Am. J. Trop. Med. Hyg., 104(6), 2021, pp. 2152–2158
doi:10.4269/ajtmh.21-0047

Association between *ABCB1* Polymorphisms and Artesunate–Mefloquine Treatment Responses of Patients with Falciparum Malaria on the Thailand–Myanmar Border

Kanyarat Boonprasert,[1,2] Nanthawat Kosa,[1] Poonuch Muhamad,[3] Anurak Cheoymang,[1] and Kesara Na-Bangchang[1,2,3]*

[1]*Chulabhorn International College of Medicine, Thammasat University (Rangsit Campus), Pathum Thani, Thailand;* [2]*Center of Excellence in Pharmacology and Molecular Biology of Malaria and Cholangiocarcinoma, Chulabhorn International College of Medicine, Thammasat University (Rangsit Campus), Pathum Thani, Thailand;* [3]*Drug Discovery and Development Center, Thammasat University (Rangsit Campus), Pathum Thani, Thailand*

Abstract. A decrease in the clinical efficacy of a 3-day artesunate–mefloquine combination treatment was reported in the areas of multidrug-resistant *Plasmodium falciparum* along the Thailand–Myanmar border. The current study investigated the possible contribution of genetic polymorphisms of the three major genes encoding drug efflux transporters, *ABCB1*, *ABCG2*, and *ABCC1*, to responses to the aforementioned treatment in 91 patients with acute uncomplicated falciparum malaria residing along the Thailand–Myanmar border. Patients carrying homozygous mutant genotype *ABCB1 c.1236C>T* (TT) were found to have a three-times higher chance of successful treatment with this combination compared with other genotypes (CC and CT). Furthermore, whole blood mefloquine concentrations in these patients with the TT genotype were significantly lower than those of patients carrying the CC genotype. Patients with heterozygous mutant genotype (CT), however, were three-times more likely to experience treatment failure. No significant association was found with the *ABCG2* and *ABCC1* gene polymorphisms. The results suggest that *ABCB1 c.1236C>T* polymorphisms could be useful genetic markers for predicting responses to the 3-day artesunate–mefloquine treatment; however, studies using larger sample sizes in different malaria-endemic areas are necessary to confirm this finding. This study highlights the impact of pharmacogenetic factors on antimalarial treatment responses and the basis for the application of control policies in various malaria-endemic areas.

INTRODUCTION

Malaria remains one of the most significant public health problems worldwide. In 2019, there were 229 million diagnosed cases and 409,000 deaths globally.[1] It is the leading cause of morbidity and mortality in several developing countries, where young children, pregnant women, and nonimmune migrants are at higher risk for contracting malaria and experiencing severe complications than other groups. Recently, the number of malaria cases has markedly decreased in some Asian countries, and strategic policies have shifted from control to elimination.[1] This accomplishment is accredited to the introduction of artemisinin-based combination therapies (ACTs) by the World Health Organization (WHO) as first-line treatment of uncomplicated *Plasmodium falciparum* malaria in all malaria-endemic areas in an effort to limit the emergence and spread of multidrug-resistant strains.[2] In Thailand, a 3-day artesunate–mefloquine combination was recommended for clinical use during the period from 1995 to 2014.[3–5] However, high failure rates have been reported in the endemic areas along the Thailand–Myanmar and Thailand–Cambodia borders since 2009.[6,7] Our previous study conducted during 2011 to 2012 in the Tak province, an endemic area along the Thailand–Myanmar border, revealed that 32% of patients had late parasitological failure (LPF).[7] Pharmacokinetic factors (alone and together with genuine parasite resistance to mefloquine and/or artemisinins) contributed to most of the LPF cases (58.8%), and unidentified host-related factors contributed to LPF in one case.[7] Parasite resistance to mefloquine (decreased in vitro sensitivity and increased *pfmdr1* gene copy number) and reduced sensitivity to artesunate (decreased in vitro sensitivity) without contributions from pharmacokinetic factors accounted for only 35.3% of the LPF cases. It could be beneficial to further investigate genetic contributions of other host-related factors in LTF cases. Currently, the Ministry of Public Health of Thailand endorses another ACT, dihydroartemisinin–piperaquine, to replace the artesunate–mefloquine combination. However, the artesunate–mefloquine combination is still used as an alternative treatment in areas with dihydroartemisinin–piperaquine resistance.[8,9] Furthermore, this ACT regimen is being used in some countries such as Brazil, Peru, Venezuela, Myanmar, and Cambodia.[1] It is evident that despite the evidence of a decrease in the sensitivity of *P. falciparum* to artemisinins, ACTs would be expected to remain the key antimalarial regimens for combating multidrug-resistant *P. falciparum*. Effective treatment of malaria depends on parasite sensitivity to antimalarial drugs, pharmacokinetic and pharmacodynamic characteristics of antimalarial drugs, and host factors, particularly pharmacokinetic factors. Subtherapeutic drug levels attributable to pharmacokinetic variability result in treatment failure and selective pressure for the development and spread of resistant *P. falciparum* strains. Continued monitoring and active surveillance of the clinical efficacy of ACTs, including identification of host factors contributing to treatment failure, are essential to excluding parasite factors from pharmacokinetic and other host-related factors.

ABCB1 (multidrug resistance 1 [MDR1] or P-glycoprotein), ABCG2 (breast cancer resistance protein [BCRP]), and ABCC1 (multidrug resistance protein 1 [MRP1]) are the major efflux transport proteins that have important roles in the pharmacokinetic processing of xenobiotics, especially absorption and elimination. The genetic polymorphisms in these protein transporters have been linked to interindividual variability in the pharmacokinetic and pharmacodynamic profiles of several clinically relevant drugs, including antimalarial drugs

* Address correspondence to Kesara Na-Bangchang, Center of Excellence in Pharmacology and Molecular Biology of Malaria and Cholangiocarcinoma, Chulabhorn International College of Medicine, Thammasat University (Rangsit Campus), 99 Moo 18 Phaholyothin Rd. Klong Luang, Pathum Thani 12120, Thailand. E-mail: kesaratmu@yahoo.com

such as mefloquine.[10–13] MDR1 or P-gp, encoded by the *ABCB1* gene, belongs to the ATP-binding cassette (ABC) transporter gene family B.[14] The gene is commonly expressed in the blood–brain barrier, intestine, liver, kidney, hematopoietic stem cells, peripheral blood mononuclear cells, and placenta.[15–17] MDR1 acts as a transmembrane efflux pump, moving xenobiotics from the intracellular to the extracellular compartment.[18] Several drugs are substrates of MDR1, and variations in the expression and function of MDR1 can influence the pharmacokinetics and therapeutic efficacy of substrate drugs. The *ABCB1* gene is highly polymorphic, with more than 50 single nucleotide polymorphisms (SNPs) reported in the coding region; of these, *c.1236C>T* (rs1128503), *c.2677G>T* (rs2032582), and *c.3435C>T* (rs1045642) are the most common. The association between *ABCB1* polymorphisms and drug pharmacokinetic variations remains controversial. The SNPs of this gene have been associated with various diseases, including cancer, epilepsy, respiratory diseases, malaria, asthma, and cardiovascular diseases.[19–22] However, their impact on antimalarial drug resistance and treatment response remains inconclusive.[23] Another ABC family transporter, ABCG2, confers high levels of resistance to a variety of chemotherapeutic agents.[24,25] In normal human tissue, ABCG2 is highly expressed in the placenta, colon, small intestine, and liver.[26] The *ABCG2* gene is a highly polymorphic transporter with more than 80 SNPs noted in the gene[27]; the most frequent of these are *c.34G>A* (*rs2231137*) and *c.421C>A* (rs2231142). These polymorphisms are associated with decreased expression and transport activity of the ABCG2 protein.[28–30] The ABCC1 protein transports various molecules across extracellular and intracellular membranes. Certain polymorphisms of the *ABCC1* gene have been shown to be connected with an increased susceptibility to certain types of cancer.[28–30] The present study aimed to identify the genetic polymorphisms in *ABCB1*, *ABCG2*, and *ABCC1* in patients with acute uncomplicated *P. falciparum* malaria residing in the malaria-endemic areas of the Thailand–Myanmar border. The relationships with antimalarial drug concentrations, systemic exposure (artesunate/dihydroartemisinin and mefloquine), and treatment responses after a 3-day artesunate–mefloquine combination were also investigated.

METHODS

Patients and treatment. The present study was a part of the previously published research conducted during 2008 to 2009 involving migrant workers and residents of the malaria-endemic areas along the Thailand–Myanmar border with highly multidrug-resistant *P. falciparum*.[7] Approval of the study protocol was obtained from the Ethics Committee of the Ministry of Public Health of Thailand. Written informed consents were obtained from all patients before study participation. In brief, the study population consisted of 91 Burmese patients (47 males and 44 females between ages 16 and 57 years) with acute uncomplicated *P. falciparum* malaria. Inclusion criteria for patient enrollment were set according to the WHO protocol for areas with low to moderate malaria transmission.[31] All were treated with a 3-day combination regimen of artesunate and mefloquine together with the gametocytocidal drug primaquine (4 mg/kg body weight of artesunate daily for 3 days plus 15 and 10 mg/kg body weight of mefloquine on the first day and second day, respectively, plus 0.6 mg/kg body weight primaquine on the third day). The study procedures and clinical outcomes have been described in detail elsewhere.[7]

Blood sample collection. Before treatment, a blood sample (5 mL) was collected from each patient into a sodium heparinized tube for genetic analysis and determination of baseline antimalarial drug concentrations. Blood samples were collected at various time points after the first dosing of the artesunate–mefloquine combination for the determination of artesunate/dihydroartemisinin (at 1, 6, and 12 hours) and mefloquine (at 1, 6, 12, 24, 48, 72, and 168 hours) concentrations.

Drug analysis. Plasma concentrations of artesunate and its active metabolite dihydroartemisinin were measured using liquid chromatography mass spectrometry.[32] Whole blood concentrations of mefloquine were determined using high-performance liquid chromatography with ultraviolet detection.[33] The quantification limits for both assays were 2 ng/mL.

Detection of *ABCB1, ABCG2,* and *ABCC1* polymorphisms. Genomic DNA (gDNA) was extracted from whole blood samples using QIAamp® DNA Mini Kit (Qiagen, Valencia, CA). Detection of the polymorphisms of *ABCB1* (*c.1236C>T*, *c.2677G>T*, and *c.3435C>T)*, *ABCG2* (*c.34G>A* and *c.421C>A*), and *ABCC1* (*c.218C>T*, *c.2168G>A*, and *c.3173G>A*) in all samples was performed using the PCR restriction fragment-length polymorphism (PCR-RFLP) method with some modifications.[28,34–37] Details of the primers, PCR conditions, and restriction enzymes used are summarized in Table 1. The digested products were electrophoresed in 2.0% agarose gel along with the DNA marker.

Data analysis. The area under plasma concentration-time curves (AUCs) of artesunate and dihydroartemisinin were calculated based on the trapezoidal rule. Statistical analyses were performed using SPSS version 17.0 (SPSS, Chicago, IL). Quantitative data are presented as the median (95% confidence interval [CI]). The odds ratio (OR) was estimated for the genotype association with treatment response. Comparisons of the differences in qualitative variables were performed using the χ^2 test. Comparisons of the differences in quantitative variables among the three independent groups (for data not conforming to normal distribution) were performed using the Kruskal-Wallis test, followed by the Mann-Whitney *U* test to detect the difference between the two groups. Genotype and allele frequency distributions for various SNPs were evaluated according to the Hardy-Weinberg equilibrium using the χ^2 test. The statistical significance level was set at $\alpha = 0.05$ for all tests.

RESULTS

The analysis of *ABCB1*, *ABCG2*, and *ABCC1* genetic polymorphisms was performed in blood samples collected from 91 Burmese patients with acute uncomplicated *P. falciparum* malaria; 62 of these cases involved an adequate clinical and parasitological response (ACPR) and 29 of these cases involved LPF or late clinical failure. The median (95% CI) admission parasitemia values in the ACPR and LPF groups were 5,267.5/μL (4,760–6,930/μL) and 7,350/μL (5,040–12,300/μL), respectively.[7]

The allele and genotype frequencies of the three *ABCB1* polymorphisms (*c.1236C>T*, *c.2677G>T*, and *c.3435C>T*), two *ABCG2* polymorphisms (*c.34G>A* and *c.421C>A*), and

TABLE 1
Primers and PCR conditions used for the genotyping of *ABCB1*, *ABCG2*, and *ABCC1* polymorphisms

Gene/SNPs	Primer sequence	PCR condition denaturation/annealing/extension	Enzyme
MDR1, c.1236C>T (rs1128503)	F: 5′-TGTGTCTGTGAATTGCCTTGA-3′ R: 5′-ATCTCACCATCCCCTCTGTG-3′	94°C 60 s/56°C 60 s/72°C 60 s	*HaeIII*, 37°C/16 h
MDR1, c.2677G>T (rs2032582)	F: 5′-TGCAGGCTATAGGTTCCAGG-3′ R: 5′-TTTAGTTTGACTCACCTTCCCG-3′	94°C 30 s/58°C 30 s/72°C 45 s	*BanI*, 37°C/16 h
MDR1, c.3435C>T (rs1045642)	F: 5′-TTGATGGCAAAGAAATAAAGC-3′ R: 5′-CTTACATTAGGCAGTGACTCG-3′	94°C 90 s/56°C 60 s/72°C 90 s	*MboI*, 37°C/16 h
BCRP, c.34G>A (rs2231137)	F: 5′-CAGTAATGTCGAAGTTTTTATCGCA-3′ R: 5′-AAATGTTCATAGCCAGTTTCTTGGA-3′	94°C 30 s/58°C 30 s/72°C 60 s	*BseMI*, 55°C/16 h
BCRP, c.421C>A (rs2231142)	F: 5′-GTTGTGATGGGCACTCTGATGGT-3′ R: 5′-CAAGCCACTTTTCTCATTGTT-3′	94°C 30 s/58°C 30 s/72°C 60 s	*TaaI*, 65°C/16 h
MRP1, c.218C>T (rs41494447)	F: 5′-TCAGATGACACCTCTCAACAGAA-3′ R: 5′-CCAGTTTTCACCTCCCACATTAT-3′	94°C 30 s/56.5°C 30 s/72°C 30 s	*Hinf I,* 37°C/16 h
MRP1, c.2168G>A (rs4148356)	F: 5′-GCCTGGATTCAGAATGATTCTCTTC-3′ R: 5′-TACTGACCTTCTCGCCAATCTCTGT-3′	94°C 30 s/52°C 30 s/72°C 30 s	*Taq I,* 65°C/16 h
MRP1, c.3173G>A (rs41410450)	F: 5′-TCTGCATTGTGGAGTTTT-3′ R: 5′-GACGAAGAAGTAGATGAGGC-3′	94°C 60 s/53°C 60 s/72°C 60 s	*Pst I*, 37°C/16 h

three *ABCC1* polymorphisms (*c.218C>T*, *c.2168G>A*, and *c.3173G>A*) in patients with ACPR and LPF responses are summarized in Table 2. All SNP loci of the three genes undergoing investigation in the population complied with the Hardy-Weinberg equilibrium ($P > 0.05$).

The frequency of the homozygous mutation (TT) of the *c.1236C>T* was significantly higher in patients with ACPR than in those with LPF ($P = 0.026$). This group of patients had an approximately three-times higher chance of successful treatment (ACPR) compared with those with other (CC and CT) genotypes (OR, 3.03; 95% CI, 1.12–8.22; z-score = 2.183; $P = 0.029$). However, the frequency of the heterozygous mutation (CT) was significantly higher in patients with LPF than in patients with ACPR ($P = 0.022$). This group of patients had an approximately three-times higher risk of treatment failure (LPF) compared with those with other (CC and TT) genotypes (OR, 2.90; 95% CI, 1.15–7.31; z-score = 2.250; $P = 0.024$). A similar trend of association was also observed with *MDR1 c.2677G>T*, but statistical significance was not reached. The allele and genotype frequencies of the SNPs of the other two genes, *ABCG2* and *ABCC1*, in patients with ACPR and LPF were not significantly different.

The relationships among *ABCB1 c.1236C>T* polymorphisms and blood concentrations of artesunate/dihydroartemisinin and mefloquine and systemic exposure after treatment with the artesunate–mefloquine combination are summarized in Table 3. No significant differences in the plasma concentrations of artesunate and dihydroartemisinin were found at all investigated time points. Patients carrying homozygous (TT) and heterozygous (CT) genotypes had significantly lower mefloquine concentrations on day 1 (24 hours after the first dose) compared with those with the wild-type genotype (CC) ($P = 0.002$ and 0.022, respectively). The plasma dihydroartemisinin concentration at 1 hour (399 ng/mL) and the AUC of dihydroartemisinin of patients carrying the TT genotype appeared to be lower than those of patients carrying the CC and CT genotypes, but statistical significance was not reached (Table 3).

DISCUSSION

The current study suggests the significant contribution of the MDR1 transporter to treatment response after the artesunate–mefloquine combination. These results provide evidence for the possible contribution of the *ABCB1 c.1236C>T* and, to a lesser extent, *c.2677G>T* polymorphisms on treatment responses after a 3-day artesunate–mefloquine combination. Patients carrying the homozygous mutant genotype (TT) of *c.1236C>T* were more likely (approximately three-times) to have successful treatment outcomes compared with patients with other (CC and CT) genotypes. Interestingly, the reverse was found for individuals with the heterozygous mutant genotype (CT) of *ABCB1 c.1236C>T*, who had an approximately three-times higher risk of treatment failure (LPF) compared with patients with other genotypes. Contrary to the expected increase in mefloquine concentrations (low gene expression and efflux activity), the concentrations in both groups of patients (TT and CT genotypes) were significantly lower than those of individuals carrying the wild-type genotype. Such a trend was also observed for plasma concentrations and $AUC_{0-12hours}$ of dihydroartemisinin, the active metabolite of artesunate; however, statistical significance was not obtained (Table 4). Most studies of the functional significance of *ABCB1* polymorphisms have focused on *c.3435C>T*, with very little information reported for the *c.1236C>T* and *c.2677G>T* polymorphisms. The findings here are in agreement with those of previous studies of the tyrosine kinase inhibitor, imatinib.[38] Individuals with the homozygous T

TABLE 2

Genotype/allele frequencies of the SNPs of *ABCB1*, *ABCG2*, and *ABCC1* genes in patients with acute uncomplicated *P. falciparum* with the adequate clinical and parasitological response (ACPR) and late parasitological failure (LPF) after treatment with a 3-day artesunate–mefloquine combination

		Artesunate–mefloquine treatment response	
Gene/SNP	Genotype/allele	ACPR	LPF
MDR1, c.1236C>T (rs1128503)	CC	21.1 (12/57)	20.7 (6/29)
	CT	29.8 (17/57)*	55.2 (16/29)
	TT	49.1 (28/57)†	24.1 (7/29)
	C	36.0 (41/114)	48.3 (28/58)
	T	64.0 (73/114)	51.7 (30/58)
MDR1, c.2677G>T (rs2032582)	GG	23.3 (14/60)	32.1 (9/28)
	GT	58.3 (35/60)	57.1 (16/28)
	TT	18.3 (11/60)	10.7 (3/28)
	G	52.5 (63/120)	60.7 (34/56)
	T	47.5 (57/120)	39.3 (22/56)
MDR1, c.3435C>T (rs1045642)	CC	21.3 (13/61)	27.6 (8/29)
	CT	70.5 (43/61)	62.1 (18/29)
	TT	8.2 (5/61)	10.3 (3/29)
	C	56.6 (69/122)	58.6 (34/58)
	T	43.4 (53/122)	41.4 (24/58)
BCRP, c.34G>A (rs2231137)	GG	37.7 (23/61)	41.4 (12/29)
	GA	47.5 (29/61)	44.8 (13/29)
	AA	14.8 (9/61)	13.8 (4/29)
	G	61.5 (75/122)	63.8 (37/58)
	A	38.5 (47/122)	36.2 (21/58)
BCRP, c.421C>A (rs2231142)	CC	61.0 (36/59)	59.3 (16/27)
	CA	28.8 (17/59)	29.6 (8/27)
	AA	10.2 (6/59)	11.2 (3/27)
	C	75.4 (89/118)	74.1 (40/54)
	A	24.6 (118)	25.9 (14/54)
MRP1, c.218C>T (rs41494447)	CC	100.0 (31/31)	88.9 (8/9)
	CT	0.0 (0/31)	11.1 (1/9)
	TT	0.0 (0/31)	0.0 (0/9)
	C	100.0 (62/62)	94.4 (17/18)
	T	0.0 (0/62)	5.6 (1/18)
MRP1, c.2168G>A (rs4148356)	GG	91.7 (33/36)	91.7 (11/12)
	GA	8.3 (3/36)	8.3 (1/12)
	AA	0.0 (0/36)	0.0 (0/12)
	G	95.8 (69/72)	95.8 (23/24)
	A	4.2 (3/72)	4.2 (1/24)
MRP1, c.3173G>A (rs41410450)	GG	73.1 (19/26)	62.5 (5/8)
	GA	26.9 (7/26)	37.5 (3/8)
	AA	0.0 (0/26)	0.0 (0/8)
	G	86.5 (45/52)	81.3 (13/16)
	A	13.5 (7/52)	18.7 (3/16)

Data are presented as percentage (number of cases/total cases).
* Statistically significant difference from LPF ($P = 0.022$, χ^2 test).
† Statistically significant difference from LPF ($P = 0.026$, χ^2 test).

allele of *c.1236C>T* were found to have more rapid clearance of imatinib and a lower $AUC_{0-\infty}$ of CGP74588 (the major active metabolite of imatinib) compared with wild-type allele carriers. Moreover, significantly lower plasma concentrations of methadone and, thus, the requirement for higher doses were reported for patients using methadone who carried the homozygous T allele compared with individuals carrying homozygous wild-type alleles.[39] Our previous study[7] suggested that in addition to the parasite factors (resistance or reduced sensitivity to mefloquine or artesunate), pharmacokinetic and other host-related factors have significant impacts on 58.8% of patients with LTF. The results of the current study found that the five patients with LTF had inadequate whole blood mefloquine concentrations, and that four of those patients carried the TT genotype of the *MDR1* gene. Other patients with LTF with adequate mefloquine concentrations carried the CT or CC genotype. Interestingly, during the current study, the TT genotype of *MDR1* was confirmed to, at least in part, contribute to one LTF that was previously reported to be caused by other host-related factors.[7]

Mefloquine has been reported to be a substrate of both the ABCB1 (MDR1), and ABCG2 transporters, and an increased concentration of it in the brain was found when the drug was administered along with inhibitors of both transport proteins.[11,12] Because MDR1 is highly expressed in the gastrointestinal tract, liver, and blood–brain barrier, the influence of *MDR1* polymorphisms on mefloquine treatment outcomes would depend on drug concentrations at particular sites of action. One of the most serious adverse effects of mefloquine is its impact on the central nervous system, resulting in insomnia, fatigue, and psychosis. *MDR1* polymorphisms may be more relevant to safety controls of the central nervous system effects of mefloquine at the blood–brain barrier, whereas the blood concentration (target site of action) is more relevant to antimalarial activity and therapeutic efficacy. ABCG2, MRP1, and MRP4 have been reported to have a more significant role in controlling mefloquine levels in the target red blood cells. The genetic polymorphism *c.1236C>T* of the *MDR1* gene was shown to affect stereoselectivity of the MDR1

TABLE 3

ABCB1 c.1236C>T polymorphism and relationships with plasma concentrations of artesunate/dihydroartemisinin and whole blood concentrations of mefloquine

Drug	Time	Drug concentration (ng/mL)		
		CC	CT	TT
Artesunate	H0	0 (0–0) [17]	0 (0–0) [31]	0 (0–0) [19]
	H1	326.0 (300.0–444.0) [17]	313.5 (278.0–369.0) [30]	345.0 (258.0–425.0) [18]
	H6	1.0 (0.0–10.0) [16]	0.0 (0.0–7.0) [29]	1.0 (0.0–7.0) [18]
	H12	0 (0–0) [16]	0 (0–0) [29]	0 (0–0) [19]
Dihydro-artemisinin	H0	0 (0–0) [17]	0 (0–0) [31]	0 (0–0) [19]
	H1	511.0 (488.0–552.0) [17]	502.0 (446.0–620.0) [30]	399.0 (356.0–560.0) [18]
	H6	89.5 (62.0–104.0) [16]	100.0 (78.0–120.0) [29]	99.5 (65.0–120.0) [18]
	H12	9.5 (6.0–18.0) [16]	10.0 (5.0–13.0) [29]	10.0 (0.0–20.0) [19]
Mefloquine	H1	247.0 (222.0–350.0) [17]	240.0 (159.0–250.0) [31]	287.5 (240.0–308.0) [18]
	H6	1,302.0 (1,110.0–1,565.0) [15]	1,400.0 (1,320.0–1,562.0) [29]	1,452.0 (1,325.0–1,756.0) [17]
	H12	1,655.0 (1,345.0–1,745.0) [15]	1,745.0 (1,650.0–1,789.0) [28]	1,649.0 (1,420.0–1,781.0) [18]
	H24 (D1)	1,900.0 (1,370.0–3,012.0) [7]*	1,460.0 (1,009.0–1,779.0) [13]	1,360.0 (1,232.0–1,513.0) [19]
	D2	1,230.0 (1,017.0–1,432.0) [7]	1,250.0 (1,112.0–1,560.0) [22]	1,211.0 (1,011.0–1,450.0) [13]
	D3	1,400.0 (1,010.0–1,493.0) [3]	1,340.0 (1,200.0–1,676.0) [5]	1,076.0 (855.0–2,104.0) [7]
	D7	1,150.0 (496.0–1,250.0) [3]	825.0 (441.0–1,212.0) [6]	890.0 (719.0–988.0) [13]

CC = wild-type genotype; CT = heterozygous mutation genotype; TT = homozygous mutation genotype. Data are presented as median (95% confidence interval) [number of cases].
* Statistically significant difference from CT and TT genotypes ($P = 0.022$ and 0.002, respectively; Mann-Whitney *U* test).

protein and, consequently, the cerebral or blood ratio of mefloquine enantiomers (R/S) without changing the total blood concentration.[40] These observations were supported by our findings of the relatively low mefloquine concentrations in patients with successful treatment (ACPR) who carried the homozygous TT genotype. The increased risk of treatment failure (LPF) for patients carrying the heterozygous CT genotype was unexpected; therefore, further studies involving larger sample sizes are required to confirm this association. Caucasian travelers carrying the *ABCB1 1236TT/2677TT/3435TT* haplotypes and using mefloquine as prophylaxis were at higher risk for neuropsychiatric adverse drug reactions that were unrelated to mefloquine blood concentrations.[41] In addition, Gupta *et al.*[42] suggested that the *ABCB1 c.1236C>T* polymorphism may be associated with the overexpression of P-gp in patients with complicated malaria and, as a consequence, the development of resistance. In this group of patients, higher doses of antimalarial drugs may be required to improve clinical efficacy. ABCB1 is a major biliary efflux pump, particularly for lipophilic drugs like mefloquine, and the structurally related antimalarial drug lumefantrine.[43,44] The difference in functionality between *ABCB1* variants appears to depend on the particular antimalarial drug being acted upon. A previous study showed that *ABCB1 c.3435CC* (wild-type) was significantly associated with recurrent infection-free status and uncomplicated falciparum malaria in Angola after treatment with the ACT artemether–lumefantrine.[45] This wild-type genotype was found most frequently (76%), followed by the heterozygous CT (15.8%) and homozygous TT (7.9%) genotypes. However, a possible positive association was suggested for the *ABCB1 c.3435C>T* genotype and lumefantrine exposure among HIV-positive patients with uncomplicated falciparum malaria who received concurrent treatment with artemether–lumefantrine and efavirenz-based therapy.[46] An altered rate of protein synthesis was demonstrated in those with the CT genotype.[46]

In conclusion, our results suggest that *ABCB1 c.1236C>T* polymorphisms could be useful genetic markers for predicting treatment responses to 3-day artesunate–mefloquine combination treatment. However, studies with larger sample sizes in different malaria-endemic areas, particularly those using this ACT regimen, are necessary to confirm the results. This study highlights the impact of pharmacogenetic factors on antimalarial treatment responses and the basis for the application of malaria control policies in various malaria-endemic areas.

Received January 12, 2021. Accepted for publication March 8, 2021.

Published online May 3, 2021.

Acknowledgments: We thank all patients for participating in the study. We thank Ms. Kalaya Ruengweerayut and the staff of Mae Tao Clinic, Mae Sot, Tak Province, for their kind support during the study. We thank Mr. Ethan Vindvamara for English editing.

Financial support: This study was supported by Thammasat University (Center of Excellence in Pharmacology and Molecular Biology of Malaria and Cholangiocarcinoma), Thammasat University, Thailand, and the National Research Council of Thailand. Kesara Na-Bangchang is supported by the National Research Council of Thailand (Ministry of Higher Education, Science, Research, and Innovation) under the Research Team Promotion grant (grant number 820/2563).

Authors' addresses: Kanyarat Boonprasert, Nanthawat Kosa, Anurak Cheoymang, and Kesara Na-Bangchang, Chulabhorn International College of Medicine (CICM), Thammasat University (Rangsit

TABLE 4

ABCB1 c.1236C>T polymorphism and relationships with the area under the artesunate and dihydroartemisinin concentration-time curve ($AUC_{0–12hours}$)

Drug	$AUC_{0–12\ hours}$ (ng/mL)		
	CC	CT	TT
Artesunate	999.3 (744.0–1,337.5) [16]	942.0 (859.0–1,123.0) [29]	1,067.5 (779.5–1,313.5) [18]
Dihydroartemisinin	2,111.5 (1,925.5–2,261.0) [16]	2,089.0 (1,875.5–2,370.0) [29]	1,825.5 (1,414.5–2,560.0) [18]

CC = wild-type genotype; CT = heterozygous mutation genotype; TT = homozygous mutation genotype. Data are presented as median (95% confidence interval) [number of cases/total cases].

Campus), Klong Luang, Pathum Thani 12120, Thailand, E-mails: noei_noey@hotmail.com, nantha.ko@hotmail.com, anurak_ch9@yahoo.com, and kesaratmu@yahoo.com. Poonuch Muhamad, Drug Discovery Center, Thammasat University (Rangsit Campus), Klong Luang, Pathum Thani 12120, Thailand, E-mail: nurah_ab@yahoo.com.

REFERENCES

1. World Health Organization, 2020. *World Malaria Report 2020*. Geneva, Switzerland: WHO Press.
2. World Health Organization, 2001. *Antimalarial Drug Combination Therapy: Report of a WHO Technical Consultation*. Geneva, Switzerland: WHO Press.
3. World Health Organization, 2018. *Status Report on Artemisinin Resistance and ACT Efficacy (August 2018)*. Geneva, Switzerland: WHO Press.
4. Carrara VI et al., 2009. Changes in the treatment responses to artesunate-mefloquine on the northwestern border of Thailand during 13 years of continuous deployment. *PLoS One 4:* e4551.
5. Hassett MR, Roepe PD, 2019. Origin and spread of evolving artemisinin-resistant *Plasmodium falciparum* malarial parasites in Southeast Asia. *Am J Trop Med Hyg 101:* 1204–1211.
6. Na-Bangchang K, Ruengweerayut R, Mahamad P, Ruengweerayut K, Chaijaroenkul W, 2010. Declining in efficacy of a three-day combination regimen of mefloquine-artesunate in a multidrug resistance area along the Thai-Myanmar border. *Malar J 9:* 273.
7. Na-Bangchang K, Muhamad P, Ruaengweerayut R, Chaijaroenkul W, Karbwang J, 2013. Identification of resistance of *Plasmodium falciparum* to artesunate-mefloquine combination in an area along the Thailand-Myanmar border: integration of clinico-parasitological response, systemic drug exposure, and in vitro parasite sensitivity. *Malar J 12:* 263.
8. World Health Organization, 2017. *Status Report on Artemisinin and ACT Resistance (April 2017)*. Geneva, Switzerland: WHO Press.
9. Duru V, Witkowski B, Ménard D, 2016. *Plasmodium falciparum* resistance to artemisinin derivatives and piperaquine: a major challenge for malaria elimination in Cambodia. *Am J Trop Med Hyg 95:* 1228–1238.
10. Ferreira PE et al., 2008. Polymorphism of antimalaria drug metabolizing, nuclear receptor, and drug transport genes among malaria patients in Zanzibar, East Africa. *Ther Drug Monit 30:* 10–15.
11. Pham YT, Régina A, Farinotti R, Couraud P, Wainer IW, Roux F, Gimenez F, 2000. Interactions of racemic mefloquine and its enantiomers with P-glycoprotein in an immortalised rat brain capillary endothelial cell line, GPNT. *Biochim Biophys Acta 1524:* 212–219.
12. Barraud de Lagerie S, Comets E, Gautrand C, Fernandez C, Auchere D, Singlas E, Mentre F, Gimenez F, 2004. Cerebral uptake of mefloquine enantiomers with and without the P-gp inhibitor elacridar (GF1210918) in mice. *Br J Pharmacol 141:* 1214–1222.
13. Nies AT, Schwab M, Keppler D, 2008. Interplay of conjugating enzymes with OATP uptake transporters and ABCC/MRP efflux pumps in the elimination of drugs. *Drug Metab Toxicol 4:* 545–568.
14. Kerb R, 2006. Implications of genetic polymorphisms in drug transporters for pharmacotherapy. *Cancer Lett 234:* 4–33.
15. Ieiri I, 2012. Functional significance of genetic polymorphisms in P-glycoprotein (MDR1, ABCB1) and breast cancer resistance protein (BCRP, ABCG2). *Drug Metab Pharmacokinet 27:* 85–105.
16. Ernest S, Bello-Reuss E, 1999. Secretion of platelet-activating factor is mediated by MDR1 P-glycoprotein in cultured human mesangial cells. *J Am Soc Nephrol 10:* 2306–2313.
17. Annese V, Valvano MR, Palmieri O, Latiano A, Bossa F, Andriulli A, 2006. Multidrug resistance 1 gene in inflammatory bowel disease: a meta-analysis. *World J Gastroenterol 12:* 3636–3644.
18. Higgins CF, Gottesman MM, 1992. Is the multidrug transporter a flippase? *Trends Biochem Sci 17:* 18–21.
19. Haerian BS, Roslan H, Raymond AA, Tan CT, Lim KS, Zulkifli SZ, Mohamed EH, Tan HJ, Mohamed Z, 2010. ABCB1 C3435T polymorphism and the risk of resistance to antiepileptic drugs in epilepsy: a systematic review and meta-analysis. *Seizure 19:* 339–346.
20. Kiyotani K, Mushiroda T, Nakamura Y, Zembutsu H, 2012. Pharmacogenomics of tamoxifen: roles of drug metabolizing enzyme and transporters. *Drug Metab Pharmacokinet 27:* 122–131.
21. Gréen H, Falk IJ, Lotfi K, Paul E, Hermansson M, Rosenquist R, Paul C, Nahi H, 2012. Association of ABCB1 polymorphisms with survival and in vitro cytotoxicty in de novo acute myeloid leukemia with normal karyotype. *Pharmacogenomics J 12:* 111–118.
22. Milojkovic M, Milacic N, Radovic J, Ljubisavlijevic S, 2015. MDR1 gene polymorphism and P-glycoprotein expression in respiratory diseases. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 159:* 341–346.
23. Chinn LW, Kroetz DL, 2007. ABCB1 pharmacogenetics: progress, pitfalls, and promise. *Clin Pharmacol Ther 81:* 265–269.
24. Sugimoto Y, Tsukahara S, Ishikawa E, Mitsuhashi J, 2005. Breast cancer resistance protein: molecular target for anticancer drug resistance and pharmacokinetics/pharmacodynamics. *Cancer Sci 96:* 457–465.
25. Cusatis G, Sparreboom A, 2008. Pharmacogenomic importance of ABCG2. *Pharmacogenomics 9:* 1005–1009.
26. Maliepaard M, Scheffer GL, Faneyte IF, van Gastelen MA, Pijnenborg AC, Schinkel AH, van De Vijver MJ, Scheper RJ, Schellens JH, 2001. Subcellular localization and distribution of the breast cancer resistance protein transporter in normal human tissues. *Cancer Res 61:* 3458–3464.
27. Tamura A et al., 2007. In vitro evaluation of photosensitivity risk related to genetic polymorphisms of human ABC transporter ABCG2 and inhibition by drugs. *Drug Metab Pharmacokinet 22:* 428–440.
28. Kobayashi D et al., 2005. Functional assessment of ABCG2 (BCRP) gene polymorphisms to protein expression in human placenta. *Drug Metab Dispos 33:* 94–101.
29. Imai Y, Nakane M, Kage K, Tsukahara S, Ishikawa E, Tsuruo T, Miki Y, Sugimoto Y, 2002. C421A polymorphism in the human breast cancer resistance protein gene is associated with low expression of Q141K protein and low-level drug resistance. *Mol Cancer Ther 1:* 611–616.
30. Kasza I, Várady G, Andrikovics H, Koszarska M, Tordai A, Scheffer GL, Németh A, Szakács G, Sarkadi B, 2012. Expression levels of the ABCG2 multidrug transporter in human erythrocytes correspond to pharmacologically relevant genetic variations. *PLoS One 7:* e48423.
31. World Health Organization, 2003. *Assessment and Monitoring of Antimalarial Drug Efficacy for the Treatment of Uncomplicated Falciparum Malaria*. Geneva, Switzerland: WHO Press.
32. Thuy LD, Hung LN, Danh PT, Na-Bangchang K, 2008. Development and validation of a liquid chromatography-mass spectrometry method for the simultaneous quantification of artesunate and dihydroartemisinin in human plasma. *Southeast Asian J Trop Med Public Health 39:* 963–977.
33. Karbwang J, Molunto P, Na Bangchang K, Bunnag D, 1989. Determination of mefloquine in biological fluids using high performance liquid chromatography. *Southeast Asian J Trop Med Public Health 20:* 55–60.
34. Ryu HC, Kwon HY, Choi IK, Rhee DK, 2006. Analyses of single nucleotide polymorphisms and haplotype linkage of the human ABCB1 (MDR1) gene in Korean. *Arch Pharm Res 29:* 1132–1139.
35. Kim HJ, Kim HK, Kwon JT, Lee SH, El Park S, Gil HW, Song HY, Hong SY, 2016. Effect of MDR1 gene polymorphisms on mortality in paraquat intoxicated patients. *Sci Rep 6:* 31765.
36. Pongstaporn W, Pakakasama S, Chaksangchaichote P, Pongtheerat T, Hongeng S, Permitr S, 2015. MDR1 C3435T and C1236T polymorphisms: association with high-risk childhood acute lymphoblastic leukemia. *Asian Pac J Cancer Prev 16:* 2839–2843.
37. Yin JY, Huang Q, Yang Y, Zhang JT, Zhong MZ, Zhou HH, Liu ZQ, 2009. Characterization and analyses of multidrug resistance-associated protein 1 (MRP1/ABCC1) polymorphisms in Chinese population. *Pharmacogenet Genomics 19:* 206–216.

38. Gurney H et al., 2007. Imatinib disposition and ABCB1 (MDR1, P-glycoprotein) genotype. *Clin Pharmacol Ther 82:* 33–40.
39. Levran O, O'Hara K, Peles E, Li D, Barral S, Ray B, Borg L, Ott J, Adelson M, Kreek MJ, 2008. ABCB1 (MDR1) genetic variants are associated with methadone doses required for effective treatment of heroin dependence. *Hum Mol Genet 17:* 2219–2227.
40. Barraud de Lagerie S et al., 2004. Cerebral uptake of mefloquine enantiomers with and without the P-gp inhibitor elacridar (GF1210918) in mice. *Br J Pharmacol 141:* 1214–1222.
41. Aarnoudse AL, van Schaik RH, Dieleman J, Molokhia M, van Riemsdijk MM, Ligthelm RJ, Overbosch D, van der Heiden IP, Stricker BH, 2006. MDR1 gene polymorphisms are associated with neuropsychiatric adverse effects of mefloquine. *Clin Pharmacol Ther 80:* 367–374.
42. Gupta H, Chaudhari S, Rai A, Bhat S, Sahu PK, Hande MH, D'Souza SC, Shashikiran U, Satyamoorthy K, 2017. Genetic and epigenetic changes in host ABCB1 influences malaria susceptibility to *Plasmodium falciparum*. *PLoS One 12:* e0175702.
43. Raju KN, Singh SP, Taneja I, 2014. Investigation of the functional role of P-glycoprotein in limiting the oral bioavailability of lumefantrine. *Antimicrob Agents Chemother 58:* 489–494.
44. Oga EF, Sekine S, Shitara Y, Horie T, 2012. Potential P-glycoprotein-mediated drug-drug interactions of antimalarial agents in Caco-2 cells. *Am J Trop Med Hyg 87:* 64–69.
45. Kiaco K, Rodrigues AS, do Rosário V, Gil JP, Lopes D, 2017. The drug transporter ABCB1 c.3435C>T SNP influences artemether-lumefantrine treatment outcome. *Malar J 16:* 383.
46. Maganda BA, Minzi OM, Ngaimisi E, Kamuhabwa AA, Aklillu E, 2016. CYP2B6*6 genotype and high efavirenz plasma concentration but not nevirapine are associated with low lumefantrine plasma exposure and poor treatment response in HIV-malaria-coinfected patients. *Pharmacogn J 16:* 88–95.