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Characterization of the most common CYP2C9 and CYP2C19 allelic variants in the population from the Republic of Macedonia

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The aim of this study was to evaluate the most common CYP2C9 and CYP2C19 polymorphisms in the population of Macedonia and compare them with the global geographic data reported from different ethnic populations. In total, 184 healthy volunteers from the general population were included. Genotypes for the CYP2C9 (*2 [rs1799853] and*3[rs1057910]) and CYP2C19 (*2 [rs4244285] and*17 [rs12248560]) polymorphisms were detected by Real-Time PCR using TaqMan SNP genotyping assay. The CYP2C9 wild-type allele (*1) was the most frequent (78.8%) and the non-functional alleles *2 and *3 had a frequency of 13.9% and 7, 3%, respectively. Seven subjects (2.97%) were poor metabolites (PMs) for CYP2C9 because of the *2/*2 and *3/*3 genotype. For CYP2C19, the frequencies of the*1 (wild-type) and the non-functional alleles (*2 and*17) were 65.4%, 14.4% and 20.1%, respectively. The *2/*2 genotype, corresponded to the predicted frequency of 2.7% for the CYP2C19 PM phenotype. The total of 59 out of 184 subjects (32.0%) was determined as UMs because of the *1/*17 and *17/*17 genotypes. The compound heterozygote (*2/*17), which is associated with a difficult-to-predict phenotype, was detected in 8 subjects (4.34%). The CYP2C9 and CYP2C19 are polymorphic in the population of the Republic of Macedonia. The frequencies of the most common CYP2C9 and CYP2C19 allelic variants are similar to those reported for Caucasians of European descendant, but differ from those of North America Caucasians. Our results suggest that the genetically determined capacity of CYP2C9 and CYP2C19 has to be taken into account in order to improve the individual risk /benefit ratio of the drug therapy in Macedonia.

1. Introduction

Genetic variation in drug metabolizing enzymes (DMEs) have long been recognized as one of the main causes of adverse drug reactions (ADRs), toxicity or reduced therapeutic benefit in individual patients or subpopulations of patients (Sim et al. 2013). It is estimated that 20–25% of all drug therapies are influenced by such genetic variations to an extent that the therapy outcome is affected, and the cytochrome P450 enzymes (CYP) play a critical role, as these enzymes are responsible for about 80% of all Phase I DME (yang et al. 2010; Sarah et al. 2011). The completion of the human genome sequence revealed the presence of 57 CYP genes organized in 18 gene families with 44 subfamilies; however members of the CYP2 and CYP3 families have a significant importance since they contribute to the metabolism of the majority of clinically important drugs (Evans and Johnson 2001). The mass of variability in CYP450 activity is due to Single Nucleotide Polymorphisms (SNPs) that can create altered splice site, frame shift mutations, premature stop codone, gene deletion/duplication or missense mutation. As a result, the molecular forms of many CYP isoenzymes show a differentiated degree of a specific catalytic activity. Accordingly, four phenotype patients can be classified: poor metabolizer (PM-abolished activity), intermediate metabolizer (IM-reduced activity), extensive metabolizer (EM-normal activity), and ultra-rapid metabolizer (UM-enhanced activity) (Evans and Johnson 2001; Ma et al. 2002).

The CYP2C subfamily, composed of CYP2C8, CYP2C9 and CYP2C19 enzymes, is encoded by a cluster of polymorphic genes on chromosome 10q23.33. These enzymes constitute about 20% of the CYP protein content in the human liver and metabolize about 20–30% of all medications (Zhou et al. 2009; Subramanian et al. 2012).

CYP2C9, the most abundant among CYP2C isoforms in the human liver, contributes to the metabolism of 15% of clinically important drug classes such as nonsteroidal antiinflamatory drugs, angiotensin II receptor antagonist, antidiabetic drugs, diuretic torsemide as well as the narrow therapeutic index drugs as phenytoin and warfarin (Sarah et al. 2011; Subramanian et al. 2012; Goldstein 2001). There have been 1199 SNPs found in the CYP2C9 upstream sequence, introns, and nine exons in NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/, access date: 12.12.2012). SNPs within the CYP2C9 coding region produce 36 variant alleles (http://www.cypalleles.ki.se/, access date: 12.12.2012). The most common allelic variants, CYP2C9*2 and CYP2C9*3 are decreased-function alleles. CYP2C9*2 is a mis-

sense mutation of 430T > C causing a substitution of R144C which is associated with a decrease in the enzyme activity toward CYP2C9 substrates. CYP2C9*3 is a missense mutation of 1075A > C on exon 7 that leads to a I359L substitution in the CYP2C9 active site and it is involved in substrate recognition (Sim et al. 2013).

The CYP2C19 enzyme plays a critical role in the oxidative biotransformation of approximately 10% of the commonly used drugs belonging to drug classes such as proton pump inhibitors, tricyclic antidepressants, selective serotonin reuptake inhibitors, benzodiazepines, barbiturates, phenytoin, voriconazole, nelfinavir, and proguanil (Sarah et al. 201; Goldstein 2001). In total, 2069 SNPs have been found in the CYP2C19 upstream sequence, introns, and nine exons (http://www.ncbi.nlm.nih.gov/, access date:12.12.2012). To date, 28 alleles of the human CYP2C19 gene have been identified (http://www.cypalleles.ki.se/, access date: 12.12.2012). The most common allelic variants, CYP2C19*2 and CYP2C19*3, are non-functional alleles, resulting in a complete loss of enzyme activity. CYP2C19*2, the dominant defective allele, containing 681G > A on exon 5, causes a splicing defect resulting in a complete loss of enzyme activity (Sim et al. 2013). The majority of PMs of CYP2C19 are due to these variant alleles. CYP2C19*17, characterized by -806C > T change in the 5'flanking region of the gene, is associated with a ultrarapid enzyme activity through an enhanced enzyme expression (Kearns et al. 2010).

The distribution of the common variant alleles of CYP2C9 and CYP2C19 has been found to vary across populations and different ethnic groups. The extent of variation is directly related to the genetic distance between populations and the allele being examined (Martis et al. 2012). The CYP2C9*2 allele is not found in the Asian population, prevalent in approximately 15% in Caucasians and variable in populations with African ancestry (1–3.6% African Americans, 4.3% Ethiopians). CYP2C9*3 is estimated to 0.5–2.3% in Africans and 1.1–6.8% in Asians, but it is more common in Caucasians (3.3–17%) (Scordo et al. 2001; Mc Graw and Waller 2012; Sistonen et al. 2009).

Unlike other CYP450 s, Caucasians and Africans share similar overall frequencies of the CYP2C19*2 allele (15% and 17%, respectively), while Asians show a higher prevalence of approximately 30%. *CYP2C19*3* has been shown to be more frequent in the Chinese (5%) and less frequent in African Americans (0.4%) and Caucasians (0.04%). The PM phenotype of CYP2C19 is inherited as an autosomal recessive trait, and the distribution of traits varies greatly at an inter-population level; Asians (13–23%), Caucasians (1–6%) and Africans (1–7.5%). CYP2C19*17 is found with a significantly higher frequency in Caucasians (15–25%) and Africans (10–26%) compared to the Asian population (0.15–0.44%) (Martis et al. 2012; Mc Graw and Waller 2012).

A recent study, examining the global patterns of genetic diversity and signals of natural selection for human genes in 283 DME across 62 worldwide ethnic groups, points to a positive selection of ADME genes. Furthermore, it suggests the genetic differentiation as a contributing factor to the population heterogeneity in drug response. The authors also state that a comprehensive understanding of a population differentiation in the genetic determinants of a drug response is vital for the extrapolation of drug usage to diverse ethnicities (Li et al. 2011). In the context of population stratification, this "gene geography" calls for more detailed information on ethnicity than such broad conventional categories, such as Caucasian, African American, Hispanic, Asian, and Other (Henn et al. 2010).

The observed intraethnic variability reinforces the need for proper selection of control subjects and points against the use of surrogate control groups for studies involving the association of CYP450 alleles with adverse drug reactions or spontaneous diseases (García-Martín et al. 2006).

In Europe, the European Medicine Agency (EMA) has a critical role in the implementation of the pharmacogenetic knowledge in clinical practice and drug development. The Committee for Medicinal Products for Human Use (CHMP) at EMA issued guidelines concerning the use of pharmacogenetics in the pharmacokinetic evaluation of medicinal products in which considerations and requirements for the design and conduct of investigations during drug development and drug evaluation are encompassed. Initial screening for polymorphic enzymes has to be done by the pharmaceutical companies in the early phase of the drug development process (Committee for Medicinal Products for Human Use - CHMP, 2011). If it is impossible to avoid the interactions with the polymorphic enzyme, drug is released to the market following an appropriate pharmacogenetic test. Of the Food and Drug Administration (FDA) approved drug labels referring to human genomic biomarkers, 62% pertain to polymorphisms in the CYP enzymes, with CYP2D6 (35%), CYP2C19 (17%), and CYP2C9 (7%) being the most common (Li and Bluth 2011).

The aim of this study was to evaluate the prevalence of the most common CYP2C9 and CYP2C19 allelic variants in the healthy population in the Republic of Macedonia compared to the global geographic data reported from different ethnic populations.

2. Investigations and results

In total, 184 healthy volunteers from the general population were included. Genotypes for the CYP2C9 (*2 [rs1799853] and*3[rs1057910]) and CYP2C19 (*2 [rs4244285] and*17 [rs12248560]) polymorphisms were detected by Real-Time PCR method [MxPro 3005P, Stratagene, La Jolla, CA, USA] using TaqMan SNP genotyping assay according to the manufacturer's instructions [Applied Biosystems, Foster City, CA, USA]. All statistical analyses were performed using the *SISA* statistical platform.

2.1. CYP2C9 genotyping

Genotyping for the two CYP2C9 alleles, CYP2C9 *2 (3608C > T) and CYP2C9*3 (42164A > C) was successively performed in 179 subjects. The wild type allele (*1) had the highest frequency (0.788), while CYP2C9*2 and *3 alleles had frequencies of 0.139 and 0.073, respectively. The distributions of the CYP2C9 allele and genotype frequencies are summarized in Tables 1 and 2. The most frequent genotype indicative for the extensive metabolizer (EM) phenotype was *1/*1 (0.578) followed by the intermediate metabolizer (IM) genotypes (31.9%) *1/*2 (0.196), *1/*3 (0.103) and *2/*3 (0.010). The frequencies of the genotypes associated with poor metabolizer (PM) phenotypes (2.97%), *2/*2 and *3/*3 were found to be 0.026 and 0.010, respectively. All results were in accordance with the expected genotype distributions, calculated with the Hardy–Weinberg equilibrium (X^2 test; P = 0.451).

2.2. CYP2C19 genotyping

CYP2C19 allele frequencies in the population study of 184 subjects from R. Macedonia were as follows: 0.654 for CYP2C19*1, 0.144 for CYP2C19*2 and 0.201 for CYP2C19*17. The *3 allele was not analyzed in this representative study due to its very rare occurrence in our population (Kapedanovska Nestorovska et al. 2010).

The distributions of CYP2C19*2 (19154 G > A) and *17 (-806C > T) alleles and genotype frequencies are summarized in

Table 1: Genotype distribution of the tested variants in a healthy population from the Republic of Macedonia

Gene	Genotype	Predicted phenotype	n	Observed frequency (CI95%)	Predicted frequency by HW eq.	P(X^2test)
	*1/*1	EM	112	0.578 (0.490−0.641)	0.620	0.451
	*1/*2	IM	38	0.196 (0.138−0.253)	0.220	
CYP2C9 (N = 179)*	*1/*3	IM	20	0.103 (0.064−0.154)	0.114	
	*2/*3	IM	2	0.010 (0.001−0.036)	0.020	
	*2/*2	PM	5	0.026(0.011−0.066)	0.019	
	*3/*3	PM	2	0.010 (0.001−0.036)	0.005	
CYP2C19 (N = 184)	*1/*1	EM	77	0.418(0.349−0.490)	0.428	0.714
	*1/*2	EM	35	0.190(0.139−0.252)	0.188	
	*2/*2	PM	5	0.027(0.011−0.061)	0.038	
	*1/*17	UM	52	0.283(0.222−0.352)	0.263	
	*17/*17	UM	7	0.038(0.018−0.076)	0.041	
	*2/*17	Dificult to predict	8	0.043(0.021−0.082)	0.058	

* Missing data (n = 5)- no PCR amplification

Tables 1 and 3 . All six possible genotypes with the corresponding phenotypes were found: *1/*1 (0.418) and *1/*2 (0.190), both specifying EM (60.8%), *1/*17 (0.283) and *17/*17 (0.038) indicative for UM (32.0%), *2/*2 (0.027) predicting PM (2.71%) and *2/*17 genotype (0.043) with a difficult to predict phenotype. The distribution of the various genotypes was in agreement with that predicted by the Hardy-Weinberg Law (X^2 test; P = 0.714).

The frequencies of the CYP2C9 and CYP2C19 variant alleles in the healthy population of Macedonia compared to data reported from various ethnic groups and global geographic regions are presented in Tables 1 and 3, respectively.

3. Discussion

Nowadays, it is clear that pharmacogenetics has the potential to allow clinical pharmacists to develop individualized drug regimens for patients, based on their genetic information. Recent pharmacogenetic studies using a variety of drugs suggest that many of the clinically observed differences in drug response between ethnic groups may be due to differences in the frequency of polymorphisms associated with the drug response in question (Frye et al. 2009). The CYP2C9 and CYP2C19 polymorphisms continue to be evaluated with respect to their effects on toxicity and efficacy of clinically used drugs. The frequencies of these allelic variations are well studied and occur at different frequencies among populations and subpopulations of different ethnic or racial origin. Some of them are unique in certain ethnic subpopulations. Our study is the first to have documented the distribution of CYP2C9 and CYP2C19 genotypes and alleles in a population of the Republic of Macedonia.

According to our results, the allelic frequencies of CYP2C9*2 (13.9%) and CYP2C9*3 (7.3%) in our population study were similar to the general frequencies reported for the Caucasian population throughout Europe (13% and 7%, respectively) (Sistonen et al. 2009). However, we observed a significant interethnic difference compared to the frequency of CYP2C9*3 found in the Spanish ethnic group (16.2%) (García-Martín et al. 2001). Within the Caucasian population, the frequencies of both CYP2C9*2 and CYP2C9*3 allelic variants were found to be significantly more frequent compared to the general data reported for the North American population (1% and 3%) Sistonen et al. 2009), specifically for CYP2C9*2 within the Canadian ethnic group (3%) (Gaedigk et al. 2001)(p values for ethnic groups listed in Table 2).

Omitting the Egyptian ethnic group, CYP2C9*2 and CYP2C9*3, respectively, occurred at a significantly higher frequency compared to the frequency data reported for the African (2% and 1%) and African American (1% and 0.5%) populations as well as the Hispanic population from Central America (7% and 4%) and Southern America (6% and 4%). The prevalence of CYP2C9*2 and CYP2C9*3 variants in our population was higher compared to the global prevalence found within the Eastern (0.01% and 0.3%) and Southeastern Asia (0.6% and 3%) populations where these variants have been reported to be rare or even absent (p values for ethnic groups listed in Table 2) (Sistonen et al. 2009; Martis et al. 2012).

The detected frequency of the CYP2C19*2 variant allele (14.4%) was in a range comparable with the frequencies reported among other European populations (ranging from 9.1% - 16%), North America populations (12.9–19.1%), as well as different ethnic groups originating from Africa (11–18.2%). As it was expected, the CYP2C19*2 allele frequency in the Macedonian population is significantly lower than that of the Asian populations (24%-31.2%) and higher compared to the global frequency reported for Hispanics from Central and South America (9% and 8%, respectively) (p values listed in Table 3). In our study, the CYP2C19*17 variant allele occurred in a frequency of 20.1%, which is similar to those of other Caucasian, African, Asian, Hispanic and other mixed populations (Table 3).

Comparing our data with those reported on a microgeographic scale, the frequencies of the analyzed CYP2C9 and CYP2C19 variant alleles were interpolated between the global values reported for Southern (14% for CYP2C9*2 and 9% for CYP2C9*3; 13% for CYP2C19*2) and Western (12% CYP2C9*2 and 7% for CYP2C9 *3; 14% for CYP2C19*2) European countries. The global frequency of CYP2C19*17 in Europe has not been reported yet.

The inherited differences in individual DMEs are typically monogenic traits. Whether a genetic polymorphism has any relevance for a drug therapy depends mainly on the characteristics of the drug in question. The quantitative role of a DME in the overall pharmacokinetic and pharmacologic effects of medications will determine the level of the dose adjustment in poor metabolisers or ultrarapid metabolisers.

However, the overall pharmacologic effects of medications are more often polygenic traits determined by numerous genes encoding proteins involved in multiple pathways of drug metabolism, disposition, and effects. Several lines of evidence suggest that the plasma concentration of antiepileptic drugs (phenobarbital, carbamazepine, phenitoin) can be affected by the presence of both CYP2C9 and CYP2C19 variant alleles

Table 2: Comparison of CYP2C9 allelic frequencies reported from different ethnic groups and different geographic regions

	Ethnic group	N (study subjects)	n (total alleles)	CYP2C9 variant allele			Reference
				Frequency, (p-value; NS-non dignificant)			
				*1	*2	*3	
	R.Macedonia	179	358	0.788 (referent)	0.139 (referent)	0.073 (referent)	present study
	Spanish	157	314	0.7	0.14(NS)	**0.16 (0.001)**	García-Martín et al. (2001)
	Italy	360	720	0.77	0.12(NS)	0.09(NS)	Scordo et al. (2004)
	Slovenia	129	258	0.82	0.12(NS)	0.06(NS)	Herman et al. (2003)
	Croatia	200	400	0.74	0.16(NS)	0.09(NS)	Bozina et al. (2003)
	Greece	283	566	0.79	0.13(NS)	0.08(NS)	Arvanitidis et al. (2007)
	French	151	302	0.77	0.15(NS)	0.08(NS)	Yang et al. (2005)
	German	266	532	0.81	0.11(NS)	0.08(NS)	Aynacioglu et al. (1999)
Caucasians	Belgium	121	242	0.82	0.10(NS)	0.07(NS)	Allabi et al. (2003)
	Sweden	430	860	0.82	0.17(NS)	0.07(NS)	Yasar et al. (1998)
	Danmark	276	552	0.83	0.12(NS)	0.05(NS)	Pedersen et al. (2004)
	British	100	200	0.84	0.13(NS)	0.08(NS)	Stubbins et al. (1996)
	Russia	290	580	0.83	0.10(NS)	0.07(NS)	Gaikovitch et al. (2003)
	Hungarian	332	664	0.86	0.11(NS)	0.09(NS)	Buzoianu et al. (2012)
	American	100	200	0.85(NS)	0.08(NS)	0.06(NS)	Sullivan-Klose et al. (1996)
	Canada	114	228	0.81(NS)	**0.03(0.007)**	0.06(NS)	Gaedigk et al. (2001)
	Turkish	499	998	0.79(NS)	0.11(NS)	0.10(NS)	Aynacioglu et al. (1999)
Hispanic	Mexican American	169	338	0.86(NS)	**0.08(0.029)**	0.06(NS)	Kramer et al. (2008)
	Brazil	331	662	0.86(NS)	**0.07 (0.017)**	0.07(NS)	Vianna-Jorge et al. (2004)
	Africa	993	1986	0.99(NS)	**0.02(0.001)**	**0.01(0.025)**	Dandara et al. (2011)
African	African Americans	100	200	0.98(NS)	**0.01($1*10^{-7}$)**	**0.005(0.00023)**	Sullivan-Klose et al. (1996)
	Egypt	247	494	0.82(NS)	0.12(NS)	0.06(NS)	Hamdy et al. (2002)
	Vietnam	157	314	0.98 (NS)	0 (<0.0001)	**0.02(0.0017)**	Lee et al. (2005)
	Japan	218	436	**0.98(0.037)**	0 (<0.0001)	**0.021(0.0008)**	Bae et al. (2005)
Asian	Korea	574	1148	**0.99(0.011)**	0(<0.0001)	0.011 (<0.0001)	Bae et al. (2005)
	China	98	196	0.97(NS)	0 (<0.0001)	**0.026(0.031)**	Bae et al. (2005)
	South india	346	692	0.88(NS)	0.04 (**$3*10^{-7}$**)	0.08(NS)	Arun Kumar et al. (2011)

(Evans et al. 2001; Ma et al. 2002; Zhou et al. 2009). In the present population study we did not identify subjects with combined defective alleles for both CYP2C9 and CYP2C19. Moreover, we found a complete absence of the CYP2C9*2 allele in subjects homozygous for the CYP2C9*3 allele as well as a complete absence of the CYP2C19*2 allele in subjects homozygous for the CYP2C19*17 and *vice versa*.

Given the recognized ethnic differences in drug responses and the fact that many genetic polymorphisms differ in frequency on the basis of ethnicity/ancestry, questions about whether pharmacogenetics may also lead to an understanding of the ethnic differences in drug response are not surprising (Sim et al. 2013). In that respect, our study adds to the evidence regarding the microgeographic distribution of the common CYP2C9 and CYP2C19 genetic variants which can then be used in further epidemiological investigations.

Although poor compliance, environmental factors and drug-drug interactions can have a tremendous influence on therapeutic outcome, there are noteworthy examples wherein an altered gene constitution can influence the therapeutic response to such an extent that it is ethically inappropriate not to be taken into consideration in clinical practice and drug development and evaluation. This study shows that CYP2C9 and CYP2C19 are polymorphic in the population in the Republic of Macedonia. Since no data were available for the Macedonian ethnic group, we provide evidence that CYP2C9 and CYP2C19 variant allele frequencies are similar to those reported for Caucasians of European descendant, but differ from those of North America Caucasians. As the goals of personalized medicine are beginning to be realized, our results suggest that the genetically determined capacity of CYP2C9 and CYP2C19 has to be taken into account in order to improve the individual risk /benefit ratio of drug therapy in Macedonia.

4. Experimental

4.1. Study population

Peripheral blood samples were collected from 184 unrelated healthy donors (162 males and 22 females, aged 32.23 ± 9.38 years) of Caucasian origin who self reported as ethnic Macedonians. All subjects were volunteers for the clinical studies of bioequivalence and bioavailability and were included in the study after having given an informed consent. The study was approved by the Ethics Committee of the Ministry of Health of R. Macedonia and is in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All personal identifiers were removed and the isolated DNA samples were tested anonymously.

4.2. Genotyping procedures

Genomic DNA was isolated using Proteinase K digestion, phenol chloroform extraction and ethanol precipitation. DNA purity was verified by UV absorption at 260/280 nm [NanoDrop 2000, Thermo Scientific] and DNA integrity was confirmed with electrophoresis in 0,8% agarose gels, stained with ethidium bromide.

The designations of all CYP450 alleles refer to those defined by the Cytochrome P450 Allele Nomenclature Committee ***(http://www.cypalleles.ki.se/).*** The genotyping of the most common CYP2C9 variant alleles (*2 [rs1799853] and*3[rs1057910]) and CYP2C19 (*2 [rs4244285] and*17 [rs12248560]) was performed by Real-Time PCR based on the allelic discrimination method [MxPro 3005P, Stratagene, La Jolla, CA, USA] using a TaqMan SNP genotyping assay according to the manufacturer's instructions [Applied Biosystems, Foster City, CA, USA].

Table 3: Comparison of CYP2C19 allelic frequencies reported from different ethnic groups and different geographic regions

	Ethnic group	N (study subjects)	n (total alleles)	CYP2C19 variant allele			Reference
				Frequency (p-value; NS-non significant)			
				*1	*2	*17	
Caucasians	R.Macedonia	184	368	0.654 (referent)	0.144 (referent)	0.201 (referent)	present study
	Portugal	153	306	0.87	0.13(NS)	/	Hamdy et al. (2002)
	Italy	360	720	0.89	0.11(NS)	/	Scordo et al. (2004)
	Slovenia	129	258	0.83	0.16(NS)		Herman et al. (2003)
	Croatia	200	400	0.85	0.15(NS)	/	Bozina et al. (2003)
	Greece	283	566	0.67	0.13(NS)	0.20(NS)	Arvanitidis et al. (2007)
	French	172	344	0.89	0.11(NS)	/	Ibeanu et al. (1998)
	German	328	656	0.84	0.16(NS)	/	Aynacioglu et al. (1999)
	Belgium	121	242	0.91	0.09(NS)	/	Allabi et al. (2003)
	Sweden	185	370	0.64	0.16(NS)	0.20(NS)	Ramsjö et al. (2010)
	Sweden	314	628	/	/	0.18(NS)	Sim et al. (2006)
	Norwegian	332	664	/	/	0.22(NS)	Rudberg et al. (2008)
	Denmark	239	478	0.84	0.16(NS)	/	Bathum et al. (1998)
	Russia	290	580	0.89	0.11(NS)	/	Gaikovitch et al. (2003)
	Hungarian	200	400	0.64	0.14(NS)	/	Buzoianu et al. (2010)
	Polish	125	250	/	/	0.27(NS)	Kurzawski et al. (2006)
	American	105	210	0.87	0.13(NS)	/	Goldstein et al. (1997)
	Canada	115	230	0.81	0.19(NS)	/	Buzoianu et al. (2010)
	Turkis	404	808	0.84	0.12(NS)	/	Buzoianu et al. (2010)
Hispanic	Mexican American	346	692	0.9	**0.09 (0.015)**	/	Luo et al. (2006)
	Brazilian	183	366	0.74	0.10(NS)	0.16(NS)	Luo et al. (2006)
	Africa	993	1986	0.84	0.16(NS)	/	Dandara et al. (2011)
	African Americans	236	472	0.81	0.18(NS)	/	Sullivan-Klose et al. (2011)
African	Egypt	247	494	0.88	0.11(NS)	/	Hamdy et al. (2002)
	Etiopian	114	228	0.84	0.14(NS)	/	Hamdy et al. (2002)
	Etiopian	190	380	/	/	0.18(NS)	Kearns et al. (2010)
	Vietnam	165	330	0.76	**0.24 (0.008)**	/	Lee et al. (2007)
	Japan	253	506	0.74	**0.26 (0.00068)**	/	Fukushima-Uesaka et al. (2005)
	Japan	265	530	/	/	0.013(NS)	Kearns et al. (2010)
Asian	Korea	271	542	0.71	**0.28 (0.00064)**	0.015(NS)	Lee et al. (2007)
	China	121	242	0.59	**0.45(0.00045)**	/	Roco et al. (2012)
	China	384	768	/	/	0.013(NS)	Kearns et al. (2010)
	South Indian	220	440	0.59	0.41(NS)	/	Siddapuram et al. (2011)

4.3. Statistical analysis

All statistical analyses were performed using the *SISA* statistical platform. Observed genotype distributions were assessed for Hardy-Weinberg equilibrium with a X^2 test. Frequency analyses of an interpopulation diversity of the examined polymorphisms were performed on data reported for apparently healthy control populations from several different geographic regions. The difference in CYP2C9 and CYP2C19 allelic frequencies between our and other ethnic populations was evaluated using Chi-squared analysis and Fishers Exact Test. Odds ratios [OR] were calculated with 95% confidence limits [95%CI]. Factors with $p \leq 0.05$ were considered statistically significant.

References

Allabi AC, Gala JL, Desager JP, Heusterspreute M, Horsmans Y (2003) Genetic polymorphisms of CYP2C9 and CYP2C19 in the Beninese and Belgian populations. Br J Clin Pharmacol 56: 653–657.

Arun Kumar AS, Chakradhara Rao US, Umamaheswaran G, Ramu P, Kesavan R, Shewade DG, Balachandar J, Adithan C (2011) Haplotype structures of common variants of CYP2C8, CYP2C9, and ADRB1 genes in a South Indian population. Genet Test Mol Biomarkers 15:407–413.

Arvanitidis K, Ragia G, Iordanidou M, Kyriaki S, Xanthi A, Tavridou A, Manolopoulos VG (2007) Genetic polymorphisms of drug-metabolizing enzymes CYP2D6, CYP2C9, CYP2C19 and CYP3A5 in the Greek population. Fundam Clin Pharmacol 21:419–426.

Aynacioglu AS, Brockmöller J, Bauer S, Sachse C, Güzelbey P, Ongen Z, Nacak M, Roots I (1999) Frequency of cytochrome P450 CYP2C9 variants in a Turkish population and functional relevance for phenytoin. Br J Clin Pharmacol48: 409–415.

Aynacioglu AS, Sachse C, Bozkurt A, Kortunay S, Nacak M, Schröder T, Kayaalp SO, Roots I, Brockmöller J. (1999) Low frequency of defective alleles of cytochromeP450 enzymes 2C19 and 2D6 in the Turkish population. Clin Pharmacol Ther 66: 185–192.

Bae JW, Kim HK, Kim JH, Yang SI, Kim MJ, Jang CG, Park YS, Lee SY (2005) Allele and genotype frequencies of CYP2C9 in a Korean population. Br J Clin Pharmacol 60: 418–422.

Bathum L, Andersen-Ranberg K, Boldsen J, Brøsen K, Jeune B (1998) Genotypes for the cytochrome P450 enzymes CYP2D6 and CYP2C19 in human longevity. Role of CYP2D6 and CYP2C19 in longevity. Eur J Clin Pharmacol 54: 427–430.

Bozina N, Granić P, Lalić Z, Tramisak I, Lovrić M, Stavljenić-Rukavina A (2003) Genetic polymorphisms of cytochromes P450: CYP2C9, CYP2C19, and CYP2D6 in Croatian population. Croat Med J 44: 425–428.

Buzoianu AD, Trifa AP Popp RA, Militaru MS, Militaru FC, Bocşan IC, Farcaş FM, Pop IV (2010) Screening for CYP2C19*2, *3 and *4 gene variants in a Romanian population study group. Farmacia 56: 806–817.

Buzoianu AD, Trifa AP, Mureşanu DF, Crişan S (2012) Analysis of CYP2C9*2, CYP2C9*3 and VKORC1–1639 G > A polymorphisms in a population from South-Eastern Europe. J Cell Mol Med 16: 2919–2924.

Committee for Medicinal Products for Human Use (CHMP) (2011) Guideline on the use of pharmacogenetic methodologies in the pharmacokinetic evaluation of medicinal products EMA/CHMP/37646/2009.

Dandara C, Lombard Z, Du Plooy I, McLellan T, Norris SA, Ramsay M (2011) Genetic variants in CYP (-1A2, -2C9, -2C19, -3A4 and -3A5), VKORC1 and ABCB1 genes in a black South African population:a window into diversity. Pharmacogenomics 12:1663–1670.

Evans WE, Johnson JA (2001) Pharmacogenomics: the inherited basis for interindividual differences in drug response. Annu Rev Genomics Hum Genet2: 9–39.

Frye RF (2009) Pharmacogenomics - Application to Patient Care. USA: American College of Clinical Pharmacy; pp.34–49.

Fukushima-Uesaka H, Saito Y, Maekawa K, Ozawa S, Hasegawa R, Kajio H, Kuzuya N, Yasuda K, Kawamoto M, Kamatani N, Suzuki K, Yanagawa T, Tohkin M, Sawada J (2005) Genetic variations and haplotypes of CYP2C19 in a Japanese population. Drug Metab Pharmacokinet 20: 300–307.

Gaedigk A, Casley WL, Tyndale RF, Sellers EM, Jurima-Romet M, Leeder JS (2001) Cytochrome P4502C9 (CYP2C9) allele frequencies in Canadian Native Indian and Inuit populations. Can J Physiol Pharmacol 79:841–847.

Gaikovitch EA, Cascorbi I, Mrozikiewicz PM, Brockmöller J, Frötschl R, Köpke K, Gerloff T, Chernov JN, Roots I (2003) Polymorphisms of drug-metabolizing enzymes CYP2C9, CYP2C19, CYP2D6, CYP1A1, NAT2 and of P-glycoprotein in a Russian population. Eur J Clin Pharmacol 59: 303–312.

García-Martín E, Martínez C, Ladero JM, Agúndez JA (2006) Interethnic and intraethnic variability of CYP2C8 and CYP2C9 polymorphisms in healthy individuals. Mol Diagn Ther 10: 29–40.

García-Martín E, Martínez C, Ladero JM, Gamito FJ, Agúndez JA (2001) High frequency of mutations related to impaired CYP2C9 metabolism in a Caucasian population. Eur J Clin Pharmacol 57: 47–49.

Goldstein JA (2001) Clinical relevance of genetic polymorphisms in the human CYP2C subfamily. Br J Clin Pharmacol 52: 349–355.

Goldstein JA, Ishizaki T, Chiba K, de Morais SM, Bell D, Krahn PM, Evans DA (1997) Frequencies of the defective CYP2C19 alleles responsible for the mephenytoin poor metabolizer phenotype in various Oriental, Caucasian, Saudi Arabian and American black populations. Pharmacogenetics 7: 59–64.

Hamdy SI, Hiratsuka M, Narahara K, El-Enany M, Moursi N, Ahmed MS, Mizugaki M (2002) Allele and genotype frequencies of polymorphic cytochromes P450 (CYP2C9, CYP2C19, CYP2E1) and dihydropyrimidine dehydrogenase (DPYD) in the Egyptian population. Br J Clin Pharmacol 53: 596–603.

Henn BM, Gravel S, Moreno-Estrada A, Acevedo-Acevedo S, Bustamante CD (2010) Fine scale population structure and the era of next-generation sequencing. Hum Mol Genet 19(R2): R221–226.

Herman D, Dolzan V, Breskvar K (2003) Genetic polymorphism of cytochromes P450 2C9 and 2C19 in Slovenian population. Zdrav Vestn 72: 347–351.

Ibeanu GC, Goldstein JA, Meyer U, Benhamou S, Bouchardy C, Dayer P, Ghanayem BI, Blaisdell J (1998) Identification of new human CYP2C19 alleles (CYP2C19*6 and CYP2C19*2B) in a Caucasian poor metabolizer of mephenytoin. J Pharmacol Exp Ther 286:1490–1495.

Kapedanovska Nestorovska A, Dimitrovska Cvetkovska A, Suturkova Lj (2010) Association between CYP2C19*2 variant and clinical outcome in clopidogrel treated patients from Republic of Macedonia. Maced Pharm Bull 56: 37–45.

Kearns GL, Leeder JS, Gaedigk A (2010) Impact of the CYP2C19*17 allele on the pharmacokinetics of omeprazole and pantoprazole in children: evidence for a differential effect. Drug Metab Dispos 38: 894–897.

Kramer MA, Rettie AE, Rieder MJ, Cabacungan ET, Hines RN (2008) Novel CYP2C9 promoter variants and assessment of their impact on gene expression. Mol Pharmacol 73: 1751–1760.

Kurzawski M, Gawronska-Szklarz B, Wrzesniewska J, Siuda A, Starzynska T, Drozdzik M (2006) Effect of CYP2C19*17 gene variant on Helicobacter pylori eradication in peptic ulcer patients. Eur J Clin Pharmacol 62: 877–880.

Lee SS, Lee SJ, Gwak J, Jung HJ, Thi-Le H, Song IS, Kim EY, Shin JG (2007) Comparisons of CYP2C19 genetic polymorphisms between Korean and Vietnamese populations. Ther Drug Monit 29: 455–459.

Lee SS, Kim KM, Thi-Le H, Yea SS, Cha IJ, Shin JG (2005) Genetic polymorphism of CYP2C9 in a Vietnamese Kinh population. Ther Drug Monit 27: 208–210.

Li J, Zhang L, Zhou H, Stoneking M, Tang K (2011) Global patterns of genetic diversity and signals of natural selection for human ADME genes. Hum Mol Genet 20: 528–540.

Li J, Bluth MH (2011) Pharmacogenomics of drug metabolizing enzymes and transporters: implications for cancer therapy. Pharmgenomics Pers Med 4: 11–33.

Luo HR, Poland RE, Lin KM, Wan YJ (2006) Genetic polymorphism of cytochrome P450 2C19 in Mexican Americans: a cross-ethnic comparative study. Clin Pharmacol Ther 80: 33–40.

Martis S, Peter I, Hulot JS, Kornreich R, Desnick RJ, Scott SA (2012) Multi ethnic distribution of clinically relevant CYP2C genotypes and haplotypes. Pharmacogenomics J: doi: 10.1038/tpj.2012.10.

Mc Graw J, Waller D (2012) Cytochrome P450 variations in different ethnic populations. Expert Opin Drug Metab Toxicol 8: 371–382.

Pedersen RS, Verstuyft C, Becquemont L, Jaillon P, Brøsen K (2004) Cytochrome P4502C9 (CYP2C9) genotypes in a Nordic population in Denmark. Basic Clin Pharmacol Toxicol 94: 151–152.

Ramsjö M, Aklillu E, Bohman L, Ingelman-Sundberg M, Roh HK,Bertilsson L (2010) CYP2C19 activity comparison between Swedes and Koreans: effect of genotype, sex, oral contraceptive use, and smoking. Eur J Clin Pharmacol 66: 871–877.

Roco A, Quiñones L, Agúndez JA, García-Martín E, Squicciarini V, Miranda C, Garay J, Farfán N, Saavedra I, Cáceres D, Ibarra C, Varela N (2012) Frequencies of 23 functionally significant variant alleles related with metabolism of antineoplastic drugs in the Chilean population: comparison with Caucasian and Asian populations. Front Genet 3: 229.

Rudberg I, Mohebi B, Hermann M, Refsum H, Molden E (2008) Impact of the ultrarapid CYP2C19*17 allele on serum concentration of escitalopram in psychiatric patients. Clin Pharmacol Ther 83: 322–327.

Santos PC, Soares RA, Santos DB, Nascimento RM, Coelho GL, Nicolau JC, Mill JG, Krieger JE, Pereira AC. (2011) CYP2C19 and ABCB1 gene polymorphisms are differently distributed according to ethnicity in the Brazilian general population. BMC Med Genet 12: 13.

Sarah C. Sim, Russ B. Altman, Magnus Ingelman-Sundberg (2011) Databases in the Area of Pharmacogenetics. Hum Mutat 32: 526–531.

Scordo MG, Aklillu E, Yasar U, Dahl ML, Spina E, Ingelman-Sundberg M (2001) Genetic polymorphism of cytochrome P450 2C9 in a Caucasian and a black African population. Br J Clin Pharmacol 52: 447–450.

Scordo MG, Caputi AP, D'Arrigo C, Fava G, Spina E (2004) Allele and genotype frequencies of CYP2C9, CYP2C19 and CYP2D6 in an Italian population. Pharmacol Res 50: 195–200.

Siddapuram SP, Banerjee R, Tandan M, Prathap N, Mittal S, Duvvuru NR (2011) CYP2C19 polymorphism as a predictor of personalized therapy in South Indian population. J Assoc Physicians India 59: 490–493.

Sim SC, Risinger C, Dahl ML, Aklillu E, Christensen M, Bertilsson L, Ingelman-Sundberg M (2006) A common novel CYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. Clin Pharmacol Ther 79: 103–113.

Sim SC, Kacevska M, Ingelman-Sundberg M (2013) Pharmacogenomics of drug-metabolizing enzymes:a recent update on clinical implications and endogenous effects. Pharmacogenomics J 13: 1–11.

Sistonen J, Fuselli S, Palo JU, Chauhan N, Padh H, Sajantila A (2009) Pharmacogenetic variation at CYP2C9, CYP2C19, and CYP2D6 at global and microgeographic scale. Pharmacogenet Genomics 19: 170–179.

Stubbins MJ, Harries LW, Smith G, Tarbit MH, Wolf CR (1996) Genetic analysis of the cytochrome P450 CYP2C9 locus. Pharmacogenetics 6: 429–439.

Subramanian M, Agrawal V, Sandee D, Tam HK, Miller WL, Tracy TS (2012) Effect of P450 oxidoreductase variants on themetabolism of model substrates mediated by CYP2C9.1, CYP2C9.2, and CYP2C9.3. Pharmacogenet Genomics 22: 590–597.

Sullivan-Klose TH, Ghanayem BI, Bell DA, Zhang ZY, Kaminsky LS, Shenfield GM, Miners JO, Birkett DJ, Goldstein JA (1996) The role of the CYP2C9-leu359 allelic variant in the tolbutamide polymorphism. Pharmacogenetics 6: 341–349.

Vianna-Jorge R, Perini JA, Rondinelli E, Suarez-Kurtz G (2004) CYP2C9 genotypes and the pharmacokinetics of tenoxicam in Brazilians. Clin Pharmacol Ther 76: 18–26.

Yang JQ, Morin S, Verstuyft C, Fan LA, Zhang Y, Xu CD, Barbu V,Funck-Brentano C, Jaillon P, Becquemont L (2003) Frequency of cytochrome P450 2C9 allelic variants in the Chinese and French populations. Fundam Clin Pharmacol 17: 373–376.

Yang X, Zhang B, Molony C, Chudin E, Hao K, Zhu J, Gaedigk A, Suver C, Zhong H, Leeder JS, Guengerich FP, Strom SC, Schuetz E, Rushmore TH, Ulrich RG, Slatter JG, Schadt EE, Kasarskis A, Lum PY (2010) Systematic genetic and genomic analysis of cytochrome P450 enzyme activities in human liver. Genome Res 20: 1020–1036.

Yasar Ü, Eliasson E, Dahl ML, Johansson, Ingelman-Sundberg M, Sjöqvist F (1999) Validation of methods for CYP2C9 genotyping: Frequencies of mutant alleles in a Swedish population. Biochem Biophys Res Commun 254: 628–631.

Zhou SF, Liu JP, Chowbay B (2009) Polymorphism of human cytochrome P450 enzymes and its clinical impact. Drug Metab Rev 41:89–295.