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GENOTYPE VARIABILITY AND HAPLOTYPE PROFILE OF *ABCB1 (MDR1)* GENE POLYMORPHISMS IN MACEDONIAN POPULATION

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Abstract

The aim of this study was to evaluate the most common *ABCB1* (*MDR1*, P-glycoprotein) polymorphisms in the population of R. Macedonia and compare the allele and haplotype frequencies with the global geographic data reported from different ethnic populations. The total of 107 healthy Macedonian individuals from the general population was included.

Genotypes for the *ABCB1* for three polymorphisms C1236T [rs1128503], G2677A/T [rs2032582] and C3435T [rs1045642] were analyzed by Real-Time PCR. Obtained allele frequencies for these three SNPs were similar to those observed in other European Caucasians. The detected genotype frequencies were 33.6% for 1236CC, 44.9% for 1236CT and 21.5% for 1236TT in exon 12; 32.7%, 44.9% and 22.4% for 2677GG, 2677GT and 2677GT consecutively in exon 21; and 25.2% for 3435CC, 52.3% for 3435CT and 22.5% for 3435TT in exon 26.Strong LD was observed in our study among all three SNPs with the highest association confirmed for C1236T and G2677T (($D\hat{o} = 0.859$, $r^2 = 0.711$). Eight different haplotypes were identified and the most prominent was the CGC haplotype (45.3%). Our study was the first to have documented the distribution of *ABCB1* alleles, genotypes and haplotypes in the population of R. Macedonia. The obtained results can help in the prediction of different response to the drugs that are P-glycoprotein substrates. Additionally, in the era of individualized medicine the determination of the P-glycoprotein genotype might be a good predictive marker for determination of the subpopulations with higher risk to certain diseases.

Key words: *ABCB1 (MDR1)*, P-glycoprotein, C1236T, G2677A/T, C3435T, interethnic differences, haplotypes Macedonian population

Introduction

ABC transporters play a major role in host detoxification and protection against xenobiotics and their importance is highly substrate-dependent. P-gp is a pivotal member of ABC transporters for pharmacological profile of many drugs. Among the 48 genes, forming 7 different subfamilies (A-G) based on sequence similarities, in the ATP-binding cassette (ABC) *ABCB1* (*MDR1*, P-glycoprotein) gene is the first identified and the best characterized. This gene encodes transmembrane protein that mediates ATP-dependent transport of various molecules. It is located in the chromosome 7q21.1 and consists of 28 translated exons and 27 introns with over 100 kb. It is 1280 amino acid polypeptide assembled in two nearly homogeneous halves, each containing six transmembrane spanning domains and one ATP binding domain [1]. P-gp is 170kDa transmembrane

protein and is widely expressed in normal tissue. The distribution of P-gp includes the epithelial cells lining the luminal surface of enterocytes in the lower gastrointestinal tract (jejunum, ileum and colon), influence the absorption and limit the bioavailability of variety of structurally diverse drugs. P-glycoprotein expression on the canicular surface of hepatocytes and the apical surface of epithelial cells of proximal renal tubules influence the metabolism and secretion of toxic xenobiotic, drugs and their metabolites. P-gp distribution on epithelial cells of placenta on the maternal blood flow side plays protective role for the child, whereas P-gp expression in the luminal surface of capillary endothelial cells of the blood-brain barrier (BBB) transport the toxic compounds out of the brain and effectively prevent the uptake [1, 2]. This transport protein is expressed and translocated to membranes of lymphocytes and hematopoietic stem cells where it contributes to decreased response in HIV and leukemia treatment [3–5]. P-gp can influence bioavailability and therapeutic response of many drugs, such as drugs including anticancer, antiarrhythmic, glucocorticoids, antipsychotics, antiepileptic's, antidepressants, opioids and many other [6, 7].

The *MDR1* gene is highly polymorphic. To date, 4456 SNPs are listed in the NCBI database, out of which 367 are in the coding region. According to PharmGKB, 41 are missense SNPs and 124 polymorphic sites have allele frequency higher than 5% [3, 8]. Polymorphic variations on *ABCB1* (*MDR1*) gene influence on its expression [9, 10], on their association with pharmacokinetics and bioavailability of drugs [11, 12] and on their association with clinical effects [13, 14]. Among 68 SNPs genotyped in various ethnical populations, 17 SNPs are in 5'-region, 19 in exonic regions (14 missense and 5 silent SNPs), 25 in intronic regions and 13 in the 3'-untranslated region (UTR) [3]. Intronic SNPs usually alter mRNA generation, integrity or processing. On the other hand SNPs on the coding region may alter the protein structure directly by amino-acid substitution (non-synonymous SNPs) or altering the mRNA sequence, its structure and stability despite coding the same amino acid (synonymous SNPs) [15]. DNA variations may influence the inter-individual differences in treatment response, alternation of drug efficacy and onset of adverse side effects in patients with specific genetic profile.

Beside the huge number of identified SNPs in *ABCB1* gene, most studies are focused on three SNPs, namely 1236C > T in exon 21 and 3435C > T in exon 26 that are synonymous, and 2677G > T/A in exon 21 that is non-synonymous triallelic polymorphism responsible for Ser to Ala/Thr amino acid substitution at position 893. They are in high linkage disequilibrium [16] and the frequencies of these allelic variations occur at different frequencies among populations and subpopulations of different ethnic or racial origin. 1236C > T SNP refers to the glycine residue located in the external surface of the N-terminal domain (NBD). 3435C > T correlates to isoleucine residue located in the internal regions of C-terminal NBD. This non-synonymous variation is associated with altered P-gp activity. To date there have been vast number of reports correlating the presence of these three SNPs with treatment response and disease predisposition and progression (renal tumor, Chron's disease and ulcerative colitis, Parkinson's disease, schizophrenia, Alzheimer, HIV infection) [9, 17–21]. Most common approach in determination of the influence of gene variation is haplotype assessment because it may provide a better understanding of the observed inconsistences and are promising predictor of the functional consequences of *ABCB1* polymorphisms. It has been confirmed that the use of *ABCB1* haplotypes is superior in prediction of the pharmacokinetics of digoxin [22], cyclosporine [23] and fexofenadine [24], as well as intestinal expression of *ABCB1* mRNA [25]. A correlation was observed between the 1236T, 2677T and 3435T haplotype and decreased irinotecan clearance in Japanese patients with cancer. Also, 2677GG genotype and 2677G-3435C haplotype were associated with significantly better chemotherapy response [26].

The aim of our study is to estimate the genotype and haplotype frequencies for C1236T, G2677T/A and C3435T polymorphisms in healthy Macedonian individuals in order to provide useful findings that might help in future studies on bioavailability and pharma-

cokinetics of drugs, interindividual treatment response variations, as well as prevalence of some certain pathologies.

Martials and methods

The study included 107 unrelated healthy ethnical Macedonians of both sexes (76 males and 31 females) from Republic of Macedonia. DNA samples were selected from the DNA bank in the Centre for Bimolecular and Pharmaceutical Analysis (CBPA) in the Faculty of Pharmacy in Skopje from individuals that were previously enrolled in other research studies that were approved by Ethical committee of the Faculty of Pharmacy University "Ss. Cyril and Methodius", Skopje, Republic of Macedonia. DNA samples were tested anonymously with previously removed personal data. All procedures were conducted in accordance with Declaration of Helsinki.

DNA isolation

The genomic DNA was extracted from peripheral lymphocytes in the blood samples obtained in EDTA vacutainers, using Proteinase K digestion, phenol chloroform extraction and ethanol precipitation. DNA yields and purity were measured at 260 nm and 260/280 nm respectively (NanoDrop 2000, Thermo Scientific) and DNA integrity was confirmed with electrophoresis on 1% agarose gels, stained with ethidium bromide.

Genotyping

The genotyping was performed with Real-Time PCR based on the allelic discrimination method (MxPRo 3005P, Staratgene, La Jolla, CA, USA) using TaqMan SNP genotyping assay for C1236T (rs1128503 assay ID C_7586662_10), G2677A/T (rs2032582 assay ID C_11711720_C_30 and C_11711720_C_40) and C3435T (rs1045642 assay ID C_7586657_20) according to the guidelines of the manufacturer (Life Technologies, USA).

Data Analysis

Statistical analysis was performed using SPSS software (v. 22). The genotype distributions were assessed for the Hardy-Weinberg equilibrium (HWE) with χ^2 test using an online calculator (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). Statistical analysis for allele and genotype frequencies between our and other ethnic populations was evaluated with Chi-squared analysis and Fisher exact probability test. Odds ratios (OR) were calculated with 95% confidence interval limits (95% CI). The level of statistical significant was defined as $p \leq 0.05$. Linkage disequilibrium (LD) between SNP pairs in the population was estimated by Lewontin's coefficient (*D'*) and Pearson 's correlation (r^2) (Lewontin and Kojima, 1960; Lewontin 1964). The statistical analyses were performed with SHEsis software platform for analysis of LD, haplotype and genetic association at polymorphism loci (http://analysis2.bio-x.cn/myAnalysis.php) [27, 28].

Results

The distributions of the *ABCB1* allele, genotype and haplotype frequencies of C1236T, G2677T/A and C3435T genetic variations in *ABCB1* gene for Macedonian healthy population are summarized in Table 1. All the determined distributions were in agreement with those predicted by the Hardy-Weinberg equilibrium. According to our results, the frequencies of wild-type alleles for C1236T (56%), G2677T/A (55%) and C3435T (51%) in our study were similar to the general frequencies reported for the Caucasians of European descendant, but differ from those of Asian and African population.

Frequencies of the wild-type C and mutant T alleles on 1236 locus were 56.07% and 43.93% respectively. The observed genotype frequencies were 33.6%, 44.9% and 21.5% for 1236CC, 1236CT and 1236TT in exon 12. None of the examined individuals was carrier of mutant A allele at position 2677, so the allelic distribution for this position were 55.14% for G allele and 44.86% for the mutant T allele. The observed genotype frequencies were 32.7%, 44.9% and 22.4% for 2677GG, 2677GT and 2677GT consecutively. Allelic frequencies in exon 26 were 51.4% for C allele and 48.6% for mutant T allele, whereas the observed genotype frequencies were 25.2% for 3435CC, 52.3% for 3435CT and 22.5% for 3435TT.

When analyzing the linkage between C1236 and G2677T the most frequent genotype was CT-GT (39%) fallowed by CC-GG (29%)

Table 1

Allele, genotype and haplotypes frequencies of ABCB1 C1236T, G2677T/A and C3435T polymorphisms identified in Macedonian population

SNP	Position	Exon	Allele	n=214 (Total chromosomes)	Allele freq	p	Genotype	n=107 (Total individuals)	Freq. (%)		Genotype combinations				Frquency estimated on the base of genotyping results	
									obs	exp	C1236T-G2677T-C3435T			Freq (%)	C1236T-G2677T-C3435T	Freq (%)
rs.1128503	1236	12	C	120 (56.07)	0.56 +/-0.035	p=0.355	CC	36	33.64	31.36	CC	GG	CC	21.50	CGC	45.33
			T	94 (43.93)			CT	48	44.86	49.26	CT	GT	CT	31.78	TTT	37.85
							TT	23	21.50	19.30	TT	TT	TT	15.89	CGT	7.48
rs.2032583	2677	21	G	118 (55.14)	0.55 +/-0.036	p=0.335	GG	35	32.71	30.40	CC	GG	CT	7.48	CTT	2.34
			W (T/A)	96 (44.86)			GT	48	44.86	49.47	CT	GT	TT	5.61	CTC	1.87
							TT	24	22.43	20.12	CC	GT	CT	3.74	TTC	2.34
rs.1045642	3435	26	C	110 (51.4)	0.51 +/-0.033	p=0.623	CC	27	25.23	26.42	CT	TT	CT	2.80	TGC	1.40
			T	104 (48.6)			CT	56	52.34	49.96	TT	TT	CT	2.80	TGT	1.40
							TT	24	22.43	23.62	CT	GG	CT	1.87	Total	100.00
											CT	GT	CC	1.87		
											CC	GT	CC	0.93		
											CT	TT	TT	0.93		
											TT	GG	CC	0.93		
											TT	GG	CT	0.93		
											TT	GT	CT	0.93		
											Total			100.00		

and TT-TT (20%) (Table 2). Considering the genotype observed between G2677T/C3435T and C1236T/C3435T SNPs we obtained exactly the same frequencies. The highest frequencies were CT-GT (36%), than CC-GG (22%) and TT-TT (17%). The haplotype combinations and their frequencies estimated on the base of genotype combination are presented in the table 2. The most frequent haplotypes were 1236C-2677G (51%), 1236C-3435C (47%) and 2677C-3435T (47%), followed by the mutant variants 1236T-2677T (41%), 1236T-3435T (41%) and 2677T-3435T (40%).

Table 2

Frequencies of different genotype combinations and haplotypes between different pairs of C1236T, G2677T and C3435T Polymorphisms identified in Macedonian population

	2677GG	Freq (%)	2677GT	Freq (%)	2677TT	Freq (%)	Haplotype	Frquency estimated on the base of genotyping results (%)
1236 CC	31	28,97	5	4,67	0	0,00	1236C-2677G	51,87
1236 CT	2	1,87	42	39,25	4	3,74	1236C-2677T	4,21
1236 TT	2	1,87	1	0,93	20	18,69	1236T-2677G	3,27
							1236T-2677T	40,65
	2677GG	Freq (%)	2677GT	Freq (%)	2677TT	Freq (%)	Haplotype	Frquency estimated on the base of genotyping results (%)
3435CC	24	22,43	3	2,80	0	0,00	2677G-3535C	47,20
3435CT	11	10,28	39	36,45	6	5,61	2677G-3435T	7,94
3435TT	0	0,00	6	5,61	18	16,82	2677T-3435C	4,21
							2677T-3435T	40,65
	3435CC	Freq (%)	3435CT	Freq (%)	3435TT	Freq (%)	Haplotype	Frquency estimated on the base of genotyping results (%)
1236 CC	24	22,43	12	11,21	0	0,00	1236C-3435C	47,20
1236 CT	2	1,87	39	36,45	7	6,54	1236C-3435T	8,88
1236 TT	1	0,93	5	4,67	17	15,89	1236T-3435C	4,21
							1236T-3435T	39,72

Pairwise LD profile for the three SNPs using D' and r^2 values are presented in Figure 1, and summarized in (Table 3). All three SNPs in Macedonian population are in high Linkage Disequilibrium. The strongest correlation was observed between C1236T and G2677T (D' = 0.859, r^2 = 0.711) followed by G2677T and C3435T (D' = 0.802, r^2 = 0.534) and C1236T and C3435T (D' = 0.795, r^2 = 0.802).

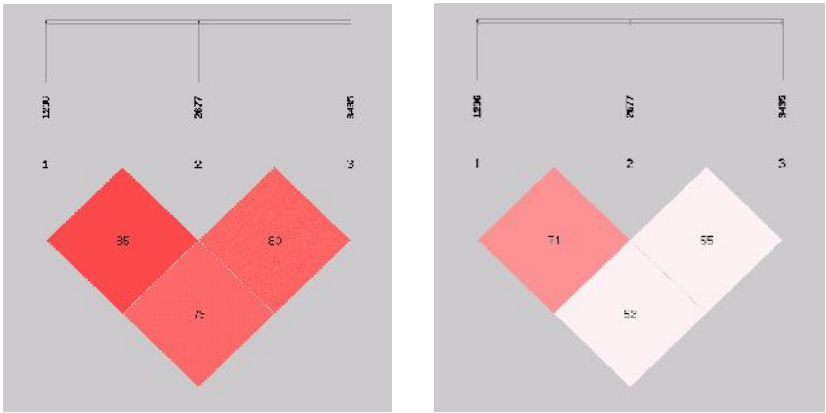
D'  r^2

Figure 1 – Pairwise LD profile for C1236T, G2677T and C3435T SNPs

Table 3

Tabular presentation of LD profile for C1236T, G2677T and C3435T SNPs

	C1236T	G2677T	C3435T	
C1236T		0.859	0.795	D'
G2677T	0.711		0.802	
C3435T	0.524	0.554		
	r2			

In our study 15 different genotype combinations among C1236, G2677T and C3435T SNPs were found, as far as we didn't identified carrier of 2677A mutant allele. Genotype combinations that were observed with frequencies higher than 3% were the following CT-GT-CT (31,8%), CC-GG-CC(21.5%), TT-TT-TT (15, 9%), CC-GG-CT (7.5%), CT-GT-TT (5.6%) and CC-GT-CT (3.7%) (Table1). In a population of healthy subjects of Macedonian ethnicity these three SNPs were structured in eight different haplotypes. The haplotype combinations CGC with 45.3%, TTT with 37.8% and CGT with 7.5% in our examined population were the most prominent.

Discussion

In recent years, there are vast numbers of published data that report interesting evidence for the influence of SNPs in the *ABCB1* gene on P-gp function. These polymorphic variants are potential determinants of inter-individual and inter-ethnical variability in drug response [29–31] and susceptibility to certain diseases.

In this study, we analyzed the three most common C1236T, G2677T/A and C3435T SNPs of *ABCB1* gene and we present the allele, genotype and haplotype frequencies in comparison with the general frequencies reported for other population.

The detected frequency of the wild type allele 1236C in exon 12 in population of ethnic Macedonians was 56.07% and it was similar to the general frequencies reported for the various ethnic groups throughout Europe; (52–58%). (Table 4) The literature data point to similar allele frequency of 1236C in our population (56%) and those reported for the Czech (51%), Serbians (0.53%), Hungarians (55%), Polish and French (57%), German and Polish (58%) populations, but in disagreement with the frequencies reported for the Romanian population (44%) ($\chi 2 = 5.79$, $p = 0.01505$) and Slovenian population (39%) ($\chi 2 = 5.79$, $p = 0.01614$). Wild type allele is most frequently observed in Moroccans (62.5%) and other African population, such as in Sub-Saccharin Africans where it estimates 88%. In Asian population the mutant T allele is more common and its frequency ranges among 59% in Chinese Uygur, 65% in Chinese Han and Japanese and 71% in Chinese population.

Homozygous mutant 1236TT genotype has been linked with reduced clearance of docetaxel [45] as well as, with better response to imatnib in patients with chronic myeloid leukemia (CML) [18].

The allelic frequencies of 55% for 2677G allele on exon 21 was found in our population. We fail to detect the mutant A allele in locus 2677, and this finding is in line with previously published data where this allele is absent in most of the European populations [32, 30]. The absence of this allele might have been result of small population size of study group. The 2677A allele is shows higher frequency in East Asia population such as Chinese [23], Japanese [33] and Korean population [34]. As the frequency of mutant A allele in European population is very low (1–3%), in our discussion the A allele was pulled with mutant T allele in "W" allele (Table 4). The frequency of G allele in our population is similar to the frequency of Germans (56%) White Italians (56%), Bulgarian and Hungarians (55%), Russians (54%), Serbians (53%), French (53%), and no statistically significant difference was observed between the allele frequency in Macedonians and other Europeans. The significant difference was confirmed only for Chinese Han population ($\chi^2 = 3.86$ with $p = 0.049$), Indian ($\chi^2 = 14, 59$, $p = 0.00013$), Vietnamese ($\chi^2 = 7.20$, $p = 0.007$), Brazilians intermediate ($\chi^2 = 10.69$, $p = 0.00108$) and the highest difference was confirmed for Brazilians-black ($\chi^2 = 32.29$, $p < 0.0000001$). Pharmacogenetic studies of this locus have confirmed that patients with non-small cell lung cancer that have 2677GG variant have better treatment response to chemotherapy in comparison to carriers of other genotypes [35]. Gonzales et al., [36] didn't confirm association between *ABCB1* polymorphisms with systemic lupus erythematosus (SLE), the evaluation of these polymorphisms is suggested, and especially the SNP2677 since the mutant allele can affect clinical features of this disease.

Table 4

Comparison of allele and genotype frequencies of ABCB1 C1236T, G2677T/A and C3435T polymorphisms reported for different ethnic population

		C1236T			G2677W (A/T)					C3435T			ref
	n	allale freq C (%)	allale freq T (%)	p value	allele freq G (%)	allale freq T (%)	allale freq A (%)	allele freq W (%)	p value	allele freq C (%)	allele freq T (%)	p value	
Macedonian healthy individuals	107	56,07	43,93		55,14	45,00	0,00	44,86		51,40	48,60		this study
European descedants	60	60,83	39,17		60,83	37,00	2,00	39,17		n.d	n.d		HapMap-CEU
Caucasians, Germany	188	62,77	37,23		n.d.	n.d.	n.d.	n.d.		51,60	47,87		[9]
Germans	461	58,57	40,56		56,51	41,00	2,00	43,49		46,10	54,12		[57]
Czech	189	51,85	41,27		54,23	45,80	0,30	46,30		43,65	56,35		[32]
Polish	139	58,63	41,37		60,43	39,00	1,00	40,00		32,73	55,76		[58]
Polish	96	57,29	42,71		59,90	40,00	n.d	40,10		53,13	46,88		[37]
Polish	204	n.d.	n.d.		60,54	38,00	2,00	40,00		47,55	52,45	**χ2=7.72 p=0.00545**	[50]
Russians	290	n.d.	n.d.		54,48	41,90	3,30	45,52		45,52	54,48		[59]
Slovenian	355	38,73	61,27	**χ2=5.79 p=0.01614**	59,72	40,00	n.d	40,28		47,18	53,10		[46]
Caucasians, UK	190	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.		48,16	51,84		[60]
British	280	n.d.	n.d.		57,86	39,60	2,50	42,10		46,07	53,93		[61]
Scottish	370	n.d.	n.d.		51,62	49,00	n.d	49,46		47,43	52,57		[62]
French	223	57,62	42,38		53,81	42,40	2,60	44,84		53,36	46,19		[63]
Spanish	408	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.		51,96	48,04		[64]
Spanish	204	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.		52,45	47,55		[65]
White Italians	106	n.d.	n.d.		56,13	44,00	n.d	43,87		53,77	46,23		[66]
Italian	450	n.d.	n.d.		55,00	42,30	2,20	44,50		52,22	47,78		[67]
Portuguese	100	n.d.	n.d.		52,50	47,00	n.d	47,00		43,00	57,00		[60]
Serbians	158	53,16	46,20		53,48	44,30	2,22	46,52		45,89	54,11		[44]
Bulgarian	160	n.d.	n.d.		55,94	44,00	n.d	44,06		49,06	50,94		[68]
Romanians	465	44,19	50,22	**χ2=5.91 p=0.01505**	52,58	45,40	2,00	47,40		51,83	48,17		[69]
Hungarian	503	55,67	44,30		54,77	44,10		45,20		47,32	52,68		[37]
Asians													
Japanese	154	34,42	65,58	**χ2=4.87 p=0.02726**	42,86	40,58	16,56	57,14		61,36	40,58	**χ2=4.00 p=0.04542**	[73]
Japanese	117	n.d	n.d		36,75	35,47	13,25	48,72		61,54	38,46	**χ2=4.68 p=0.03055**	[74]
Chinese Han	200	34,25	65,75	**χ2=27.29 p=1.755e-07**	41,75	45,00	13,25	58,25	**χ2=3.86 p=0.04934**	56,75	43,25		[75]
Chinese Uygur	161	40,68	59,32	**χ2=12.23 p=0.00047**	45,03	50,62	4,35	54,97		47,20	52,80		[76]
Chinese Kazakh	108	32,87	67,13	**χ2=23.44 p=1.286e-06**	50,93	38,89	10,19	49,07		60,19	39,81		[76]
Chinese Han	165	36,36	63,64	**χ2=20.46 p=6.095e-06**	44,55	42,12	13,33	55,45		62,12	37,88		[76]
Chinese	206	36,17	63,83	**χ2=22.78 p=1.814e-06**	52,18	36,41	11,41	47,82		65,29	34,71	**χ2=11.37 p=0.00074**	[58]
Chinese	96	28,13	71,88	**χ2=32.28 p=1.333e-08**	n.d.	n.d.	n.d.	n.d.		n.d	n.d		[23]
Malay	92	34,24	65,76	**χ2=6.19 p=0.01285**	n.d.	n.d.	n.d.	n.d.		n.d	n.d		[23]
Indian	87	32,76	67,24	**χ2=71.38 p=2.941e-17**	33,33	59,77	6,90	66,67	**χ2=14.59 p=0.00013**	n.d	n.d		[23]
Chinese	45	31,11	68,89	**χ2=15.80 p=0.00007**	n.d	n.d	n.d	n.d		60,00	40,00		[37]
Korean	632	n.d.	n.d.		43,83	39,08	17,09	56,17		60,68	39,32	**χ2=6.53 p=0.01061**	[34]
Vietnamese	142	n.d.	n.d.		59,51	34,15	6,34	40,49	**χ2=7.20 p=0.00729**	63,38	36,62		[34]
Africans													
Maroccans	100	67,50	32,50	**χ2=17.27 p=0.00003**	72,50	28,00	0,00	27,50	**χ2=13.45 p=0.00025**	64,50	35,50	**χ2=7.27 p=0.00702**	[78]
Sudanese	51	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.		74,51	29,41	**χ2=40.40 p=2.064e-10**	[71]
Kenyan	80	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.		83,13	16,88	**χ2=5.05 p=0.02469**	[71]
Ghanaian	206	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.		83,98	16,99	**χ2=71.35 p=2.990e-17**	[71]
African-American	88	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.		83,52	16,48		[77]
Sub Sacharan African	57	87,72	12,28	**χ2=33.72 p=6.350e-09**	n.d.	n.d.	n.d.	n.d.		88,60	11,40	**χ2=44.84 p=2.136e-11**	[78]
Other													
Iranian	131	n.d.	n.d.		n.d	n.d	n.d	n.d		46,18	64,50	**χ2=4.65 p=0.03114**	[70]
Egyptians	200	n.d.	n.d.		n.d	n.d	n.d	n.d		59,75	40,25	**χ2=3.96 p=0.04658**	[71]
Turkish	107	45,79	54,21		45,79	48,60	5,61	54,21		47,66	52,34	**χ2=12.01p=0.00053**	[51]
Turkish	100	45,50	54,50		52,00	48,00	0,00	48,00		n.d	n.d		[72]
Ashenazi Jewish	101	57,92	42,08	**χ2=44.34 p=2.762e-11**	59,41	41,00	0,00	40,59		64,36	34,65	**χ2=7.85 p=0.00509**	[80]
Brazilians (white)	106	58,96	41,04		61,26	38,00	1,00	37,74		54,72	45,28		[81]
Brazilians (intermediate)	114	66,67	33,33	**χ2=4.28 p=0.03866**	70,18	28,00	2,00	29,82	**χ2=10.69 p=0.00108**	66,67	34,21	**χ2=9.88 p=0.00167**	[81]
Brazlians (black)	100	66,50	33,50	**χ2=4.73 p=0.02968**	82,00	18,00	1,00	19,00	**χ2=32.29 p=1.329e-08**	70,50	29,50	**χ2=15.80 p=0.00007**	[81]

The detected frequency of the 3435T variant allele (49%) was in a range comparable with the frequencies reported among other European populations (ranging from 46%–56%). As it was expected, the variant allele frequency 3435T in the R. Macedonia population is significantly higher compared to that of the Asian populations (34%–43%) except Chinese Uygur where it estimates 52% and African population, Sudanese (30%), Kenyan, Gahanna and African-Americans (17%), and Sub-Saharan Africans (11%). The exception was the frequency reported for Egyptians (40.3%, 50.2%) which is similar to the frequencies observed in Caucasian population (p values listed in Table 4).

The synonymous C3435T SNP has been associated with altered P-gp activity. The homozigocity of wild type 3435CC genotype is highest among African population and lowest in sought-west Asians [37]. The high frequencies of the C-allele in Africans imply overexpression of P-gp [38] which may influence drug absorption and bioavailability especially in CNS. So, this polymorphism is important therapeutic and prognostic factor for use of p-gp dependent drugs [35]. Homozygous mutant 3435TT in exon 26 was associated with higher plasma levels of digoxin and lower P-gp expression compared to wild type 3435CC [9]. Lower expression of P-gp in 3435TT carriers gives better prediction for treatment response to palliative chemotherapy in woman with breast cancer [39]. Simon et al. [40] reported that patients with acute myocardial infarction that are on clopidogrel and are carrying 3435T mutant variant have more than five times greater rate for development of adverse events than wild type carriers. On the other hand 3435CC genotype is associated with better prognosis and complete remission of acute myeloid leukemia (AML) [41] and higher predisposition to drug-resistant epilepsy [42]. Similar findings were obtained with fexofenadine in individuals with 3435TT/2677TT haplotype carriers [43], but some other authors failed to confirm this results for the same drugs [41, 44]. 3435CC/2677GG haplotype has been indicated to be in significant correlation with higher response rate to docetaxel/cisplatin [45] treatment of non-small cell lung cancer. This wild type haplotype also was associated with better response to etoposide-cisplatin treatment of small cell lung cancer. On the other hand Potocnik et al. [46], have reported that patients with 1236TT/3435TT genotypes are associated to higher microsatellite instability in colorectal cancer compared to controls. Beside C3435T is silent mutation, in many studies is reported that mutant 3435T allele influence mRNA stability [47]. On the other hand this variant shouldn't be analyzed separately because it is in high linkage disequilibrium with other the non-synonymous polymorphism, such as G2677T/A and it should be taken in consideration that the variability on the other locus could be associated with altered therapeutic response or disease predisposition [48]. That is the reason why the haplotype determination of *ABCB1* gene facilitates better estimation of functional role of *ABCB1* gene in treatment response and development and clinical picture of certain diseases. Online available date at www.hapmap.org confirm individual LD pattern for each population for number of different SNPs. Strong LD has been confirmed between the three SNPs in Chinese, Malays and Indian [16], and in Hispanic and non- Hispanic [49]. The results from our study give the evidence that the pairwise haplotype frequencies observed in Macedonian population are in agreement with the reported data for other Europeans (Table 2) [50]. Namely, almost complete LD was observed between C1236T and 2677T (D' = 0.91 r^2 = 0.83) and G2677T (D' = 0.0.89 r^2 = 0.55) and C3435T in CEU (Utah residents with Northern and Western European ancestry from the CEPH collection). In ethnical Macedonians the LD was only slightly lower D' = 0.859, r^2 = 0.711 and D' = 0.795, r^2 = 0.802 for C1236T and G2677T and G2677T and C3435T, respectively. (Fig. 1, Table 3)

A great inter-ethnical variability in the total number and frequencies of *ABCB1* haplotypes has been reported. In the study conducted on 48 *ABCB1* variants in different ethnic groups, 64 haplotypes that were identified, but 33 of them account for 92% of chromosomes analyzed. In Caucasian population 25 haplotypes were found compared to 55 haplotypes confirmed in African-Americans, and only 20 haplotypes were identified in both populations [51]. When analyzing the three most common SNPs C1236T, G2677T/A and C3435T in Asian population, 10 haplotypes were identified in Chinese, 9 in Indians and 6 in Malays [16]. Twelve different haplotypes were identified in Roma,

and 11 in Hungarian population [37]. The most frequent haplotype in our population was CGC (45.3%). This finding was similar to haplotype frequency of Ashkenazy Jewish population but it differs from the frequencies reported for the other population Caucasians (36.8%), Uygur Chinese (30.1%), Indian (28.5%) and Turkish (25%) [52]. The higher frequency of this haplotype is observed because of the highest distribution of the following genotype combinations CT-GT-CT (31.8%), CC-GG-CC (21.5%), and CC-GG-CT (7.5%) found in Macedonians. The second most frequent haplotype in ethnical Macedonian population is TTT with frequency of 37.8%. This haplotype is considered as the most frequent haplotype in most other populations, Caucasians 41%, Czech 39.3%, Roma 36%, and Hungarian 37.5% [37, 52]. The observed difference is probably observed because genotype TT-TT-TT is reported in 15.9%, of our population. The third most prominent haplotype in Macedonian population was CGT with frequency of 7.5% which is in agreement with most other populations.

Dulucq et al. [18] confirmed that carriers of 1236C-2677G-3435C haplotype have poorer response to imatinib in treatment of chronic myeloid leukemia (CML). Bandur et al. [53] reported that wild type haplotype of these three SNPs increases the risk of acute rejection of the graft in renal transplant patients. Some clinical studies have shown that 1236T-2677T-3435T haplotype is associated with reduced P-gp expression and activity [54], and in this manner Panczyk et al. [55] confirmed greater risk for colorectal cancer development in population with TTT haplotype. Sai et al. [56] associated this mutant haplotype with poorer irinotecan clearance in various cancers.

Published reports are based mostly on nationality rather than ethnic origin and these results in bias. Interethnic variability, presented in the nations reinforces the need for proper selection of control subjects and points against the use of surrogate control groups for studies involving the association of *ABCB1* alleles with adverse drug reactions or predisposition to certain diseases.

Conclusion

This is the first study reporting *ABCB1* polymorphisms and haplotypes in ethnical Macedonian population. Our findings suggest that the allele frequencies in Macedonians are similar to those reported for Caucasians of European descendant and have shown statistically significant differences in comparison with the Asians and African population. We have identified eight different haplotypes in our population but, three haplotypes CGC, TTT and CGT represent almost 90% of ethnical Macedonians. This study may contribute to population specific data on *ABCB1* gene and has to be taken into account in order to for future establishment of the association and functional impact of *ABCB1* polymorphisms with various drug responses and disease predispositions in R. Macedonia.

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Резиме

ГЕНОТИПСКА ВАРИЈАБИЛНОСТ И ХАПЛОТИПСКИ ПРОФИЛ НА ПОЛИМОРФИЗМИТЕ ВО *АБЦБ1* (*МДР1*) ГЕНОТ ВО МАКЕДОНСКАТА ПОПУЛАЦИЈА

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Главна цел на оваа студија е да направи евалуација на најчесто испитуваните полиморфизми на *АБЦБ1* (*МДР1*, П-гликопротеин) генот во популацијата во Република Македонија и да ги спореди алелните и хаплотипските фреквенции со глобалните географски податоци за пријавените фреквенции во различни етнички популации.

Во студијата беа вклучени 107 здрави индивидуи со етничко македонско потекло. Со метода на Real-Time PCR беше направена анализа на генотиповите на АБЦБ1 за три полиморфизми C1236T [rs1128503], G2677A/T [rs2032582] и C3435T [rs1045642]. Добиените алелни фреквенции за овие три снипа во македонската етничка

популација беа слични со веќе публикуваните за други европски народи од бела раса. Генотипските фреквенции што беа добиени изнесува 33,6% за 1236CC, 44,9% за 1236CT и 21,5% за 1236TT во егзон 12; 32,7%, 44,9% и 22,4% за 2677GG, 2677GT и 2677GT соодветно во егзон 21; и 25,2% за 3435CC, 52,3% за 3435CT за 22,5% за 3435TT во егзон 26. Силен Linkage Disequilibrium (LD) беше потврден со резултатите од нашата студија помеѓу трите еднонуклеотидни полиморфизми (Single Nucleotide Polymorphisms-SNPs), при што највисока асоцираност беше потврдена за C1236T и G2677T ((D'= 0,859, r^2 = 0,711). Идентификувани се осум различни хаплотипови со најголема застапеност на CGC-хаплотипот (45,3%). Нашата студија е прва која ја пријавува дистрибуцијата на АБЦБ1 алелните, геотипските и хаплотипските фреквенциивo популацијата со етничкомакедонско потекло во Република Македонија. Добиените резултати може да помогнат во предвидувањето на различниот терапевтски одговор при употреба на лекови што се супстрати на П-гликопротеинот. Дополнително, во ерата на индивидуализирана медицина познавањето на генотипот на П-гликопротеинот може да биде добар предиктивен маркер за определување на суп-популациите со зголемена предиспозиција кон одредени заболувања.

Клучни зборови: АБЦБ1 (МДР1), П-гликопротеин, C1236T, G2677A/T, C3435T, интеретнички разлики, хаплотипови, македонска популација.