the frequency of *MDR1* C3435T, G2677T/A and C1236T polymorphisms in the Serbian population. Considering the number and significance of PGP substrates, determination of the frequency of functionally important SNPs in the *MDR1* gene provides substantial information for the assessment of inter-individual differences in drug response and for the prediction of side effects and the likelihood of adverse reactions during treatment with PGP-modulated therapeutics. Our results for the Serbian population could be relevant to further investigations of PGP-substrate drugs and for studies of interethnic diversity in *MDR1* polymorphism frequency.

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The authors declare no conflict of interest.

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