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)
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; CYP2C9*2 and/or CYP2C9*3 alleles are present in about 85% of poor metabolizers. Of the polymorphic enzyme CYP2C19, which hydroxylates (S)-mephenytoin on the 4’ position, 15 variant alleles have been identified. Marked inter racial 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 (NaEDTA) 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 endonucleases (Roche Diagnostics, Mannheim, Germany), respectively (26).

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

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 withMsp 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, CYP2C9*2 and CYP2C9*3, were 0.165 and 0.095, respectively (Table 2). The proportion of subjects homozygous for the wild type allele (extensive metabolizer), 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

<p>| Table 1. Most frequent alleles, nucleotide changes, and enzyme activities of CYP2C9, CYP2C19, and CYP2D6 according to previous investigations |</p>
<table>
<thead>
<tr>
<th>Allele</th>
<th>Nucleotide changes</th>
<th>Effect</th>
<th>Enzyme activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9*1 (wt)</td>
<td>none</td>
<td>normal</td>
<td>decreased</td>
</tr>
<tr>
<td>CYP2C9*2</td>
<td>C 430 T</td>
<td>R 144 C</td>
<td>normal</td>
</tr>
<tr>
<td>CYP2C9*3</td>
<td>A1075 C</td>
<td>I 359 L</td>
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</tr>
<tr>
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<td>decreased</td>
</tr>
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</tr>
<tr>
<td>CYP2C9*3</td>
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</tr>
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</tr>
<tr>
<td>CYP2D6*2 (1XN)</td>
<td>none</td>
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<td>none</td>
</tr>
<tr>
<td>CYP2D6*3</td>
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<tr>
<td>CYP2D6*6</td>
<td>T1707 del frameshift</td>
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</table>

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 polymerase chain reaction (PCR) amplification. 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 CYP2C19 *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% Dutch (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 ultra-rapid 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 CYP2D6, has been estimated in the Croatian population. The prevalence values of polymorphic alleles CYP2D6 *6, *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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