AAC
Journals.ASM.org

Effect of Single Nucleotide Polymorphisms in Cytochrome P450 Isoenzyme and *N*-Acetyltransferase 2 Genes on the Metabolism of Artemisinin-Based Combination Therapies in Malaria Patients from Cambodia and Tanzania

Eva Maria Staehli Hodel,[a] Chantal Csajka,[b,c] Frédéric Ariey,[d] Monia Guidi,[b,c] Abdunoor Mulokozi Kabanywanyi,[e] Socheat Duong,[f] Laurent Arthur Decosterd,[b] Piero Olliaro,[g] Hans-Peter Beck,[a] Blaise Genton[a,h]

Swiss Tropical and Public Health Institute, University of Basel, Basel, Switzerland[a]; Division of Clinical Pharmacology, Department de Medicine, University Hospital, Lausanne, Switzerland[b]; Department of Pharmaceutical Sciences, University of Geneva, Geneva, Switzerland[c]; Molecular Epidemiology, Pasteur Institute of Cambodia, Phnom Penh, Cambodia[d]; Ifakara Health Institute, Dar es Salaam and Ifakara, Tanzania[e]; National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia[f]; UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, Geneva, Switzerland[g]; Division of Infectious Diseases and Department of Ambulatory Care and Community Medicine, University Hospital, Lausanne, Switzerland[h]

The pharmacogenetics of antimalarial agents are poorly known, although the application of pharmacogenetics might be critical in optimizing treatment. This population pharmacokinetic-pharmacogenetic study aimed at assessing the effects of single nucleotide polymorphisms (SNPs) in cytochrome P450 isoenzyme genes (*CYP*, namely, *CYP2A6*, *CYP2B6*, *CYP2C8*, *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A4*, and *CYP3A5*) and the *N*-acetyltransferase 2 gene (*NAT2*) on the pharmacokinetics of artemisinin-based combination therapies in 150 Tanzanian patients treated with artemether-lumefantrine, 64 Cambodian patients treated with artesunate-mefloquine, and 61 Cambodian patients treated with dihydroartemisinin-piperaquine. The frequency of SNPs varied with the enzyme and the population. Higher frequencies of mutant alleles were found in Cambodians than Tanzanians for *CYP2C9*3*, *CYP2D6*10* (100C→T), *CYP3A5*3*, *NAT2*6*, and *NAT2*7*. In contrast, higher frequencies of mutant alleles were found in Tanzanians for *CYP2D6*17* (1023C→T and 2850C→T), *CYP3A4*1B*, *NAT2*5*, and *NAT2*14*. For 8 SNPs, no significant differences in frequencies were observed. In the genetic-based population pharmacokinetic analyses, none of the SNPs improved model fit. This suggests that pharmacogenetic data need not be included in appropriate first-line treatments with the current artemisinin derivatives and quinolines for uncomplicated malaria in specific populations. However, it cannot be ruled out that our results represent isolated findings, and therefore more studies in different populations, ideally with the same artemisinin-based combination therapies, are needed to evaluate the influence of pharmacogenetic factors on the clearance of antimalarials.

Use of artemisinin-based combination treatment (ACT) is the current World Health Organization (WHO) recommendation for uncomplicated malaria (1). While generally very effective and safe, variations in ACT efficacy are found across the areas in the world where malaria is endemic. Parasite, human, and drug factors combined contribute to such variance. Both drug efficacy and safety depend upon appropriate drug levels being achieved and maintained long enough (2–4); insufficient exposure is associated with risk of failure and emergence of resistance (5), while excessive exposure carries the risk of toxicity (1, 2, 6–8). All other factors being equal, human genetics can alter drug exposure. Understanding the role of genetic polymorphisms in genes encoding proteins and enzymes involved in drug absorption, distribution, metabolism, elimination, and action is important for better understanding the interindividual differences in drug pharmacokinetics and pharmacodynamics (9). Depending on the alleles carried by an individual, drug metabolism can be either enhanced, decreased, or abolished (10). The cytochrome P450 enzyme family (*CYP* genes) and the phase II *N*-acetyltransferase 2 enzyme (*NAT2* gene) are involved in the metabolism of various antimalarial drugs (Table 1). For some antimalarial drugs (i.e., piperaquine [11], pyrimethamine [12, 13], and pyronaridine [14]), the metabolic pathways are still not well understood, while for others essentially no metabolism takes place at all (i.e., atovaquone [15] and doxycycline [16]).

With varied frequencies of polymorphisms in different ethnic groups, variations in drug metabolism and hence drug efficacy and safety are expected between distinct populations (17). In this study, we analyzed and compared malaria patients who were enrolled in Cambodia and Tanzania. Artemether-lumefantrine (AL) has been the first-line treatment for uncomplicated malaria since the end of 2006 and displays good efficacy in Tanzania (18). In Cambodia, however, its efficacy since 2002 has been reported in all but one study to be considerably and consistently lower (19, 20, 21).

While the influence of nongenetic factors, such as food intake, age, or body weight, on the pharmacokinetics of antimalarial drugs has received some attention, only more recently have the effects of polymorphisms in genes encoding enzymes responsible for antimalarial drug metabolism (such as *CYP*, *NAT2*, and UDP-glucuronosyltransferase [*UGT*] gene), received attention (22–28). Better knowledge of the ethnic variability of genes encoding metabolizing enzymes, and of the impact of this variability on the

Received 17 August 2012 Returned for modification 28 November 2012
Accepted 1 December 2012
Published ahead of print 10 December 2012
Address correspondence to Hans-Peter Beck, hans-peter.beck@unibas.ch.
H.-P.B. and B.G. contributed equally to this work.
Copyright © 2013, American Society for Microbiology. All Rights Reserved.
doi:10.1128/AAC.01700-12

TABLE 1 Cytochrome P450 isoenzyme genes and *N*-acetyltransferase 2 genes involved in metabolism of antimalarial drugs

Enzyme(s)	Antimalarial drugs (reference[s])
CYP2A6 and CYP2B6	Artemisinins (25, 28, 62–67)
CYP2C8	Amodiaquine (25, 28, 68–70), chloroquine (25, 64, 71, 72), and dapsone (64)
CYP2C9 and NAT2	Dapsone (25, 64), sulfamethoxazole (69, 72, 73), and sulfadoxine (74)
CYP2C19	Artemether (75), dapsone (63), and proguanil (23, 25, 62, 76, 77)
CYP2D6	Chloroquine (5, 63, 71) and halofantrine (78)
CYP3A4	Artemisinins (25, 28, 62, 64, 65, 76), chloroquine (25, 63, 70, 71), dapsone (25, 63), halofantrine (25, 76, 78), lumefantrine (28, 48, 76), mefloquine (25, 28, 79–81), primaquine (25), and quinine (25, 75, 80–84)
CYP3A5	β-Arteether (25, 64), artemether (48), chloroquine (25, 70), halofantrine (25), lumefantrine (25), mefloquine (79), and quinine (80)

pharmacokinetic profiles of antimalarial drugs, could help tailor treatments for specific populations.

The objectives of the present study were thus to characterize the allelic frequencies of the main genetic variants of the *CYP* and *NAT2* genes involved in currently used ACTs in Tanzania (artemether-lumefantrine almost exclusively) and Cambodia (artesunate-mefloquine and dihydroartemisinin-piperaquine) and to evaluate whether genetic polymorphisms influence the pharmacokinetic profile of ACTs, in addition to nongenetic factors.

MATERIALS AND METHODS

Study areas, design, and populations. This study was part of an *in vivo* drug efficacy study which enrolled mostly unrelated patients with uncomplicated *Plasmodium falciparum* malaria who were of all ages in northern and western Cambodia (64 patients in 2007 at Phnom Dék Health Centre, Rovieng District, Preah Vihear Province, and 61 patients in 2008 at Pramoy Health Centre, Veal Veng District, Pursat Province) and in central Tanzania (149 patients in 2008 at Kibaoni Health Centre, Kilombero District, Morogoro Region). Details of the study protocol will be reported elsewhere by E. M. Staehli Hodel et al. (submitted for publication). In brief, all suspected malaria cases were screened either by a rapid diagnostic test (RDT) or microscopy. After clinical examination and informed consent, venous blood samples (anticoagulated using EDTA) were taken before and during supervised treatment (days 1, 2, and 7 in all locations, and also in Cambodia 1 h after the first dose and on day 14) with the standard national first-line treatment against uncomplicated *P. falciparum* malaria. In Tanzania, patients were given six doses of artemether-lumefantrine (Coartem; Novartis Pharma, Switzerland) over 3 days. In Cambodia, patients received three doses of artesunate (Arsumax; Sanofi-Aventis, France) and mefloquine (Eloquine; Medochemie Ltd., Cyprus) over 3 days in Phnom Dék and three doses of dihydroartemisinin-piperaquine (Duo-Cotecxin; Zhejiang Holley Nanhu Pharmaceutical Co., Ltd., China) over 3 days in Pramoy.

In Cambodia, more than 90% of the people are classified in the Khmer ethnic group. The ethnic minorities include Chinese, Chams (Muslim descendants of Cham refugees who fled to Cambodia after the fall of Champa), Khmer Loeu (a collective term for Mon-Khmer- or Austronesian-speaking hill tribes), and Vietnamese. In the Preah Vihear and Pursat Provinces, Khmer Loeu people form the most represented minority, and they tend to live in separate villages. The study in Tanzania was conducted at Kibaoni Health Center in Ifakara town in Kilombero District. The town lies in the flood plain of the Kilombero River Valley at approximately 36.41°E 8.8°S. The total population of the study area was approximately 90,000. The inhabitants of the Kilombero River Valley live in widely scattered households in the rice field plains. Most people are subsistence farmers of various ethnic groups, and recently highly mobile pastoralist ethnic groups, i.e., Masaai, Barbaig, and Sukuma people, have migrated to the area.

As ethnicity is a sensitive issue and was not necessary for the population pharmacokinetic-pharmacogenetic analysis, this information was not collected. In order to simplify the genetic epidemiological analyses, subsequent testing for Hardy-Weinberg equilibrium showed that the ethnic distributions among patients at both Cambodian study sites were similar.

Laboratory procedures. Blood samples were kept on ice for no longer than 6 h after withdrawal, then aliquoted into whole blood, plasma, and pellet and immediately stored in liquid nitrogen or in a −80°C freezer. Plasma drug concentrations of 14 antimalarial drugs and their metabolites, i.e., artemether (AM), artesunate (AS), dihydroartemether (DHA), amodiaquine, *N*-desethyl-amodiaquine, lumefantrine (LF), desbutyl-lumefantrine (DLF), piperaquine (PPQ), pyronaridine, mefloquine (MQ), chloroquine, quinine, pyrimethamine, and sulfadoxine, were determined simultaneously by using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method that required 200 μl of plasma (29).

A sequencing strategy described in detail by Hodel et al. (30) was adopted to assess the following SNPs: *CYP2A6*2*, *CYP2B6*5*, *CYP2B6*6* (only 516G→T, also called *CYP2B6*9*), *CYP2C8*3* (only 416G→A), *CYP2C9*3*, *CYP2C9*5*, *CYP2C19*3*, *CYP2D6*4*, *CYP2D6*10* (100C→T and 4180G→C), *CYP2D6*17* (1023C→T and 2859C→T), *CYP3A4*1B*, *CYP3A5*3*, *NAT2*5*, *NAT2*6*, *NAT2*7*, and *NAT2*14.*

Sample size. Since prevalence rates of the different polymorphisms varied from 0 to 100%, there was no *a priori* sample size that could be estimated. The sample size of 150 patients represents one of the largest sample sizes so far reported for population pharmacokinetic and pharmacogenetic studies of antimalarial agents.

Data analysis. Sequences were analyzed using the Prism AutoAssembler version 1.4.0 (Applied Biosystems) for assembly. The genotype of each patient was then assessed visually. Hardy-Weinberg equilibrium was tested using the χ^2 Hardy-Weinberg equilibrium test calculator for biallelic markers of the Online Encyclopedia for Genetic Epidemiology studies (http://www.oege.org; last accessed 12 January 2012). Differences of allele frequencies between populations were tested using two-by-two tables and Fisher's exact test. A Bonferroni correction for multiple comparisons was performed for both tests, and a P value of <0.003 was considered significant. The fixation index (F_{ST}) was calculated using Arlequin version 3.1 (31) in order to measure population differentiation based on the investigated SNPs.

Population pharmacokinetic modeling. The population pharmacokinetic-pharmacogenetic analysis was performed using NONMEM version 6 (32). This method uses mixed (fixed and random) effects regression to estimate population means and variances of the pharmacokinetic parameters and to identify factors that influence them. The structural and statistical models for AM, LF, MQ, and PPQ were adopted from our models to be described elsewhere, as elaborated for the same study population and elsewhere by Staehli Hodel et al. (submitted). In brief, the population pharmacokinetic model for LF, AM, their metabolites DLF and DHA, and MQ were described using a one-compartment model with first-order absorption, whereas a two-compartment model was used for PPQ. A fixed allometric power function of body weight was used on clearance (CL) and the volume of distribution of the central compartment (V_C) to capture difference in kinetics according to age. Except for body weight, which influenced CL and V_C, only CYP-inhibiting medications taken concomitantly showed a significant effect on CL. The average estimates from the final model, along with interindividual variability (Staehli Hodel et al., submitted) are summarized in Table 2. Owing to the limited

TABLE 2 Parameters estimated for the base models describing the population pharmacokinetics of artemether, lumefantrine, mefloquine, and piperaquine and estimates from the bootstrap evaluation in 200 replicates

	Population mean				Bootstrap evaluation		
Drug and pharmacokinetics parameter[a]	Estimate	SE[b]	IIV[c]	SE[d]	Mean of 200	SE	95% CI
Artemether							
CL (liters/h/kg)	$24.7 \times BW^{0.75}$	10%	44%	17%	$24.4 \times BW^{0.75}$	10%	19–29
θ_{INH}	−0.3	19%			−0.27	60%	0.5–1.1
V_C (liters/kg)	129	20%			133	26%	88–232
V_M (liters)	(Fixed to V_C)						
k_a (h^{-1})	0.27	11%	119%	11%	0.27	14%	0.21–0.35
k_{23} (h^{-1})	5.86	21%	68%	9%	5.83	25%	3.6–9.7
CL_{met} (liters/h)	419	30%			440	42%	213–927
σ_C (μmol/liter)	74%	9%[d]			73%	11%[d]	59–86%
σ_M (μmol/liter)	119%	11%[d]			116%	9%[d]	88–149%
Lumefantrine							
CL (liters/h/kg)	$0.84 \times BW^{\theta BWCL}$	28%	38%	14%	$0.87 \times BW^{\theta BWCL}$	24%	0.51–1.37
θ_{BWCL}	0.52	19%			0.51	14%	0.36–0.65
V_C (liters/kg)	$59.9 \times BW^{\theta BCVC}$	28%	33%	11%	$59.5 \times BW^{\theta BCVC}$	24%	35.1–91.9
θ_{BCVC}	0.35	28%			0.34	19%	0.1–0.45
V_M (liters)	(Fixed to V_C)						
k_a (h^{-1})	0.54	31%			0.48	43%	0.11–0.88
F_0 (mg)	2.53	14%	103%	14%	2.45	15%	1.58–3.28
k_{23} (h^{-1})	3.7×10^{-4}	12%	38%	15%	3.7×10^{-4}	9%	$(30–44) \times 10^{-4}$
CL_{met} (liters/h)	4.8	10%			4.6	13%	3.4–5.7
σ_C (μmol/liter)	72%	9%[d]			6%	3%	55–77%
σ_M (μmol/liter)	0.013	4%[c]			0.013	45%	0.010–0.016
Mefloquine							
CL (liters/h/kg)	$0.10 \times BW^{0.75}$	5%	12%	88%	$0.10 \times BW^{0.75}$	5%	(0.09–0.11)
V_C (liters/kg)	8.93 × BW	6%	19%	96%	9.01 × BW	6%	(8.04–10.20)
k_a (h^{-1})	0.15	14%			0.15	14%	0.12–0.19
F_0 (mg)	33.1	56%	175%	48%	31.0	43%	11.8–48.1
σ_C (μmol/liter)	43%	6%[d]			43%	6%[d]	0.14–0.22
Piperaquine							
CL (liters/h/kg)	$4.50 \times BW^{0.75}$	13%	45%	61%	$4.26 \times BW^{0.75}$	22%	3.24–5.76
V_C (liters/kg)	346 × BW	12% × BW	65%	48%	347 × BW	13%	260–432
Q (liters/h)	122	13%			126	13%	86–158
V_P (liters)	18,600	22%	50%	77%	20,053	37%	8,778–28,422
k_a (h^{-1})	0.93	28%			1.00	34%	0.35–1.52
F_0 (mg)	123	18%			125	18%	75–171
σ_C (μmol/liter)	41%	10%[d]			41%	6%[d]	0.14–0.21%

[a] CL, clearance; BW, body weight; θ_{INH}, inhibitor effect on CL, determined as $(1 - [\theta_{INH} \times INH])$; V_C, central volume of distribution; Q, intercompartment clearance; V_M, volume of distribution of the metabolite; V_P, peripheral volume of distribution; k_a, first-order absorption rate constant; F_0, residual amount from the previous treatment; k_{23}, metabolism rate constant; CL_{met}, metabolite clearance; σ_C, exponential residual error for the central compartment; σ_M, exponential residual error for the metabolite compartment.

[b] Standard error of the estimate θ_i defined as (SE estimate)/estimate × 100%.

[c] IIV, interindividual variability.

[d] Standard error of the coefficient of variation defined as $\sqrt{[(\text{SE estimate})/\text{estimate}]} \times 100\%$.

number of samples for AS and its metabolite, DHA, no pharmacokinetic analysis could be performed for this drug and its metabolite.

Genetic polymorphisms in the enzymes responsible for the metabolism of the respective antimalarial agent (Table 3) were included in the covariate analysis if their allele frequency was not equal to 0% or 100% among the study populations (see Tables 4 and 5, below). For PPQ, no data on the metabolic pathways were available, and thus all available SNPs were tested, i.e., *CYP2B6*5*, *CYP2B6*6*, *CYP2C9*3*, *CYP2C19*3*, *CYP2D6*10* (100C→T and 4180G→C), *CYP2D6*17* (2859C→T), *CYP3A4*1B*, *CYP3A5*3*, *NAT2*5*, *NAT2*6*, and *NAT2*7*. Patients lacking pharmacogenetic information for a specific SNP were excluded from the analysis of the respective SNP.

In these analyses, each genotype was entered solo into the model. Several models were tested and compared to the richest possible model, which assigned a separate fixed effect to each genotypic group, i.e., homozygous for the reference allele (Hom-REF) or heterozygous (Het-VAR) or homozygous (Hom-VAR) for the mutated allele, as follows: $CL = \theta I_1 + \theta I_2 + \theta I_3$, where θ is the population pharmacokinetic estimate of CL and I_i is an indicator variable that takes the value of 1 if an individual carries the ith genotypic score (i.e., I_1, Hom-REF; I_2, Het-VAR; I_3, Hom-VAR) and 0 otherwise. The impacts of genotypes were further explored to distinguish the differences between the genotypic groups by using two reduced models, in which the same genotyping group was assigned to Hom-VAR and Het-VAR or Hom-REF and Het-VAR.

The models were fitted using the first-order conditional method with interaction. Goodness-of-fit statistics were used to compare models. For goodness-of-fit statistics, NONMEM uses the objective function, which is approximately equal to −2 logs of the maximum likelihood. A ΔOFV (−2

TABLE 3 SNP tested in the population pharmacokinetic model

Drug	Allele
Artemether	*CYP2B6*5*, *CYP2B6*6*, *CYP2C19*3*, *CYP3A4*1B*, *CYP3A5*3*
Lumefantrine	*CYP3A4*1B*, *CYP3A5*3*
Mefloquine	*CYP3A4*1B*, *CYP3A5*3*
Piperaquine[a]	*CYP2C9*3*, *CYP2C19*3*, *CYP3A4*1B*, *CYP3A5*3*, *CYP2D6*10* (100C→T and 4180G→C), *CYP2D6*17* (2850C→T), *NAT2*5*, *NAT2*6*, *NAT2*7*

[a] For piperaquine, no data on the metabolic pathways were available, and thus all available SNP were tested if their allele frequency was not equal to 0% or 100% among the study population.

log likelihood; approximate χ^2 distribution) of 3.84 points for 1 additional parameter and 5.99 for 2 additional parameters, respectively, was used for determining statistical significance ($P < 0.05$) of the differences between two models. Model comparison and evaluations were also assessed by goodness-of-fit plots and visual predictive checks (VPC). The figures were generated with GraphPad Prism (version 4.03).

Ethical approval. All the applied protocols were approved by the ethics committees of the two cantons of Basel (Ethikkommission beider Basel) and the responsible local authorities (Medical Research Coordination Committee of the National Institute for Medical Research in Tanzania and the National Ethics Committee for Health Research in Cambodia). Blood samples were obtained after written informed consent in the local language (Khmer or Swahili) from the participants or their responsible guardians.

RESULTS

The SNPs of interest for the *CYP* and *NAT2* genes were determined by sequencing DNAs from 125 Cambodian and 150 Tanzanian malaria patients. The allele frequencies for each population are shown in Tables 4 and 5 (sequence data were not always available for all samples). Most allele frequencies were found to be in Hardy-Weinberg equilibrium, except for the Tanzanian study population for *CYP2B6*5* and *CYP2D6*4* and in the Cambodian study population for *CYP2B6*5*, *CYP2C9*3*, *CYP2D6*10* (4180G→C), and *CYP2D6*17* (2850C→T).

The frequency of mutations varied with the enzyme and the population (Tables 4 and 5). Higher frequencies of mutant alleles were found in Cambodians than in Tanzanians for *CYP2C9*3* (6.6% versus 0%), *CYP2D6*10* (100C→T; 60.6% versus 6.6%), *CYP3A5*3* (64.5% versus 18.4%), *NAT2*6* (39.8% versus 26.7%), and *NAT2*7* (20.5% versus 2.4%). In contrast, higher frequencies of mutant alleles were found in Tanzanians for *CYP2D6*17* (1023C→T; 20.3% versus 0%), *CYP2D6*17* (2850C→T; 60.6% versus 7.7%), *CYP3A4*1B* (73.5% versus 3.7%), *NAT2*5* (36.4% versus 6.6%), and *NAT2*14* (14.5% versus 2.5%). Significant differences in frequencies were found in 10 SNPs, while no significant differences were observed in 8 SNPs (Tables 4 and 5). The F_{ST} value was 0.16 at the 5% significance level. Many studies are in agreement that F_{ST} values in world samples of human populations are between 0.10 and 0.15 (33). The somewhat higher figure in the present study indicates that the two populations are distinct.

The population pharmacokinetic-pharmacogenetic analysis was performed on the data collected from 143 and 135 of the total of 150 patients recruited in Tanzania for LF and AM, respectively. In Cambodia, 63 out of 64 were included in the analysis of MQ and 60 of 61 were included in the analysis of PPQ. Reasons for exclusion were low hemoglobin levels, inability to swallow the drug, impossibility to take a blood sample, and withdrawal of consent (details are provided in another report by Staehli Hodel et al. (submitted). The pharmacogenetic profile of the patients included in the analysis is summarized in Table 6.

For LF, inclusion of genotype *CYP3A4*1B* did not significantly improve the fit, suggesting a very small change in CL of 13% (95% confidence interval [CI_{95}], −83% to 110%) in Hom-VAR compared to Het-VAR and Hom-REF carriers (ΔOFV = −2.2; P = 0.13). The inclusion of genotype *CYP3A5*3* did not improve the model fit either (ΔOFV = −1.3; P = 0.25), with an estimated 13% decrease (CI_{95}, −48% to 230%) in CL in Hom-VAR compared to Hom-REF individuals. For AM, no statistical improvement of the model was observed after inclusion of *CYP2B6**5 and *CYP2B6*6* variants (ΔOFV < −2.9; P = 0.09), yielding a modest 20% increase (CI_{95}, −17% to 70%) in *CL* Hom-VAR compared to the other genotypic groups. No effect of *CYP3A4*1B* nor of *CYP2C19*3* was observed either (ΔOFV = 0). Neither *CYP3A5*3* nor *CYP3A4*1B* influenced MQ significantly (ΔOFV < −2; P = 0.16), with less than a 10% change (CI_{95}, −15% to 35%) in the CL estimated between Het-VAR and Hom-REF genotypic groups. Finally, among all tested covariates of PPQ CL, a small 15% decrease (CI_{95}, −68% to 39%) in elimination was estimated in the individuals carrying the *CYP2D6*17* (2859C→T) allele, which did not reach statistical significance (ΔOFV = −0.8). No effects of other genetic variants were found to significantly affect PPQ elimination (ΔOFV < 0.6; P > 0.4).

DISCUSSION

This study investigated the effects of single nucleotide polymorphisms in *CYP* and *NAT2* genes on the population pharmacokinetic profiles of ACTs. For this purpose, alleles of *CYP* and *NAT2* genes involved in drug metabolism of antimalarials (*CYP2A6*, *CYP2B6*, *CYP2C8*, *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A4*, *CYP3A5*, and *NAT2*) were assessed in malaria patients from Cambodia and Tanzania. We found alleles that showed similar frequencies in Cambodians and Tanzanians and others that varied greatly between the two populations. The largest differences were found for *CYP2D6*, *CYP3A4*, *CYP3A5*, and *NAT2*. These results are in agreement with previous reports that allele frequencies of *CYP2D6* and *NAT2* generally vary considerably between continents or even countries (34, 35). However, there is little information available on allele frequencies of *CYP* and *NAT2* gene polymorphisms specifically for Tanzanian or Cambodian populations. Sistonen et al. investigated *CYP2D6* haplotype frequencies in single populations, and in Cambodia they found no polymorphisms in *CYP2D6*4* or *CYP2D6*17* genes but a 54.5% mutation rate in *CYP2D6*10* (34), which is comparable with our data. A study on *CYP2D6* genotypes in Tanzania also reported a low allele frequency for *CYP2D6*4* (1%) and an intermediate frequency for *CYP2D6*17* (17%) (36), while for the latter we found a much higher frequency (60.6%). As for *CYP2C19*3*, the observed low frequency confirmed previous findings from a study for Tanzania, where no *CYP2C19*3* alleles were found (37). The allele frequencies we have reported for *CYP2B6*6* (516G→T), *CYP3A4*1B*, and *CYP3A5*3* are very similar to those previously described for three Tanzanian populations: one study from Zanzibar reported allele frequencies of 32.0% for *CYP2B6*6* (516G→T), 69.2% for *CYP3A4*1B*, and 15.8% for *CYP3A5*3*, and two studies from mainland Tanzania reported allele frequencies of 73.2% for *CYP3A4*1B* and 19.0% for *CYP3A5*3* (38–40).

In general, the allele frequencies found in our study show high

TABLE 4 Allele frequencies in cytochrome P450 isoenzyme genes in Cambodia and Tanzania[a]

		Cambodia				Tanzania				
Gene	SNP	*n*	% Allele	χ^2	P_χ	*n*	% Allele	χ^2	P_χ	P_F
*CYP2A6*2*	T/T	123				148				
	T/A	0	0.0			0	0.0			
	A/A	0				0				
*CYP2B6*5*	C/C	116				145				
	C/T	2	3.3	66.52	<0.003	1	1.7	93.91	<0.003	0.108
	T/T	3				2				
*CYP2B6*6*	G/G	38				56				
	G/T	40	38	0.93	0.335	47	34	2.31	0.129	0.056
	T/T	16				18				
*CYP2C8*3*	G/G	75				69				
	G/A	0	0.0			0	0.0			
	A/A	0				0				
*CYP2C9*3*	A/A	109				131				
	A/C	10	6.6	13.38	<0.003	0	0.0			<0.003
	C/C	3				0				
*CYP2C9*5*	C/C	122				129				
	C/G	0	0.0			2	0.8	0.01	0.920	0.268
	G/G	0				0				
*CYP2C19*3*	G/G	117				139				
	G/A	7	2.8	0.10	0.752	2	0.7	0.01	0.920	0.049
	A/A	0				0				
*CYP2D6*4*	G/G	74				126				
	G/A	0	0.0			5	4.1	37.07	<0.003	0.007
	A/A	0				3				
*CYP2D6*10* (100C→T)	C/C	19				119				
	C/T	33	61	2.30	0.129	16	6.6	0.31	0.578	<0.003
	T/T	38				1				
*CYP2D6*10* (4180G→C)	G/G	17				13				
	G/C	36	71	9.78	<0.003	49	74	1.79	0.181	0.065
	C/C	69				80				
*CYP2D6*17* (1023C→T)	C/C	66				74				
	C/T	0	0.0			37	20	0.02	0.888	<0.003
	T/T	0				5				
*CYP2D6*17* (2850C→T)	C/C	46				9				
	C/T	4	7.7	10.92	<0.003	19	61	1.10	0.294	<0.003
	T/T	2				19				
*CYP3A4*1B*	A/A	87				8				
	A/G	7	3.7	0.14	0.708	55	74	0.39	0.532	<0.003
	G/G	0				71				
*CYP3A5*3*	A/A	16				98				
	A/G	56	65	0.02	0.888	39	18	1.39	0.238	<0.003
	G/G	52				7				

[a] *n* indicates the number of patients, % SNP is the mutated allele frequency, χ^2 is the result from the Hardy-Weinberg equilibrium test, and P_χ is the resulting one-tailed *P* value. P_F is the *P* value based on Fisher's exact test for differences between populations.

concordance with data from the National Center for Biotechnology Information (NCBI) SNP home page (note: data for Asians and Africans are available only for *CYP2A6*2*, *CYP2B6*5*, *CYP2B6*6* [516G→T], *CYP2C8*3*, *CYP2C9*3*, *CYP2C19*3*, *CYP3A4*1B*, and *CYP3A5*3*) (41). Furthermore, the F_{ST} value of 0.16 at the 5% significance level implies that the degree of differentiation between the two populations is moderate. The fact that the majority of samples were found to be in Hardy-Weinberg

TABLE 5 Allele frequencies in *N*-acetyltransferase 2 genes in Cambodia and Tanzania[a]

		Cambodia				Tanzania				
Gene	Allele	*n*	% SNP	χ^2	P_χ	*n*	% SNP	χ^2	P_χ	P_F
*NAT2*5*	T/T	106				58				
	T/C	16	6.6	0.60	0.439	66	36.4	0	1.000	<0.003
	C/C	0				19				
*NAT2*6*	G/G	44				84				
	G/A	59	40	0.01	0.920	49	27	3.51	0.061	<0.003
	A/A	19				15				
*NAT2*7*	G/G	78				141				
	G/A	38	21	0.24	0.624	7	2.4	0.09	0.764	<0.003
	A/A	6				0				
*NAT2*14*	G/G	115				107				
	G/A	6	2.5	0.08	0.777	27	15	7.42	0.006	<0.003
	A/A	0				7				

[a] *n* indicates the number of patients, % SNP is the mutated allele frequency, χ^2 is the result from the Hardy-Weinberg equilibrium test, and P_χ is the resulting one-tailed *P* value. P_F is the *P* value from Fisher's exact test for differences between populations.

equilibrium indicates that sampling was unbiased. The few cases where population data were found not to reflect Hardy-Weinberg proportions were most likely due to the rather small sample size (and thus might represent chance findings) or due to the Wahlund effect, i.e., the reduction in heterozygosity because of highly diverging subpopulations.

A limitation of our SNP analysis was the small number of genotyping results for some of the *CYP2D6* loci, which may be ascribed to the PCR products being too short or the PCR efficiency being too low. Short PCR products tend to fail when sequencing due to stuttering at the beginning of sequencing. The PCR primers were initially designed for use in a DNA microarray, and length of the PCR product was not considered. Due to budgetary constraints, a redesign was not feasible. It is also possible that PCR efficiency was reduced by mismatches incorporated into the primer in order to avoid amplification of the pseudogene *CYP2D7*. For future studies, primers should be redesigned, especially for *CYP2D6*.

To investigate the effects of SNPs in *CYP* and *NAT2* genes on the CL of ACTs, and thus to address the main question of this study, the individual pharmacogenetic profiles of the patients acquired by sequencing were incorporated into population pharmacokinetic models for AM, LF, MQ, and PPQ (Staehli Hodel et al., submitted).

For AM, the effects of *CYP2B6*, *CYP2C9*, *CYP2C19*, and *CYP3A4/5* genotypes were assessed. Previous studies of AM conversion to DHA showed that the turnover was highest for human recombinant *CYP2B6* (42). In the present study, *CYP2B6*6* polymorphism was not associated with AM elimination. While intestinal *CYP3A4* might play a role in the presystemic metabolism of AM (43, 44), interaction studies indicated that liver *CYP3A4* is not important in the *in vivo* metabolism of AM (28). This is in line with our finding that there was no difference in CL according to genetic variations of *CYP3A4*. In addition, it has been shown that *CYP3A4* genotyping only poorly reflects the variability of CYP3A4 activity (45). Likewise, no significant difference in the pharmacokinetic profile of AM was seen in carriers of *CYP2C19*3* compared to carriers of the respective reference allele. In our study, about one-third of patients were carriers of at least one *CYP3A5*3* allele, which is the most frequent and functionally important SNP for the *CYP3A5* gene (46). The mutation confers low *CYP3A5* protein expression as a result of improper mRNA splicing and reduced translation of a functional protein (46, 47). However, including the pharmacogenetic profile of *CYP3A5* did not significantly improve the fit.

LF, the long-acting component of artemether-lumefantrine, is metabolized by *CYP3A4* (48). In our findings, no influence of *CYP3A4*1B* was detected. Inconclusive data on alterations of the metabolism in *CYP3A4*1B* carriers are reported in the literature.

TABLE 6 Allele frequencies in patients included in the population pharmacokinetic model

	Frequency of allele in patients who received indicated drug(s) (*n*)[a]				
Gene	AM/DHA[b] (135)	LF/DLF[b] (143)	AS/DHA[b] and MQ (63)	DHA (56)	PPQ (60)
*CYP2A6*2*	0 (134)	0 (142)	0 (59)	0 (55)	0 (59)
*CYP2B6*5*	1.9 (134)	1.8 (142)	5.2 (58)	1.8 (56)	1.7 (60)
*CYP2B6*6*	34 (110)	34 (116)	42 (43)	33 (44)	33 (46)
*CYP2C8*3*	0 (62)	0 (66)	0 (36)	0 (33)	0 (37)
*CYP2C9*3*	0 (118)	0 (125)	8.5 (59)	4.5 (56)	4.2 (60)
*CYP2C9*5*	0.9 (118)	0.8 (125)	0 (59)	0 (56)	0 (60)
*CYP2C19*3*	0.8 (128)	0.7 (136)	3.3 (60)	2.7 (56)	2.5 (60)
*CYP2D6*4*	4.1 (122)	4.3 (128)	0 (50)	0 (20)	0 (22)
*CYP2D6*10* (100C→T)	6.4 (125)	6.9 (130)	65 (52)	53 (32)	51 (35)
*CYP2D6*10* (4180G→C)	75 (128)	75 (136)	72 (58)	69 (56)	70 (60)
*CYP2D6*17* (1023C→T)	22 (105)	21 (112)	0 (50)	0 (13)	0 (14)
*CYP2D6*17* (2859C→T)	65 (36)	62 (38)	11 (19)	7.1 (28)	6. 5 (31)
*CYP3A4*1B*	73 (121)	73 (129)	4.7 (53)	1.3 (39)	2.4 (41)
*CYP3A5*3*	19 (130)	19 (138)	70 (59)	59 (56)	61 (60)
*NAT2*5*	36 (129)	37 (137)	5.9 (59)	8.0 (56)	7.5 (60)
*NAT2*6*	27 (134)	27 (142)	35 (58)	46 (56)	44 (60)
*NAT2*7*	2.2 (134)	2.5 (142)	23 (59)	19 (55)	20 (59)
*NAT2*14*	14 (127)	14 (135)	5.1 (59)	0 (56)	0 (60)

[a] Frequencies are percentages of patients in whom the SNP was found, from among the total patient population that received the drug(s). Values in parentheses are numbers of patients for whom SNP data were available for each group (compared to the total *n*, show at the top of each column).
[b] Parent drug/metabolite.

Some investigators have suggested enhanced drug elimination associated with variations in this gene (49, 50), whereas others have reported decreased metabolism of drugs in carriers of *CYP3A4*1B* among cancer patients (51, 52). It is uncertain whether interindividual differences in drug metabolism can be attributed to the allelic variant *CYP3A4*1B* (47, 49, 53–58); it has also been suggested that a linkage disequilibrium between *CYP3A4*1B* and *CYP3A5*1A* (and thus increased *CYP3A5* expression) could be the actual cause of the altered metabolism (47, 58, 59). No effect of *CYP3A5*3* could be detected in our study.

For MQ and PPQ, none of the SNPs tested seemed to affect their kinetics. Only one individual carrying the Hom-VAR *CYP2D6*17* showed a modest decrease in PPQ elimination. This is in line with previous study results, indicating diminished *CYP2D6* activity (60, 61) in carriers of *CYP2D6*17* alleles. The PPQ elimination mechanism is still unknown, and the influence of this SNP needs to be confirmed in a larger population.

To the best of our knowledge, this is the first study to assess the impact of pharmacogenetic profiles on the clearance of several antimalarial drugs currently in use in Southeast Asia and Africa. Pharmacogenetics could not explain differences in treatment outcomes with AL between Tanzanians and Cambodians (18, 19). We cannot exclude the possibility that other genetic factors not tested in this candidate approach might contribute to treatment outcome. Thus, our results suggest that pharmacogenetic data need not be included in determining appropriate first-line treatments for uncomplicated malaria in specific populations. Possible reasons for our results might be that (i) the pharmacogenetic profiles were not different enough for the panel tested, or (ii) the roles of tested *CYP* genes in metabolism of drugs used in our study populations are not large enough to show effects *in vivo*. Furthermore, DHA, the primary metabolite of AM, is more potent than the parent drug, and the very high intrapatient variability might have limited the possibility to detect genetic association with drug disposition. The limitation of this study was the low allelic frequency of poor metabolizers, which prevented us from detecting a true difference of 30% in drug elimination with most alleles. Further studies might be more relevant if blood is taken from patients with treatment failure and to compare their pharmacogenetic profiles with those of patients with an adequate clinical and parasitological response (ACPR).

ACKNOWLEDGMENTS

Support for this work was provided by grant 320000-112479 from the Swiss National Science Foundation.

We thank the patients who participated in the study. Furthermore, we thank the staff from the National Center for Parasitology, Entomology and Malaria Control in Cambodia, namely, Rithea Leang, Mey Bouth Denis, Chivv Lim, Va Soch, and Oung Chavvin, the staff from Phnom Dék and Pramoy Health Centre in Cambodia, the staff from the Ifakara Health Institute in Tanzania, namely, Hassan Mshinda, Aggrey Malila, and Fidelis Mbena, the staff of the Kibanoi Health Centre in Tanzania, and all the drivers for their help in the sample collection. We also acknowledge Serej Ley, who did most of the primer design and PCR optimization. We are grateful for critical reading of the manuscript by Simon Staehli and Chin B. Eap. The computations were performed at the Vital-IT (http://www.vital-it.ch) Center for high-performance computing of the Swiss Institute of Bioinformatics, and we are grateful for their support.

The opinions expressed in this paper are ours and may not reflect those of our employing organizations. P.O. is a staff member of the WHO; we alone are responsible for the views expressed in this publication, and they do not necessarily represent the decisions, policy, or views of the WHO.

REFERENCES

1. **World Health Organization.** 2010. Guidelines for the treatment of malaria, 2nd ed. WHO, Geneva, Switzerland.
2. **Barnes KI, Lindegardh N, Ogundahunsi O, Olliaro P, Plowe CV, Randrianarivelojosia M, Gbotosho GO, Watkins WM, Sibley CH, White NJ.** 2007. World Antimalarial Resistance Network (WARN). IV: clinical pharmacology. Malaria J. **6**:122. doi:10.1186/1475-2875-6-122.
3. **Sevene E, Gonzalez R, Menendez C.** 2010. Current knowledge and challenges of antimalarial drugs for treatment and prevention in pregnancy. Expert Opin. Pharmacother. **11**:1277–1293.
4. **White NJ, Stepniewska K, Barnes K, Price RN, Simpson J.** 2008. Simplified antimalarial therapeutic monitoring: using the day-7 drug level? Trends Parasitol. **24**:159–163.
5. **Watkins WM, Mosobo M.** 1993. Treatment of Plasmodium falciparum malaria with pyrimethamine-sulfadoxine: selective pressure for resistance is a function of long elimination half-life. Trans. R. Soc. Trop. Med. Hyg. **87**:75–78.
6. **AlKadi HO.** 2007. Antimalarial drug toxicity: a review. Chemotherapy **53**:385–391.
7. **Efferth T, Kaina B.** 2010. Toxicity of the antimalarial artemisinin and its dervatives. Crit. Rev. Toxicol. **40**:405–421.
8. **Li Q, Hickman M.** 2011. Toxicokinetic and toxicodynamic (TK/TD) evaluation to determine and predict the neurotoxicity of artemisinins. Toxicology **279**:1–9.
9. **Ingelman-Sundberg M, Rodriguez-Antona C.** 2005. Pharmacogenetics of drug-metabolizing enzymes: implications for a safer and more effective drug therapy. Philos. Trans. R. Soc. B Biol. Sci. **360**:1563–1570.
10. **Relling MV.** 1989. Polymorphic drug metabolism. Clin. Pharm. **8**:852–863.
11. **Tarning J, Bergqvist Y, Day NP, Bergquist J, Arvidsson B, White NJ, Ashton M, Lindegardh N.** 2006. Characterization of human urinary metabolites of the antimalarial piperaquine. Drug Metab. Dispos. **34**:2011–2019.
12. **Cavallito JC, Nichol CA, Brenckman WD, Deangelis RL, Stickney DR, Simmons WS, Sigel CW.** 1978. Lipid-soluble inhibitors of dihydrofolate-reductase. 1. Kinetics, tissue distribution, and extent of metabolism of pyrimethamine, metoprine, and etoprine in rat, dog, and man. Drug Metab. Dispos. **6**:329–337.
13. **Klinker H, Langmann P, Righter E.** 1996. Plasma pyrimethamine concentrations during long-term treatment for cerebral toxoplasmosis in patients with AIDS. Antimicrob. Agents Chemother. **40**:1623–1627.
14. **Lee J, Son J, Chung SJ, Lee ES, Kim DH.** 2004. In vitro and in vivo metabolism of pyronaridine characterized by low-energy collision-induced dissociation mass spectrometry with electrospray ionization. J. Mass Spectrom. **39**:1036–1043.
15. **Rolan PE, Mercer AJ, Tate E, Benjamin I, Posner J.** 1997. Disposition of atovaquone in humans. Antimicrob. Agents Chemother. **41**:1319–1321.
16. **Agwuh KN, MacGowan A.** 2006. Pharmacokinetics and pharmacodynamics of the tetracyclines including glycylcyclines. J. Antimicrob. Chemother. **58**:256–265.
17. **Meyer UA, Zanger UM.** 1997. Molecular mechanisms of genetic polymorphisms of drug metabolism. Annu. Rev. Pharmacol. Toxicol. **37**:269–296.
18. **Kabanywanyi AM, Mwita A, Sumari D, Mandike R, Mugittu K, Abdulla S.** 2007. Efficacy and safety of artemisinin-based antimalarial in the treatment of uncomplicated malaria in children in southern Tanzania. Malaria J. **6**:146. doi:10.1186/1475-2875-6-146.
19. **Denis MB, Tsuyuoka R, Lim P, Lindegardh N, Yi PTop Socheat SND, Fandeur T, Annerberg A, Christophel EM, Ringwald P.** 2006. Efficacy of artemether-lumefantrine for the treatment of uncomplicated falciparum malaria in northwest Cambodia. Trop. Med. Int. Health **11**:1800–1807.
20. **Song J, Socheat D, Tan B, Seila S, Xu Y, Ou F, Sokunthea S, Sophorn L, Zhou C, Deng C, Wang Q, Li G.** 2011. Randomized trials of artemisinin-piperaquine, dihydroartemisinin-piperaquine phosphate and artemether-lumefantrine for the treatment of multi-drug resistant falciparum malaria in Cambodia-Thailand border area. Malar. J. **10**:231. doi:10.1186/1475-2875-10-231.
21. **Krudsood S, Chalermrut K, Pengruksa C, Srivilairit S, Silachamroon U, Treeprasertsuk S, Kano S, Brittenham GM, Looareesuwan S.** 2003.

Comparative clinical trial of two-fixed combinations dihydroartemisinin-napthoquine-trimethoprim (DNP) and artemether-lumefantrine (Coartem/Riamet) in the treatment of acute uncomplicated falciparum malaria in Thailand. Southeast Asian J. Trop. Med Public Health **34**:316–321.

22. **Cavaco I, Stromberg-Norklit J, Kaneko A, Msellem MI, Dahoma M, Ribeiro VL, Bjorkman A, Gil JP.** 2005. CYP2C8 polymorphism frequencies among malaria patients in Zanzibar. Eur. J. Clin. Pharmacol. **61**:15–18.
23. **Kaneko A, Bergqvist Y, Taleo G, Kobayakawa T, Ishizaki T, Bjorkman A.** 1999. Proguanil disposition and toxicity in malaria patients from Vanuatu with high frequencies of CYP2C19 mutations. Pharmacogenetics **9**:317–326.
24. **Kerb R, Fux R, Morike K, Kremsner PG, Gil JP, Gleiter CH, Schwab M.** 2009. Pharmacogenetics of antimalarial drugs: effect on metabolism and transport. Lancet Infect. Dis. **9**:760–774.
25. **Mehlotra RK, Henry-Halldin CN, Zimmerman PA.** 2009. Application of pharmacogenomics to malaria: a holistic approach for successful chemotherapy. Pharmacogenomics **10**:435–449.
26. **Mehlotra RK, Ziats MN, Bockarie MJ, Zimmerman PA.** 2006. Prevalence of CYP2B6 alleles in malaria-endemic populations of West Africa and Papua New Guinea. Eur. J. Clin. Pharmacol. **62**:267–275.
27. **Parikh S, Ouedraogo JB, Goldstein JA, Rosenthal PJ, Kroetz DL.** 2007. Amodiaquine metabolism is impaired by common polymorphisms in CYP2C8: implications for malaria treatment in Africa. Clin. Pharmacol. Ther. **82**:197–203.
28. **Piedade R, Gil JP.** 2011. The pharmacogenetics of antimalaria artemisinin combination therapy. Expert Opin. Drug Metab. Toxicol. **7**:1185–1200.
29. **Hodel EM, Zanolari B, Mercier T, Biollaz J, Keiser J, Olliaro P, Genton B, Decosterd LA.** 2009. A single LC-tandem mass spectrometry method for the simultaneous determination of 14 antimalarial drugs and their metabolites in human plasma. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. **877**:867–886.
30. **Hodel EM, Ley SD, Qi W, Ariey F, Genton B, Beck HP.** 2009. A microarray-based system for the simultaneous analysis of single nucleotide polymorphisms in human genes involved in the metabolism of antimalarial drugs. Malaria J. **8**:285. doi:10.1186/1475-2875-8-285.
31. **Excoffier L, Laval G, Schneider S.** 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol. Bioinform. Online **1**:47–50.
32. **Beal SL, Sheiner LB, Boeckmann AJ (ed).** 2006. NONMEM users guides *(1989–2006)*. Icon Development Solutions, Ellicott City, MD.
33. **Harpending H.** 2002. Kinship and population subdivision. Popul. Environ. **24**:141–147.
34. **Sistonen J, Sajantila A, Lao O, Corander J, Barbujani G, Fuselli S.** 2007. CYP2D6 worldwide genetic variation shows high frequency of altered activity variants and no continental structure. Pharmacogenet. Genomics **17**:93–101.
35. **Sabbagh A, Langaney A, Darlu P, Gerard N, Krishnamoorthy R, Poloni ES.** 2008. Worldwide distribution of NAT2 diversity: implications for NAT2 evolutionary history. BMC Genetics **9**:21. doi:10.1186/1475-2156-9-21.
36. **Wennerholm A, Johansson I, Massele AY, Jande M, Alm C, Aden-Abdi Y, Dahl ML, Ingelman-Sundberg M, Bertilsson L, Gustafsson LL.** 1999. Decreased capacity for debrisoquine metabolism among black Tanzanians: analyses of the CYP2D6 genotype and phenotype. Pharmacogenetics **9**:707–714.
37. **Bathum L, Skjelbo E, Mutabingwa TK, Madsen H, Horder M, Brosen K.** 1999. Phenotypes and genotypes for CYP2D6 and CYP2C19 in a black Tanzanian population. Br. J. Clin. Pharmacol. **48**:395–401.
38. **Ferreira PE, Veiga MI, Cavaco I, Martins JP, Andersson B, Mushin S, Ali AS, Bhattarai A, Ribeiro V, Bjorkman A, Gil JP.** 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.
39. **Mirghani RA, Sayi J, Aklillu E, Allqvist A, Jande M, Wennerholm A, Eriksen J, Herben VMM, Jones BC, Gustafsson LL, Bertilsson L.** 2006. CYP3A5 genotype has significant effect on quinine 3-hydroxylation in Tanzanians, who have lower total CYP3A activity than a Swedish population. Pharmacogenet. Genomics **16**:637–645.
40. **Rodriguez-Antona C, Sayi JG, Gustafsson LL, Bertilsson L, Ingelman-Sundberg M.** 2005. Phenotype-genotype variability in the human CYP3A locus as assessed by the probe drug quinine and analyses of variant CYP3A4 alleles. Biochem. Biophys. Res. Commun. **338**:299–305.
41. **National Center for Biotechnology Information.** 2010. NCfB: SNP home. http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp.
42. **Navaratnam V, Mansor SM, Sit NW, Grace J, Li QG, Olliaro P.** 2000. Pharmacokinetics of artemisinin-type compounds. Clin. Pharmacokinet. **39**:255–270.
43. **van Agtmael MA, Gupta V, van der Graaf CAA, van Boxtel CJ.** 1999. The effect of grapefruit juice on the time-dependent decline of artemether plasma levels in healthy subjects. Clin. Pharmacol. Ther. **66**:408–414.
44. **van Agtmael MA, Gupta V, van der Wosten TH, Rutten JPB, van Boxtel CJ.** 1999. Grapefruit juice increases the bioavailability of artemether. Eur. J. Clin. Pharmacol. **55**:405–410.
45. **Eap CB, Buclin T, Hustert E, Bleiber G, Golay KP, Aubert AC, Baumann P, Telenti A, Kerb R.** 2004. Pharmacokinetics of midazolam in CYP3A4- and CYP3A5-genotyped subjects. Eur. J. Clin. Pharmacol. **60**:231–236.
46. **Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, Watkins PB, Daly A, Wrighton SA, Hall SD, Maurel P, Relling M, Brimer C, Yasuda K, Venkataramanan R, Strom S, Thummel K, Boguski MS, Schuetz E.** 2001. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. Nat. Genet. **27**:383–391.
47. **Lamba JK, Lin YS, Schuetz EG, Thummel KE.** 2002. Genetic contribution to variable human CYP3A-mediated metabolism. Adv. Drug Deliv. Rev. **54**:1271–1294.
48. **White NJ, van Vugt M, Ezzet F.** 1999. Clinical pharmacokinetics and pharmacodynamics of artemether-lumefantrine. Clin. Pharmacokinet. **37**:105–125.
49. **Amirimani B, Ning B, Deitz AC, Weber BL, Kadlubar FF, Rebbeck TR.** 2003. Increased transcriptional activity of the CYP3A4* 1B promoter variant. Environ. Mol. Mutagen. **42**:299–305.
50. **Amirimani B, Walker AH, Weber BL, Rebbeck TR.** 1999. Modification of clinical presentation of prostate tumors by a novel genetic variant in CYP3A4 - Response. J. Natl. Cancer Inst. **91**:1588–1590.
51. **Felix CA, Walker AH, Lange BJ, Williams TM, Winick NJ, Cheung NKV, Lovett BD, Nowell PC, Blair IA, Rebbeck TR.** 1998. Association of CYP3A4 genotype with treatment-related leukemia. Proc. Natl. Acad. Sci. U. S. A. **95**:13176–13181.
52. **Rebbeck TR, Jaffe JM, Walker AH, Wein AJ, Malkowicz SB.** 1998. Modification of clinical presentation of prostate tumors by a novel genetic variant in CYP3A4. J. Natl. Cancer Inst. **90**:1225–1229.
53. **Westlind A, Lofberg L, Tindberg N, Andersson TB, Ingelman-Sundberg M.** 1999. Interindividual differences in hepatic expression of CYP3A4: relationship to genetic polymorphism in the 5′-upstream regulatory region. Biochem. Biophys. Res. Commun. **259**:201–205.
54. **Spurdle AB, Goodwin B, Hodgson E, Hopper JL, Chen XQ, Purdie DM, McCredie MRE, Giles GG, Chenevix-Trench G, Liddle C.** 2002. The CYP3A4*1B polymorphism has no functional significance and is not associated with risk of breast or ovarian cancer. Pharmacogenetics **12**:355–366.
55. **He P, Court M, Greenblatt DJ, von Moltke LL.** 2005. Genotype-phenotype associations of cytochrome P450 3A4 and 3A5 polymorphism with midazolam clearance in vivo. Clin. Pharmacol. Ther. **77**:373–387.
56. **Bertrand J, Treluyer JM, Panhard X, Tran A, Auleley S, Rey E, Salmon-Ceron D, Duval X, Mentre F, Study G.** 2009. Influence of pharmacogenetics on indinavir disposition and short-term response in HIV patients initiating HAART. Eur. J. Clin. Pharmacol. **65**:667–678.
57. **Ball SE, Scatina JA, Kao J, Ferron GM, Fruncillo R, Mayer P, Weinryb I, Guida M, Hopkins PJ, Warner N, Hall J.** 1999. Population distribution and effects on drug metabolism of a genetic variant in the 5′ promoter region of CYP3A4. Clin. Pharmacol. Ther. **66**:288–294.
58. **Ando Y, Tateishi T, Sekido Y, Yamamoto T, Satoh T, Hasegawa Y, Kobayashi S, Katsumata Y, Shimokata K, Saito H.** 1999. Modification of clinical presentation of prostate tumors by a novel genetic variant in CYP3A4. J. Natl. Cancer Inst. **91**:1587–1588.
59. **Wojnowski L, Hustert E, Klein K, Goldammer M, Haberl M, Kirchheiner J, Koch I, Klattig J, Zanger U, Brockmoller J.** 2002. Re: modification of clinical presentation of prostate tumors by a novel genetic variant in CYP3A4. J. Natl. Cancer Inst. **94**:630–631.
60. **Masimirembwa C, Persson I, Bertilsson L, Hasler J, Ingelman-Sundberg M.** 1996. A novel mutant variant of the CYP2D6 gene (CYP2D6*17) com-

mon in a black African population: association with diminished debrisoquine hydroxylase activity. Br. J. Clin. Pharmacol. **42**:713–719.
61. **Oscarson M, Hidestrand M, Johansson I, Ingelman-Sundberg M.** 1997. A combination of mutations in the CYP2D6*17 (CYP2D6Z) allele causes alterations in enzyme function. Mol. Pharmacol. **52**:1034–1040.
62. **Giao PT, de Vries PJ.** 2001. Pharmacokinetic interactions of antimalarial agents. Clin. Pharmacokinet. **40**:343–373.
63. **Li XQ, Bjorkman A, Andersson TB, Gustafsson LL, Masimirembwa CM.** 2003. Identification of human cytochrome P_{450}s that metabolise anti-parasitic drugs and predictions of in vivo drug hepatic clearance from in vitro data. Eur. J. Clin. Pharmacol. **59**:429–442.
64. **Grace JM, Aguilar AJ, Trotman KM, Brewer TG.** 1998. Metabolism of beta-arteether to dihydroqinghaosu by human liver microsomes and recombinant cytochrome P450. Drug Metab. Dispos. **26**:313–317.
65. **Svensson USH, Ashton M.** 1999. Identification of the human cytochrome P450 enzymes involved in the in vitro metabolism of artemisinin. Br. J. Clin. Pharmacol. **48**:528–535.
66. **Honda M, Muroi Y, Tamaki Y, Saigusa D, Suzuki N, Tomioka Y, Matsubara Y, Oda A, Hirasawa N, Hiratsuka M.** 2011. Functional characterization of CYP2B6 allelic variants in demethylation of antimalarial artemether. Drug Metab. Dispos. **39**:1860–1865.
67. **Gil JP.** 2008. Amodiaquine pharmacogenetics. Pharmacogenomics **9**:1385–1390.
68. **Daly AK.** 2004. Pharmacogenetics of the cytochromes P450. Curr. Top. Med. Chem. **4**:1733–1744.
69. **Li XQ, Bjorkman A, Andersson TB, Ridderstrom M, Masimirembwa CM.** 2002. Amodiaquine clearance and its metabolism to N-desethylamodiaquine is mediated by CYP2C8: a new high affinity and turnover enzyme-specific probe substrate. J. Pharmacol. Exp. Ther. **300**: 399–407.
70. **Kim KA, Park JY, Lee JS, Lim S.** 2003. Cytochrome P4502C8 and CYP3A4/5 are involved in chloroquine metabolism in human liver microsomes. Arch. Pharmacol. Res. **26**:631–637.
71. **Projean D, Baune B, Farinotti R, Flinois JP, Beaune P, Taburet AM, Ducharme J.** 2003. In vitro metabolism of chloroquine: identification of CYP2C8, CYP3A4, and CYP2D6 as the main isoforms catalyzing N-desethylchloroquine formation. Drug Metab. Dispos. **31**:748–754.
72. **Alfirevic A, Stalford AC, Vilar FJ, Wilkins EGL, Park BK, Pirmohamed M.** 2003. Slow acetylator phenotype and genotype in HIV-positive patients with sulphamethoxazole hypersensitivity. Br. J. Clin. Pharmacol. **55**:158–165.
73. **Miller JL, Trepanier LA.** 2002. Inhibition by atovaquone of CYP2C9-mediated sulphamethoxazole hydroxylamine formation. Eur. J. Clin. Pharmacol. **58**:69–72.
74. **Barraviera B, Pereira PC, Machado JM, De Souza MJ, Lima CR, Curi PR, Mendes RP, Meira DA.** 1991. Isoniazid acetylating phenotype in patients with paracoccidioidomycosis and its relationship with serum sulfadoxin levels, glucose-6-phosphate dehydrogenase and glutathione reductase activities. Rev. Soc. Brasil. Med. Trop. **24**:111–114.
75. **Novartis Pharmaceuticals Corporation.** 2009. Full prescribing information for Coartem. Novartis Pharmaceuticals, Branford, CT.
76. **Khoo S, Back D, Winstanley P.** 2005. The potential for interactions between antimalarial and antiretroviral drugs. AIDS **19**:995–1005.
77. **Skjelbo E, Mutabingwa TK, Bygbjerg I, Nielsen KK, Gram LF, Brosen K.** 1996. Chloroguanide metabolism in relation to the efficacy in malaria prophylaxis and the S-mephenytoin oxidation in Tanzanians. Clin. Pharmacol. Ther. **59**:304–311.
78. **Halliday RC, Jones BC, Smith DA, Kitteringham NR, Park BK.** 1995. An investigation of the interaction between halofantrine, Cyp2D6 and Cyp3A4. Studies with human liver-microsomes and heterologous enzyme expression systems. Br. J. Clin. Pharmacol. **40**:369–378.
79. **Fontaine F, de Sousa G, Burcham PC, Duchene P, Rahmani R.** 2000. Role of cytochrome P450 3A in the metabolism of mefloquine in human and animal hepatocytes. Life Sci. **66**:2193–2212.
80. **Ridtitid W, Wongnawa M, Mahatthanatrakul W, Chaipol P, Sunbhanich M.** 2000. Effect of rifampin on plasma concentrations of mefloquine in healthy volunteers. J. Pharm. Pharmacol. **52**:1265–1269.
81. **Ridtitid W, Wongnawa M, Mahatthanatrakul W, Raungsri N, Sunbhanich M.** 2005. Ketoconazole increases plasma concentrations of antimalarial mefloquine in healthy human volunteers. J. Clin. Pharm. Ther. **30**:285–290.
82. **Zhang H, Coville PF, Walker RJ, Miners JO, Birkett DJ, Wanwimolruk S.** 1997. Evidence for involvement of human CYP3A in the 3-hydroxylation of quinine. Br. J. Clin. Pharmacol. **43**:245–252.
83. **Mirghani RA, Hellgren U, Westerberg PA, Ericsson O, Bertilsson L, Gustafsson LL.** 1999. The roles of cytochrome P450 3A4 and 1A2 in the 3-hydroxylation of quinine in vivo. Clin. Pharmacol. Ther. **66**:454–460.
84. **Mirghani RA, Yasar U, Zheng T, Cook JM, Gustafsson LL, Tybring G, Ericsson O.** 2002. Enzyme kinetics for the formation of 3-hydroxyquinine and three new metabolites of quinine in vitro; 3-hydroxylation by CYP3A4 is indeed the major metabolic pathway. Drug Metab. Dispos. **30**:1368–1371.