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Preliminary Investigation of the Contribution of *CYP2A6, CYP2B6,* and *UGT1A9* Polymorphisms on Artesunate-Mefloquine Treatment Response in Burmese Patients with *Plasmodium falciparum* Malaria

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Abstract. *CYP2A6*, *CYP2B6*, and *UGT1A9* genetic polymorphisms and treatment response after a three-day course of artesunate-mefloquine was investigated in 71 Burmese patients with uncomplicated *Plasmodium falciparum* malaria. Results provide evidence for the possible link between *CYP2A6* and *CYP2B6* polymorphisms and plasma concentrations of artesunate/dihydroartemisinin and treatment response. In one patient who had the *CYP2A6*1A/*4C* genotype (decreased enzyme activity), plasma concentration of artesunate at one hour appeared to be higher, and the concentration of dihydroartemisinin was lower than for those carrying other genotypes (415 versus 320 ng/mL). The proportion of patients with adequate clinical and parasitologic response who had the *CYP2B6*9/*9* genotype (mutant genotype) was significantly lower compared with those with late parasitologic failure (14.0% versus 19.0%). Confirmation through a larger study in various malaria-endemic areas is required before a definite conclusion on the role of genetic polymorphisms of these drug-metabolizing enzymes on treatment response after artesunate-based combination therapy can be made.

INTRODUCTION

Emergence and spread of artemisinin-resistant *Plasmodium falciparum* is a major concern for malaria control in malaria-endemic areas, particularly in areas bordering Myanmar and Cambodia.[1–5] In Thailand and most countries in Southeast Asia, a three-day artesunate-mefloquine combination is currently used as first-line treatment for acute uncomplicated *P. falciparum* malaria. However, because of the limitation in the study design of various studies, it was not possible to attribute treatment failures after this regimen in these studies to true parasite resistance.

Several factors contribute to antimalarial treatment response, including the pharmacokinetics of the drugs (plasma/blood drug concentrations) in malaria patients. In our previous study,[6] identification of treatment failure cases caused by an intrinsic parasite factor to each component of a three-day artesunate-mefloquine was reported based on integrated information on clinico-pathologic assessment, together with *in vitro* sensitivity (intrinsic parasite resistance) and systemic drug exposure (pharmacokinetic factor) in 17 patients with recrudescence response (late parasitologic failure [LPF]). Excluding the pharmacokinetic factor, results demonstrated only one confirmed case with reduced sensitivity to artesunate alone, and three cases were identified that had reduced sensitivity to artesunate and resistance to mefloquine. A pharmacokinetic factor was shown to account for approximately 59% of the total recrudescence cases, of which approximately half had inadequate dihydroartemisinin systemic drug exposure. Information on the impact of pharmacogenetics on antimalarial drug concentrations and treatment outcome after treatment with artemisinin-based combination therapy (ACT) in populations from various malaria-endemic regions, particularly the Thailand–Myanmar and Thailand–Cambodia borders, have been limited.

Cytochrome P450 (CYP) and uridine diphosphate glucuronosyltransferase (UGT) are the major enzyme superfamilies involved in biotransformation of xenobiotics in humans, including antimalarial drugs. Both enzymes are highly polymorphic and several of them, either singularly or in combination, are functionally of therapeutic significance with regards to clinical efficacy and safety.[7] After oral or parenteral route administration, artesunate is rapidly and extensively converted by the liver enzyme CYP2A6, and to a lesser extent CYP2B6, to form the major active plasma metabolite dihydroartemisinin. Elimination of dihydroartemisinin is largely through the conversion to inactive glucuronide conjugates by UGT1A9 and UGT2B7 enzymes.[8] Therefore, the polymorphisms in *CYP2A6* and *UGT1A9* genes may be of clinical relevance to treatment response after artesunate-based combination therapy as a result of inadequate plasma concentrations of dihydroartemisinin in some patients. Patients with poor metabolic activity of CYP2A6 and/or CYP2B6 may have unusually low concentrations of the active metabolite dihydroartemisinin. In addition, persons with high metabolic activity of UGT1A9 and/or UGT2B7 may also have unusually low concentrations of dihydroartemisinin.

There is no direct evidence for the association between this poor metabolic genotype and the decrease in antimalarial activity. Although, the *in vitro* antimalarial potency of both the parent drug artesunate and dihydroartemisinin have been shown to be similar, the residence time (reflected by half-life) of the later is relatively longer, and thus more sustainable antimalarial activity.[9,10] In either case, the situation may lead to the decrease in antimalarial activity and as a consequence, an increase in the potential of resistance development of *P. falciparum* parasite to artesunate.

In the present study, the genetic polymorphisms of *CYP2A6*, *CYP2B6*, and *UGT1A9*, and their relationship with plasma concentrations of artesunate/dihydroartemisinin and treatment response were investigated in Burmese patients with acute uncomplicated *P. falciparum* malaria after a three-day course of artesunate-mefloquine therapy.

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MATERIALS AND METHODS

Chemicals and reagents. Phenol, chloroform, isoamyl alcohol, and magnesium chloride were obtained from Merk (Hesse, Darmstadt, Germany). Trizma base, sodium dodecyl sulfate, sodium chloride, and 7.5 M ammonium acetate were obtained from Sigma (Missouri, St. Louis, MO). EDTA and proteinase K were obtained from Bio-Rad (Hercules, CA). *Taq* DNA polymerase and deoxyribonucletides were obtained from Fermentas (Vilnius, Lithuania). All restriction enzymes were obtained from New England BioLabs (Ipswich, MA). Agarose was obtained from Axygen Scientific Inc. (Union City, CA).

Patients, treatment, and sample collection. The study was conducted at the Mae Tao clinic for migrant workers, Tak Province, Thailand during 2008–2009. This area is along the Thailand–Myanmar border and had highly multidrug-resistant *P. falciparum*.[6] The study was approved by the Ethics Committee of the Ministry of Public Health of Thailand. Written informed consents for study participation were obtained from all patients before study.

A total of 71 (34 men and 37 women, age range = 16–50 years) Burmese patients with acute uncomplicated *P. falciparum* malaria were included in the study. Inclusion criteria for patient enrollment were according to the World Health Organization protocol for areas with low-to-moderate malaria transmission[11]: axillary temperature ≥ 37.5°C or recent history of fever; and a slide-confirmed *P. falciparum* mono-infection with a parasite density of 1,000–100,000 asexual parasites/μL. All were treated with a three-day course of artesunate-mefloquine combination therapy (4 mg/kg body weight of artesunate daily for three days; 750 and 500 mg mefloquine on the first and second day, respectively). Patients were admitted to the clinic during the three-day course of treatment or until signs and symptoms of malaria disappeared. Blood samples (3 mL each) were collected into heparinized tubes before treatment (H0) and at 1, 6, and 12 hours after the first dose for determination of artesunate/dihydroartemisinin concentrations. Plasma samples were separated within 30 minutes after collection by centrifugation at 1,200 × *g* for 15 min and stored at −180°C in a liquid nitrogen tank until analysis.

Patients were requested to return for follow-up on days 7, 14, 21, 28, and 42 or at any time if fever or symptoms suggestive of malaria developed. At each visit, a parasite count was performed (blood smear stained with Giemsa), and a detailed questionnaire for general symptoms was completed. Malaria blood smears were prepared at enrollment and thereafter, twice daily until two consecutive slides were confirmed to be negative, as well as at every follow-up visit. Thick blood films were screened for 200 oil-immersion fields before declaring a slide negative. Asexual parasites and gametocytes were separately counted against 200 leukocytes. If the parasite density was too numerous to count on the thick blood film, the number of parasites per 2,000 erythrocytes on the thin blood film was counted. The clinical outcome of a three day course of artesunate-mefloquine was evaluated in all patients who completed the 42-day follow-up period.

The classification of the therapeutic outcome was according to the World Health Organization protocol[9] as adequate clinical and parasitologic response (ACPR), early treatment failure, and late treatment failure, which was further classified as late parasitologic failure (LPF) or late clinical failure. *Plasmodium falciparum* genotyping of the three polymorphic genes for merozoite surface antigen 1, merozoite surface antigen 2, and glutamate-rich protein was performed in paired samples obtained before treatment and at the time of parasite re-appearance to distinguish between re-infection and recrudescence.[12–14]

Determination of artesunate and dihydroartemisinin concentrations in plasma. Plasma concentrations of artesunate and its active metabolite dihydroartemisinin were measured by using liquid chromatography mass-spectrometry.[15] In brief, the liquid-liquid extraction of plasma samples was carried out by using dichloromethane and *tert*.-methyl butyl ether (at a ratio of 8:2 v/v). Chromatographic separation and mass analysis were performed on the 1100 Series Liquid Chromatography/Mass Spectrometer Detector Trap System (Agilent Technologies, Santa Clara, CA) by using electrospray ionization as an interface. The stationary phase was an Elipse XDB-C18 column. The mobile phase consisted of acetonitrile and 0.003 M glacial acetic acid at a ratio of 62:38 (v/v) delivered at a flow rate of 0.5 mL per minute. Positive ion mode was selected to detect extracted ions at *m/z* 407 and 261 for artesunate, at *m/z* 307 and 261 for dihydroartemisinin, and at *m/z* 305 for artemisinin (internal standard). The limit of detection was 2 ng/mL for artesunate and dihydroartemisinin.

Detection of polymorphisms in *CYP2A6*, *CYP2B6*, and *UGT1A9* alleles. Genomic DNA was extracted from all samples using phenol/chloroform method.[16] DNA samples were analyzed for *CYP2A6* alleles *CYP2A6*1A, *1B, *4C*,[17] **2* and **3*,[18] and **6*[19]; *CYP2B6* alleles *CYP2B6*1,*2, *3, *4, *5, *6, *7*, and **9*[20]; and *UGT1A9* alleles *UGT1A9*1, *4*, and **5*[21] using a polymerase chain reaction–restriction fragment length polymorphism method.

Data analysis. Statistical analysis was performed by using the SPSS version 15 (SPSS, Chicago, IL). Median (95% confidence interval [CI]) values were used to summarize quantitative data that were not normally distributed, and statistical analysis was performed by using Kruskal Wallis and/or Mann-Whitney *U* tests. The frequencies of *CYP2A6*, *CYP2B6*, and *UGT1A9* alleles and genotypes are summarized as the number of cases and percentage values. Association between the prevalence of *CYP2A6*, *CYP2B6*, and *UGT1A9* alleles and treatment response was evaluated by using chi square and Fisher exact tests. The statistical significance level was set at $\alpha = 0.05$ for all tests.

RESULTS

Clinical response. Median (95% CI) admission parasitemia and body weights of the patients were 4,970 (4,268–5,672) parasites/μL and 53.5 (49.0–58.0) kg, respectively. Fifty (70.42%) patients had ACPR, and 21 (29.58%) patients had LPF (confirmed by polymerase chain reaction as recrudescence) during days 20–35 of treatment. Patients with LPF and ACPR were well matched with respect to sex, age, and body weight. Significantly higher admission parasitemia, longer parasite clearance time (time until the first series of negative blood smears occurred), and fever clearance time (time from start of treatment until the axillary temperature decreased to below 37.5°C and remained below this temperature for the next 48 hours) were observed in patients with LPF compared with those with ACPR (median [95% CI] admission parasitemia = 6,615 [2,407–10,823] versus 4,690 [4,160–5,220] parasites/μL; $P = 0.02$; parasite clearance time = 32 [29–35]

TABLE 1

Plasma concentrations of artesunate and dihydroartemisinin at one hour after a three-day course of artesunate-mefloquine and proportions of patients (ACPR and LPF) with inadequate concentrations*

Drug	Variable	Treatment response	
		ACPR (n = 27)	LPF (n = 14)
Artesunate	Plasma concentration at 1 hour after dosing (ng/mL)	350 (300–400)	337 (237–438)
	Proportion of patients with inadequate plasma concentrations at 1 hour† after dosing (%)	9/27 (33.3)	5/14 (35.7)
Dihydroartemisinin	Plasma concentration at 1 hour after dosing (ng/mL)	536 (479–593)	555 (461–649)
	Proportion of patients with inadequate plasma concentrations at 1 hour‡ after dosing (%)	6/27 (22.2)	5/15 (33.3)

*Values are median (95% confidence interval) or no. positive/no. tested (%). ACPR = adequate clinical and parasitologic response; LPF = late parasitologic failure.
†< 300 ng/mL (lower limit of 95% confidence interval for median concentration in the ACPR group).
‡< 479 ng/mL (lower limit of 95% confidence interval for median concentration in the ACPR group).

versus 26 [24–28] hours; $P = 0.02$; and fever clearance time = 34 [31–37] versus 26 [25–27] hours; $P = 0.01$).

Plasma concentrations of artesunate and dihydroartemisinin. Plasma artesunate and dihydroartemisinin concentrations at one hour after the first dose of artesunate were comparable between patients with LPF (n = 14) and ACPR (n = 27) (Table 1). In addition, the proportions of patients with LPF and ACPR who had inadequate concentrations of artesunate (< 300 ng/mL, the lower limit of 95% CI for median concentration in the ACPR group) and dihydroartemisinin (< 479 ng/mL, the lower limit of 95% CI for median concentration in the ACPR group) concentrations at one hour after the first dose were comparable.

***CYP2A6*, *CYP2B6,* and *UGT1A9* allele frequencies and genotypes and relationship with treatment response.** The allele frequencies of *CYP2A6, CYP2B6,* and *UGT1A9* were determined in 50 and 21 patients with ACPR and LPF, respectively (Table 2). The allele frequencies of *CYP2A6*1A* (80.0 versus 90.5%) and *CYP2A6*1B* (12.0 versus 9.5%) in patients with ACPR and LPF were similar. Heterozygous mutant of *CYP2A6*4C* was observed in one patient (1.4%).

TABLE 2

CYP2A6, *CYP2B6*, and *UGT1A9* allele frequencies in 50 patients with ACPR and 21 patients with LPF after treatment with a three-day course of artesunate-mefloquine*

Allele	Artesunate-mefloquine treatment response		
	ACPR (n = 50)	LPF (n = 21)	Total (n = 71)
CYP2A6			
*CYP2A6*1A*	86.0 (23, 20)	90.5 (14, 5)	87.3 (37, 25)
*CYP2A6*1B*	12.0 (3, 3)	9.5 (2, 0)	11.3 (5, 3)
*CYP2A6*2*	0 (0, 0)	0 (0, 0)	0 (0, 0)
*CYP2A6*3*	0 (0, 0)	0 (0, 0)	0 (0, 0)
*CYP2A6*4C*	2.0 (1, 0)	0 (0, 0)	1.4 (1, 0)
*CYP2A6*6*	0 (0, 0)	0 (0, 0)	0 (0, 0)
CYP2B			
*CYP2B6*1*	86.0 (22, 21)	81.0 (8, 9)	84.5 (30, 30)
*CYP2B6*2*	0 (0, 0)	0 (0, 0)	0 (0, 0)
*CYP2B6*3*	0 (0, 0)	0 (0, 0)	0 (0, 0)
*CYP2B6*4*	0 (0, 0)	0 (0, 0)	0 (0, 0)
*CYP2B6*5*	0 (0, 0)	0 (0, 0)	0 (0, 0)
*CYP2B6*6*	0 (0, 0)	0 (0, 0)	0 (0, 0)
*CYP2B6*7*	0 (0, 0)	0 (0, 0)	0 (0, 0)
*CYP2B6*9*	14.0 (3, 4)	19.0 (4, 0)	15.5 (7,4)
UGT1A9			
*UGT1A9*1*	100 (25, 25)	100 (12, 9)	100 (37, 34)
*UGT1A9*4*	0 (0, 0)	0 (0, 0)	0 (0, 0)
*UGT1A9*5*	0 (0, 0)	0 (0, 0)	0 (0, 0)

*Values are percentages (no. females, no. males). CYP = cytochrome P450; UGT = uridine diphosphate glucuronosyltransferase; ACPR = adequate clinical and parasitologic response; LPF = late parasitologic failure.

*CYP2A6*2*, *CYP2A6*3*, and *CYP2A6*6 alleles were not found in any patient. For *CYP2B6*, most (84.5%) had the wild-type allele *CYP2B6*1*, and 15.5% had the mutant *CYP2B6*9* allele. *CYP2B6*2*, *CYP2B6*3*, *CYP2B6*4, CYP2B6*5, CYP2B6*6*, and *CYP2B6*7* alleles were not found in any patient. For *UGT1A9*, all patients had the homozygous wild-type allele.

The genotype frequencies of *CYP2A6* and *CYP2B6* are summarized in Table 3. Four *CYP2A6* genotypes were observed with frequencies in descending order as follows: *CYP2A6*1A/*1B* (46.2%) > *CYP2A6*1B/*1B* (27.7%) > *CYP2A6*1A/*1A* (24.6%) > *CYP2A6*1A/*4C* (1.5%). The frequencies of these genotypes in patients with ACPR and LPF were not significantly different. Two *CYP2B6* genotypes were observed, i.e., *CYP2B6*1/*1* (84.6%) and *CYP2B6*9/*9* (15.4%). The proportion of patients with ACPR who had the *CYP2B6*9/*9* genotype was significantly lower than those with LPF (14.0% versus 19.0%; $P = 0.015$).

***CYP2A6* and *CYP2B6* genotypes and relationship with plasma concentrations of artesunate and dihydroartemisinin.** The *CYP2A6* and *CYP2B6* genotypes in relationship with plasma concentrations of artesunate and dihydrortemisinin at 1, 6, and 12 hours after the first dose of artesunate are summarized in Tables 4 and 5. For one patient who had the *CYP2A6*1A/*4C* genotype, plasma concentration of artesunate at one hour (415 ng/mL) appeared to be higher, and the concentration of dihydroartemisinin (320 ng/mL) was lower than in persons who had other three genotypes (*CYP2A6*1A/*1A*, *CYP2A6*1A/*1B* and *CYP2A6*1B/*1B*) (Table 4). Plasma concentrations of artesunate and dihydroartemisinin in patients who had *CYP2A6*1A/*1A*,

TABLE 3

CYP2A6 and *CYP2B6* genotypes in 46 patients with ACPR and 19 patients with LPF after treatment with a three-day course of artesunate-mefloquine*

Genotype	Artesunate-mefloquine treatment response		
	ACPR (n = 46)	LPF (n = 19)	Total (n = 65)
CYP2A6			
CYP2A6†1A/†1A	21.7 (7, 3)	31.6 (5, 1)	24.6 (12, 4)
CYP2A6†1A/†1B	47.8 (7, 15)	42.1 (3, 5)	46.2 (10, 20)
CYP2A6†1B/†1B	30.4 (10, 4)	21.1 (2, 2)	27.7 (12, 6)
CYP2A6†1A/†4C	0.0 (0, 0)	5.2 (0, 1)	1.5 (0, 1)
CYP2B6			
CYP2B6†1/†1	86 (21,19)	81.0 (8, 7)	84.6 (29, 26)
CYP2B6†9/†9	14.0 (3, 3)	19.0 (2, 2)*	15.4 (5, 5)

*Values are percentage (no. females, no. males). CYP = cytochrome P450; ACPR = adequate clinical and parasitologic response; LPF = late parasitologic failure.
†Statistically significant difference from ACPR ($P = 0.015$, by Fisher exact test).

TABLE 4
CYP2A6 genotypes and relationship with concentrations of artesunate and dihydroartemisinin*

Drug	Time	Drug concentration (ng/mL)			
		*CYP2A6*1A/*1A*	*CYP2A6*1A/*1B*	*CYP2A6*1B/*1B*	*CYP2A6*1A/*4C*
Artesunate	H0	0 (0–0) [10]	0 (0–0) [15]	0 (0–0) [10]	0 [1]
	H1	364 (285–443) [10]	311 (219–403) [15]	339 (241–437) [10]	415 [1]
	H6	1.5 (0–7) [6]	1 (0–5) [11]	7 (1–13) [5]	ND
	H12	0 (0–0) [10]	0 (0–0) [15]	0 (0–0) [10]	0 [1]
Dihydro-artemisinin	H0	0 (0–0) [11]	0 (0–0) [16]	0 (0–0) [10]	0 [1]
	H1	555 (473–637) [11]	519 (457–581) [16]	606 (439–774) [10]	320 [1]
	H6	123 (74–172) [10]	84 (69–99) [16]	99 (73–125) [9]	40 [1]
	H12	11 (0–62) [6]	10 (6–14) [13]	14 (5–22) [8]	ND

*Values are median (95% confidence interval) [no. of cases]. CYP = cytochrome P450; ND = not determined.
Data are presented as median (95% CI) [number of cases].

*CYP2A6*1A/*1B*, *CYP2A6*1B/*1B* (Table 4), *CYP2B6*1/*1* and *CYP2B6*9/*9* (Table 5) genotypes were similar.

DISCUSSION

The artemisinin group of antimalarial drugs markedly exhibit variable pharmacokinetic profiles with rapid absorption and elimination phases. Their bioavailability is highly variable depending on drug formulations, route of administration, health status, and severity of malaria disease.[22] Biotransformation into its active metabolite dihydroartemisinin occurs almost immediately after artesunate administration and the drug is considered as a prodrug. The elimination half-lives of artesunate and dihydroartemisinin range from 0.5 to 3 hours.[9,10] CYP2A6, CYP2B6, and UGT1A9 in human livers are drug-metabolizing enzymes involved in the biotransformation of artesunate. Results from the relatively limited sample size in our present study provide evidence for the possible contribution of *CYP2A6* and *CYP2B6* polymorphisms on artesunate/dihydroartemisinin concentrations and treatment response in patients with uncomplicated *P. falciparum* malaria after a three-day course of artesunate-mefloquine therapy.

The influence of *UGT1A9* polymorphism on treatment response could not be assessed because all patients had the wild-type genotype of this gene. Most of the patients had the two *CYP2A6* variants with normal (**1A*) or increased (**1B*) activity. The frequency distribution of these two allelic variants in Burmese population appears to be similar to those of other Asian ethnicities, particularly the Thai ethnic groups, where the frequencies of *CYP2A6*1A* and *CYP2A6*1B* alleles were 34.0 and 35.5%, respectively.[23] The gene allele frequencies in Japanese and Koreans were relatively lower, i.e., 20.1 versus 27.7% and 24.4 versus and 36.8%, respectively.[19] It was noted that in one patient who had the *CYP2A6*1A/*4C* genotype (decreased enzyme activity), plasma concentration of artesunate at one hour after the first dose (415 ng/mL) appeared to be higher, and the concentration of dihydroartemisinin (320 ng/mL) was lower than the median concentrations for those who had the other three genotypes. However, with adequate whole blood concentration of mefloquine and adequate parasite sensitivity to mefloquine and artesunate,[6] the patient still responded well to treatment (ACPR).

Polymorphism of *CYP2A6* has been reported at high frequency in Asian populations than in Caucasian and African populations.[24] Several polymorphisms with functional significance have been identified, and there are at least 13 *CYP2A6* variants with decreased metabolizing function and 5 variants with absent activity *in* vivo. CYP2A6*1B allele occurs when the 3′ flanking region of CYP2A6 gene is converted with CYP2A7 gene. CYP2A6*1B is believed to increase the stability of the mRNA transcript, resulting in a greater amount of the CYP2A6 enzyme.[25] Previous studies showed that persons who had the CYP2A6*1B allele tended to have higher metabolic ratios than those who did not have this allele.[26–28] Therefore, persons who had the CYP2A6*1/*1 genotype would have 100% activity of CYP2A6 enzyme. *CYP2A6*4C* is a null gene with deleted *CYP2A6*.[29,30] Persons who have one or two copies of the *CYP2A6*2* or *CYP2A6*4* alleles, together with one of *CYP2A6*9* or *CYP2A6*12* alleles were shown to have reduced enzyme activity in nicotine metabolism by 50%.[31] For *CYP2B6*, which plays a secondary role in artesunate metabolism, the defective mutant *CYP2B6*9* was the only mutant variant detected in the samples at a frequency of approximately 15.5%.

Although no direct association between *CYP2B6*9/*9* genotypes and artesunate and dihydroartemisinin plasma concentrations was found, there was a significantly lower proportion of patients with ACPR (cure) who had the *CYP2B6*9/*9*

TABLE 5
*CYP2B6**9 allele and relationship with concentrations of artesunate and dihydroartemisinin*

Drug	Time	Drug concentration (ng/mL)	
		*CYP2B6*1*	*CYP2B6**9*
Artesunate	H0	0.0 (0.0–0.0) [33]	0.0 (0.0–0.0) [8]
	H1	315.0 (259.0–371.0) [33]	402.0 (276.0–528.0) [8]
	H6	1.0 (0.0–2.0) [26]	10.0 (5.0–15.0) [7]
	H12	0.0 (0.0–0.0) [33]	0.0 (0.0–0.0) [8]
Dihydro-artemisinin	H0	0.0 (0.0–0.0) [33]	0.0 (0.0–0.0) [8]
	H1	555.0 (490.0–620.0) [34]	493.5 (395.0–592.0) [8]
	H6	98.5 (77.5–119.5) [32]	70.0 (27.0–113.0) [8]
	H12	10.0 (8.0–12.0) [26]	12.5 (0.0–28.0) [8]

*Values are median (95% confidence interval [no. cases]. CYP = cytochrome P450.

genotype compared with LPF (14% versus 19%). *CYP2B6* functional polymorphisms are highly prevalent in several African[32–35] and Asian countries.[35,36] In most of these countries, artemisinin-based combination therapy has been used as first-line treatment for acute uncomplicated *P. falciparum* malaria. The *CYP2B6*9* allele is commonly found in all ethnic groups, but more frequently in Hispanics compared with African-Americans, Caucasians, and Asians.[37] Previous studies have shown an effect of this variant on CYP2B6-mediated metabolism of non-nucleoside reverse transcriptase inhibitors.[38] The 516T allele has been associated with higher plasma exposure to efavirenz in patients infected with human immunodeficiency virus.[39]

Results from the present study provide evidence for the possible contribution of *CYP2A6* and *CYP2B6* genetic polymorphisms on clinico-parasitologic treatment response after a three-day course of artesunate-mefloquine therapy. Apart from the contribution of genetic polymorphism in drug metabolism, the possible link between treatment failure and the newly discovered K13 protein gene is also worth exploring. Whole-genome sequencing of an artemisinin-resistant parasite line from Africa and clinical parasite isolates from Cambodia has associated the mutation in this parasite gene with artemisinin resistance *in vitro* and *in vivo*.[40] Confirmation through a larger study in various malaria-endemic areas is required before a definite conclusion on the role of genetic polymorphisms in drug metabolizing enzymes on treatment response after artesunate-based combination therapy can be made and a translational use of pharmacogenetic for supporting surveillance and monitoring of their clinical efficacy can be implemented.

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