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Genetic Polymorphisms of Cytochromes P450: CYP2C9, CYP2C19, and CYP2D6 in Croatian Population

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Aim. To determine the prevalence of most common mutations of cytochrome P450 (CYP), ie, allelic variants of CYP2C9, CYP2C19, and CYP2D6, and to predict genotype frequency in the Croatian population.

Methods. CYP genotype was determined in 200 non-related Croatian citizens. DNA isolated from blood samples was used for the analysis of the most common allelic variants of CYP2C9, CYP2C19, and CYP2D6 by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

Results. For 200 subjects genotyped for CYP2C9, the allele frequencies of CYP2C9*1 (wt), CYP2C9*2, and CYP2C9*3 were 0.74, 0.165, and 0.095, respectively. Among them, 3.5% of subjects were predicted to be poor metabolizers. For CYP2C19, the most frequent alleles were CYP2C19*1 and CYP2C19*2, with frequencies of 0.85 and 0.15, respectively; 3% of subjects were predicted to be poor metabolizers. For CYP2D6, the most frequent alleles were CYP2D6*1 (frequency 0.765), CYP2D62* (0.04), CYP2D6*3 (0.0275), CYP2D6*4 (0.14), CYP2D6*5 (0.01), and CYP2D6*6 (0.015). Out of these, 3% were predicted to be poor metabolizers, and 4% were predicted to be ultra-rapid metabolizers.

Conclusion. The prevalence of allelic variants and predicted genotypes in the Croatian population is in accordance with the other European populations, and it can be interpolated between the values for mid-European and Mediterranean populations.

Key words: *alleles; Croatia; cytochrome P450 enzyme system; genotype; phenotype; polymorphism (genetics)*

Cytochrome P450 (CYP) enzymes are important catalysts for oxidative biotransformation of both endogenous and exogenous compounds, including drugs (1,2). Genetic polymorphism of CYPs can lead to severe toxicity or therapeutic failure of medications (3) as well as to a possible increase in an individual's susceptibility to certain types of chemically induced cancers and other diseases (4). Interindividual variations of drug metabolizing enzyme activity were first documented by phenotyping. In this approach, the metabolic capacity is predicted from the metabolic ratio of metabolite to parent drug (5). Phenotyping has some disadvantages, ie, complicated protocols, interactions with other drugs that the patient must take (6), a risk of adverse drug reactions, and confounding effects of disease.

The development of objective and reproducible molecular methods has made pharmacogenetic analysis possible, e.g., genotyping of polymorphic alleles and comparison/prediction of drug metabolism in variant genotypes (7). Genotyping can increase the safety and efficacy of pharmacotherapy by identifying patients susceptible to the development of harmful side effects of certain drugs, and it can predict the drug efficiency in patients prior to treatment. The completion of the human genome sequence draft revealed the presence of 90 different cytochrome P450 genes, of which 55 are functional (8). CYP2D6, debrisoquine 4-hydroxylase, is involved in the metabolism of many widely prescribed drugs (9,10). Interethnic differences in the activity of these enzymes have been reported (11,12). Poor activity of the enzyme is an autosomal recessive trait, with 2-10% prevalence in white populations (11-13), 1% in Chinese and Japanese, and between 0-2 % in the black populations. The ultraextensive metabolizer fenotype, caused by CYP2D6 gene duplications, can be found at various frequencies in different populations: 1% in Swedes, 2% in Germans, 6% in Spaniards, 8% in Turks, 20% in Arabs, 29% in Ethiopeans, and 1-2% in Asians (14,15). With standard medications, the poor metabolizer phenotype could develop adverse reactions and the ultraextensive metabolizer pheno-

type will have subtherapeutic plasma concentrations and consequently decreased drug response (16,17). Although more than 70 different allelic variants have been identified, the analyses of CYP2D6*3, *4, *5, and *6 mutant alleles and gene duplications have to be performed to allow a 99% sensitive prediction of poor or ultrarapid metabolizers in the clinical routine. The polymorphism in CYP2C family is important because these enzymes act on some very important drugs: anticonvulsants, antidiabetics, anticoagulants, antidepressants, antimalarial, nonsteroid antiinflammatory agents, and proton pump inhibitors (18,19). Polymorphisms in CYP2C9 seriously affect the toxicity of drugs with lower therapeutic indices, such as the anticonvulsant phenytoin and the common anticoagulant warfarin, causing severe and life-threatening bleeding episodes (20,21). The CYP2C9 allele in poor metabolizers has a frequency of approximately 2-6% in white populations (22). At present, 12 different alleles of CYP2C9 have been reported; CYP 2C9*2 and/or CYP2C9*3 alleles are present in about 85% of poor metabolizers. Of the polymorphic enzyme CYP2C19, which hidroxylates (S)-mephenytoin on the 4′ position, 15 variant alleles have been identified. Marked interracial differences have been documented (23): the poor metabolizer prevalence is approximately 1-5% in white populations, 13-23 % in Orientals, 6% in Ethiopians, and 70% in villagers residing in Tanna and Malakula islands (Vanuatu). CYP2C19*2 and CYP2C19*3 alleles are responsible for about 95% of poor metabolizer phenotypes.

The aim of this study was to investigate the prevalence of most common allelic variants of CYP2C9, CYP2C19, and CYP2D6 in the Croatian population and compare them with the literature data for other populations. These data, summarized by experts in pharmacogenomics, are available online (*http://www.imm.ki.se /CYPalleles*).

Subjects and Methods

Subjects

All participants were Croatian citizens residing in Zagreb urban area, but with origins from all parts of Croatia, thus representing a mixed population (100 participants originated from the continental part of Croatia and 100 participants originated from the area along the Adriatic sea and islands). All subjects were included in the study after giving informed consent. The study was approved by the Ethics Committee of the Zagreb University Hospital Center. Two hundred subjects (120 men and 80 women for 2C9; 104 men and 96 women for 2C19; and 110 men and 90 women for 2D6) participated in each genotype determination study for screening polymorphic variants (Table 1).

Genotyping Procedures

Genomic DNA was isolated from 5 mL peripheral blood collected in sodium-ethylenediaminetetraacetic acid (Na-EDTA) vacutainers according to the standard procedure (24,25). Polymerase chain reactions (PCR) were run in 0.2 mL tubes on the Perkin Elmer DNA Thermal Cycler 9600 (Norwalk, CT, USA).

CYP2C9. For the detection of CYP2C9*2 and CYP2C9*3 alleles, we performed a 50 μL polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis using Ava I and Nsi I restriction endonuclease (Roche Diagnostics, Mannheim, Germany), respectively (26).

CYP2C19. For the detection of CYP2C19*2 and CYP2 C19*3 alleles, a 25 μL tetra-primer PCR was performed (27).

Table 1. Most frequent alleles, nucleotide changes, and enzyme activities of CYP2C9, CYP2C19, and CYP2D6 according to previous investigations*

Allele	Nucleotide changes	Effect	Enzyme activity
CYP2C9*1 (wt)	none		normal
CYP2C9* 2	C 430 T	R 144 C	decreased
CYP2C9* 3	A1075 C	I 359 L	decreased
CYP2C19*1 (wt)	none	normal	
CYP2C19* 2	G 681 A	splicing defect	none
CYP2C19* 3	G 636 A	stop codon	none
CYP2D6*1 (wt)	none		normal
CYP2D6*2 (1xN)	N active genes		increased
CYP2D6* 3	A 2549 del	frameshift	none
CYP2D6* 4	G 18464 A	splicing defect	none
CYP2D6* 5	CYP2D6 deleted	gene absence	none
CYP2D6* 6	T1707 del	frameshift	none

*Data available at *http://www.imm.ki.se /CYPalleles*.

CYP2D6. For the detection of CYP2D6*2, a 50 μL long-PCR was performed (28). For the detection of CYP2D6*3 and CYP2D6*4 alleles, we used a 50 μL PCR-RFLP method with Msp I and Mva I restriction endonucleases (Roche Diagnostics, Mannheim, Germany), respectively (29,30). Long-PCR reaction was performed for CYP2D6*5 detection (31).

For the detection of CYP2D6*6, a 25 μL tetra-primer PCR was performed (30).

Statistical Analysis

MedCalc 4.10 (Frank Schoonjans, Mariakerke, Belgium) and Excel 97 SR-1 (Microsoft, USA) PC programs were used for statistical analysis. Hardy-Weinberg equilibrium was tested by the chi-square test, and 95% confidence intervals (95% CI) calculated.

Results

The frequency of polymorphic CYP2C9 alleles responsible for impaired drug metabolisms, CYP 2C9*2 and CYP2C9*3, were 0.165 and 0.095, respectively (Table 2). The proportion of subjects homozygous for the wild type allele (extensive metabolizers), heterozygous for the mutant alleles (with partially impaired enzyme activity, intermediate metabolizer), and homozygous for the mutant alleles (poor metabolizers) was 74.0%, 22.5%, and 3.5%, respectively (Table 3). The frequency of polymorphic

Table 2. Frequencies of wild type (wt) and mutant alleles of CYP2C9, CYP2C19, and CYP2D6 in the study group of 200 Croatians

Cytochrome P450 genes and alleles*	No. of alleles	% (95% CI)
CYP2C9:		
1-wt	296	74.0 (69.5-78.1)
*2	66	16.5 (13.2-20.5)
*3	38	9.5 (7.0-12.8)
total mutant	104	26.0 (21.9-30.5)
total	400	100.0
CYP2C19:		
1-wt	340	85.0 (81.2-88.2)
*2	60	15.0 (10.1-16.7)
total mutant	60	15.0 (11.8-18.8)
total	400	100.0
CYP2D6:		
1-wt	306	76.5 (72.1-80.4)
*2	16	4.0 (2.5-6.4)
*3	12	2.75 (1.7-5.2)
*4	56	14.0 (10.9-17.7)
*5	4	1.0 (0.4-2.5)
*6	6	1.5 (0.7-3.2)
total mutant	94	23.5 (19.6-27.9)
total	400	100.0

*Mutant allele.

Table 3. Prevalence of the CYP2C9, CYP2C19, and CYP2D6 genotypes in the study group of 200 Croatians*

Cytochrome P450 genotypes	No. of subjects	% (95% CI)
CYP2C9:		
wt/wt	148	74.0 (67.1-79.3)
wt/mut	45	22.5 (15.3-29.7)
mut/mut	7	3.5 (1.7-7.0)
total	200	100.0
CYP2C19:		
wt/wt	146	73.0 (66.5-78.7)
wt/mut	48	24.0 (18.6-30.4)
mut/mut	6	3.0 (1.4-6.4)
Total	200	100.0
CYP2D6:		
wt/wt	120	60.0 (53.1-66.5)
wt/mut	66	33.0 (26.9-39.8)
mut/mut	6	3.0 (1.4-6.4)
dupl	8	4.0 (2.0-7.7)
total	200	100.0

*Abbreviations: wt/wt – homozygous wild type; wt/mut – heterozygous mutant; mut/mut – homozygous mutant; dupl – duplications.

CYP2C19 *2 allele was 0.15. There were 73.0% extensive metabolizers, 24.0% intermediate metabolizers and 3.0% poor metabolizers in the CYP2C19 genotype in the Croatian population. The frequencies of polymorphic CYP2D6*2,*3, *4,* 5, and * 6 alleles were 0.04, 0.028, 0.14, 0.01, and 0.015, respectively. The most frequently observed null allele was CYP2D6*4, which accounted for 72% of all null alleles. Among the population studied, 60% of the subjects had extensive metabolizer genotype, 33% were intermediate metabolizers, and 3% exhibited the poor metabolizer genotype. Four percent exhibited the ultrarapid metabolizer genotype due to amplified CYP2D6 gene (*2 allele). The observed genotypes were in Hardy-Weinberg equilibrium.

Discussion

Our study showed the prevalence of genetic polymorphisms of important cytochromes P450, ie, CYP2C9, CYP2C19, and CYP2D6, in the Croatian population. The subjects included in the study resided in the Zagreb area but originated from different parts of Croatia and were good representatives of a mixed Croatian population. The frequency values for polymorphic alleles and genotypes corresponded to the frequencies for other European white populations (11-13,16,19). According to our results, the prevalence of CYP2C9 genotypes in Croatian population is similar to other mid-European populations (approximately 3% of poor metabolizers) (22,23). Genotyping for polymorphic CYP2C19 revealed the CYP2C19*2 mutant allele (frequency, 15%) but not the CYP2C 19*3 allele (main allelic variant in Oriental populations), which is in agreement with the results of other investigators (20,13). The frequency of the most common allelic variant of CYP2C19*2 in the Croatian population was comparable to that found in other European populations: 13.3% Duch (13), 15% German (32), 13% French (19), and 15% Swedish (33). Within the European populations, there are interethnic differences in the CYP2D6 genotype distributions (1-10%), with a decreasing frequency of poor metabolizers to the south (north-south gradient) and a corresponding increase in ultrarapid metabolizers (14,16). We have found the frequency distribution of CYP2D6*1, CYP2D6*2, CYP2D6*3, CYP2D6*4, CYP2D6*5, and CYP2D6*6 alleles to be similar to those for the other white European populations. Our CYP2D6 genotype values (3% of homozygous mutants, with predicted phenotype of poor metabolizers, and 4% of gene duplications, with predicted phenotype of ultrarapid metabolizers) were interpolated between the values for northern and mid-European countries (9,10,14) and Mediterranean countries (11,16). This is the first time the distribution of the genotypes of cytochromes P450, ie, CYP2C9, CYP2C19, and CYP2 D6, has been estimated in the Croatian population. The prevalence values of polymorphic alleles CYP2D 6*3, *4, and *6 (0.014, 0.11, and 0.010 respectively) reported by Topić et al (34) are in agreement with our results.

In conclusion, our study showed that cytochrome P450 genes – CYP2C9, CYP2C19, and CYP2D6 – were polymorphic in the Croatian population, with a similar distribution as determined in other European populations. Because these genetic polymorphisms are medically significant, genotyping could help clinicians to optimization of therapy or identification of persons at risk of adverse drug reactions before clinical trials.

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