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Cross-Ethnic Distribution of Clinically Relevant Cyp2c19 Genotypes and Haplotypes

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Abstract

Study background: Knowledge of the drug metabolism phenotype of a patient is critically important to making an informed choice about therapeutic drug prescription. The objective of this study was to determine the prevalence of common clinically relevant variants of the CYP2C19 gene in Malaysian ethnic groups and in other Asian and Middle Eastern populations, and to predict their metabolic phenotypes.

Methods: A total of 1103 subjects from six ancestral origins were genotyped for 16 single nucleotide polymorphic (SNP) markers on the CYP2C19 gene to comprehensively understand their allelic distributions. The expectation-maximisation algorithm was used to analyse the genotype data to estimate the maximum likelihood haplotypes in each sample group and to predict the clinical phenogroups.

Results: Of the 16 SNP loci genotyped in the six subpopulations studied, only four SNP markers (rs17885098, rs4986893, rs4244285 and rs3758581) showed polymorphism (minor allele frequency >1.0%). Nearly half of the Indians (55%) and Chinese (48.8%) had at least one copy of the loss-of-function allele. The incidence was relatively lower in Malays (39.3%), Orang Asli (27.3%), Javanese (23.8%) and Saudis (28%).

Conclusion: These ethnic groups pose a significant risk of drug sensitivity should they are prescribed regular doses of CYP2C19 substrate.

Keywords: CYP2C19; Polymorphism; Malaysia; Adverse drug reactions; Poor metabolisers

Introduction

Therapeutic drugs are processed by common biochemical pathways regulated by a class of genes associated with drug absorption, distribution, metabolism, and excretion (ADME). Genetic polymorphisms in these ADME genes alter their pattern of expression and functional heterogeneity and thus are responsible for differences in therapeutic drug response at the inter-individual level and inter-population levels. Among the core ADME enzymes, most phase I dependent metabolising enzymes are encoded by a repertoire of genes known as cytochrome P450 (CYP) genes, especially by the *CYP2C* family of genes [1].

CYP family-2 subfamiliy-C peptide-19 (*CYP2C19*) encodes a monoxygenase that metabolises and bioactivates a wide variety of therapeutic drugs and drug classes, including anticoagulant clopidogrel (Plavix) [2], antimalarial proguanil [3], proton pump inhibitors (e.g., omeprazole and esomeprazole) [4], anticonvulsants (S-mephenytoin and diazepam) [4,5], antidepressants (barbiturates and imipramine) [6,7], HIV-protease inhibitors (e.g., nelfinavir) [8], and β-blockers (e.g., propranolol) [9]. The *CYP2C19* gene is highly polymorphic. To date, 34 alleles have been catalogued by the Human Cytochrome P450 (CYP) Allele Nomenclature Committee [10]. However, analyses show that only 11 variants, mostly missense point mutations, encode for functionally inactive or unstable enzymes that result in significant differences in pharmacological response. Based on their oxidative and biotransformative metabolic rate of probe xenobiotics, individuals are phenotypically stratified as extensive metabolisers (EMs, representing normal drug metabolism), intermediate metabolisers (IMs), or poor metabolisers (PMs, signifying impaired drug metabolism due to deficiency of functional CYPs). A fourth phenogroup, known as ultra-rapid metabolisers (UM), also has been described. Significant interethnic differences exist in the incidence of PMs among Asians (13–23%), Africans (6%), and Caucasians (2-5%) [11].

Distribution of the *CYP2C19* alleles that define these phenogroups is highly variable from one ethnic group to another. Nevertheless, within each ethnic group only a few alleles (commonly **1*, **2*, **3*, and **17*) are expected to reach high frequencies. A meaningful clinical classification of a prospective patient into EM, IM, PM, or UM requires genotyping of the common alleles characteristic in the population. *CYP2C19*1* represents the wild type allele that encodes an enzyme with normal (extensive) activity. *CYP2C19*2* is defined by a single nucleotide variant on exon 5 (NM_000769.2:c.681G>A rs4244285) that activates a cryptic splice acceptor, and it is the most frequent defective allele in patients with a PM phenotype. *CYP2C19*3* has a point mutation on exon 4 (c.636G>A rs4986893) that produces a premature stop codon that results in no in-vitro enzyme activity. Conversely, *CYP2C19*17* has increased expression and therefore has increased activity, and it confers the UM phenotype. Ethnic Asians have a higher frequency of the **2* allele (28.9-31.2%) than African Americans (18.2%) and

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Caucasians (12.7%). For individual populations, the highest frequencies of these variants are observed in Japanese (15–22.5%) [12], Chinese (13–20%) [13], Indians (12–14%) [14,15], Koreans (12.6%) [16], Papua New Guineans (70%) [17], and individuals from the Vanuatu Islands of Melanesia [18].

Malaysia is a multi-ethnic society with great genetic, linguistic, cultural, and phenotypic diversity. These well-defined social constructs are comprised predominantly of Malays (67.4%), Chinese (24.6%), and Indians (7.3%) [19], and they remain separated as they seldom intermarry. Other ethnic minorities include an aboriginal group locally known as *Orang Asli* (OA) [19]; this group, which lives in forest fringe communities isolated from urban areas, forms an inconspicuous scant minority. To date, how the common and rare alleles on the *CYP2C19* gene are segregated in these ethnic groups has not been comprehensively studied.

Malaysia's National Pharmaceutical Control Bureau reported 4041 cases of adverse drug reactions (ADRs) during the period from 2004 to 2005 [20]. This rudimentary estimate is anticipated to increase as the population ages and drug-drug interactions increase due to the large number of medications prescribed to older people. Trial and error administration of clinical drugs when a genetic basis has been established on its pharmacokinetic is a dangerous proxy. Thus, knowledge of how clinically significant variants of drug metabolising genes are distributed in groups defined by ethnic characteristics is important for better prescription of therapeutic drugs. In this study we genotyped the 16 alleles on the *CYP2C19* gene to determine their frequencies in three major ethnic groups and the indigenous OA group in Malaysia. The study was extended to include Javanese from Indonesia and Saudis from Saudi Arabia.

Materials and Methods

Sample

The 1103 unrelated healthy subjects enrolled in this cross-sectional study consisted of 209 Malays, 201 Chinese, 200 Indians, and 176 OA from Malaysia as well as 185 Javanese from Indonesia and 132 Saudis from Saudi Arabia. Ethnicity and population were defined by all four grandparents belonging to same ethnic group. Blood samples were obtained with informed consent from all participants. This study was approved by the Ethical Committee of Research in Medical Health, Faculty of Medicine, Gadjah Mada University, Indonesia and the Research and Medical Ethics Committee of University Sains Malaysia.

Single nucleotide polymorphism (SNP) genotyping

Genomic DNA was isolated using the QIAamp DNA Blood Midi Kit (Qiagen Gmbh, Hilden, Germany) according to the manufacturer's instructions. All 1103 samples were genotyped for 16 variants (c.1A>G, rs28399504; c.50T>C, rs55752064; c.55A>C, rs17882687; c.99C>T, rs17885098; c.276G>C, rs17878459; c.395G>A, rs72552267; c.431G>A, rs17884712; c.449G>A, rs58973490; c.636G>A, rs4986893; c.680C>T, rs6413438; c.681G>A, rs4244285; c.991A>G, rs3758581; c.1228C>T, rs17879685; c.1251A>C, rs17886522; c.1297C>T, rs56337013; c.1473A>C, rs55640102) on the *CYP2C19* gene using a nested amplification-refractory mutation system (ARMS)-PCR approach.

Briefly, each DNA sample was initially subjected to two multiplex PCR reactions that amplified seven regions of interest on the CYP2C19 gene. The first multiplex reaction (*A*) was carried out with 0.25 μM of each primer (EX1Fw, EX1Rv, EX2Fw, EX2Rv, EX9Fw, and EX9Rv) and 0.6 μM of EX8Fw and EX9Rv (Table 1A). The following thermoprofile was used: initial denaturation at 94°C for 5mins, followed by 25 cycles of denaturation at 94°C for 60sec, annealing at 54°C for 45 sec, and 72°C for 45 sec with a final extension at 72°C for 5 min. The second multiplex PCR (*B*) was performed with 0.6 μM of primers EX4Fw and EX4Rv and 0.25 μM of primers EX5Fw, EX5Rv,EX7Fw, and EX7Rv using the same cycling conditions, except the annealing temperature was set at 61°C. These two reactions were carried out with 0.2 μg of genomic DNA, 1.0 U Taq DNA polymerase (Biotool®, B&M Labs, SA, Madrid, Spain), 1x PCR buffer, 2mM $MgCl_2$, and 0.2mm dNTPs (Promega Corporation, Madison, WI, USA). After confirming the presence of the expected bands from the two reactions on 3% agarose gels, the amplicons from both reactions *A* and *B* were mixed and diluted to 1-in-25 or 1-in-50 and used as templates in the subsequent ARMS-PCR.

The targeted alleles were ARMS amplified using either of the two allele-specific primers, one complementary to the mutation to be detected and the other complementary to the corresponding normal allele at the same locus. Details about the mutation-specific and wild type sequence-specific primers used are listed in Table 1B. Common characteristics of the thermoprofile used include 15 cycles of denaturation-annealing-extension with diluted PCR amplicons derived from the initial gene amplifications used as the template.

Haplotype phasing and statistical analyses

Observed and expected genotype frequencies were compared under the Hardy-Weinberg Equilibrium (HWE) theorem using the χ^2 goodness-of-fit test. The expectation-maximisation algorithm [21] was used on the genotype data that conformed to HWE to estimate the maximum likelihood haplotypes with frequencies greater than 1% in each sample group. All statistical analyses were performed using the software Haploview version 4.2 [22].

Inferred haplotypes were annotated using the criteria catalogued by the Human Cytochrome P450 (CYP) Allele Nomenclature Committee [10]. The *CYP2C19*1* haplotype was assigned by exclusion when derived alleles failed to fit into the defined variant haplotypes.

Results

All population groups studied were genotyped for 16 bi-allelic variants on the *CYP2C19* gene. Their genotype frequencies are summarised in Table 2. Three markers (rs17882687A>C, rs17878459C>T, and rs17884712G>A) in the Saudi sample showed statistically significant departure from the HWE ($p < 0.05$) assumption and were not polymorphic (minor allele frequency <1%). Together with these three loci, seven other SNPs (rs28399504, rs55752064, rs72552267, rs58973490, rs4986893, rs6413438, and rs56337013) across all ethnic groups were homozygous for the wild-type allele and hence were monomorphic.

Haplotypes deduced from expectation-maximisation algorithm with frequencies greater than 1% in each sub-populations are summarised in Table 3. Of the wild type **1* allele sub-types, *CYP2C19*1B* was invariantly the major sub-haplotype observed in all ethnic groups studied. However, **1B* sub-haplotype frequency revealed an empirical difference in the OA group relative to the other ethnic groups. This was directly attributed to the 5–6 fold increased prevalence of the *CYP2C19*1A* (31.2%) sub-haplotype in OA. Apart from this intriguing difference, **1A* and **1C* sub-types were observed at low frequencies in Malays, Chinese, Javanese and Saudis, whereas both were absent in the Indian sample, making this sub-ethnic group less diverse at the *CYP2C19* gene.

Name	Oligonucleotide sequence	Annealing temperature	Second primer	Product size (bp)	Amplification strategy
A. Primers used in the initial multiplex PCR amplifications					
Ex1Fw	AGG ACA AAG TCT CCT AAT CTT C			750	
Ex1Rv	CAA TGA TCT CTT GTA ACA TTG TAC				
Ex2Fw	TAA CTG TAT CTC CTT TTC TAG C			533	
Ex2Rv	AGG TCA GTG ATA GAG AGT ATG				
Ex4 Fw	AAG TGT TTT ATA TCT AAT GTT TAC			304	
Ex4 Rv	CTT CAG GGC TTG GTC AAT ATA G				
EX5Fw	CAG AGC TTG GCA TAT TGT ATC			321	
Ex5 Rv	GTA AAC ACA CAA CTA GTC AAT G				
Ex7 Fw	TGT TCC ATT TCT CTC CTT TTC C			272	
Ex7 Rv	GAA CAT GGA GTT GCA GTG TAG				
Ex8 Fw	CAT ATT AAC TTC CCT CAC TTC			174	
Ex8 Rv	CTT GTA CCC TGA AAC ACA AAG				
Ex9 Fw	AGT AAC TTC TCC CTA TGT TTG			312	
Ex9 Rv	GAT GAC GGG TCA GAA GAA G				
B. Allele specific Primers					
rs28399504, c.1A>G	ACA AGA GGA GAA GGC TTC AA ACA AGA GGA GAA GGC TTC AG	61	Ex 1Rv	288	Duplexed with §
c.395G>A, rs72552267	CTC CCT CAT GAC GCT GCG CTC CCT CAT GAC GCT GCA		Ex 2Rv	134	
c.50T>C, rs55752064	CTC TCA TGT TTG CTT CTC CC CTC TCA TGT TTG CTT CTC CT	61	Ex 1Rv	239	Duplexed with §
c.1297C>T, rs56337013	ATG TTT GTT ATT TTC AGG AAA AC ATG TTT GTT ATT TTC AGG AAA AT		Ex9 Rv	298	
c.55A>C, rs17882687	CAG AGC TCT GTC TCC AGA T CAG AGC TCT GTC TCC AGA G	61	Ex1Fw	554	Duplexed with §
c.1228C>T, rs17879685	CCA GAG ATG TTT GAC CCT T CCA GAG ATG TTT GAC CCT C		Ex8Rv	122	
c.99C>T, rs17885098	AAT CAC TGG GAG AGG AGT G AAT CAC TGG GAG AGG AGT A	61	Ex1Fw	598	Duplexed with §
c.636G>A, rs4986893	GTA AGC ACC CCC TGG GTA AGC ACC CCC TGA		Ex4Rv	109	
c.276G>C, rs17878459	GCC TCT TCC AGA AAA CTC G GCC TCT TCC AGA AAA CTC C	63	Ex2Fw	147 488	Duplexed with ‡
c.449G>A, rs58973490	ACT CCT CCA CAA GGC AGC ACT CCT CCA CAA GGC AGT				
c.431G>A, rs17884712	GAG GAG CAT TGA GGA CCG GAG GAG CAT TGA GGA CCA	61	Ex2Rv	98	With amplicons from PCR A+B‡
c.681G>A, rs4244285	CTA TCA TTG ATT ATT TCC CA CTA TCA TTG ATT ATT TCC CG	57	Ex5Rv	231	With amplicons from PCR A+B§
c.680C>T, rs6413438	CCA CTA TCA TTG ATT ATT TCC C CCA CTA TCA TTG ATT ATT TCC T	61	Ex5Rv	234	Duplexed with §
c.991A>G, rs3758581	GCT CCG GTT TCT GCC AAT GCT CCG GTT TCT GCC AAC		Ex7Fw	95	
c.1251A>C, rs17886522	ACT TTC TGG ATG AAG GTG GC ACT TTC TGG ATG AAG GTG GA	61	Ex8Rv	100	Duplexed with ‡
c.1473A>C, rs55640102	CAG ACC ATC TGT GCT TCT T CAG ACC ATC TGT GCT TCT G		Ex9Fw	231	
§Amplicons from A+B mixed and diluted to 1:25 with PCR buffer; ‡ Amplicons from A+B mixed and diluted to 1:50 with PCR buffer					

Table 1: Primers used in the initial multiplex PCR amplifications and allele specific PCR reactions with their cycling conditions.

The commonest loss-of-function allele observed was the *CYP2C19*2A* sub-haplotype; it was found in all ethnic groups studied at varying frequencies ranging from 9.3% in Saudis to 28.5% in Indians. The **3* allele was present at appreciable frequencies only in the three major ethnic groups from Malaysia, with the highest prevalence in Chinese (3.5%). Less frequent haplotypes **12* (c.1473A>C, rs55640102) and **13* (c.1228C>T, rs17879685) were private and found only in the Saudi population at frequencies of 1.8% and 1%, respectively. None of the populations studied had **4* (c.1A>G, rs28399504), **5* (c.1297C>T, rs56337013), **6* (c.395G>A, rs72552267), **10* (c.680C>T, rs6413438), **11*, **14* (c.1473A>C, rs55640102), and **15*.

Based on the functional genotypes (Table 3) as the determinants of metabolic phenotypes, 33.5% of Malays , 32.3% of Chinese, 44.5% of Indians, 22.7% of OA, 21.6% of Javanese, and 22% of Saudis were predicted to be IMs, whereas 5.7% of Malays, 16.4% of Chinese, 10.5% of Indians, 4.5% of OA, 2.2% of Javanese, and 1.5% of Saudis were PMs. The Saudi cohort also contained unknown phenotypes consisting of individuals heterozygous for **1/*12* and **1/*13*.

Discussion

The *CYP2C19* gene contains many variants with likely functional consequences. Acute deficiency of frequency data beyond the *CYP2C19*2* (c.681G>A, rs4244285) and **3* (c.636G>A, rs4986893) variants that are commonly assessed impelled us to further genotype 16 variants within the six Asian ethnic groups from a variety of ancestral origins to comprehensively understand their allelic distributions and predict the clinical phenogroups. As shown in Table 4, the studied loci are the defining mutations that readily characterise at least 13 alleles: **2-*6* and **9-*15*, and **1* by exclusion. Most allelic determinations are apparently straightforward and can be identified by genotyping the defined loci (e.g., c.681G>A designated for **2*, c.636G>A for **3*, and c.1A>G for **4*).

Our data show that the Malaysian ethnic groups contain a wide range of PMs that involve **2* and **3* in various genotypic combinations. In particular, greater than 10% of the Chinese and Indians subpopulations were PMs. Therefore, in our exposition, genotyping the *CYP2C19* gene, at least for the c.681G>A, rs4244285 (**2*) and c.636G>A, rs4986893 (**3*) alleles, may be crucial for reducing the incidence of ADR and for gauging the individual's response to CYP2C19 substrate drugs. For example, the inactive prodrug clopidogrel is an important CYP2C19 substrate medication used to reduce cardiovascular events; pre-treatment genetic testing to determine the metabolic status of the patient may be helpful to determine the clinical outcome of treatment with this drug. This is particularly important in a community such as Malaysian, in which the incidence of acute coronary syndrome (ACS) is high (currently 141 per 100,000 population per year, with an impatient mortality rate of 7%) [23]. Although there is no general consensus about genetic testing of patients, information about clopidogrel sensitivity clearly would be useful for determining optimised dosage if the patient needs it for management of ACS.

The results of this study clearly show ethnic heterogeneity with regard to loss of function alleles associated with the *CYP2C19* gene. Nearly half of the Indians (55%) and Chinese (48.8%) have at least one copy of the loss of function allele (Table 3). Although the incidence was relatively lower in Malays (39.3%), OA (27.3%), Javanese (23.8%), and Saudis (28%), these cohorts pose a significant risk for drug sensitivity should they are prescribed the regular doses of *CYP2C19* substrate drugs. Similar frequencies have been reported from different ethnic groups: 50% of Asians, 34% Africans, and at least 25% of Caucasians reportedly carry at least a single copy of a loss of function allele. In our samples, these differences were in principle attributed to four polymorphic SNPs loci (rs17885098, rs4986893, rs4244285, and rs3758581) used to infer the haplotypes.

The C*YP2C19*1A* sub-haplotype, which is denoted by the absence of derived alleles, was not common in any group tested except OA. The distinctly high presence of **1A* in OA made it the second most common functionally normal sub-haplotype after **1B*. This result supports the evolutionary premise that ancestral alleles are more common in aboriginal populations such as OA, which form small pockets of endogamous communities in Malaysia that live in isolation disconnected from urban sub-populations. Invariably, **1B* was the major sub-haplotype in all the six ethnic groups. This is the result of the widespread variant alleles T and G at the c.99C>T (rs17885098) and c.991A>G (rs3758581) loci, which apparently reached fixation in all ethnic groups except the aboriginal OA group. Near fixation of these variant alleles has been observed globally in Europeans, Asians, and Sub-Saharan Africans [24,25]. Increased heterozygosity at these two loci within the ancestral OA cohort probably reflects a benign stochastic process or that it confers resistance to an unknown aetiology. Despite the missense mutation c.991A>G, rs3758581 present in **1B* and **1C*, it remains functionally unimpaired and no different from the **1A*. *CYP2C19*1B(v)* observed in OA and Saudis is presumed to be a variant of **1B*, which differed by a single nucleotide at 991A>G (rs3758581). Similarly, another variant *CYP2C19*1C (v)* which was only observed in the Javanese cohort, has a single nucleotide difference from the less frequent **1C*.

*CYP2C19*1* allele determination by exclusion may overestimate the EM phenotype. A recent study on a Saudi sample revealed that nearly 46% of its sample contained the **17* allele, with a gene frequency of 26.9% [26]. However, the genotyping panel used in the present study did not include the **17* determinant (c.-806C>T, rs12248560), and hence the **1* allele determined by exclusion in our Saudi cohort likely was overestimated, resulting in overestimation of the presence of the EM phenotype. The substitution at c.-806C>T at the promoter region (**17*) increases the transcription activity and thus is associated with the UM phenotype.

Our understanding of the consequences of CYP2C19 variants is far from perfect. Some variants are apparently straightforward and their phenotype is predictable, such as **2* and **3*. However, the contributions of **12* and **13* to genotypes such as **1/*12* and **1/*13* observed in the Saudi cohort remains unclear.

The goals of this study were to identify polymorphisms of alleles on the *CYP2C19* gene and to determine the distribution of phenotypes within six different populations. *CYP2C19*2* was found at various frequencies across all sub-populations studied, thus it constituted the predominant dysfunctional allele. The data also revealed the presence of **3* in Malays, Chinese, and Indians at low frequencies but it was not present in the Javanese, OA, and Saudis. Taken together, the inferred phenotypes were due to nucleotide substitutions at four loci (rs17885098, rs4986893, rs4244285, and rs3758581). Nucleotide diversity was intuitively high in Saudis, and this population also had additional SNPs (rs17879685 (c.1228C>T) and rs55640102 (c.1473A>C), characteristic of **12* and **13,* respectively). It is unclear at present whether genotyping of *CYP2C19* will materially change the overall rate of ADRs in these sub-populations, but these results suggest that this approach would be successful.

Nucleotide change in cDNA, NCBI SNP ID	Genotype	Genotype Frequencies, N (%)					
		Malays (n=209)	Chinese (n=201)	Indians (n=200)	Orang Asli (n= 176)	Javanese (n=185)	Saudis (n=132)
c.1A>G, rs28399504	AA AG GG	209 (100%)	201 (100%)	200 (100%)	176 (100%)	185 (100%)	132 (100%)
c.50T>C, rs55752064	TT CT CC	209 (100%)	201 (100%)	200 (100%)	176 (100%)	185 (100%)	132 (100%)
c.55A>C, rs17882687	AA AC CC	209 (100%)	201 (100%)	200 (100%)	176 (100%)	185 (100%)	130 (98.5%)* 2 (1.5%)* 0
c.99C>T, rs17885098	CC CT TT	4 (1.9%) 27 (12.9%) 178 (85.2%)	3 (1.5%) 30 (14.9%) 168 (83.6%)	0 10 (5%) 190 (95%)	16 (9.1%) 82 (46.9%) 77 (44%)	1 (0.5%) 25 (13.5%) 159 (85.9%)	- 24 (18.2%) 108 (81.8%)
c.276G>C, rs17878459	GG CG CC	209 (100%)	201 (100%)	200 (100%)	176 (100%)	185 (100%)	131 (97.7%)* 1 (1.5%)*
c.395G>A, rs72552267	GG AG AA	209 (100%)	201 (100%)	200 (100%)	176 (100%)	185 (100%)	132 (100%)
c.431G>A, rs17884712	GG AG AA	209 (100%)	201 (100%)	200 (100%)	176 (100%)	185 (100%)	130 (98.5%)* 2 (1.5%)*
c.449G>C, rs58973490	GG CG CC	209 (100%)	201 (100%)	200 (100%)	176 (100%)	185 (100%)	132 (100%)
c.636G>A, rs4986893	GG AG AA	199 (95.2%) 10 (4.8%)	186 (92.5%) 14 (7.0%) 1 (0.5%)	193 (96.5%) 7 (3.5%) 0	176 (100%)	185 (100%)	132 (100%)
c.680C>T, rs6413438	CC CT TT	209 (100%)	201 (100%)	200 (100%)	176 (100%)	185 (100%)	132 (100%)
c.681G>A, rs4244285	GG AG AA	137(65.6%) 60 (28.7%) 12 (5.7%)	117 (58.2%)* 65 (32.3%)* 19 (9.5%)*	96 (48%) 84 (42%) 20 (10%)	128 (72.7%) 41 (23.3%) 7 (4.0%)	141 (76.2%) 40 (21.6%) 4 (2.2%)	102 (77.3%) 28 (21.2%) 2 (1.5%)
c.991A>G, rs3758581	AA AG GG	- 8 (3.8%) 201 (96.2%)	- 20 (10%) 181 (90%)	- 4 (2%) 196 (98%)	17 (9.6%) 83 (47.2%) 76 (43.2%)	- 12 (6.5%) 173 (93.5%)	- 38 (28.8%) 94 (71.2%)
c.1228C>T, rs17879685	CC CT TT	209 (100%)	201 (100%)	200 (100%)	176 (100%)	185 (100%)	126 (95.5%) 6 (4.5%)
c.1251A>C, rs17886522	AA AC CC	209 (100%)	201 (100%)	200 (100%)	170 (96.6%) 6 (3.4%)	174 (94.1%) 11 (5.9%) 0	132 (100%)
c.1297C>T, rs56337013	CC CT TT	209 (100%)	201 (100%)	200 (100%)	176 (100%)	185 (100%)	132 (100%)
c.1473A>C, rs55640102	AA AC CC	209 (100%)	201 (100%)	200 (100%)	176 (100%)	185 (100%)	126 (95.5%) 6 (4.5%)
*χ^2 goodness-of-fit test p values < 0.05							

Table 2: Genotype frequencies of CYP2C19 alleles in Malays, Chinese, Indians, Orang Asli, Javanese and Saudis.

Subpopulations	Phenotypes								
	Extensive	Intermediate			Poor			Unknown	
Genotypes	*1/*1	*1/*2	*1/*3	*2/*12	*2/*2	*2/*3	*3/*3	*1/*12	*1/*13
Malay, N (%)	127 (60.7%)	60 (28.7%)	10 (4.8%)	-	12 (5.7%)	-	-		
Chinese, N (%)	103 (51.2%)	61 (30.3%)	4 (2%)	-	23 (11.4%)	9 (4.5%)	1 (0.5%)	-	-
Indian, N (%)	90 (45.0%)	83 (41.5%)	6 (3.0%)	-	20 (10.0%)	1 (0.5%)	-		
OA, N (%)	128 (72.7%)	40 (22.7%)	-	-	8 (4.5%)	-	-	-	-
Javanese, N (%)	141 (76.2)	40 (21.6)	-	-	4 (2.2)	-	-		
Saudis, N (%)	95 (72.0%)	28 (21.2%)	-	1 (0.75%)	2 (1.5%)		-	4 (3.0%)	2 (1.5%)

Table 3: Comparison of CYP2C19 allele frequencies within various ethnic groups.

Haplotype	1 A>G, rs28399504	50T>C , rs55752064	55A>C , rs17882687	99C>T, rs17885098	276G>C, rs17878459	395G>A, rs72552267	431G>A, rs17884712	449G>A, rs58973490	636G>A, rs4986893	680C>T, rs6413438	681G>A, rs4244285	991A>G, rs3758581	1228C>T, rs17879685	1251A>C, rs17886522	1297C>T , rs56337013	1473A>C, rs55640102	Ethnic groups Haplotype frequencies					
																	Malays	**Chinese**	**Indians**	**Orang Asli**	**Javanese**	**Saudis**
CYP2C19*1B	A	T	A	T	G	G	G	G	G	C	G	G	C	A	C	A	72.1%	63.4%	67.2%	51.3%	79.8%	73.7%
CYP2C19*2A	A	T	A	T	G	G	G	G	G	C	A	G	C	A	C	A	17.4%	24.1%	28.5%	14.9%	12.9%	9.3%
CYP2C19*1A	A	T	A	C	G	G	G	G	G	C	G	A	C	A	C	A	1.7%	5.0%	-	31.2%	3.2%	6.6%
CYP2C19*1C	A	T	A	C	G	G	G	G	G	C	G	G	C	A	C	A	3.9%	2.5%	-	<1.0	1.0%	1.4%
CYP2C19*1 (variant of *1B)	A	T	A	T	G	G	G	G	G	C	G	A	C	A	C	A	-	-	-	1.5%	-	3.9%
CYP2C19*1 (variant of *1C)	A	T	A	C	G	G	G	G	G	C	G	G	C	C	C	A	-	-	-	-	3.0%	-
CYP2C19*2	A	T	A	C	G	G	G	G	G	C	A	G	C	A	C	A	2.5%	1.1	1.5%			
CYP2C19*3A	A	T	A	T	G	G	G	G	A	C	G	G	C	A	C	A	2.4%	3.5%	1.7%			
CYP2C19*12	A	T	A	T	G	G	G	G	G	C	G	A	C	A	C	C	-	-		-	-	1.8%
CYP2C19*13	A	T	A	T	G	G	G	G	G	C	G	G	T	A	C	A	-	-		-	-	1.0%

Table 4: Haplotypes observed for study populations. Haplotypes observed in singletons are not presented here.

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