Genetic polymorphisms of \textit{CYP2C9}, \textit{CYP2C19}, and \textit{CYP3A5} in Kosovar population

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Cytochrome P450 genetic polymorphisms are responsible for individual variations in drug metabolism and drug-drug interactions. They are very important for pharmacogenetics, and their frequency varies across different populations. There is a big gap in the knowledge about the CYP gene family polymorphisms in the population of Kosovo, and the aim of our study was to fill that gap by determining the frequency of the most important variant alleles of \textit{CYP2C9}, \textit{CYP2C19}, and \textit{CYP3A5} in 234 nonrelated Kosovars. The allele frequencies of \textit{CYP2C9}\textsuperscript{*2} and \textit{2C9}\textsuperscript{*3} were 17.52 \% and 10.89 \%, respectively. Sixteen participants (6.81 \%) were \textit{CYP2C9} poor metabolisers. The \textit{CYP2C19}\textsuperscript{*2} and \textit{*17} variant frequencies were 13.03 \% and 19.01 \%, respectively. There were 2.13 \% \textit{CYP2C19} poor and 4.27 \% ultra-rapid metabolisers (homozygous carriers of the \textit{*17} allele). With regard to \textit{CYP3A5}, the frequency of the \textit{*3} variant allele was 98.29 \% (non-expressors), while the remaining participants (1.70 \%) were expressors of \textit{CYP3A5}. These findings are comparable with other European ethnicities, specifically those of Southeast Europe.

KEY WORDS: cytochrome P450 enzyme system; drug metabolism; pharmacogenetics

Individual variability in response to the most common drugs presents one of the major challenges to pharmacotherapy today. This variability depends on a variety of factors (drug interactions, hepatorenal disorders, sex, age, and lifestyle), but the most challenging are the genetic polymorphisms that have a direct and important impact on drug-detoxifying enzymes, drug targets, and drug transporters (1-3). Phase I enzymes metabolise nearly 59 \% of the drugs reported for adverse drug reactions (ADR), and cytochromes P450 (CYP450 or CYP) make 75–86 \% of these phase I enzymes (2, 4). Their genetic variants are responsible for unintended drug metabolism and interactions (5) that can lead to toxicity or failed pharmacotherapy (6). This has been recognised by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA), as both push policies requiring that pharmacogenetic information is provided for drug development and postmarketing surveillance (7, 8).

The most important genetic variants of CYP in clinical practice include those of \textit{CYP2C9}, \textit{CYP2C19}, and \textit{CYP3A5}. Their frequencies vary across populations, and mapping them, so to speak, can be very useful in designing specific pharmacotherapy. To the best of our knowledge no such mapping has been done for the population of Kosovo, a southeast European country of over 1.7 million people, 93 \% of whom are ethnic Albanians. The aim of our study was to address this gap and determine the frequency of the pharmacologically most important variant alleles of \textit{CYP2C9}, \textit{CYP2C19}, and \textit{CYP3A5} in Kosovo population, hoping that our findings could help to optimise pharmacotherapy in the country.

PARTICIPANTS AND METHODS

This study included a mixed population of 234 randomly selected Caucasians (116 women and 118 men) with no blood relation from all parts of Kosovo. Their age ranged between 18 and 65 years (median: 36 years). The main exclusion criteria were mental illnesses or a history of serious physical illness.

The study was approved by the Ethics Committee of the University Clinical Centre of Kosovo in Prishtina (Kosovo) and by the Faculty of Pharmacy in Skopje (Macedonia), where genotyping was performed. Prior to enrolment, all participants gave their written consent for
participation. The study was performed according to the principles expressed in the Declaration of Helsinki.

We determined the frequencies of the most common variant alleles of polymorphic CYP enzymes: CYP2C9*2 (c.430C>T, rs1799853), CYP2C9*3 (c.1075A>C, rs1057910), CYP2C19*2 (c.681G>A, rs4244285), CYP2C19*17 (c. 806C>T, rs12248560), and CYP3A5*3 (g.6986A>G, rs776746). The CYP2C19*3 (c.636G>A, rs57081121) allele was excluded as very rare in the European population (9, 10).

For genotyping we collected 5 mL of peripheral blood from each subject into test tubes with the anticoagulant ethylene diamine tetra-acetic acid (EDTA). Genomic DNA was extracted from whole blood using a QIAGEN DNA extraction kit (QIAGEN AS, Oslo, Norway). The extracted DNA concentrations were determined with a NanoDrop 2000™ spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA).

CYP2C9*2 (C_25625805_10), CYP2C9*3 (C_27104892_10), CYP2C19*2 (C_25986767_70), CYP2C19*17 (C_469857_10), and CYP3A5*3 (C_26201809_30) were genotyped using a TaqMan® drug metabolism genotyping assay or a TaqMan® single nucleotide polymorphism (SNP) genotyping assay (Applied Biosystems, Foster City, CA, USA) on an Mx3005P real-time quantitative polymerase chain reaction (QPCR) system with pertaining MxPro software (Agilent Technologies, Santa Clara, CA, USA) following the manufacturer’s instructions.

In order to multiply a DNA segment, we mixed 2 µL of DNA with 10.5 µL of PCR and incubated the mixture under the following conditions: uracil-N-glycosylase incubation (2 min at 50 °C), AmpliTaq Gold® activation (10 min at 95 °C), PCR [50 cycles: denaturation (15 sec at 92 °C); annealing/extension (60 sec at 90 °C)].

Statistical analysis

The variables were analysed with an SPSS statistical program (SPSS Inc., Chicago, IL, USA, 20.0 version) and online calculator (available at https://www.allto.co.uk/tools/statistic-calculators/confidence-interval-for-proportions-calculator). Allele and genotype frequencies were estimated using the gene counting method, and their distribution was tested with the chi-squared test for the Hardy-Weinberg equilibrium.

Using the chi-squared test with Yate’s correction we compared our allele frequency distribution with other studies to establish differences or similarities with other populations, Caucasian in particular. The threshold of significance was set at a 5 %, where all differences above p=0.05 were considered non-significant between our results and the reported frequencies in other populations.

RESULTS AND DISCUSSION

Our findings of the CYP gene polymorphisms in the Kosovar sample are similar to other European populations reported in several publications (11–13). Tables 1–3 show genotype distribution and the predicted phenotypes of CYP2C9, CYP2C19, and CYP3A5. All genotype frequencies were in line with the Hardy-Weinberg principle.

CYP2C9

The CYP2C9 allele frequencies compare to other European Caucasians, such as Croats (Southeast Europe) and the French (West Europe) (14, 15). The CYP *2 and *3 allele frequencies in our sample were 17.52 % and 10.89 %, respectively. The frequency of CYP2C9 poor metabolisers was 6.8 %, which is similar to the Spanish (5.0 %) (16) and Macedonian (5.1 %) population, but differs from England’s population (3.2 %) (17, 18). The following frequencies of CYP2C9*2 have been reported for the European Mediterranean and other European countries: Spain (16.0 %), Greece (12.9 %), Croatia (14.0 %), Romania (11.3 %), Germany (14.0 %), France (15.0 %), and Sweden (10.7 %) (19–25).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of participants (N)</th>
<th>Frequency (%)</th>
<th>95 % confidence interval</th>
<th>Predicted phenotype</th>
<th>Investigated allele</th>
<th>No. of alleles (2n) / (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9*1/*1</td>
<td>117</td>
<td>50.00</td>
<td>43.59 - 56.41</td>
<td>EM</td>
<td>CYP2C9*1</td>
<td>335 (71.58)</td>
</tr>
<tr>
<td>CYP2C9*1/*2</td>
<td>62</td>
<td>26.49</td>
<td>20.84 - 32.14</td>
<td>IM</td>
<td>CYP2C9*2</td>
<td>82 (17.52)</td>
</tr>
<tr>
<td>CYP2C9*1/*3</td>
<td>39</td>
<td>16.66</td>
<td>11.89 - 21.43</td>
<td>IM</td>
<td>CYP2C9*3</td>
<td>51 (10.89)</td>
</tr>
<tr>
<td>CYP2C9*2/*2</td>
<td>8</td>
<td>3.41</td>
<td>1.08 - 5.74</td>
<td>PM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C9*2/*3</td>
<td>4</td>
<td>1.70</td>
<td>0.04 - 3.36</td>
<td>PM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C9*3/*3</td>
<td>4</td>
<td>1.70</td>
<td>0.04 - 3.36</td>
<td>PM</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PM - poor metabolisers; IM - intermediate metabolisers; EM - extensive metabolisers
receiving carriers of the CYP2C19*17 allele were reported for increased risk of bleeding, the highest risk being for those who carried the CYP2C19*17/*17 genotype. Eight participants (3.41 %) in our sample were the carriers of the CYP2C19*2/*17 combined genotype. Their metabolic phenotype is difficult to predict and remains a matter of debate. Some data suggest that the presence of the CYP2C19*17 allele may not compensate for the inactivating effect of CYP2C19*2 (36).

Finally, our results indicate a significant difference from other Caucasians, such as Russian (CYP2C19*1/*1; p<0.001) (37).

CYP3A5

Over 98.3 % of our participants had the CYP3A5*3/*3 genotype and are classified as non-expressors. This finding corresponds to the other reports for Caucasians (18, 24, 28), as it varies from 85 to 96 % (38). The CYP3A5*3 allele plays an important role in the pharmacokinetics of tacrolimus (basic therapy for immunosuppression in patients who undergo solid organ and haematopoietic stem cell transplantation) and imatinib (a tyrosine kinase inhibitor, which is the drug of choice in treating blood malignancies such as chronic myeloid leukaemia) (39). Statistical analysis showed a significant similarity with Croats (95.5 %; p=0.072), Basques (96.0 %; p=0.143), and Poles (96.3 %; p=0.180) (16, 24, 40).

CONCLUSION

Even though our study is limited to the most important CYP alleles (SNPs) and a relatively small sample, it clearly

Table 2 CYP2C19 genotype frequencies and predicted phenotype in the Kosovar population sample (N=234)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of participants (N)</th>
<th>Frequency (%)</th>
<th>95 % confidence interval</th>
<th>Predicted phenotype</th>
<th>Investigated allele (2n) / (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C19*1/*1</td>
<td>107</td>
<td>45.72</td>
<td>39.34 - 52.1</td>
<td>EM</td>
<td>CYP2C19*1</td>
</tr>
<tr>
<td>CYP2C19*1/*2</td>
<td>43</td>
<td>18.37</td>
<td>13.41 - 23.33</td>
<td>IM</td>
<td>CYP2C19*2</td>
</tr>
<tr>
<td>CYP2C19*2/*2</td>
<td>5</td>
<td>2.13</td>
<td>0.28 - 3.98</td>
<td>PM</td>
<td>CYP2C19*17</td>
</tr>
<tr>
<td>CYP2C19*2/*17</td>
<td>8</td>
<td>3.41</td>
<td>1.08 - 5.74</td>
<td>IM</td>
<td></td>
</tr>
<tr>
<td>CYP2C19*1/*17</td>
<td>61</td>
<td>26.06</td>
<td>20.44 - 31.68</td>
<td>UM</td>
<td></td>
</tr>
<tr>
<td>CYP2C19*17/*17</td>
<td>10</td>
<td>4.27</td>
<td>1.68 - 6.86</td>
<td>UM</td>
<td></td>
</tr>
</tbody>
</table>

PM - poor metabolisers; IM - intermediate metabolisers; EM - extensive metabolisers; UM - ultra-rapid metabolisers

the following frequencies: France 8.0 %, Belgium 7.4 %, Hungary 8.8 %, Croatia 9.5 %, Greece 8.1 %, and Macedonia 7.3 % (14, 15, 23, 26–28).

CYP2C19

Genotyping the CYP2C19 alleles in Kosovars revealed the *2 and *17 allele frequencies of 13.03 % and 19.01 %, respectively, which are comparable to other Caucasians. CYP2C19*2 is the most common variant allele in Caucasians, and its frequency varies from 13.1 % to 18.7 % (23, 29). We found high similarity of our distribution with both Macedonia (14.9 %; p=0.55) and Greece (13.1 %; p=1.00) (23, 28).

As the CYP2C19*2 is an inactivating allele, knowing its distribution in a population is important when prescribing medicines such as clopidogrel, which is a pro-drug that must be activated by CYP2C19 (30). In this study, five participants (2.13 %) were discovered to be homozygous carriers of the *2 allele (CYP2C19*2/*2 genotype).

Our findings of the CYP2C19*17 frequency in Kosovars are very similar to reports from the neighbouring countries, Greece (19.61 %, p=0.69) and Macedonia (20.2 %, p=0.84) in particular (23, 24, 31). In Caucasians its frequency varies from 15.4 % to 20.2 % (17). The CYP2C19*17 allele was documented to be more frequent in the Mediterranean and Middle East than in Eastern Asia (32). Even though carriers of the CYP2C19*1/*17 and CYP2C19*17/*17 genotype were predicted to be ultra-rapid metabolisers, only homozygous carriers of the *17/*17 genotype have shown a higher rate of metabolism of the CYP2C19 substrates, such as tricyclic antidepressants and esomeprazole when compared to extensive metabolisers (33-35). Clopidogrel

Table 3 CYP3A5 genotype frequencies and predicted phenotype in the Kosovar population sample (N=234)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of participants (N)</th>
<th>Frequency (%)</th>
<th>95 % confidence interval</th>
<th>Predicted phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP3A5*1/*1</td>
<td>4</td>
<td>1.7</td>
<td>0.04 - 3.36</td>
<td>expressor</td>
</tr>
<tr>
<td>CYP3A5*1/*3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>expressor</td>
</tr>
<tr>
<td>CYP3A5*3/*3</td>
<td>230</td>
<td>98.3</td>
<td>96.64 - 99.96</td>
<td>non-expressor</td>
</tr>
</tbody>
</table>
shows a distribution that is consistent with the Caucasian ethnicity, especially with the Southeast and Mediterranean European populations.

Knowing the distribution of these gene variants in Kosovo may prove very useful for the future research of certain complex diseases and for determining the impact of geographical and climatic conditions in their pathogenesis. Our data will form the basis for detecting the genetic risk factors related to specific diseases, including the toxic potential of numerous environmental pollutants. In addition, they can prove relevant to clinical pharmacokinetic studies and dosage recommendations for the Kosovo population.

REFERENCES

Genski polimorfizam CYP2C9, CYP2C19 i CYP3A5 u populaciji Kosova

Vrlo visok stupanj polimorfnosti, zbog interindividualne genske varijabilnosti, enzima citokromska P450 (CYP) značajno pridonosi raznolikosti u odnosima između primijenjene doze lijeka, koncentracije lijeka u serumu, kao i terapijskog odgovora, ali i interakcije lijekova. Podaci o poznatim polimorfizmima CYP vrlo su važni u kliničkoj primjeni saznanja o genetičkim rasporedima u populaciji. Genski polimorfizam CYP u populaciji Kosova te je cilj ovog istraživanja bio utvrđiti učestalost najvažnijih genskih varijanti alela i genotipova u godišnjim podacima. Učestalost alela i genotipova u populaciji Kosova te je bitno provesti istraživanja učestalosti u pojedinim populacijama. Do sada nema objavljenih podataka o farmakogenetici tijekom farmakoterapije. Učestalost pojedinih polimorfizama može značajno varirati između različitih geografskih populacija.