REVIEW ARTICLE

DISTRIBUTION OF THE MOST COMMON GENETIC VARIANTS ASSOCIATED WITH A VARIABLE DRUG RESPONSE IN THE POPULATION OF THE REPUBLIC OF MACEDONIA

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ABSTRACT

Genetic variation in the regulation, expression and activity of genes coding for Phase I, Phase II drug metabolizing enzymes (DMEs) and drug targets, can be defining factors for the variability in both the effectiveness and occurrence of drug therapy side effects. Information regarding the geographic structure and multi-ethnic distribution of clinically relevant genetic variations is becoming increasingly useful for improving drug therapy and explaining inter-individual and inter-ethnic differences in drug response.

This study summarizes our current knowledge about the frequency distribution of the most common allelic variants in three broad gene categories: the Phase I oxidation-cytochrome P450 (CYP450) family *(CYP2C9, CYP2C19, CYP3A5, CYP2D6)*; the Phase II conjugation *(GSTT1, SULT1A1; UGT1A1)* and drug target *(TYMS-TSER, MTHFR* and *VKORC1)* in the population of the Republic of Macedonia and compares the information obtained with data published for other indigenous European populations.

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Our findings define the population of the Republic of Macedonia as an ethnic group with a highly polymorphic genetic profile. These results add to the evidence regarding the distribution of clinically important variant alleles in DME and drug target genes in populations of European ancestry.

Keywords: Drug metabolizing enzymes (DMEs); Drug target; Allelic variants; Republic of Macedonia

INTRODUCTION

Patient-to-patient variability in drug response is one of the major problems in clinical practice and drug development. Aberrant drug responses, such as a lack of therapeutic effect and adverse drug reactions, have been associated with severe medical and economic consequences. The inter-individual differences in drug disposition cannot be explained satisfactorily by factors such as a renal and/or liver function, patients' age and co-morbidity, life style, or patients' co-medication and compliance [1]. Therefore, genetic variations in the regulation, expression and activity of genes coding for Phase I, Phase II drug metabolizing enzymes (DMEs), drug targets and drug transporters, can be important determinants for drug efficacy and toxicity.

The growing body of consistent, reproducible findings for an increasing number of genetic markers for drug effectiveness and adverse drug reactions has resulted in an increasing number of pharmacogenetic effects being included in drug labels. Of the Food and Drug Administration (FDA) approved drug labels referring to human genomic biomarkers, 62.0% pertain

to the above discussed polymorphisms in the *CYP* enzymes, with *CYP2D6* (35.0%; risperidone, tamoxifen, codeine, clozapine, metoprolol, *etc.*), *CYP2C19* (17.0%; clopidogrel, voriconazole, omeprazole), and *CYP2C9* (7.0%; celecoxib, warfarin) being the most common, as well as to polymorphisms in *UGT1A1* (irinotecan, nilotinib) and *VKORC1* (warfarin) genes. The European Medicine Agency (EMA) also has a significant role in the implementation of the pharmacogenetic evidence in drug development, emphasizing the pharmacogenetic considerations and requirements for the pharmacokinetic characterization of medicinal products as a crucial step in designing and conducting drug development and drug evaluation investigations.

Besides the recognized inter-individual difference in drug response, the sequencing of the human genome has renewed and strengthened the interest in biological differences between racial and ethnic populations, as genetic variants associated with disease susceptibility, environmental response, and drug metabolism are identified, and frequencies of these variants in different populations are reported [2]. The availability of data from various genome re-sequencing projects has shown that the largest part of genetic variability within the human population is due to differences in individuals within populations, rather than to differences between populations [3]. A recent study, examining the global patterns of genetic diversity and signals of natural selection for human genes in 283 DMEs across 62 worldwide ethnic groups, suggests that genetic variants in absorption, distribution metabolism and elimination (ADME) genes could contribute to the intra-population heterogeneity in drug response [4].

The most prevalent allelic variants in three broad gene categories: the Phase I oxidation, cytochrome P450 (CYP450) family (*CYP2D6, CYP2C19, CYP2C9, CYP3A5*), the Phase II conjugation (*GSTT1, SULT1A1, UGT1A1*) and drug target (*TYMS-TSER, MTHFR* and *VKORC1*). Their function, resulting enzyme activity and global frequencies in the European population have been extensively studied.

In the present study, our objective was to summarize the current knowledge about the frequency distribution of these genetic variants in the population of the Republic of Macedonia (Supplementary materials and methods), resulting from ongoing studies (unpublished data) as well as from studies already published, and compare the information received with data reported for other populations of European origin. We emphasize that the genetic variants presented and analyzed in this study are only the described ones, not all from Phase I, Phase II and drug target genes.

GENETIC VARIANTS ASSOCIATED WITH A VARIABLE DRUG RESPONSE

Phase I: Oxidation, *CYP450* Family (*CYP2D6, CYP2C19, CYP2C9, CYP3A5*). The *CYP450* isoform activity has emerged as a major determinant of the variability in drug metabolism and response as these enzymes are responsible for about 80.0% of all Phase I DMEs. Despite the large number of *CYP* genes and enzymes, 57 individual *CYP* genes organized in 18 gene families comprising 44 subfamilies, only the members of the *CYP2* and *CYP3* gene families have a significant importance since they contribute to the metabolism of the majority of clinically important drugs [5,6]. The *CYP2D6* enzyme, al-though expressed at low levels, constituting only 4.0-6.0% of the *CYP* content in the liver, metabolizes 25.0 to 30.0% of all clinically used drugs including anti-arithmics, anti-depressants, anti-psychotics, beta blockers and codeine. The enzyme is the only one among the *CYP*s DMEs that is not inducible, and therefore, the genetic variation contributes largely to the inter-individual variation in the enzyme activity [7].

The *CYP2C19* enzyme plays a critical role in the oxidative biotransformation of approximately 10.0% of the commonly used drugs belonging to drug classes such as proton pump inhibitors, tricyclic anti-depressants, selective serotonin reuptake inhibitors, benzodiazepines, barbiturates, phenytoin, voriconazole, nelfinavir and proguanil [7,8], whereas, the *CYP2C9* enzyme contributes to the metabolism of 15.0% of clinically important drug classes, such as nonsteroidal anti-inflamatory drugs, angiotensin II receptor antagonist, anti-diabetic drugs, diuretic torsemide as well as the narrow therapeutic index drugs such as phenytoin and warfarin [7,9]. The *CYP3A* enzymes are responsible for the metabolism of approximately 50.0% of the currently marketed drugs, including steroids, anti-depressants, immunosuppressive agents and anti-biotics. The *CYP3A5* is the primary extrahepatic *CYP3A* isoform, and typically, displays decreased catalytic activity compared to *CYP3A4* [5,9]. The *CYP450* allelic variant and numerous subvariants

reported to date are summarized at the Human *CYP Allele Nomenclature Committee* website (http:// www.cypalleles.ki.se/).

Currently, over 100 allelic variants and numerous subvariants have been identified within the *CYP2D6* gene. The most important non functional alleles that are predominantly responsible for poor metabolizers (PMs) and account for 90.0-95.0% of PMs in indigenous Europeans are *CYP2D6*3, CYP2D6*4* and *CYP2D6*6* [10]. The *CYP2D6*4* with an allele frequency of 12.0-21.0%, is the most common allele associated with PMs. Other detrimental alleles, *CYP2D6*3* and *CYP2D6*6*, are frequently found in indigenous Europeans (1.0-2.0%), but are very rare or absent in other populations. The most common alleles with severely reduced activity are represented by *CYP2D6*9, CYP2D6*10, CYP2D6*17* and *CYP2D6*41*. The frequency of *CYP2D6* deletions ranges from 2.0-7.0% in indigenous Europeans, 6.0% in Blacks and less than 1.0% in Asians and 2.0-10.0% of the populations carry multiple copies of the functional *CYP2D6* allele [7,11].

The majority of PMs of *CYP2C19* are due to the *CYP2C19*2* (681G>A) and *CYP2C19*3* (636G>A) allelic variants that are non functional alleles, resulting in a complete loss of enzyme activity. They account for 95.0% of PMs. The frequency of the *CYP2C19*2* allele ranges between 15.0-17.0% in indigenous Europeans and Blacks and up to 30.0% in the Asian population. The most common allele associated with ultrarapid enzyme activity, *CYP2C19*17* (–806C>T), has a similar allelic frequency of 15.0-25.0% in both the indigenous European and Black populations, and much reduced frequency (4.0%) in Asians [7,12].

The most prevalent CYP2C9 alleles in the European population are *CYP2C9*2* (430C>T) and *CYP2C9*3* (1075A>T). Both are decreased-function alleles, lead to significant reduction in the enzymatic activity of the *CYP2C9* enzyme and account for 85.0% of PM phenotypes. The reported frequencies of *CYP2C9*2* and *CYP2C9*3*, ranges between 13.0-22.0% and 4.0-6.0%, respectively, in indigenous Europeans, but are much lower in Blacks and Asians [7,9,12].

CYP3A5 is a highly polymorphic enzyme as a result of mutations that severely diminish the synthesis of the functional *CYP3A5* protein. The *CYP3A5*3C* (6986A>G) mutant is the major defective allele, leading to alternative splicing and protein truncation, which in turn results in the absence of the enzyme activity. It is widely detectable in the indigenous European population (85.0-98.0%) with a lower frequency in the Black (27.0-48.0%) and Asian (60.0-75.0%) populations [7,12,13].

Distribution of the *CYP450* (*CYP2D6, CYP2C19, CYP2C9, CYP3A5*) Genetic Variants in the Population of the Republic of Macedonia. The allelic and genotype frequency distributions in our population and the correlated *CYP450* phenotype status for the investigated DMEs are presented in Table 1. The *CYP2D6* gene is considered a challenge for genotyping because its genetic variants include not only single nucleotide polymorphisms (SNPs), but copy number variations (CNVs) as well. The optimum amount of genotyping required to accurately predict the *CYP2D6* phenotype remains debatable. A total of 11 different *CYP2D6* variant alleles have been identified in our population. The prevalence of both inactive (*3,*4* and *6*) and decreased activity)*9, *10* and *41*) alleles was estimated to be 0.324. The most frequently observed variant allele was *4*, accounting for 0.575 of all variant alleles. A total of 33 different genotypes were identified. The geno-type-phenotype association was made according to the *CYP2D6 DPWG Genotype Phenotype Classification System* (http://www.pharmgkb.org). Table 2 summarizes the resulting *CYP2D6* genotypes and corresponding phenotypes recognized in the population of the Republic of Macedonia. Our results regarding the *CYP2D6* profile were generally in accordance with the frequencies reported for other European populations (Supplementary Table 1A; references 5-18). Statistically significant differences were found in comparison to the prevalence of the *CYP2D6* gene duplication and deletion. We report a higher frequency of *CYP2D6* duplications/deletions in the Republic of Macedonia compared to that of Germany, France, Russia and Croatia [5,14,17]. These findings regarding the distribution of the *CYP2D6* CNV status corresponds to the north-south gradient theory, suggesting a decreasing prevalence of *CYP2D6* CNV from the Mediterranean countries towards Northern Europe. The frequency distribution of *CYP2D6* PMs is similar to that of the other European populations [10,11,15-23].

All six possible *CYP2C19*2* and *17* genotypes with the corresponding phenotypes [*1/*1* and *1/*2*, both specifying extensive metabolizers (EMs); *1/*17* and *17/*17* indicative for ultrarapid metabolizers (UMs); *2/*2* predicting PM and the *2/*17* com-

Table 1. Allelic frequencies and genotype/phenotype distributions of the evaluated Phase I-oxidation, CYP450 genetic variants in the healthy population of the Republic of Macedonia.

Gene	n	Variant Alleles[a]	Frequency	Genotype (phenotype)	Frequency	References
CYP2D6	184	*1[b]	0.249	EM[c,d]	0.658	this study
		*2	0.108			
		*3	0.008			
		*4	0.187			
		*6	0.000	IM[e,r]	0.320	
		*9	0.016			
		*10	0.027			
		*41	0.086			
		*33	0.019	PM[g]	0.054	
		*35	0.100			
		*34	0.054			
		*39	0.146			
		N >2 (duplication)	0.059	UM[h]	0.043	
		N <2 (deletion)	0.091			
CYP2C19	184	*1[b]	0.654	*1/*1 (IM)	0.418	23
				*1/*2 (IM)	0.190	
		*2	0.144	*2/*2 (PM)	0.027	
				*2/*17 (difficult to predict)	0.043	
		*17	0.202	*17/*17 (UM)	0.038	
				*1/*17 (UM)	0.283	
CYP2C9	179	*1[b]	0.788	*1/*1 (EM)	0.610	23
				*1/*2 (IM)	0.210	
		*2	0.139	*2/*2 (PM)	0.027	
				*2/*3 (PM)	0.010	
		*3	0.073	*3/*3 (PM)	0.010	
				*1/*3 (IM)	0.110	
CYP3A5	174	*1[b]	0.092	*1/*1 (EM)	0.011	25
				*1/*3 (IM)	0.160	
		*3	0.908	*3/*3 (PM)	0.827	

EM: extensive metabolizers; IM: intermediate metabolizers; PM: poor metabolizers; UM: ultrarapid metabolizers.
[a] Labeling and terminology according to DPWG genotype-Phenotype table (http://www.pharmgkb.org).
[b] Defined by the absence of variant alleles.
[c] Carrier of two active alleles.
[d] Carrier of one active and one decreased-activity allele.
[e] Carrier of two decreased-activity alleles.
[f] Carrier of one inactive and one decrease or active allele.
[g] Carrier of two inactive alleles.
[h] Carrier of CNV, N >2.

bined heterozygous genotype with a difficult to predict phenotype] have been identified [23] (Table 1). The detected frequency of the most common CYP2C19 variant alleles in the population of the Republic of Macedonia is within the range comparable with other European populations (Supplementary Table 1A; references 7,11-14,18-36). According to the previously reported data [24], the CYP2C19*3 allele has a very rare occurrence in the population of the Republic of Macedonia and therefore, it was not a subject of comparison in this study.

The allelic frequencies for CYP2C9*2 and CYP2C9*3 found in our population correspond to the general frequencies reported for the various ethnic groups throughout Europe (Supplementary Table 1B; references 11-14,20,23, 34-47). The frequencies of the

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CYP2C9 genotypes associated with PM phenotypes (0.297), *2/*2 and *3/*3 were found to be 0.026 and 0.010 [23], respectively (Table 1).

The *CYP3A5*3* allele was abundantly present in the specimens of our study with a frequency of 0.908. We found no statistically significant difference compared to the frequencies reported for other Europeans (Supplementary Table 1B; references 13,36,48-52). Since heterozygotes and wild types for *CYP3A5*3* may have a *CYP3A5* activity, we estimate that 0.82 of the population of the Republic of Macedonia do not have a *CYP3A5* enzymatic activity [25]. This is in agreement with the finding that 10.0% of the indigenous European population are high expressers of *CYP3A* [13].

Phase II: Conjugation (*GSTT1, SULT1A1; UGT1A1*). Extensive polymorphism also occurs in the majority of Phase II drug metabolizing enzymes. *Sulfo-transferases* and *UDP-glucuronosyltransferases* are two important phase II enzyme families that catalyze the sulfate and glucuronide conjugation of many endogenous and exogenous substances. The most frequent polymorphism in the *SULT1A1* gene is the *SULT1A1*2* (638G>A) that is correlated with a diminished capacity to sulphate *SULT1A1* substrates. The greatest clinical significance of *SULT1A1* is its role in the metabolism of estrogens and tamoxifen [26]. *UGT1A1* pharmacogenetics has been studied more extensively than that of other *uridine 5'-diphospho-glucuronosyltransferases (UGTs)*. The greatest clinical significance has the *UGT1A1*28* variant allele correlated with an altered level of the enzyme expression and therefore a low enzymatic activity. It has been associated with adverse drug reactions (ADRs) due to reduced clearance, most notably life-threatening toxicity of chemotherapy with a high-dose irinotecan [27].

Ethnic variations in allele frequencies observed for *SULT1A1*2* ranges from 0.332 in indigenous Europeans, 0.080 in Asians and 0.294 in African-Americans. The frequency of the *UGT1A1*28* allele varies among ethnicities, being the highest in those of African (43.0%) or European (39.0%) descent and the lowest in those of Asian (16.0%) descent [26,27].

Glutathione S-transferases (*GST*s), are active in the detoxification of a wide variety of potentially toxic and carcinogenic electrophiles in conjugation with glutathione (GSH). The most common polymorphism is caused by a gene deletion that results in the absence of *GSTT1* enzyme activity and has been associated

Table 2. Predicted phenotypes from the CYP2D6 genotype and genotype frequencies in a sample from the population of the Republic of Macedonia (n = 184).

Predicted Phenotype[a]	Genotype[a] (*n*)	Observed Frequency
EM	Normal/Normal (87)	
	*1/*1	0.028
	*1/*2	0.262
	*1/*35	0.112
	*2/*2	0.009
	*34/*34	0.047
	*39/*39	0.243
	*2/*33	0.028
	*2/*35	0.019
	*34/*35	0.047
	*35/*35	0.009
	*39/*39-*33/*33	0.009
EM	Normal/Decreased (18)	
	*1/*10	0.028
	*1/*41	0.047
	*35/*10	0.028
	*2/*9	0.009
	*35/*41	0.037
	*35-*41/*41	0.009
	*2/*4/*35	0.009
EM	Normal/Inactive, Duplication (2)	
	*35/*4N	0.009
	*1/*4N	0.009
IM	Normal/Inactive (41)	
	*1/*4	0.576
	*2/*3	0.017
	*35/*3	0.034
	*35/*4	0.034
	*33/*4	0.034
IM	Decreased/Decreased (10)	
	*9/*10	0.017
	*10/*10	0.017
	*10/*41	0.017
	*41/*41	0.119
IM	Decreased/Inactive (8)	
	*9/*4	0.068
	*41/*4	0.051
	*41/*41-*4 (score=1)	0.017
PM	Inactive/Inactive (10)	
	*4/*4	1.000
UM	Normal/Normal, Duplication (8)	
	*1/*2	0.250
	*1/*41	0.125
	*34/*35	0.125
	*35/*35	0.125
	*34/*34	0.250
	*39/*39	0.125

[a] Labeling and terminology according to DPWG Geno-type-Phenotype table (http://www.pharmgkb.org).

Table 3. Allelic frequencies and genotype/phenotype distributions of the evaluated Phase II: conjugation of genetic variants in the healthy population of the Republic of Macedonia.

Gene	Individuals	Variant Alleles	Frequency	Genotype	Frequency	References
SULT1A1	200	*1 (638G) *2 (638A)	0.633 0.367	GG GA AA	0.435 0.395 0.17	29
UGT1A1	96	*1 (TA6) *28 (TA7)	0.690 0.310	*1/*1 *1/*28 *28/*28	0.500 0.375 0.125	30
GSTT1	128			no null null	0.870 0.130	this study

with a reduction of the relapse risk in several tumor types (acute lymphoblastic leukemia, acute myeloblastic leukemia, breast cancer, ovarian cancer and lung cancer) treated with chemotherapy. Reported frequencies for the *GSTT1* null genotype from different ethnic groups show a range of 16.0-60.0% for Asians, 15.0-26.0% for Africans and 10.0-20.0% for the European population [26,28].

Distribution of the Phase II Conjugation (*GSTT1, SULT1A1; UGT1A1*) Genetic Variants in the Population of the Republic of Macedonia. For the *SULT1A1* (638G>A) polymorphism, approximately one-third of the population from the Republic of Macedonia carry the low-activity *SULT1A1*2* allele and 17.0% are predicted to be homozygous for the *SULT1A1*2* genetic variant [29] (Table 3). The distributions of the *UDP-glucuronosyltransferase (UGT)1A1*28* allele and genotype frequencies in our population are summarized in Table 3 [30]. Regarding the *GSTT1* genotype distribution, 16 (0.13) out of 128 study subjects were predicted to carry the null genotype.

We found no significant difference in *SULT1A1*2* allelic and genotype distribution in our population compared to the one reported for other Europeans (Supplementary Table 2; references 53-57). Our findings regarding allelic and genotypic frequencies for *UGT1A1*28* genetic variant are comparable to those observed in the Framingham Heart Study that comprises subjects of almost entirely European descent from England, Ireland, France and Italy, and to the variant allele frequency reported for the Turkish population (Supplementary Table 2; references 58-63). The results concerning the population frequency of *GSTT1* null genotypes corroborate the regional frequency gradients of the *GSTT1 null* allele that exist within the European populations (Supplementary Table 2; references 18, 64-70).

Drug Target Genetic Variants (*MTHFR, TYMS-TSER, VKORC1*). Polymorphisms in drug targets are also an important area for pharmacogenetic studies, since an over-expression or under-expression of drug targets could also lead to resistance or toxicity in standard chemotherapy regimens. Two polymorphisms of folate cycle enzymes, *methylenetetrahydrofolate reductase (MTHFR) C677T* and *thymidylate synthase (TYMS) enhancer region (TSER)* 28 bp tandem repeat, are the two most important intracellular targets for several chemotherapeutic drugs, including antifolates (metotrexate) and fluoropirimidines (5FU, capecitabine), respectively [31]. The most common *MTHFR 677C>T* variant is associated with a decreased enzyme activity and increased metotrexate intolerance. The polymorphism of a 28 bp sequence in the 5' promoter enhancer region of the *TS* gene (*TSER*) varies from two (*TSER*2*) to nine (*TSER*9*) copies of the tandem repeat, with *TSER*2* and *TSER*3* being the most common alleles. The second SNP within the second repeat of *TSER*3* (*TSER*3RG>C*), up-regulates the *TS* expression and is associated with resistance to *TS*-targeted chemotherapy. The *MTHFR C677T* polymorphism shows wide regional and ethnic variations among the European population (23.0-41.0%), Asian countries (40.0-44.0%) and Africans (12.0, 6.0%) [31,32]. Inter-population differences have also been documented for *TYMS-TSER* genetic variants; the frequencies reported for TSER*3 allele within the Asian, indigenous European and African populations were 0.81, 0.60 and 0.52, respectively [32,33].

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Table 4. Allelic frequencies and genotype distributions of the evaluated drug target genetic variants in the healthy population of the Republic of Macedonia.

Gene	Individuals	Variant Alleles	Frequency	Genotype	Frequency	References
MTHFR	185	677C 677T	0.586 0.414	CC CT TT	0.350 0.470 0.180	35
TYMS-TSER	105	2R 3C 3G	0.376 0.395 0.228	2R/2R 2R/3C 2R/3G 3C/3C 3C/3G	0.120 0.300 0.200 0.190 0.100	36
VKORC1	101	−1639G −1639A	0.624 0.376	GG GA AA	0.396 0.455 0.149	this study

The *VKORC1* gene encodes for the enzyme vitamin K epoxide reductase that is the pharmacodynamic target of coumarinic oral anticoagulants. The *VKORC1 (–1639 G>A)* variant, the one SNP included in the International Warfarin Pharmacogenetics Consortium (IWPC) dosing algorithm, has been shown to confer a high risk of bleeding, predisposing the individuals carrying these polymorphisms to hemorrhagic incidents. In general, the A allele has an increasing frequency from Western Europe (40.0%) towards Eastern Asia (90.0%) [34].

Distribution of the Drug Target Genetic Variants in the Population of the Republic of Macedonia. The allelic frequencies and the genotype distributions of the *MTHFR 677C>T* [35], *TYMS-TSER *2/*3 (2R/3R; 3C/3G) [36] and *VKORC1 –1639G>A* genetic variants in our population are presented in Table 4. Both *MTHFR C677T* and *TYMS-TSER* genetic variants show wide regional and ethnic variations among the European population (Supplementary Table 2; references 71-90). Significantly higher frequencies of the *MTHFR 677T* allele was observed when compared to the frequencies reported in the populations of the Nordic countries, Turkey and Croatia. There are few population studies of the *TYMS-TSER* locus in the literature and the frequencies reported for the TSER*3 allele correspond to that found in our population (Supplementary Table 2; references 80-90). The prevalence of *VKORC1 –1639G>A* in the population of the Republic of Macedonia is in accordance with the values reported for other European populations (Supplementary Table 2; references 42,91-94).

CONCLUSIONS

Different populations or ethnic groups are likely to be more similar than different to each other. However, inter-individual differences in drug responses are well recognized and may be due to genetic or environmental influences. These contributing factors may also result in inter-ethnic or inter-geographic differences in drug response.

In addition, regional differences in the frequency of *CYP450* genetic variants leading to population specific DME activity patterns have been observed. Altogether, at least 28 out of 42 DMEs show differences between ethnically defined populations [12]. Several lines of evidence imply that the effect of demography on pharmacogenetic variations should be considered even on a microgeographic scale, especially when population sizes are small [5]. Thus, information regarding geographic structure and multi-ethnic distribution of clinically relevant genetic variations is becoming increasingly useful for improving drug therapy and explaining inter-individual and inter-ethnic differences in drug response [4,5].

Given the recognized ethnic differences in drug responses and the fact that many genetic polymorphisms differ in frequency on the basis of ethnicity/ancestry [7], it is not surprising that the inter-ethnic comparisons of drug response have become a major aspect of pharmacogenetics. Furthermore, the experts advise a careful consideration of the appropriate usage of drugs in populations where known ethnic differences occur and where genetic determinants may or may not be elucidated. Most of the European ethnic

groups have already reported the frequency of the above mentioned genetic polymorphisms, but data regarding the distribution in the normal population of the Republic of Macedonia are deficient.

This study is the first to have reviewed and summarized the current knowledge about the frequency distribution of the most common allelic variants in three broad gene categories: Phase I-oxidation, CYP450 family (*CYP2C9, CYP2C19, CYP3A5, CYP2D6*), Phase II conjugation (*GSTT1, SULT1A1, UGT1A1*) and drug target (*TYMS-TSER, MTHFR* and *VKORC1*) in a population of the Republic of Macedonia compared to those reported for European populations.

Our findings define the population of the Republic of Macedonia as an ethnic group with a highly polymorphic genetic profile. These results add to the evidence regarding the distribution of clinically important variant alleles in DME and drug target genes in populations with European ancestry. They may serve as a background for comparison with other population samples and could be included in case-control studies as a reference for Macedonian population.

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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