Regular Article

**CYP2C19 Genetic Polymorphism in Thai, Burmese and Karen Populations**

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**Summary:** The genetic polymorphism of CYP2C19 was examined in three Southeast Asian populations. This study was conducted in 774 Thais, 127 Burmeses and 131 Karens. Genomic DNA was extracted from leucocytes and analyzed by the PCR-RFLP technique. Genotype analysis revealed that the allele frequencies of CYP2C19*1, CYP2C19*2 and CYP2C19*3 in the Thais were 0.68, 0.29 and 0.03, respectively, and those of the Burmese population were 0.66, 0.30 and 0.04, respectively. For Karens, the minority ethnic in Mynmar, the allele frequencies of CYP2C19*1, CYP2C19*2 and CYP2C19*3 were 0.71, 0.28 and 0.01, respectively. The prevalence of PM estimated from genotype data among these three ethnic populations were 9.2%, 11.0%, and 8.4%, respectively. The PM phenotype and the frequencies of CYP2C19 defective alleles, particularly CYP2C19*3 among these three Southeast Asian ethnics appeared to be lower than other Asian populations. Lower prevalence of CYP2C19 PM suggests that these ethnics may have different capacity to metabolize drugs that are substrates of CYP2C19. Certain drug dosage regiments should be considered differently for Asian populations.

**Key words:** CYP2C19; genetic polymorphism; Thai; Burmese; Karen; southeast asian

**Introduction**

One of the common causes of individual variations in drug response is genetic polymorphisms of drug metabolizing enzymes. Cytochrome P4502C19 (CYP2C19) is a major enzyme responsible for metabolism of a number of therapeutic drugs, including barbiturates, diazepam, lansoprazole, mephenytoin, omeprazole, proguanil and propranolol.¹² The metabolic activity of CYP2C19 is genetically controlled which to date at least 20 different allelic variants have been identified. Clinical significance of CYP2C19 has been a major interest of many scientists since the discovery of this genetic polymorphism. The most significant evidence appears to be the association between proton pump inhibitors and CYP2C19, which suggested the pharmacoeconomic value of CYP2C19 genotyping prior to using of these drugs in certain patients.³¹ Two allelic variants of CYP2C19 have been revealed as major mutations contributing to the PM phenotype. The most common defect, CYP2C19*2, is a splice mutation in exon 5 which accounts for 75–83% of the defective alleles in PMs.⁴ A second defect, CYP2C19*3 consisting of a premature stop codon in exon 4,⁵² is rarely found in Caucasians but accounts for nearly all the remaining mutant alleles in Asian populations.

Individuals have been characterized as either extensive (EM) or poor (PM) metabolizers by which the gene-dose effects of CYP2C19 genotype on metabolism of the drugs can be predicted. The prevalence of PM are estimated to be 2–5% in Caucasians,⁶ 4–8% in Africans,⁷⁸ 11–23% in Asians⁶⁹ and as high as 70% in the residents of Vanuatu in Melanesia.¹⁰ Since CYP2C19 is involved in the metabolism of several anti-malarial agents including proguanil,¹¹¹² the increasing spread of this malarial parasite in Southeast Asia countries suggests that alternative anti-
malarial treatment regimens containing proguanil may become necessary in the near future. Although the clinical implications of CYP2C19 genotype on clinical efficacy of proguanil are still unclear, the concentration of proguanil is positively correlated with the degree of gastrointestinal adverse effects of the drug. Thus, knowledge of CYP2C19 polymorphism in the endemic areas of malaria such as Mynmar and Thailand may be useful as a basis for selection of anti-malarial treatment regimens in these populations.

We have recently reported that the prevalence of PM and the frequency of CYP2C19 defective allele, particularly CYP2C19*3 in a Northeastern Thai population are significant lower than those of other Asian populations. In contrast, the prevalence of CYP2C19 PM in Thais reported by Yamada et al. was more than two fold higher than those reported by our group (15.7 vs 6.5%). Heterogeneity among the Thai ethnic is well recognized, particularly those who reside in different parts of the country. In addition, the influence of migrants such as Chinese and Laos may also contribute to this uncertainty of this genetic polymorphism. Moreover, there is no report about this genetic polymorphism in Burmese and Karen, a minor ethnic, populations of Mynmar. In order to verify the polymorphism of CYP2C19 in these three populations, the present study was therefore carried out in well-defined subjects whom belong to these ethnics and compare with other populations.

**Materials and Methods**

**Subjects:** Seven hundred and seventy-four unrelated Thais (313 females and 461 males, mean aged 38.1 ± 12.1 yr) residing in different regions of Thailand were recruited to participate in this study. The other two Southeast Asian populations recruited in this study were 127 Burmeses (69 females, and 58 males, mean aged 29.3 ± 9.6 yr) and 131 Karens (50 females and 81 males, mean aged 29.3 ± 8.7 yr). The subjects were classified as Native Thais, Burmeses or Karens according to history of their parents and grandparents. All of them were healthy as defined by medical history and physical examination. All subjects were informed, both verbally and in writing, about the experimental procedures and the purposes of the study. Written informed consents were obtained from all of them for their participation in the study. The study protocol was approved by the Khon Kaen University Ethics Committee for Human Research, Khon Kaen University, Thailand and the Ethics Committee, Ministry of Public Health, Thailand.

**CYP2C19 genotyping procedures:** Five milliliters of venous blood were collected into a tube containing EDTA. Peripheral blood leucocytes were separated by centrifugation at 3,000 g for 15 min. Genomic DNA was purified from peripheral blood leukocytes by a standard phenol-chloroform extraction procedure. Genotype analyses of CYP2C19 alleles were performed as previously described. Briefly, for determination of CYP2C19*2 and CYP2C19*3, PCR amplifications of exon 5 and exon 4 were performed using specific primers. PCR amplification was performed using a Gradient Robocycler (Stratagene®, La Jolla, CA, USA). An initial denaturation step at 94°C for 5 min was followed by 37 cycles consisting of denaturation step at 94°C for 45 seconds, annealing at 53°C for 45 seconds, and extension at 72°C for 45 seconds. The final extension was subsequently performed at 72°C for 7 min. The PCR products for exon 5 or exon 4 were then digested with SmaI or BamHI, respectively, and subsequently analyzed using a 3% agarose gel electrophoresis and staining with ethidium bromide.

**Data analysis:** Genotype and allele frequencies were calculated by counting. Expected genotype frequencies were calculated using the Hardy-Weinberg equation from the allele frequencies ($p^2 + 2pq + q^2 = 1$), where $q$ was the combined allele frequency of CYP2C19*2 and CYP2C19*3. It should be noted that only common defective alleles, CYP2C19*2 and CYP2C19*3 were determined in this study therefore $p$ was combination frequency of CYP2C19*1 allele and other rare defective alleles. Goodness of fit between observed and estimated genotype frequencies according to the Hardy-Weinberg equilibrium was determined by a Chi-square test. Allele frequencies from other populations were compared using the Z-test. Statistical analysis of the data was performed using the Stata statistical software package (version 6, Stata Corp, TX, USA).

**Results**

The allele frequencies of CYP2C19*1, CYP2C19*2, CYP2C19*3 and genotype frequencies in the three Southeast Asian populations are shown in Fig. 1. The most common defective allele in these ethnics was CYP2C19*2 with observed frequencies ranging between 0.28–0.30. The CYP2C19*3 allele were found between 0.01–0.04.

Based on the Hardy-Weinberg equilibrium, the prevalence of PM estimated from genotype data in the Thai, Burmese and Karen populations were 9.2% (95% CI 7.2–11.4), 11.0% (95% CI 5.6–16.5) and 8.4% (95% CI 3.6–13.1), respectively (Table 1). Nevertheless, there was no statistical significant difference in the prevalence of PM among the three ethnics. To gain more insight into this pharmacogenetic variation, further CYP2C19 genotype analysis was carried out among these ethnics and compared with other Asian populations (Table 2). Between Thai and Burmese populations, the allele frequencies of CYP2C19*1 (0.68, CI 0.65–0.70 vs. 0.66, 95%CI 0.61–0.72), CYP2C19*2 (0.29, 95%CI 0.27–0.31 vs. 0.30, 95%CI 0.24–0.36), and CYP2C19*3
Table 1. Frequencies of CYP2C19 genotype in three Southeast Asian ethnics

<table>
<thead>
<tr>
<th>Genotype</th>
<th>% Observed genotype [% predicted genotype]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thai</td>
</tr>
<tr>
<td>*1/*1</td>
<td>44.5 [45.7]</td>
</tr>
<tr>
<td>*1/*2</td>
<td>42.6 [39.3]</td>
</tr>
<tr>
<td>*1/*3</td>
<td>3.7 [4.5]</td>
</tr>
<tr>
<td>*2/*2</td>
<td>6.7 [8.5]</td>
</tr>
<tr>
<td>*2/*3</td>
<td>2.1 [1.9]</td>
</tr>
<tr>
<td>*3/*3</td>
<td>0.4 [0.1]</td>
</tr>
</tbody>
</table>

% PM (95% CI) 9.2 (7.2–11.4) 11.0 (5.6–16.5) 8.4 (3.6–13.1)

Data in parentheses represent genotype frequency predicted according to the Hardy-Weinberg equation. *PM calculated from homozygous mutant genotype data. Numbers of Thais, Burmeses and Karens used in this study were 774, 127 and 131, respectively.

(0.03, 95% CI 0.02–0.04 vs. 0.04, 95% CI 0.01–0.06), were not significantly different (p>0.05). In contrast, the allele frequency of CYP2C19*3 in the Karen population (0.01, 95% CI 0–0.02) was significantly lower than those observed in Thai (0.03, 95% CI 0.02–0.04) and Burmese (0.04, 95% CI 0.01–0.06) populations (p≤0.05).

Discussions

Previous reports on CYP2C19 genetic studies in Thai population had shown discrepancy results regarding this polymorphism. Our recent study report demonstrated that the PM phenotype in 107 Northeastern Thai subjects was 6.5% and the frequency of CYP2C19*3 defective alleles was only 0.02 which significantly lower than other Asian populations. In contrast, Yamada et al. have reported in 121 healthy Thais that the prevalence of CYP2C19 PM is 15.7% and the allele frequencies of both defective alleles, CYP2C19*2 and CYP2C19*3, are similar to those of Chinese and Japanese populations. It should be noted that the heterogeneity among the Thai population is recognized therefore in the present study large scale analysis of this genetic polymorphism was performed in Thai subjects who resided in different regions of Thailand (n=774). The prevalence of PM in Thai population found in this study (9.2%, 95% CI 7.2–11.4) was not different from our previous report in the Northeastern Thai subjects (6.5%, 95% CI 1.86–11.22%) but still markedly lower than those previously reported by Yamada et al. Consistently, the allele frequency of CYP2C19*3 defective allele in Thais observed in the present study was significantly lower than those reported by Yamada et al., This discrepancy may be due to the difference in sample size and selection bias between the two studies. The subjects who participated in our study were native Thais, whereas the origin of the subjects who participated in the study by Yamada’s group were not well defined. It is noteworthy that the Chinese-Thai admixture is contributed to the major populations in Thailand. Nevertheless, with larger number of subjects participated and distribution sampling across Thailand, the results obtained from the present study provides a reliable prevalence of PM and allele frequencies of CYP2C19 for the Thai population.
The low frequencies of PM phenotype and of CYP2C19*2 and CYP2C19*3 alleles in Chinese-Dai was close to those observed in the Thais. Thus, this finding has confirmed the close genetic relationship between the two ethnic populations as suggested by other means of study such as anthropological and historical approaches. The prevalence of PM and the frequency of defective alleles, particularly CYP2C9*3 in these three Asian populations was significantly lower than those reported in other Asian population (Table 2) as well as those of the data obtained from meta-analysis in Chinese and Japanese population.

Although the prevalence of PM of CYP2C19 in Thai (9.2%), Burmese (11.0%) and Karen (8.4%) populations found in the present study were not significant different, the allele frequency of CYP2C19*3 observed in Karens, a minority Ethnic of Mynmar was significantly lower than those observed in Burmese and Thai populations. Lower frequency of this defective allele in these three ethnics may explain the lower prevalence of PM in these populations as compared to other Asian populations. Hence, our results suggest that these Southeast Asian populations may have higher capacity to metabolize clinically important CYP2C19 substrates compared to people from other Asian countries.

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References


