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Prevalence of *CYP2B6* alleles in malaria-endemic populations of West Africa and Papua New Guinea

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Abstract

Objective—Cytochrome P450 2B6 (CYP2B6) is involved in the metabolism of artemisinin drugs, a novel series of antimalarials. Our aim was to analyze the prevalence of the most commonly observed *CYP2B6* alleles in malaria-endemic populations of West Africa (WA) and Papua New Guinea (PNG).

Methods—Using a post-PCR ligation detection reaction-fluorescent microsphere assay, frequencies of *CYP2B6*1A*, **2*, **3*, **4*, **5*, **6*, **7*, and **9* were determined in WA (*n*=166) and PNG (*n*=174). To compare with the results of previous studies, we also determined the allele frequencies in 291 North Americans of various ethnic groups.

Results—Significant differences were observed between WA and PNG for the frequencies of alleles *CYP2B6*1A* (45% vs 33%, *P* = 0.003), **2* (4% vs. 0%, *P*<0.001), **6* (42% vs 62%, *P*<0.001), and **9* (8% vs 1%, *P*<0.001), and genotypes **1A/*9* (9% vs 0%, *P*<0.001) and **6/*6* (17% vs 43%, *P*<0.001). The frequencies of *CYP2B6* genotypes in the populations were in Hardy-Weinberg equilibrium, except for PNG where an overall significant deficit of heterozygosity was observed (H_O=0.431, H_E=0.505, *P*=0.004). The allele frequencies in Asian-Americans and Caucasians-Americans were comparable to those documented for Japanese and Caucasian populations.

Conclusions—*CYP2B6* variants, previously shown to affect metabolism of a variety of drugs, occur in WA and PNG, and there are significant genetic differences at the *CYP2B6* locus in these populations. It may be important to determine if these differences alter the efficacy of artemisinin drugs.

Keywords

Artemisinin; *CYP2B6* alleles; Malaria; Papua New Guinea; West Africa

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Introduction

Among infectious diseases, acquired immunodeficiency syndrome (AIDS), tuberculosis (TB), and malaria are the top three causes of mortality, killing over 6 million people each year (http://www.theglobalfund.org/en/). Despite increased efforts and resources, the number of people infected and dying from these diseases continues to rise. The effectiveness of chemotherapeutic treatment of these diseases is threatened by the emergence of drug-resistant pathogens. In addition, our knowledge of the basic mechanisms underlying the pharmacokinetic and pharmacodynamic behaviors of major classes of drugs used to treat these infections in various populations, is very limited.

Human cytochrome P450 2B6 (CYP2B6) is involved in the biotransformation of a variety of clinically important drugs. Among these are antiretrovirals nevirapine (NVP) and efavirenz (EFV), agents used to treat AIDS and/or stop the spread of the HIV infection [1, 2], and the novel antimalarial drug artemisinin [3–5] and its derivatives artesunate [4] and β-arteether [6]. Artemisinin drugs are unique and valuable, as they act rapidly and effectively against severe and multidrug-resistant falciparum malaria [7, 8]. Dihydroartemisinin (DHA) is the major active metabolite of artemisinin derivatives [9], and most of the antimalarial activity of artesunate is attributed to DHA [10]. However, parent drugs may also contribute to the antimalarial effect [11]. DHA is further metabolized to DHA-glucuronide by polymorphic uridine diphosphate glucuronosyltransferase (UGT) enzymes UGT1A9 and UGT2B7 [10]. While these drugs have been found to be virtually void of any serious side effects in humans, their neurotoxicity in animal models has raised some concern about their use [12]. Monotherapy with these drugs leads to high recrudescence [13]. Therefore, artemisinin-based combination therapies (ACTs) are viewed as the most promising drug strategies for combating malaria morbidity and mortality, especially in children and pregnant women [14, 15]. The World Health Organization (WHO) is now actively encouraging malaria-endemic countries to switch to ACT (http://www.rbm.who.int/). So far, 43 countries have adopted ACT either as the first-line or as the second-line malaria treatment, and 5–10 more countries are in the process of a malaria treatment policy change to ACT [16]. The WHO recommended ACTs are: artemether + lumefantrine, artesunate + amodiaquine, artesunate + mefloquine, and artesunate + sulfadoxine-pyrimethamine; DHA + piperaquine is under consideration [16]. The WHO, however, warns that as more people gain access to these life-saving malaria medicines, it is vital that countries closely monitor their effectiveness (http://www.mosquito.who.int/).

The expressed level of the CYP2B6 protein in the human liver is about 0.1–1.0% of the total CYP content, and both protein expression levels and enzyme activities in liver microsomes vary more than 100-fold among individuals [17]. This inter-individual variability has been associated with CYP2B6 genetic polymorphism, which can influence expression and function of the enzyme [18], and therapeutic outcomes of CYP2B6-metabolized drugs [19].

The *CYP2B6* gene is located on the chromosome 19q13.2 [20], spans a region of ~26 kb, is composed of 9 exons, and encodes a 491 amino acid protein [20–22]. Initially, six nonsynonymous mutations were found in the *CYP2B6* gene, located in exon 1 (64C>T) (nucleotide position based on *CYP2B6* mRNA sequence, GenBank accession number

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M29874), exon 3 (415A>G), exon 4 (516G>T), exon 5 (777C>A and 785A>G), and exon 9 (1,459C>T). Based on these mutations, the wild type *CYP2B6*1* [21] (now designated as **1A*, http://www.imm.ki.se/CYPalleles/cyp2b6.htm) and 8 variant alleles (*CYP2B6*2* to **9*) were identified [18, 23]. The most prevalent variant allele was *CYP2B6*6* in Caucasian (26%) [18] and Japanese (16%) [24] populations. Allele *CYP2B6*8* was reported at a low frequency (~1%) in Caucasians only [23, 25], and was not found in African-American, Hispanic, and Asian subjects [23]. Recently, very low frequency novel variant alleles *CYP2B6*10* to **15* in Caucasian (0.2%–1%) [25] and *CYP2B6*23* to **25* in Japanese (0.25%–0.75%) [26] populations have been identified. Some of these variant alleles, particularly *CYP2B6*5*, **6*, **7*, and **9*, could have a significant impact on the CYP2B6 expression level and activity [18, 23], whereas CYP2B6*8, *11, *12, and *15 were characterized as very low or null activity variants [25]. Beside nonsynonymous mutations, several single nucleotide polymorphisms (SNPs) have been found in the *CYP2B6* promoter region [23, 27–29]. Some of these, either by themselves or in combination with certain nonsynonymous mutations, may alter the enzyme expression level and activity [23, 27, 29].

The aim of the present study was to analyze the prevalence of the most commonly observed alleles of *CYP2B6* (*CYP2B6*1A* to **7* and **9*) in the populations from malarious areas of West Africa and Papua New Guinea (PNG). In these countries, malaria, HIV/AIDS, and TB are significant public health threats, and the use of artemisinin drugs has been recently approved (http://www.mosquito.who.int/ and http://www.dndi.org/). Our genotyping assay involves a multiplexed oligonucleotide ligation detection reaction (LDR) [30] and flow cytometric analysis of fluorescent microspheres (FM) [31]. Using this assay, we also determined *CYP2B6* allele and genotype frequencies in Asian, Caucasian, Hispanic, and African individuals from North America to compare with previous findings. Recently, a similar assay has been used for analyzing the most common mutant alleles of *CYP2C9* and *CYP2C19* [32].

Materials and methods

Study populations and genomic DNA extraction

DNA samples from West African individuals (*n*=166) from Senegal (*n*=10, 1 village), Guinea (*n*=21, 1 village), Sierra Leone (*n*=52, 6 villages), Ivory Coast (*n*=41, 6 villages), and Ghana (*n*=42, 3 villages), kindly provided by Dr. B.A. Boatin, were isolated as a part of the Onchocerciasis Control Program [33]. These samples were encoded to conceal sample identity such as precise village of residence, sex, age, etc. However, all these samples were obtained from the West Atlantic sub-group of the Niger-Congo linguistic group of individuals, implying that all the individuals belong to the same ethnicity. Blood samples from PNG (*n*=174) were collected from the Wosera area of East Sepik Province as a part of the malaria epidemiology study as previously described [34]. Samples from PNG were obtained from the Abelam linguistic group of individuals. Although these samples were collected from onchocerciasis-endemic (West Africa) and malaria-endemic (PNG and West Africa) regions, to the best of our knowledge, there is no known association between *CYP2B6* polymorphisms and onchocerciasis or malaria. North American random blood donors (*n*=291), whose race or ethnicity was self-identified at the time of sample collection,

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were obtained from the National Histocompatibility Laboratory, American Red Cross/University of Maryland Medical System, Baltimore, MD [35]. Among these North American donors were individuals of Asian (*n*=61), Caucasian (*n*=60), Hispanic (*n*=77), and African (*n*=93) ethnicities. Samples from all individuals from the three different groups (West African, Papua New Guinean, and North American) were collected under protocols, including the procedures for informed consent, approved by the corresponding institutional review boards.

Blood samples were collected in K^+-EDTA-coated Vacutainer tubes, and stored at −20°C until DNA extraction could be performed. DNA was extracted from 200 μl of whole blood from study subjects using the QIAamp 96 spin blood kit (QIAGEN, Valencia, CA).

Polymerase chain reaction (PCR) and agarose gel electrophoresis

For *CYP2B6* exon 4 and exon 5 amplification, PCR primers of Lang et al. [18] were used. PCR primers were designed (based on *CYP2B6* genomic DNA sequence, GenBank accession number AC023172) to selectively amplify *CYP2B6* exon 1 and exon 9. Since the nucleotide sequence of *CYP2B6* is approximately 95% similar to that of the *CYP2B7* pseudogene [21], sequence homology and specificity of all the primer sequences were checked by using BLASTn (http://www.ncbi.nlm.nih.gov). The primer sequences, PCR buffer, amplification conditions, and method to perform agarose gel electrophoresis are provided in supplemental Table 1.

Cloning and sequencing

Exon-specific PCR amplification products were purified using the QIAquick PCR purification kit (QIAGEN, Valencia, CA). These products were cloned into pCR2.1-TOPO plasmid vector using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA). Clones were amplified, purified, and DNA sequencing was performed using fluorescence-based sequencing protocols on the ABI PRISM 377 automated sequencer (Applied Biosystems, Foster City, CA). Sequences were analyzed using the Sequencher software v4.1.4 (Gene Codes Corporation, Ann Arbor, MI). Cloned and sequenced DNA templates were subjected to PCR and used in all steps of our SNP genotyping assay to serve as positive controls.

SNP genotyping

Our SNP genotyping assay is divided into three steps: (1) LDR, (2) FM hybridization, and (3) detection using the Bio-Plex suspension array system, which includes a fluorescence reader and the Bio-Plex Manager analytical software (Bio-Rad Laboratories, Hercules, CA). For each SNP, the LDR uses two allele-specific primers and a common primer. Each allele-specific primer incorporates a unique 24 bp tag sequence at the 5′ end, which is complementary to anti-tag sequences attached to unique fluorescent Luminex Flex-MAP microspheres (Luminex Corporation, Austin, TX). The common primers are 5′-phosphorylated and 3′-biotinylated by the manufacturer (Integrated DNA Technologies, Coralville, IA). In the LDR, only the annealed allele-specific primer is ligated to the common primer to generate a biotin-labeled product. The other primer does not ligate nor label due to the 3′ mismatched base. When microspheres are added, specific hybridization occurs between tag and anti-tag sequences. After adding streptavidin-R-phycoerythrin

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conjugate, the hybridized microspheres are sorted according to their unique fluorescence, and the phycoerythrin signal is measured as median fluorescence intensity (MFI).

This multiplex genotyping assay was used for the simultaneous detection of all the SNPs in each sample. Equal volumes of each PCR product from each sample were mixed. The LDRs were performed in a solution (total vol. 15 μl) containing 20 mM Tris-HCl (pH 7.6), 25 mM potassium acetate, 10 mM magnesium acetate, 10 mM dithiothreitol, 1 mM NAD^+, 0.1% Triton X-100, 10 nM (150 fmol) of each LDR primer, 2 units of Taq DNA ligase (New England BioLabs, Beverly, MA), and 1 μl of the mixed PCR products. LDR primers (based on *CYP2B6* genomic DNA sequence, GenBank accession number AC023172) for each SNP are described in supplemental Table 2. All reactions were performed in the 96-well PCR plates using the Peltier Thermal Cycler PTC-225 (MJ Research, Watertown, MA). The LDR conditions were 95°C 1 min, 95°C 15 sec (denaturation) and 60°C 2 min (annealing/ligation) (32×). Following this reaction, the 5 μl LDR products were hybridized to microspheres by adding 60 μl of TMAC hybridization solution (3 M tetramethyl-ammonium chloride, 50 mM Tris-HCl [pH 8.0], 3 mM EDTA [pH 8.0], 0.1% SDS) containing 250 of each microsphere. Hybridization reactions were performed in the 96-well Costar plates using the Peltier Thermal Cycler, under conditions 95°C 90 sec (denaturation) and 37°C 40 min. Following hybridization, 6 μl of TMAC hybridization solution containing 120 ng streptavidin-R-phycoerythrin conjugate (Molecular Probes, Eugene, OR) was added to the above reaction mix, and incubated at 37°C for 40 min. After the incubation, detection was completed on the Bio-Plex system by sorting and quantifying MFI for 75 of each microsphere.

Statistical analysis

The MFI values were used to calculate the allelic ratio for each SNP by dividing the allele-specific MFI value by the sum of the MFI values for that SNP (allele A/A+B=A_n, and allele B/A+B=B_n), where A and B are the 2 alleles of a SNP. To be homozygous for a particular allele of the SNP, the allelic ratio must be >0.75. To be heterozygous, each allele must have a ratio between 0.25 and 0.75. Consequently, an allele with a ratio of <0.25 is considered negative (i.e., not present). Normalized values, A_n and B_n, were further divided (A_n/B_n), and the quotient was log-transformed. The mean and 95% confidence interval (CI) of the log-transformed quotients are presented in Table 1.

CYP2B6 allele (or haplotype) and genotype (or diplotype) frequencies were calculated and Hardy-Weinberg (H-W) exact test (estimation of *P*-values by the Markov chain method) was performed for each population using the Genepop web version 3.4 (http://www.wbiomed.curtin.edu.au/genepop/). Differences in allele and genotype frequencies between two populations were measured using the Fisher's exact test (http://www.matforsk.no/ola/fisher.htm). In order to analyze linkage disequilibrium (LD) between SNPs, we calculated Lewontin's *D'* using SHEsis (http://www.nhgg.org/analysis/) [36]. Further, the observed and expected allele and genotype numbers for the West African (all five populations pooled together) and PNG populations were compared. For this, the expected numbers were calculated from a contingency table using the formula (row total×column total)/grand total, where the grand total is the total number of individuals in these two populations. We then performed the chi-square analysis to test for fit of data to H-

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W proportions. For all statistical analyses, a *P*-value of <0.05 was considered to be significant.

Results

Polymerase chain reactions were performed on genomic DNA preparations from a total of 631 individuals from six populations to amplify *CYP2B6* exons 1, 4, 5, and 9. The most commonly observed 5 SNPs of *CYP2B6* (64C>T [exon 1], 516G>T [exon 4], 777C>A and 785A>G [exon5], and 1459C>T [exon 9]) were analyzed by a post-PCR ligation detection reaction-fluorescent microsphere assay (LDR-FMA). Using this assay, the observed frequencies of *CYP2B6*1A* and variant alleles in the 6 populations are presented in Table 2. Note that for this analysis, all five populations from West Africa were pooled together. The frequency of *CYP2B6*1A* was lowest (33%) in Papua New Guineans and highest (64%) in Asian-Americans. In our study, variant alleles **2* (3%–7%),**4* (2%–6%), **7* (1%–3%), and **9* (1%–8%) were present in four or five (out of six) populations. We did not observe *CYP2B6*3* in any of the study subjects. Previously, the frequency of this variant allele in a Caucasian population was 0.5% [18], suggesting that *CYP2B6*3* is a rare allele. Alleles *CYP2B6*5* (2%–5%) and **6* were found in all the populations analyzed here. Among the variant alleles, *CYP2B6*6* had the highest prevalence in all the populations. The frequencies of this allele ranged from 23% in Asian-Americans to 62% in Papua New Guineans. The frequency of *CYP2B6*6* in Papua New Guineans was significantly higher than that in any other population (P<0.001, Fisher's exact test). Previously, *CYP2B6*6* was reported as the most frequent variant allele in Caucasian (26%) [18] and Japanese (16%) [24] populations. Our results on Asian-Americans and Caucasian-Americans using the LDR-FMA were comparable to those previously reported for Japanese [24] and Caucasian [18] populations using a PCR-RFLP assay. One study has reported CYP2B6 allele frequencies, determined by sequencing, in Hispanics (n=7) and African-Americans (n=29) [23]. Due to significant differences in the number of subjects, we did not compare our results on Hispanic-Americans (n=77) and African-Americans (n=93) with the results of that study [23].

We then analyzed the West African samples (n=166) for each represented country. As shown in Table 3, the frequency of *CYP2B6*1A* ranged from 30% (Senegal) to 50% (Ivory Coast). Alleles *CYP2B6*3*, **4*, and **7* were not observed in any of the samples. Allele *CYP2B6*5* (2%–4%) was present in three (Guinea, Ivory Coast, and Sierra Leone) out of five populations. Alleles *CYP2B6*6* and **9* (2%–11%) were found in all the populations. As seen for other populations, *CYP2B6*6* was the most prevalent variant allele, with frequencies ranging from 36% (Sierra Leone) to 60% (Senegal). Despite some allele frequency differences between some of the populations, our overall quantitative assessment of *CYP2B6* alleles in West Africa does not change.

Table 4 shows frequencies of *CYP2B6* genotypes in the study populations. Except for the PNG population, these results were in accordance with the expected genotype distribution (data not shown), suggesting that the study populations were in H-W equilibrium. In further analysis, we found an overall significant deficit of heterozygosity in the PNG population (H_O=0.431, H_E=0.505, P=0.004, Markov chain method). Further SNP-by-SNP analysis showed a significant deficit of heterozygosity at positions 516 (P=0.016) and 1459

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(P=0.003) in the PNG population. Heterozygosity is a measure of genetic variation in natural populations. If the observed heterozygosity (H_O) is lower than the expected heterozygosity (H_E), it indicates that the population in question may be inbred. In fact, there does seem to be reduced genetic diversity in many populations in PNG [37].

A comparison between West Africans (n=166) and Papua New Guineans (n=174) showed significant differences in the allele frequencies of *CYP2B6*1A* (P=0.003, Fisher's exact test), **2* (P<0.001, Fisher's exact test), **6* (P<0.001, Fisher's exact test), and **9* (P<0.001, Fisher's exact test). A comparison of the observed and expected numbers of alleles showed that *CYP2B6*1A* occurred in H-W proportion in both the populations (data not shown). *CYP2B6*6* was significantly lower than expected in West Africans (chi-square$_{1df}$=6.61, P=0.01), but was significantly higher than expected in Papua New Guineans (chi-square$_{1df}$=6.31, P=0.01). On the other hand, *CYP2B6*2* and **9* were significantly higher than expected in West Africans (chi-square$_{1df}$=7.51 and 8.3, P=0.006 and 0.004, respectively), but were significantly lower than expected in Papua New Guineans (chi-square$_{1df}$=7.16 and 7.92, P=0.007 and 0.005, respectively). Further, the genotype frequencies of **1A/*1A*, **1A/*6*, and **6/*9* were not significantly different between West Africans and Papua New Guineans, but the frequencies of **1A/*9* (P<0.001, Fisher's exact test) and **6/*6* (P<0.001, Fisher's exact test) were significantly different between the two populations. A comparison of the observed and expected numbers of genotypes showed that genotypes **1A/*1A, *1A/*6*, and **6/*9* occurred in H-W proportions in both the populations (data not shown). However, we observed a significant departure of genotypes **1A/*9* (higher than expected in West Africans, chi-square$_{1df}$=8.05, P=0.005; lower than expected in Papua New Guineans, chi-square$_{1df}$=7.68, P=0.006) and **6/*6* (lower than expected in West Africans, chi-square$_{1df}$=9.54, P=0.002; higher than expected in Papua New Guineans, chi-square$_{1df}$=9.1, P=0.003) from H-W proportions. The results of allelic and genotypic analyses indicate significant regional and ethnic differences between West African and Papua New Guinean populations.

In order to further analyze the haplotype data, we performed LD analysis among pairs of SNPs in each population. We did 6 pairwise analyses. Since position 777 did not show C>A change in any of the study samples, this SNP was not included in the LD analysis. As shown in Table 5, the pattern and extent of LD varied among the populations, suggesting that LD may not be uniformly maintained in different populations. Although the magnitude of values of D'<1 has no clear interpretation, it may suggest that there are blocks of LD interrupted by regions of recombination in Asian-Americans, Caucasian-Americans, Hispanic-Americans, and in African-Americans. These North American populations are the result of very recent human migration and mixture. Although it might provide some valuable information (e.g., the prevalence of *CYP2C8* alleles found in malaria-endemic African populations were recently found not to differ significantly from that in African-Americans [38]), genetic data from these populations should be evaluated with caution. For the West African (all five populations pooled together) and PNG populations, D' values were between 0.881 and 1.00 for all pairs, indicating strong to complete LD.

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Discussion

CYP2B6 is involved in the metabolism of a number of drugs used to treat infectious diseases, including artemisinin and its derivatives, a novel class of highly effective antimalarials [3–6]. Unfortunately, only limited information is available regarding *CYP2B6* pharmacogenetic variation in malaria-endemic populations, where these drugs are either being used or might be used in the near future [28, 39]. Here, we demonstrated that genetic variants of *CYP2B6* occur in the populations from two different malaria-endemic regions, West Africa and PNG. We observed allelic variants *CYP2B6*2*, **5*, **6*, and **9* in West Africa (frequencies 4%, 2%, 42%, and 8%, respectively), and **5*, **6*, **7*, and **9* in PNG (frequencies 2%, 62%, 1%, and 1%, respectively). While we observed significant allelic and genotypic differences between these two populations, the functional significance of *CYP2B6* polymorphisms in artemisinin drug metabolism, and their overall clinical impact on the outcome of treatment with these antimalarial drugs remain to be determined.

Previous in vitro heterologous expression studies have shown that the polymorphisms found in alleles *CYP2B6*5*, **6*, **7*, and **9* can alter the enzyme expression level and/or activity [40–42]. Human liver microsome studies have also yielded similar results [18, 23]. The functional significance of these *CYP2B6* variants has been shown for a variety of drugs. For example, in AIDS clinical studies, they were associated with 2- to 4-fold higher plasma EFV and NVP concentrations [43–46], and, in HIV-infected subjects, 2-fold higher plasma EFV concentrations were associated with neuropsychological adverse effects [43, 44, 47, 48]. Beside antiretroviral drugs, these variants have also been found to affect the metabolism and pharmacokinetics of bupropion (an antidepressant) [27], cyclophosphamide (an anticancer and immunosuppressive) [49], and mephobarbital (an anticonvulsant) [50].

Artemisinin drugs belong to a different class of compounds; they are rapidly absorbed and eliminated, and their peak plasma concentrations occur for only hours [51–53]. Yet *CYP2B6* polymorphisms may be pharmacologically important in the case of artemisinin drugs. Several studies have reported large inter-individual variations in the main pharmacokinetic parameter values, including the area under the concentration-time curve (5- to 45-fold), of artemisinin, artesunate, and DHA among both healthy subjects and malaria patients [52, 54–58]. In malaria patients, given a single oral dose (25 to 250 mg) of artesunate, DHA concentrations at 2 h varied from 2-fold to at least 17-fold, depending on the dose of artesunate given [59]. Data suggest that doses of artesunate up to 2 mg/kg are generally correlated with both plasma DHA and parasite and fever clearance [59]. However, taking into account the considerable variance between individuals in both pharmacokinetics and pharmacodynamics, use of a larger dose was suggested [59]. The basis for such a large inter-individual pharmacokinetic variability was not addressed in these studies [52, 54–59], and may involve *CYP2B6* polymorphisms. Despite large variations in the pharmacokinetic properties of artemisinin drugs, parasite and fever clearance was observed in these malaria patients [55, 57, 58, 59]. This observation is difficult to interpret because of the fact that other antimalarial drugs, such as mefloquine [55, 59], sulfadoxine-pyrimethamine [57, 58], or chloroquine [58], were also given to the patients in the same studies. Therefore, it is possible that the observed parasite and fever clearance was primarily due to the other antimalarial drug given.

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Since the West African and PNG populations compared in this study are significantly different at the *CYP2B6* locus, phenotypic differences might occur between these populations for drugs that are metabolized primarily via CYP2B6. For example, significant differences in *CYP2B6* allele frequencies have been observed between Korean and other populations [28]. In vitro evidence has suggested that CYP2B6 may be involved in the metabolism of primaquine, another antimalarial effective against latent liver stages of *Plasmodium vivax* [4]. In Korean patients, the mean peak plasma concentration of primaquine was 5-fold higher and carboxyprimaquine (the active metabolite of the drug) 2.5- to 5-fold lower compared with Indian and Thai patients, suggesting interethnic differences in the metabolism of this drug [60].

Another line of evidence suggests that differences in frequencies of *CYP* alleles between Papua New Guinean and African individuals may lead to metabolic differences. *CYP2C8* variant alleles (**2*, **3*, and **4*) are prevalent in Africans (cumulative frequency 16.6%) [38], but have not been seen in Papua New Guineans [61]. In the case of amodiaquine, which is metabolized to desethylamodiaquine via CYP2C8, the peak concentration and time to reach the peak concentration mean values were higher for Papua New Guinean (369 ng/ml, 3 days) than those for African (161 ng/ml, 3.9 h) patients [61], again suggesting interethnic differences. Similarly, *CYP2C19* null allele frequencies, associated with the poor metabolism phenotype of proguanil, differ significantly between Papua New Guinean (36%) and most of the African (<10%) populations [62].

Finally, it is important to acknowledge that the current study has exclusively focused on the most prevalent *CYP2B6* variant alleles. Since polymorphisms of functional significance also occur in *UGT1A9* and *UGT2B7* genes [63], whose products are involved in the DHA metabolism [10], it is important to include these genes in future studies. It is probable that the outcome of treatment with artemisinin drugs is associated with a combined effect of polymorphisms in both *CYP* and *UGT* genes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Genotype-specific fluorescence parameters

Polymorphism	Genotype	*n*	Mean	95% CI
64	CT	34	0.34	0.04
	CC	597	1.34	0.02
516	TT	147	−1.79	0.07
	GT	285	−0.29	0.02
	GG	199	1.43	0.05
777/785	CC/GG	133	−1.73	0.06
	CC/AG	291	0	0.01
	CC/AA	207	1.53	0.05
1459	TT	4	−1.77	0.15
	CT	47	−0.12	0.03
	CC	580	1.34	0.01

n number of individuals

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Table 2

CYP2B6 alleles in various populations

Alleles	Frequencies					
	Asian-American (*n*=61)	**Caucasian-American (*n*=60)**	**Hispanic-American (*n*=77)**	**Afiican-American (*n*=93)**	**West Afiican (*n*=166)**	**PNG (*n*=174)**
**1A*[a]	0.64	0.56	0.53	0.56	0.45	0.33
**2*	0.07	0.03	0.03	0.03	0.04	0
**3*	0	0	0	0	0	0
**4*	0.04	0.06	0.03	0.02	0	0
**5*	0.03	0.03	0.05	0.05	0.02	0.02
**6*	0.23	0.28	0.30	0.34	0.42	0.62
**7*	0	0.03	0.02	0.01	0	0.01
**9*	0	0.01	0.05	0.01	0.08	0.01

[a]wild type

[*2]64C>T (R22C);

[*3]777C>A (S259R);

[*4]785A>G (K262R);

[*5]1,459C>T (R487C);

[*6]516G>T (Q172H) and 785A>G (K262R);

[*7]516G>T (Q172H), 785A>G (K262R), and 1,459C>T (R487C);

[*9]516G>T (Q172H)

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Table 3

CYP2B6 alleles in West African populations

Alleles	Frequencies				
	Ghana (*n*=42)	Guinea (*n*=21)	Ivory Coast (*n*=41)	Sierra Leone (*n*=52)	Senegal (*n*=10)
**1A*	0.42	0.45	0.50	0.45	0.30
**2*	0.02	0.02	0.06	0.06	0
**3*	0	0	0	0	0
**4*	0	0	0	0	0
**5*	0	0.02	0.04	0.02	0
**6*	0.46	0.48	0.38	0.36	0.60
**7*	0	0	0	0	0
**9*	0.09	0.02	0.02	0.11	0.10

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Table 4

CYP2B6 genotypes in various populations

Genotypes	Frequencies					
	Asian-American (*n*=61)	**Caucasian-American (*n*=60)**	**Hispanic-American (*n*=77)**	**African-American (*n*=93)**	**West African (*n*=166)**	**PNG (*n*=174)**
**1A/*1A*	0.46	0.28	0.25	0.28	0.18	0.13
**1A/*2*	0.05	0.02	0.03	0.03	0.02	0
**1A/*4*	0.05	0.07	0.03	0.02	0	0
**1A/*5*	0.05	0.07	0.06	0.05	0	0.02
**1A/*6*	0.21	0.33	0.39	0.44	0.42	0.36
**1A/*7*	0	0.07	0.04	0.01	0	0.02
**1A/*9*	0	0	0.03	0	0.09	0
**2/*4*	0.02	0.02	0	0	0	0
**2/*5*	0	0	0	0.01	0.04	0
**2/*6*	0.07	0.02	0.03	0.01	0.02	0
**4/*5*	0	0	0	0.01	0	0
**4/*6*	0.02	0.03	0.03	0	0	0
**5/*5*	0	0	0	0.01	0	0.01
**5/*7*	0	0	0	0	0	0.01
**5/*9*	0	0	0.04	0	0	0
**6/*6*	0.08	0.08	0.06	0.11	0.17	0.43
**6/*9*	0	0.02	0.03	0.01	0.06	0.02

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Table 5

Lewontin's *D'* values in various populations

Population	*n*	SNP pairs					
		64–516	64–785	64–1,459	516–785	516–1,459	785–1,459
Asian-American	61	0.083	0.206	1.00	1.00	1.00	1.00
Caucasian-American	60	0.975	0.510	1.00	0.838	0.098	0.974
Hispanic-American	77	0.023	0.897	1.00	0.875	0.987	0.997
African-American	93	0.996	0.997	0.105	0.975	0.999	0.998
West African	166	0.999	0.999	1.00	1.00	1.00	1.00
PNG	174	1.00	1.00	1.00	0.987	0.884	0.881