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

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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.

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^{o}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*2* allele results from a C→T mutation at the 416 nucleotide position in exon 3 (11). The forward primer, 5'-TTTGGGATGGGGAAGAGGAGCATTG***GGTA C***(***C***→T)-3', and the reverse primer, 5'-GCTAACAACC AGACTCATAATGAAAGA-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 homozygotes for the wild type allele (**1/*1*) will be seen as a fragment of 168 bp, homozygotes for the *CYP2C9*2* 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*3* allele results from an A→C mutation at the 1061 nucleotide position in exon 7 (11). The forward primer 5'-CCCCTGAATTGCTACAACAAATGTGCC-3' and the reverse primer 5'-CATGGGGCAGGCTGG TGGGGA-GAAG***ATTAA(T→G)***-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 homozygotes for the wild type allele (**1/*1*) will be seen as a fragment of 183 bp, homozygotes for the *CYP2C9*3* allele (**3/*3*) will not contain the Vsp1 site, and so will be seen as a fragment of 213 bp, and heterozygotes (**1/*3*) will display fragments of 183 bp and 213 bp.

The detection of *CYP2C19*17* allele was carried out using a forward primer, 5'-GGTTCTATTT AATGTGAA GCC-3' and a reverse primer, 5'- TGGCGCATTATCTCTT ACATCAGACAT-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*17* 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*2* allele, three individuals were heterozygous *2C9*1/*3*, no subject was homozygous *2C9*3/*3*, and nine were heterozygous *2C19*1/*17*. The *CYP2C19*17/*17* and *CYP2C9*3/*17* allele was absent in the Buginese. There was no *CYP2C9*2* allele in the sample, while the frequency of the *CYP2C9*3* and *CYP2C19*17* allele in the Buginese was 1.56 % and 4.68 %, respectively. The allele frequency of *CYP2C9*3* 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*17* 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 homozygotefor *CYP2C9*3* is expected in every 5000 Buginese, whereas, one homozygote for *CYP2C19*17* is expected in every 476 Buginese.

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

A

Variant allele	Males (%)	Females (%)	*P-value*
*CYP2C9*1*	98.95	97.92	
CYP2C9*2	0	0	-
*CYP2C9*3*	1.05	2.08	0.557
*CYP2C19*1*	98.95	91.67	
*CYP2C19*17*	1.05	8.33	0.014

B

Genotype	Male		Female	
	N	(%)	(N)	(%)
*CYP2C9*1/*1*	47	97.92	46	95.83
*CYP2C9*1/*2*	0	0	0	0
*CYP2C9*2/*2*	0	0	0	0
*CYP2C9*1/*3*	1	2.08	2	4.17
*CYP2C9*3/*3*	0	0	0	0
*CYP2C19*1/*1*	47	97.92	40	83.33
*CYP2C19*1/*17*	1	2.08	8	16.67
*CYP2C19*17/*17*	0	0	0	0
*CYP2C19*3/*17*	0	0	0	0

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, specially 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.

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