Allele Frequency Distributions of the Drug Metabolizer Genes CYP2C9*2, CYP2C9*3, and CYP2C19*17 in the Buginese Population of Indonesia

Zullies Ikawati1,* , Theresia D. Askitosari2, Lukman Hakim1, Joseph Tucci3 and John Mitchell4

1Faculty of Pharmacy Universitas Gadjah Mada, Yogyakarta, Indonesia; 2University of Surabaya, Biotechnology Faculty, Raya Kalirungkut, Tenggilis, 60293 Surabaya, Indonesia; 3School of Pharmacy and Applied Science, La Trobe University, Bendigo Campus PO Box 199 Bendigo Victoria 3552, Australia; 4Faculty of Science, Technology and Engineering, School of Molecular Sciences, Department of Genetics, La Trobe University, Australia

Abstract: The present study is part of the genetic mapping of Indonesia focusing on drug metabolizing enzymes, which started with the Buginese population of Makassar, South Sulawesi. The two CYP450 gene subfamilies, i.e. CYP2C9 and CYP2C19 are of interest as they exhibit wide inter-individual variation in expression, which influence the drug metabolism capacity. The CYP2C9 alleles of interest in this study were CYP2C9*2 and *3, and of CYP2C19 was CYP2C19*17. The study aimed to determine the frequencies of the CYP2C9 genotype, which contains *1, *2 and *3 alleles, and the CYP2C19 genotype, which comprises the *1 and *17 alleles in the Buginese. Ninety six Buginese subjects, comprising 48 males and 48 females were studied. CYP2C9 and CYP2C19 alleles were detected by a PCR-RFLP assay method. Results showed that there was no CYP2C9*2 allele present, while the frequencies of CYP2C9*3 and CYP2C19*17 overall were 1.56 % and 4.68 %, respectively. The frequency of the CYP2C9*3 allele in females was 2.08%, and not statistically different from that in males (1.05%). The frequency of the CYP2C19*17 allele in females (8.33%), was significantly different (P<0.05) from that in males (1.05%). No subject carried the CYP2C9*2/*2, CYP2C9*3/*3, CYP2C19*17/*17, or CYP2C9*3/CYP2C19*17 genotype. The study is the first to describe the drug metabolizing enzyme polymorphisms, CYP2C9 and CYP2C19, in the Indonesian Buginese population.

Keywords: Buginese, CYP2C9*2, CYP2C9*3, CYP2C19*17, Indonesia.

1. INTRODUCTION

Genetic polymorphisms of drug metabolizing enzymes are one of the major determinants of inter-individual variability in drug response and are becoming a rapidly growing field in pharmacogenetics [1-3]. The cytochrome P450 (CYP450) family are the primary metabolic pathway for the metabolism of most drugs. The two CYP2C subfamilies, i.e. CYP2C9 and CYP2C19, exhibit wide inter-individual variation in expression that affects drug metabolism capacity [4]. Among these functional variants, CYP2C9*2 (rs1799853) and CYP2C9*3 (rs1057910) have been extensively studied. The CYP2C9*2 allele encodes a moderately defective protein whereas the CYP2C9*3 allele has lower affinity and markedly lower intrinsic clearance for numerous drugs, both in vitro and in vivo [5]. These alleles have reduced catalytic activity compared to that of the wild type CYP2C9*1 [6]. The reduced activity may decrease the clearance of the parent drug and therefore increase its toxicity. In the case of CYP2C19, the variant allele of interest is CYP2C19*17 (rs12248560). This allele is associated with an increase in transcription rate and drug metabolism compared to those seen in the wild type CYP2C19*1 [7], and this results in a lack of response to the parent drug due to its low availability. In contrast, it causes an extensive response when the parent drug is activated to the more active metabolites by this enzyme (as in prodrug). CYP2C9 and CYP2C19 variant alleles show considerable variation across different ethnic and continental populations [7-9].

The archipelago of Indonesia consists of hundreds of different ethnic groups. Sulawesi, is located near the Wallace line, which marks the transition zone between Asia and the Australia continent. Sulawesi, therefore, has different flora, fauna, and also ethnic diversity, compared to other islands within Indonesia. One ethnic group in Sulawesi is the Buginese. Buginese is the third biggest ethnic in Indonesia, after Javanese and Sundanese which may have specific physical features and a socio-culture compared to other ethnic groups in Indonesia [10]. To date, however, there is still a lack of data on polymorphism in the CYP450 genes in Indonesians. This paper is one of the first to focus on the polymorphism of CYP450 in Indonesia, especially on the distribution and frequency of CYP2C9 and CYP2C19 variants in Buginese population.

2. MATERIALS AND METHODS

2.1. Samples

Blood samples were taken from 96 healthy subjects of Buginese ancestry, consisting of 48 males and 48 females. Informed consent was obtained from all subjects and the
study protocol was approved by the Ethics Committee of Faculty of Medicine, Universitas Gadjah Mada. DNA samples were prepared from 200 µl of blood using 20% Chelex (Bio-Rad) following the manufacturer’s instructions, then stored at -4°C until genotyping was performed.

2.1. Genotyping Strategy

The genotyping strategies involved polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis, based on previously developed techniques for *CYP2C9* and *CYP2C19* genes [11, 12], with modification in the primer sequences. The primers were designed to contain a mismatch site to generate the digestion site of the respective restriction enzyme. This digestion site was disrupted if the subject DNA contained the mutation.

The *CYP2C9* allele results from an A→T mutation at the 416 nucleotide position in exon 3 (11). The forward primer, 5'-TTTGGATGGGAAGAGGAAGG-3', and the reverse primer, 5'-GCTGAGCAGGTGGTGGGAAGG-3', were designed utilising two mismatches to generate the digestion site of the *Kpn1* enzyme, if the sample is wild type. The digestion site is destroyed if the sample contains the C→T mutation. The amplicon is 198 base pairs (bp) in size. Following PCR and digestion by the *Kpn1* enzyme homoygotes for the wild type allele (*1/*1) will be seen as a fragment of 168 bp, homozygotes for the *CYP2C9* allele (*2/*2) will not contain the *Kpn1* site, and so a fragment of 198 bp will be seen, and heterozygotes (*1/*2) will display fragment sizes of 168 bp and 198 bp.

The *CYP2C9* allele results from an A→C mutation at the 1061 nucleotide position in exon 7 (11). The forward primer 5'-CCCCTGATGCTACAAACATGCCC-3' and the reverse primer 5'-CATGGGGCCAGCTGGTGAGGAAG-3', were designed utilising two mismatches to generate the digestion site of the *Vsp1* enzyme, if the sample is wild type. The site is destroyed if the sample contains the A→C mutation. The amplicon is 213 bp in size. Following PCR and digestion with the *Vsp1* enzyme homoygotes for the wild type allele (*1/*1) will be seen as a fragment of 183 bp, homozygotes for the *CYP2C9* allele (*2/*2) will not contain the *Vsp1* site, and so will be seen as a fragment of 213 bp, and heterozygotes (*1/*2) will display fragments of 183 bp and 213 bp.

The detection of *CYP2C19* allele was carried out using a forward primer, 5'-GGTCTCTATTGAAAGGG-3' and a reverse primer, 5'-TGGGGCATCAGCTTTACATCAGACAT-3'. This is a novel strategy whereby there is a mismatch in the reverse primer (mismatch occurs 24 bases from 5' end of primer) to introduce a *Hsp9211* digestion site if DNA is wild type, resulting in a 153 bp fragment. If DNA has the *CYP2C19* mutation, then the *Hsp9211* site is destroyed and the 177 bp fragment will be seen after digestion with the *Hsp9211* enzyme.

3. RESULTS

The *CYP2C9* and *CYP2C19* alleles as well as the genotype frequencies in the Buginese population are summarized in Table 1. No individuals carried the *CYP2C9* allele, three individuals were heterozygous 2C9*1/*3, no subject was homozygous 2C9*3/*3, and nine were heterozygous 2C9*1/*17. The *CYP2C19* allele was absent in the Buginese. There were no *CYP2C9* allele in the sample, while the frequency of the *CYP2C9* allele in the Buginese was 1.56 % and 4.68 %, respectively. The allele frequency of *CYP2C9* allele was 1.05% in males and was not significantly different from that (2.08%) seen in females. On the other hand, the frequency of *CYP2C19* allele in females was 8.33% which was significantly greater than that seen in males (1.05%), (P<0.05). Under Hardy-Weinberg equilibrium conditions one homoyzogote for *CYP2C9* is expected in every 5000 Buginese, whereas, one homoyzogote for *CYP2C19* is expected in every 476 Buginese.

Table 1. Allele (A) and genotype (B) frequencies of *CYP2C9* and *CYP2C19* in male and female Buginese populations.

<table>
<thead>
<tr>
<th>Allele</th>
<th>Male (%)</th>
<th>Female (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9*1</td>
<td>98.95</td>
<td>97.92</td>
<td></td>
</tr>
<tr>
<td>CYP2C9*2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>CYP2C9*3</td>
<td>1.05</td>
<td>2.08</td>
<td>0.557</td>
</tr>
<tr>
<td>CYP2C9*17</td>
<td>98.95</td>
<td>91.67</td>
<td></td>
</tr>
<tr>
<td>CYP2C9*17</td>
<td>1.05</td>
<td>8.33</td>
<td>0.014</td>
</tr>
</tbody>
</table>

4. DISCUSSION

Our genotyping approach was designed for the utilization of inexpensive and commercially available digestion enzymes. In order to improve the genotyping, one of each primer pairs was designed to incorporate a restriction endonuclease digestion site at its 3’ end, and each primer was designed to be around 30 bp in length, which ensured a large
enough difference in band size to easily differentiate between wild type alleles and those with mutations of CYP2C9 and CYP2C19 genes when run on a 3% agarose gel.

These results are novel, as ethnic groups from Indonesia have not previously been assessed for CYP450 status. The data showed a similar general trend as that seen for other Asian groups, namely, a negligible frequency of CYP2C9*2, and the presence of the CYP2C9*3 allele at a frequency between 2%-5% [9, 13, 14]. This observation is in line with the report that the CYP2C9*2 allele is primarily restricted to European, Middle Eastern and Central/South Asian populations, but is absent or found at very low frequencies in other geographic regions (Africa, East Asia, Oceania and America). The CYP2C9*3 allele has a broader geographic distribution, but the highest allele frequencies are also found in European and Central/South Asian populations [15].

In the case of CYP2C19, we only found heterozygous 2C19*1/*17, individuals, and no homozygous 2C19*17/*17. These data are also similar to that of other Asian populations, especially Korean and Chinese [7, 8]. The prevalence of the CYP2C19*17 allele was very low [16], which therefore has no association with adverse clinical outcomes after percutaneous coronary intervention and clopidogrel. On the contrary, the CYP2C19*17 allele was found in a relatively high frequency in European population, especially Greeks, in which it was reported at 19.61% [17].

There is ongoing debate about the health consequences of such polymorphisms as those studied here. It is possible that they may be associated with adverse clinical outcomes for patients taking medications whose metabolism is controlled by the products of these genes. This is especially the case for patients taking drugs with a narrow therapeutic range (warfarin, theophylline, digoxin), and saturable kinetics (phenytoin), where small alterations in plasma levels may result in disproportionate toxicity [11, 18] or when taking other drugs which may have CYP450 inducing or inhibiting properties [19, 20].

The polymorphism also has implications for drug dose adjustment. Tentative estimates of how CYP2C9 genotyping might be applied to dose adjustments in clinical therapy were based on dose-related pharmacokinetic parameters, especially the clearance or trough drug concentrations. Mean clearances in homozygous carriers of the *3 allele were below 25% of that of the wild type for S-warfarin, tolbutamide, glipizide, celecoxib, and fluvastatin. In the more frequent heterozygote genotype *1/*3, the clearances were between 40% and 75%. In these cases in which individual dosages are derived from clinical drug effects, such as for the oral anticoagulants, the pharmacogenetics-based dose adjustments showed a good correlation with the genotype-specific empirically derived doses [21].

In the case of polymorphism in CYP2C19, examples of clinical consequences are linked to clopidogrel, a pro drug. Carriers of the CYP2C19*2 loss-of-function allele present higher platelet reactivity and worse clinical outcomes compared to that seen in non-carriers. The hyper-function allele CYP2C19*17 increases the biotransformation of clopidogrel to its active metabolite, which in turn increase the effect to inhibit platelet aggregation. The simultaneous occurrence of the CYP2C19*17 polymorphism seems to offset the negative impact of the CYP2C19*2 polymorphism on platelet aggregation [22]. For other drugs, like escitalopram, the homozygous CYP2C19*17 genotype is associated with lower serum concentration of escitalopram, which might imply an increased risk of therapeutic failure in psychiatric patients [23].

Such clinical issues highlight the importance of pharmacogenomic screening. Further, wider application of such testing is being advocated in order to establish whether inter individual variation in metabolic capacity exists between participants in clinical trials [24], a factor which could influence outcomes of these studies and subsequent acceptance or rejection of new therapeutic substances.

CONCLUSION

The study is the first to describe the genetic polymorphism of drug metabolizing enzymes, CYP2C9 and CYP2C19, in an Indonesian population. The findings are similar to those seen in other Asian populations, especially Korean and Chinese, and contribute to genetic polymorphism mapping of drug metabolizing enzymes in Asian population.

LIST OF ABBREVIATIONS

CYP450 = Cytochrome P450
PCR = Polymerase chain reaction
RFLP = Restriction fragment length polymorphism

ETHICS STATEMENT

Study protocol was approved by the Ethics Committee of Faculty of Medicine, Universitas Gadjah Mada.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

The authors wish to thank Prof. Nasrum Massi from Hasanuddin University for DNA isolation of samples, The Ministry of Education for financial support and Muhammad Novrizal Abdi Sahid for assistance in writing.

REFERENCES

Allele Frequency Distributions of the Drug Metabolizer Genes

Current Pharmacogenomics and Personalized Medicine, 2014, Vol. 12, No. 4 239


