

SCREENING FOR *CYP2C19**2, *3 AND *4 GENE VARIANTS IN A ROMANIAN POPULATION STUDY GROUP

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Abstract

CYP2C19, a member of the cytochrome P450 enzymes family, metabolizes a range of clinically significant drugs. Its homologous gene has been shown to be polymorphic; two major alleles, *CYP2C19**2 and *3 and several other less frequent, among which the allele *4, are responsible for the poor metabolizer (PM) phenotype. Based on Polymerase Chain Reaction- restriction Fragment Length Polymorphism (PCR-RFLP) and tetra-primer PCR techniques, *CYP2C19**2, *3 and *4 variants were studied in 200 healthy, unrelated Romanian volunteers. 48 individuals (24%) were *CYP2C19**2 heterozygotes, while a homozygous genotype *CYP2C19**2/*2, has been demonstrated in 3 individuals (1.5%). No *CYP2C19**3 variant has been found in this study. *CYP2C19**4 was found only in a *CYP2C19**2/*4 compound heterozygous individual. The allele frequencies for *CYP2C19**2, *3 and *4 were 13.75%, 0% and 0.25%, respectively. Overall, the distribution of the *CYP2C19* alleles in the population studied matches data from reports on other Caucasian populations. Our study can represent the basis of establishing the best *CYP2C19* genotyping protocol in individuals who are prescribed *CYP2C19* substrates in Romania.

Rezumat

CYP2C19, un membru al sistemului enzimatic citocrom P450, metabolizează o serie de medicamente relevante din punct de vedere al importanței clinice. Gena ce codifică *CYP2C19* este polimorfică, cunoscându-se la ora actuală faptul că două alele majore, respectiv *CYP2C19**2 și *3, și altele mai puțin frecvente, printre care și alela *4, sunt asociate cu un fenotip *CYP2C19* metabolizator lent. Am studiat distribuția alelelor *CYP2C19**2, *3 și *4 într-un grup de 200 de voluntari români sănătoși, neînruțiți, utilizând tehnici de genetică moleculară, respectiv PCR-RFLP și tetra-primer PCR. În grupul investigat, 48 de indivizi (24%) au fost heterozigoți *CYP2C19**2, în vreme ce 3 indivizi (1,5%) au fost homozigoți *CYP2C19**2. Alela *CYP2C19**3 nu a fost identificată nici în stare heterozigotă, nici în stare homozigotă. Alela *CYP2C19**4 a fost identificată la un singur individ, heterozigot compus *CYP2C19**2/*4. Astfel, frecvențele alelice *CYP2C19**2, *3 și *4 au fost 13,75%, 0 și respectiv 0,25%. În general, distribuția alelelor *CYP2C19**2, *3 și *4 observată în studiul nostru este similară cu cea observată în alte populații caucasiene. Studiul nostru ar putea reprezenta primul pas în stabilirea protocolului optim de genotipare în țara noastră pentru alelele *CYP2C19*, înainte de începerea tratamentului cu medicamente - substrat pentru *CYP2C19*.

Keywords: *CYP2C19*, allelic variants, genotyping, Romanian population

Introduction

Serious inter-ethnic and inter-individual differences regarding the drug response has been shown to be due, at least in part, to genetic polymorphisms involving the drug metabolizing enzymes.

Cytochrome P450 (CYP) superfamily enzymes metabolize a wide range of clinically significant drugs, as well as other xenobiotics. CYP2C19, a member of the cytochrome P450, is involved in the biotransformation of important drugs, such as: PPIs (proton pump inhibitors) [2, 39, 42] anticonvulsants (S-mephenytoin and diazepam) [27, 40], and major antidepressants (tricyclic antidepressants and SSRIs - selective serotonin reuptake inhibitors) [15]. Regarding the metabolic rates for these drugs, individuals can be classified as ultraextensive metabolizers (UMs) extensive metabolizers (EMs), intermediate metabolizers (IMs) and poor metabolizers (PMs). The frequency of CYP2C19 PMs varies from 3 -5% in Caucasians, 13-23% in Oriental populations [23] and as high as 70% in some Oceanian islands populations [24]. PM status is related to the genetic polymorphism exhibited by the *CYP2C19* gene (MIM *124020) and is inherited as an autosomal recessive trait [9]. From the multitude of detrimental *CYP2C19* alleles, *CYP2C19*2* and *CYP2C19*3* account together for >99% of Oriental PMs, but only around 87% of Caucasians PMs [9, 11, 12]. The principal detrimental allele, *CYP2C19*2*, results from a guanine (G) to adenine (A) transition at position 681 in exon 5 (rs4244285), producing an aberrant splice site [11] and it represents the most frequent CYP2C19 defect in all the populations. The other well characterized allele, *CYP2C19*3*, which results from a G to A transition at position 636 in exon 4 (rs57081121), leading to a premature stop codon [12] accounts for around 25% of all inactive forms in Orientals, [9] being by converse very rare in Caucasians.

*CYP2C19*4* allele, which is the result of an A to G transition in the initiation codon (rs28399504) of the CYP2C19 gene, explains in part the difference between Caucasians and Orientals regarding the prevalence of *CYP2C19*2* and *3 among the CYP2C19 PMs [13].

This study was a first attempt to determine the distribution of *CYP2C19*2*, *3 and *4 alleles in Romania, as there were no such data reported on the Romanian population. Meanwhile, the study provides a comparison between Romanians and different other populations, regarding the distribution of the major *CYP2C19*2* and *3 alleles and of the PMs prevalence.

Materials and methods

Study group

200 healthy, unrelated volunteers of Romanian ethnical origin have been included in the study, after their written, informed consent has been given by each one of them. This study was reviewed and approved by the Ethics Committee of the University of Medicine and Pharmacy «Iuliu Hatieganu», Cluj-Napoca.

*CYP2C19**2, *3 and *4 genotyping

After admission to the study, 3 mL of peripheral blood were withdrawn on EDTA (ethylenediaminetetraacetic acid) from each participant by venous puncture. DNA was extracted from peripheral blood leukocytes using a commercially available kit (Wizard Genomic DNA Purification Kit, Promega, Madison, USA).

The *CYP2C19**2, *3 and *4 alleles were studied using the PCR-RFLP (Polymerase Chain Reaction - Restriction Fragment Length Polymorphism) technique, after partially modified, previously described protocols [11, 12, 13]. The PCR reactions were carried out in an Eppendorf thermocycler (Mastercycler Gradient, Eppendorf, Germany). The amplification products were then digested overnight, with the appropriate restriction endonuclease, *Sma*I (5U at 30°C) for *CYP2C19**2, *Bam*HI (10U at 37°C) for *CYP2C19**3 and *Pst*II (5U at 37°C), respectively (Fermentas MBI, Vilnius, Lithuania). The digested PCR products were resolved by electrophoresis in 2.5% agarose gels stained with ethidium bromide.

In addition, some samples were randomly selected and genotyped for *CYP2C19**2 and *3, by two tetra-primer PCR assays, which are described in detail elsewhere [21]. The tetra-primer PCR amplification products were analyzed by electrophoresis in 2.5% agarose gels stained with ethidium bromide. The results obtained by PCR-RFLP technique were 100% reproducible using the tetra-primer PCR assays.

Statistical analysis

The observed genotypes and alleles frequencies were compared with those expected, in order to verify the Hardy-Weinberg equilibrium. Alleles frequencies and PMs prevalence were compared between Romanian and other populations using the Fisher's exact test. The statistical analysis has been performed using the software GraphPad InStat version 3.0 (GraphPad Software, San Diego, California, USA). A value of $p < 0.05$ was considered statistically significant.

Results and discussion

CYP2C19 variants in Romanians

*CYP2C19**2 allele was present in 52 individuals, of which 48 (24%) were *CYP2C19**1/*2 heterozygotes, 3 (1.5%) were *CYP2C19**2/*2 homozygotes, while 1 individual (0.5%) was a *CYP2C19**2/*4 compound heterozygote. Meanwhile, *CYP2C19**3 was not found in any individual, neither in heterozygous, nor in homozygous state. Therefore, 4 individuals – 3 *CYP2C19**2/*2 homozygotes and 1 *CYP2C19**2/*4 compound heterozygote (2%) are predicted to be *CYP2C19* PMs. The allele frequencies for *CYP2C19**2, *3 and *4 were 13.75%, 0% and 0.25%, respectively. The frequencies of *CYP2C19* genotypes in the Romanian population were found not to deviate from the Hardy-Weinberg equilibrium. Genotypes distribution and allele frequencies for *CYP2C19**2, *3 and *4 are shown in detail in tables I and II.

Table I
CYP2C19 genotypes distribution in the Romanian population

<i>CYP2C19</i> genotypes	Observed		95% CI*	Expected number by Hardy- Weinberg law
	n	%		
*1/*1	148	74	67.5-79.6	147.9
*1/*2	48	24	18.08- 9.92	47.3
*1/*3	0	0	0	0
*1/*4	0	0	0	0.9
*2/*2	3	1.5	0.3-0.4	3.78
*2/*3	0	0	0	0
*2/*4	1	0.5	0-2.4	0.14
*3/*3	0	0	0	0
*3/*4	0	0	0	0
*4/*4	0	0	0	0

C*I= confidence interval

Table II
Allele frequencies for *CYP2C19* variants in the Romanian population

<i>CYP2C19</i> allele	Frequency		95%
	n= 400 chromosomes	%	
*1	344	86	82.6-89.4
*2	55	13.75	10.7-17.5
*3	0	0	0
*4	1	0.25	0-0.74

***CYP2C19**2 and *3 alleles in other populations versus Romanian population**

The allele frequency of *CYP2C19**2 in the Romanian population was not statistically different ($p>0.05$) from that found in most of the European ethnical groups, a similar distribution being found in Danish, Germans, Croatians, Swedish, Portuguese, Russians, Italians and Belgians. No significant differences regarding the *CYP2C19**2 allele frequency were found when comparing Romanians to South-West Asian groups (Turkish, Saudi Arabians, Jewish Israel) and to African ethnic groups (Tanzanians, Ethiopians, Beninese and Egyptians). In East and South Asian groups (Chinese, Japanese, Koreans and North Indians), in Australian aborigenes and Oceanians, *CYP2C19**2 was significantly more frequent than in the Romanian population ($p<0.05$). Significantly lower *CYP2C19**2 allele frequencies ($p<0.05$) were found in some South American groups (Bolivians and Columbians).

In the case of *CYP2C19**3 allele, a similar distribution as in Romanians have been found in most of the European, African, South-West Asian, North and South American ethnic groups ($p>0.05$). This allele was much more frequent ($p<0.0001$) than in Romanians in East and South Asians, Australian aborigenes and Oceanians.

Table III
Allele frequencies and PMs prevalence in different populations compared to Romanians (NS = statistically non-significant)

Population	Sample size	<i>CYP2C19</i> allele frequency %, (p-value)		PM genotype %, (p-value)	Reference
		*2	*3		
Romania	200	13.7	0	1.5	Present study
Europe					
Faroe Islands	310	18.8 (0.04)	0 (NS)	3.2 (NS)	Halling <i>et al.</i> , 2005
Denmark	239	16 (NS)	0 (NS)	3.8 (NS)	Bathum <i>et al.</i> , 1998

Germany	328	15.9 (NS)	0.2 (NS)	4.3 (NS)	Aynacioglu <i>et al.</i> , 1999
Croatia	200	15 (NS)	0 (NS)	3 (NS)	Bozina <i>et al.</i> , 2003
Sweden	83	14 (NS)	0.1 (NS)	1.2 (NS)	Yamada <i>et al.</i> , 1998
Portugal	153	13 (NS)	0 (NS)	1 (NS)	Ruas and Lechner, 1997
Russia	290	11.4 (NS)	0.3 (NS)	2 (NS)	Gaikovitch <i>et al.</i> , 2003
Italia	360	11.1 (NS)	0 (NS)	1.7 (NS)	Scordo <i>et al.</i> , 2004
Belgium	121	9.1 (NS)	0 (NS)	1.6 (NS)	Allabi <i>et al.</i> , 2003
Asia					
Saudi Arabia	97	15 (NS)	0 (NS)	2 (NS)	Goldstein <i>et al.</i> , 1997
Jewish Israel	140	15 (NS)	1 (NS)	2.8 (NS)	Sviri <i>et al.</i> , 1999
Iran	200	14 (NS)	0 (NS)	3 (NS)	Zand <i>et al.</i> , 2005
Turkish	404	12 (NS)	0.4 (NS)	1 (NS)	Aynacioglu <i>et al.</i> , 1999
China	121	45.5 (<0.0001)	4.5 (<0.0001)	24 (<0.0001)	Yamada <i>et al.</i> , 2001
Philippines	52	39 (<0.0001)	8 (<0.0001)	23 (<0.0001)	Goldstein <i>et al.</i> , 1997
North India	121	29.7 (<0.0001)	0 (NS)	7.4 (0.01)	Lamba <i>et al.</i> , 2000
Japan	217	27.4 (<0.0001)	10.8 (<0.0001)	15.2 (<0.0001)	Takakubo <i>et al.</i> , 1996
Korea	103	20.9 (0.02)	11.7 (<0.0001)	11.7 (0.0002)	Roh <i>et al.</i> , 1996
Australia and Oceania					
Australian Aborigines	227	35.5 (<0.0001)	14.3 (<0.0001)	25.6 (<0.0001)	Griese <i>et al.</i> , 2001
Vanuatu+other Pacific islands	5 538	63.3 (<0.0001)	14.4 (<0.0001)	61 (<0.0001)	Kaneko <i>et al.</i> , 1999

North America						
Canadian Indian	Native	115	19.1 (NS)	0 (NS)	7 (0.04)	Nowak <i>et al.</i> , 1998
South America						
Columbia		189	8.7 (0.03)	0	1.1 (NS)	Isaza <i>et al.</i> , 2007
Bolivia		778	7.8(0.0004)	0.1 (NS)	1 (NS)	Bravo-Villalta <i>et al.</i> , 2005
Africa						
Tanzania		251	18 (NS)	1 (NS)	3.2 (NS)	Herrlin <i>et al.</i> , 1998
Ethiopia		114	14 (NS)	3 (0.0008)	5.3 (NS)	Persson <i>et al.</i> , 1996
Benin		111	13 (NS)	0 (NS)	0 (NS)	Allabi <i>et al.</i> , 2003
Egypt		247	10.9 (NS)	0.2 (NS)	0.8 (NS)	Hamdy <i>et al.</i> , 2002

PMs in other populations versus Romanians

As we had no data for most of the other populations regarding the frequency of the *CYP2C19**4 allele, and thus its contribution to the PMs prevalence, we compared the PMs prevalences determined by the major alleles *CYP2C19**2 and *3 only. When comparing the prevalence of PMs in different other populations to that found in Romanians, no significant differences ($p>0.05$) have been demonstrated among Romanians and most of European, South American and African populations. A significantly higher ($p<0.05$) number of PMs than in Romanians has been observed in South and East Asians, Australian aborigenes and Oceanians.

Table III presents the detailed comparison between Romanians and different populations regarding the *CYP2C19**2 and *3 allele frequencies and PMs prevalence.

The distribution of *CYP2C19* variants in the Romanian population is consistent with the relatively high frequency of *CYP2C19**2 allele all around the world, suggesting that this mutation is old and has occurred before the separation of Caucasians, Oriental and Black populations [31]. By contrast, the *CYP2C19**3 allele, which was absent in our population group, is specific to Asian and Oceanian ethnical groups. The low frequency, or sometimes absence of this allele in different Caucasians

populations suggests that this allele occurred quite recently, after the separation of Caucasian and Oriental groups [33].

For *CYP2C19**4, the allelic frequency of 0.25% we observed in our group is close to the value of 0.6% reported on 173 Caucasian individuals [13]. In that report, *CYP2C19**4 contributed to around 3% of the *CYP2C19* PM alleles.

Based on the results of our study, by extrapolation, it would be expected that more than 400,000 Romanians are PMs for *CYP2C19* drug substrates. Nevertheless, taken into consideration other *CYP2C19* defective alleles, such as *CYP2C19**5, *6, *7 or *8, which have been reported to contribute to the PM phenotype in the Caucasian populations, but haven't been studied in our population yet, the number of PMs could be even higher in our country. Another concern would be the number of *CYP2C19* UMs, associated with the newly described *CYP2C19**17 allele [34] which was reported to have high frequencies in the Caucasians and to contribute to treatment failure in the case of *CYP2C19* substrates.

According to the results of multiple studies, *CYP2C19* genetic variation represents an important factor affecting the pharmacokinetics of most of the PPIs, values of intragastric pH and eradication rates of *Helicobacter pylori* [32].

CYP2C19 has a role in the biotransformation of the antiepileptic drug phenytoin, especially with increasing doses and concurrent *CYP2C9* deficiency; in PMs who are prescribed high doses, a reduced phenytoin clearance is expected to occur [4, 15].

The treatment for depression comprises some agents that are metabolized by *CYP2C19*, such as the tricyclic antidepressants amitriptyline, imipramine and clomipramine and the SSRIs citalopram, escitalopram and sertraline. *CYP2C19* PMs may manifest weaker tolerance to tricyclic antidepressants and SSRIs and could be susceptible to adverse reaction at usual doses, especially those in whom other routes of elimination for these drugs are suppressed [10, 15].

CYP2C19 metabolizes some benzodiazepines as well, including diazepam, flunitrazepam, quazepam and clobazam. Diazepam is the most studied benzodiazepine in relation with *CYP2C19* genetic variation. Several studies indicated that the overall defect in PMs is an increased exposure to diazepam and thus a prolonged effect [6, 29, 35], which could lead to excessive central nervous system depression, especially with long-term regimens [15].

For all above mentioned drugs, which are currently frequently used in practice in Romania, *CYP2C19* genotyping prior to the therapy

initialization could be a reasonable approach, with respect to the optimal dosage adjustments and treatment cost-effectiveness. The results of our study indicate that such an attitude would be suitable for *CYP2C19**2 and perhaps *CYP2C19**4 alleles, but probably not for the *CYP2C19**3 allele, which was absent in our group. Thus, the magnitude of adverse drug reactions and/or lack of efficacy of treatment could be substantially reduced.

Conclusions

In conclusion, this is the first report on the Romanian population regarding the distribution of the 2 most studied detrimental alleles of the *CYP2C19* gene and of the less characterized across different populations *CYP2C19**4 allele. Overall, the distribution of the *CYP2C19**2 and *3 variants observed in the Romanian population is in agreement with the frequencies of these 2 variants reported in other ethnic groups. Taken into consideration that other alleles associated with an abnormal *CYP2C19* enzymatic activity do exist in Caucasians, it would be of interest to assess their frequencies in the Romanian population as well, in order to obtain a more comprehensive *CYP2C19* PMs alleles profile of our population. This would serve as a valuable tool in establishing the optimal *CYP2C19* genotyping protocol in individuals who are prescribed *CYP2C19* substrates.

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