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Prevalence of *UGT1A9* and *UGT2B7* nonsynonymous single nucleotide polymorphisms in West African, Papua New Guinean, and North American populations

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Abstract

Objective—UDP-glucuronosyltransferases (UGTs) UGT1A9 and UGT2B7 are involved in the metabolism of antimalarial dihydroartemisinin and antiretroviral zidovudine. Our aim was to analyze the prevalence of *UGT1A9* (chromosome 2) and *UGT2B7* (chromosome 4) nonsynonymous single nucleotide polymorphisms (SNPs) in West African (WA), Papua New Guinean (PNG), and North American (NA) populations.

Methods—Using a post-PCR ligation detection reaction-fluorescent microsphere assay, frequencies of *UGT1A9* (8G>A, 98T>C, 766G>A) and *UGT2B7* (211G>T, 802C>T, 1192G>A) SNPs were determined in WA (*n*=133, 5 countries), PNG (*n*=153), and NA (*n*=350, 4 ethnic groups) individuals.

Results—The *UGT1A9* variant alleles were not common in the study populations. None of the SNPs were present in WA and PNG. Among NA, all 3 SNPs were present (1% each) in Asian-Americans, while 98T>C was present only in Caucasian-Americans (1%) and Hispanic-Americans (1%). Regarding *UGT2B7* SNPs, the prevalence of 802C>T was 21% in WA, 28% in PNG, and 28–; 52% in NA. The SNP 211G>T was present only in Asian-Americans (9%) and Hispanic-Americans (2%), while 1192G>A was not present in any of the subjects. No significant linkage was observed at *UGT1A9*, *UGT2B7*, and between both the loci in any of the study populations.

Conclusions—Taken together, the *UGT1A9-UGT2B7* polymorphism profile in WA and PNG populations is similar to African-Americans, but different from Asian-Americans. It is important to determine if these differences, along with previously reported differences in *cytochrome P450 2B6* allele frequencies, are associated with altered metabolism/ effectiveness of artemisinin drugs.

Keywords

Dihydroartemisinin; UGT1A9; UGT2B7; Papua New Guinea; West Africa

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Introduction

Glucuronidation, catalyzed by UDP-glucuronosyltransferases (UGTs), is the major phase II conjugation pathway in the biotransformation and elimination of endogenous and xenobiotic compounds. UDP-glucuronosyltransferases transfer the glucuronic acid moiety of UDP-glucuronic acid to the substrate, thereby increasing the polarity of the substrate and facilitating its excretion in bile or urine [1,2]. Although glucuronidation is generally considered to be a detoxification mechanism, active and/or toxic glucuronide-conjugated metabolites are known to occur [3].

In humans, UGT1A9 and UGT2B7 catalyze glucuronidation of dihydroartemisinin (DHA), which belongs to a novel class of highly effective antimalarials called artemisinin drugs [4]. Dihydroartemisinin is a derivative of the parent compound artemisinin and the major metabolite of artemisinin derivatives artesunate, artemether, β-arteether, and artelinic acid [5-7]. Artemisinin-based combination therapies, including those with DHA, are viewed as the most promising drug strategies for combating malaria-related morbidity and mortality [8,9]. In Vietnamese adult malaria patients given artesunate, α-DHA-β-glucuronide was found to be the major urinary product of DHA glucuronidation, and, using V79 cells expressing human UGT1A1, 1A6, 1A9, or 2B7, α-DHA-β-glucuronide formation was observed with UGT1A9 and UGT2B7, but not with UGT1A1 and UGT1A6 [4].

In addition to DHA, UGT2B7 also plays an important role in the phase II biotransformation of zidovudine (AZT), one of the most commonly used antiretrovirals [10]. Using HEK293 cells expressing human UGT2B4, 2B7, 2B10, 2B11, 2B15, or 2B17, it was observed that only UGT2B7 catalyzed the formation of AZT-glucuronide [10]. The DHA glucuronidation [4] and AZT glucuronidation [10] studies were not performed with all 18 known human UGTs, and it is possible that other UGTs might be important for the glucuronidation of these anti-infectious drugs. Although efavirenz and its phase I hydroxyl metabolites [11] and phase I hydroxyl metabolites of nevirapine [12] have been shown to undergo glucuronidation, indicating the involvement of phase II pathway through UGTs, there is currently no information, to our knowledge, on the identity of the UGT involved in the glucuronidation of these commonly prescribed antiretroviral drugs.

The entire *UGT1* family (subfamily *UGT1A*) has been localized to chromosome 2q37 and spans ~210 kb [13]. All *UGT1A* isoforms, including *UGT1A9*, are composed of a unique first exon and 4 common exons 2–5 [13]. The *UGT1A9* cDNA is 1593 bp in length. So far, 3 nonsynonymous single nucleotide polymorphisms (SNPs) (8G>A [C3Y], 98T>C [M33T], and 766G>A [D256N]) and a SNP at 726T>G resulting in a premature termination codon TAG (Y242X) have been reported in exon–1 of this gene (http://galien.pha.ulaval.ca/labocg/alleles/UGT1A/UGT1A9.htm). Among these SNPs, C3Y is located in the signal peptide of UGT1A9 protein, which is cleaved when the protein is integrated into the endoplasmic reticulum membrane [2,14]. Using HEK293 cells expressing *UGT* allelic variants, this polymorphism was not found to alter glucuronidation activity for anticancer drugs [14]. The reported frequencies of *UGT1A9* polymorphisms are: 8G>A (2.5%, African-Americans) [14], 98T > C (2.2–3.6%, Caucasians) [14], 766G>A (<1%, Japanese) [15], and 726T>G (0.5%, Japanese) [16].

UGT2B7, along with other members of the *UGT2B* subfamily, is localized to chromosome 4q13, spans a region of ~16 kb, and is composed of 6 exons [17]. So far, 3 nonsynonymous SNPs have been reported in this gene. These are: 211G>T (A71S, exon–1), 802C>T (H268Y, exon–2), and 1192G>A (D398N, exon–5) (http://galien.pha.ulaval.ca/labocg/alleles/UGT2B/UGT2B7.htm). The reported frequencies

of these polymorphisms are: 211G>T (14.8–18.5%, Japanese) [18-20], 802C>T (24.4–29.3%, Japanese; 48.9–53.7%, Caucasians) [18-22], and 1192G>A (<1%, Japanese) [19].

Malaria and/or HIV/AIDS pose significant health threats to the individuals living in West Africa, Papua New Guinea (PNG), and North America. Recently, the use of artemisinin drugs has been approved in many countries in West Africa and in PNG (http://malaria.who.int/ and http://www.dndi.org/). However, the World Health Organization warns that as more people gain access to these life-saving malaria medicines, it is vital that countries closely monitor their effectiveness (http://malaria.who.int/). Recently, pharmaco-vigilance of artemisinin-based combination therapy in Africa has been suggested in order to assess the safety of these drugs when used widely [23]. Unfortunately, only limited information is available regarding *UGT1A9* and *UGT2B7* pharmacogenetic variations in various populations; the information pertains mainly to Caucasian and Japanese populations [14,15,18-22]. Here, for the first time, we present a comprehensive account of *UGT1A9* and *UGT2B7* nonsynonymous SNPs in populations from two different malaria-endemic regions, West Africa (Ghana, Guinea, Ivory Coast, Sierra Leone, and Senegal) and PNG, and in four major North American ethnic groups (Caucasian, African, Asian, and Hispanic).

Methods

Study populations and genomic DNA extraction

DNA samples from West African individuals (*n*=133) [Ghana (*n*=23, 3 villages), Guinea (*n*=22, 1 village), Ivory Coast (*n*=36, 6 villages), Sierra Leone (*n*=43, 6 villages), and Senegal (*n*=9, 1 village)] were kindly provided by Dr. B.A. Boatin as a part of the Onchocerciasis Control Program [24]. These samples were encoded to conceal sample identity such as name, sex, age, precise village of residence, etc. All these samples were obtained from the West Atlantic sub-group of the Niger-Congo linguistic group of individuals, implying that all these West African individuals belong to the same ethnicity. Blood samples from PNG (n=153) were collected from the Wosera area of East Sepik Province as a part of the malaria epidemiology study previously described [25]. Samples from PNG were obtained from the Abelam linguistic group of individuals. Blood samples from North American random donors (*n*=350), whose ethnic group was self-identified at the time of sample collection, were obtained through the National Histocompatibility Laboratory, American Red Cross/University of Maryland Medical System, Baltimore, Mary [26]. Among these North American donors were Caucasian (*n*=94), African (*n*=86), Asian (*n*=83), and Hispanic (*n*=87) individuals. Samples from all West Africans, Papua New Guineans, and North Americans were collected under protocols, including the procedures for informed consent, approved by the corresponding institutional review boards.

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

Polymerase chain reaction (PCR) and agarose gel electrophoresis

PCR primers were designed based on *UGT1A9* sequence (GenBank accession number AF297093) and *UGT2B7* sequence (GenBank accession number AC111000) to selectively amplify *UGT1A9* exon–1 and UGT2B7 exon–2 regions. For *UGT2B7* exon–1 and exon–5 amplification, PCR primers of Saeki et al. [19] were used. Sequence homology and specificity of all primer sequences were checked by using BLASTn (http://www.ncbi.nlm.nih.gov). The primer sequences, PCR buffer, amplification conditions, and method to perform agarose gel electrophoresis are provided in supplemental Table 4.

SNP genotyping

Genotyping of *UGT1A9* and *UGT2B7* SNPs was performed using a high throughput, post-PCR oligonucleotide ligation detection reaction-fluorescent microspheres assay (LDR-FMA) [27]. Our SNP genotyping assay is divided into 3 steps: (1) LDR, (2) FM hybridization, and (3) streptavidin-R-phycoerythrin (SA-PE) signal detection using the Bio-Plex suspension array system, which includes a fluorescence reader and the Bio-Plex Manager analytical software (Bio-Rad Laboratories, Hercules, Calif.). The basic principle of this assay and buffers/conditions to perform these steps has been recently described [27]. This multiplex genotyping assay was used for the simultaneous detection of all 6 nonsynonymous SNPs (3 in *UGT1A9* and 3 in *UGT2B7*) in each sample. Equal volumes of PCR products from each sample were mixed. LDR primers were designed based on *UGT1A9* and *UGT2B7* sequences in GenBank, and the primer sequences are provided in supplemental Table 5. The LDR conditions were 95° C 1 min, 95°C 15 sec (denaturation) and 62°C 2 min (annealing/ligation) (32×). After the SA-PE incubation step, detection was completed on the Bio-Plex system by sorting and quantifying median fluorescence intensity (MFI) for 75 microspheres of each type.

Statistical analysis

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

UGT1A9 and *UGT2B7* allele frequencies and genotype numbers for each population were calculated using the Genepop web version 3.4 (http://wbiomed.curtin.edu.au/genepop/). Differences in allele frequencies between two populations were measured using the Fisher's exact test (http://www.matforsk.no/ola/fisher.htm). In order to analyze linkage disequilibrium (LD) between SNPs, we calculated Lewontin's *D'* and correlation coefficient (r^2) values using SHEsis (http://www.nhgg.org/analysis/)[28]. Values of *D'* and r^2 can range from 0 (indicating no LD) to 1 (indicating complete LD). The relative magnitude of values of <1 has no clear interpretation. Nevertheless, it indicates that the complete ancestral LD has been disrupted. For all statistical analyses, a *P* value <0.05 was considered to be significant.

Results

Using LDR-FMA, the observed frequencies of *UGT1A9* and *UGT2B7* wild type and variant allele at each position in West Africans, Papua New Guineans, and North Americans are presented in Table 2. Overall, 629 out of 636 (98.9%) individuals carried *UGT1A9* wild type alleles at all 3 positions. These include all African-Americans (*n*=86), West Africans (*n*=133), and Papua New Guineans (*n*=153). *UGT1A9* variant alleles were observed only in 7 individuals (1.1%). Among the variant alleles, the frequency of allele 8A was 1% in Asian-Americans (*n*=2/83, heterozygous). The frequency of allele 98C was 1% in Caucasian-Americans (*n*=2/94, heterozygous), 1% in Asian-Americans (*n*=1/83, heterozygous), and 1% in Hispanic-Americans (*n*=1/87, heterozygous). Finally, the frequency of allele 766A was 1% in Asian-Americans (*n*=1/83, heterozygous). Asian-Americans carrying 8A (*n*=2), 98C (*n*=1), or 766A (*n*=1) alleles were different individuals. Our results, by LDR-FMA, validate previous findings [14,15]that *UGT1A9* nonsynonymous SNPs are relatively uncommon.

Among the *UGT2B7* variant alleles, 802T had the highest prevalence in all study populations. The frequency of this variant allele was 21% in West Africans and 28% in Papua New Guineans. This difference was, however, not significant (P=0.09, Fisher's exact test). The frequency of 802T in North Americans ranged from 28% (Hispanic-Americans) to 52% (Caucasian-Americans). The frequency in Caucasian-Americans was significantly higher than that in any other population ($P < 0.001$, Fisher's exact test). Our data regarding 802C>T frequency in Caucasian-Americans are concordant with previous findings [21,22]. However, the frequency was higher (41%) in our Asian-Americans compared with Japanese (24.4–29.3%) [18-21] and another Asian population from North America (26.6%) [22], suggesting ethnic and spatial differences, respectively. The allele 211T was observed only in two North American ethnic groups, 9% in Asian-American [n=13/83 (all heterozygous), n=1/83 (homozygous)] and 2% in Hispanic-American (n=4/87, heterozygous). The frequency of 211G>T in Asian-Americans was significantly higher (P=0.05, Fisher'sexact test) than the frequency in Hispanic-Americans. However, the frequency was lower (9%) in our Asian-Americans compared with Japanese populations (14.8–18.5%) [18-20], suggesting ethnic differences. Finally, we did not observe the variant allele 1192A in any study sample, suggesting that 1192A is a rare allele. Overall, our results are in accordance with the results of previous studies that 802T is the most common *UGT2B7* nonsynonymous SNP in various populations [18-22].

Among 13 Asian-Americans and 4 Hispanic-Americans, who were heterozygous at position 211, 8 Asian-Americans and 1 Hispanic-American was also heterozygous at position 802 (211GT-802CT), while the remaining 5 Asian-Americans and 3 Hispanic-Americans were wild type at position 802 (211GT-802CC). One Asian-American individual was 211TT-802CC. Using the HAP algorithm for haplotype resolution from genotype data (http://research.calit2.net/hap/) [29], presence of *UGT2B7* haplotype, a combination of 211T and 802C, was inferred in Asian-Americans (haplotype frequency 9%) and Hispanic-Americans (haplotype frequency 2.3%), which warrants validation by cloning and sequencing.

We further analyzed the 802C>T polymorphism among West African samples (n=133) for each represented country. The frequencies of 802T allele were 18% [Guinea (n=22), Ivory Coast (n=36), and Sierra Leone (n=43)], 28% [Ghana (n=23)], and 45% [Senegal (n=9)]. Since the limitation of this analysis is the relatively small number of samples (from 9 to 43) from each country, it may therefore not be meaningful to compare these frequencies. The very high frequency of 802T allele in the Senegalese population could be due to low sample size and/or sub-ethnic differences compared with the other West African populations.

We calculated *UGT1A9* and *UGT2B7* expected genotype numbers for the positions which were polymorphic in the study populations (see Table 2). The expected numbers did not significantly differ from the observed numbers (data not shown), suggesting that the study populations were in Hardy-Weinberg equilibrium.

We investigated LD distributions for UGT1A9 and *UGT2B7* SNPs by calculating both D' and r^2 values. We analyzed populations that showed polymorphisms at 2 or more positions. Since African-Americans, West Africans, and Papua New Guineans were polymorphic at only 1 position (802C>T), they were not included in the analysis. As presented in Table 3, high D' but low r^2 values were observed for *1A9* 98–*2B7* 802 pair in Caucasian-Americans, for all SNP pairs in Asian-Americans, and for *UGT2B7* 211–802, *1A9* 98–*2B7* 211, and *1A9* 98–*2B7* 802 pairs in Hispanic-Americans, suggesting that there is no significant linkage at *UGT1A9*, *UGT2B7*, and between both the loci in the study populations.

Discussion

In this study, using LDR-FMA, we analyzed the distribution of known genetic variants of *UGT1A9* and *UGT2B7* among West Africans, Papua New Guineans, and North Americans (Caucasians, Africans, Asians, and Hispanics). While we observed allelic differences among these populations/ethnic groups, the functional significance of *UGT1A9* and *UGT2B7* polymorphisms in antimalarial and antiretroviral drug metabolism and their overall clinical impact on the outcome of treatment with these drugs remains to be determined.

In our study, *UGT2B7* 802C>T emerged as the highest frequency variant allele in all the populations. The frequency of this variant allele ranged from 21% in West Africans to 52% in Caucasian-Americans. In spite of its high prevalence, results of previous studies yield conflicting views regarding the contribution of the 802C>T (H268Y) polymorphism to altered glucuronidation of certain drugs. In the human liver, AZT glucuronidation varies >1 order of magnitude [30]. Heterologous expression of the UGT2B7 enzyme variant Y268 in HEK293 cells resulted in 1.9-fold lower AZT glucuronidation efficiency after normalization of the apparent V_{max} values by the level of protein expression [10]. However, AZT glucuronidation activity, normalized to UGT2B7 protein content, was not significantly different between human liver microsomes expressing wild type H268 or variant Y268 enzyme [31]. Large interindividual variations in morphine 3-glucuronide: morphine (up to 48-fold) and morphine 6-glucuronide: morphine (up to 146-fold) ratios have been seen in cancer patients [32,33]. Although UGT2B7 is the major isoform responsible for the 3- and 6-glucuronidation of morphine [34], no correlation was observed between the H268Y polymorphism and morphine glucuronidation using human liver microsomes [31], or between the H268Y polymorphism and glucuronide:morphine ratios in patients [32,33]. It seems that the H268Y polymorphism has no significant effect on the glucuronidation of the large majority of UGT2B7 substrates. Nevertheless, a negative impact of this polymorphism on the protein content [10,35] and specific activity [21] has been suggested. It is also possible that H268Y in association with *UGT2B7* promoter polymorphisms −161C>T [36]and −79G>A [37] may have functional significance. Finally, phenotypic consequences of UGT2B7 H268Y polymorphism may be substrate-specific [38,39].

The other *UGT2B7* polymorphism, frequently observed in Japanese populations [18-20] and in Asian-American and Hispanic-American subjects in our study, is 211G>T (A71S). However, the functional significance of this polymorphism is not yet reported.

Even though *UGT1A9* polymorphisms are not commonly seen, they may be associated with altered metabolism/pharmacokinetics of certain drugs. UGT1A9 enzyme variant M33T, heterologously expressed in HEK293 cells, showed 1.7-fold reduced intrinsic clearance (V_{max}/K_m) for mycophenolic acid 7-O-glucuronide (mycophenolic acid is the active metabolite of a standard immunosuppressive drug mycophenolate mofetil) [40], and 26-fold reduced intrinsic clearance for SN-38 glucuronide (SN-38 is the active metabolite of an anticancer drug irinotecan) [14]. The other UGT1A9 enzyme variant D256N, heterologously expressed in COS-1 cells, showed 22-fold reduced intrinsic clearance for SN-38 glucuronide [15]. In addition to M33T and D256N, *UGT1A9* promoter polymorphisms −275T>A and −2152C>T, associated with 2.3-fold higher protein expression and 2.1-fold higher glucuronidation activity in human liver samples [41], may influence the pharmacokinetics of mycophenolate mofetil in transplant recipients [42].

While there is limited information available regarding the role of *UGT2B7* and *UGT1A9* polymorphisms in the metabolism/pharmacokinetics of AZT, morphine, mycophenolic acid, and irinotecan, nothing is known regarding their effect on artemisinin drug metabolism. Artemisinin drugs belong to a unique class of compounds; they are rapidly absorbed and

eliminated, their peak concentrations in plasma occur in <30 min to 2 h after administration, and then decline rapidly [43-45]. Although it could be argued that these drugs are metabolized so rapidly that drug metabolism polymorphisms may not have pharmacological significance, a number of observations suggest otherwise. Several studies, involving Southeast Asian, Papua New Guinean, and African malaria patients given artesunate or artemether, have reported large interindividual variations in the main pharmacokinetic parameter values of DHA, depending on the dose and route of administration. These include the area under the concentration-time curve (2- to 26-fold) [43,46-48], the peak concentrations of DHA in plasma (2- to 54-fold) [43,44,46-48], and the clearance/bioavailability for DHA (2- to 22-fold) [43,46,48,49]. Large interindividual variations in the pharmacokinetics of DHA have also been seen in healthy Southeast Asian and Caucasian subjects [50-53]. The basis for such a large inter-individual pharmacokinetic variability observed in these studies [43,44,46-49] may include drug metabolism polymorphisms. One explanation for these observations may center on the joint contribution of both *cytochrome P450 2B6* (*CYP2B6*)and *UGT2B7* polymorphisms. CYP2B6 is involved in the phase I metabolism of artemisinin drugs [27 for references], and *CYP2B6* 516G>T, associated with reduced CYP2B6 protein expression and activity [54], is the most commonly observed SNP among West Africans (50%) and Papua New Guineans (64%) [27]. We found that 33% of West Africans and 42% of Papua New Guineans carried both *CYP2B6* 516G>T and *UGT2B7* 802C>T SNPs. Although phenotypic consequences of these polymorphisms are yet to be determined, the individuals carrying both *CYP2B6* 516G>T (phase I) and *UGT2B7* 802C>T (phase II) polymorphisms might have entirely different pharmacokinetics/effectiveness of artemisinin drugs than those carrying only one (either *CYP2B6* 516G>T or *UGT2B7* 802C>T) polymorphism.

It was interesting to note that *UGT1A9* 98T>C and 766G>A polymorphisms were present in the Asian-American population. Also, *UGT2B7* 211G>T and 802C>T polymorphisms were observed in ~10% of Asian-American individuals. It is likely that the Asian-American population studied here is a heterogeneous mixture of individuals, and we have no information to further classify this population. Although the role of *UGT1A9* (98T>C and 766G>A) and *UGT2B7* (211G>T and 802C>T) polymorphisms in the metabolism of DHA is yet to be explored, our data suggest that it may be of interest to analyze these polymorphisms in the populations of Asian countries where artemisinin drugs, including DHA, are either commonly used or are under clinical trials (http://rbm.who.int/rbminfosheets.html). Finally, we did not observe any of the *UGT1A9* and *UGT2B7* 211G>T polymorphisms in African-Americans or in the samples from malaria-endemic West Africa and PNG. Depending on the prevalence of *UGT1A9*, *UGT2B7*, and *CYP2B6* polymorphisms and their functional significance, it is possible that metabolic/phenotypic differences for artemisinin drugs might occur between Asian and African and between Asian and Papua New Guinean populations.

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References

1. Guillemette C. Pharmacogenomics of human UDP-glucuronosyltransferase enzymes. Pharmacogenomics J 2003;3:136–158. [PubMed: 12815363]

2. Nagar S, Remmel RP. Uridine diphosphoglucuronosyltransferase pharmacogenetics and cancer. Oncogene 2006;25:1659–1672. [PubMed: 16550166]

3. Burchell B, Coughtrie MW. UDP-glucuronosyltransferases. Pharmacol Ther 1989;43:261–289. [PubMed: 2506584]

4. Ilett KF, Ethell BT, Maggs JL, Davis TM, Batty KT, Burchell B, Binh TQ, Thu le TA, Hung NC, Pirmohamed M, Park BK, Edwards G. Glucuronidation of dihydroartemisinin in vivo and by human liver microsomes and expressed UDP-glucuronosyltransferases. Drug Metab Dispos 2002;30:1005–1012. [PubMed: 12167566]

5. Price RN. Artemisinin drugs: novel antimalarial agents. Expert Opin Investig Drugs 2000;9:1815–1827.

6. Balint GA. Artemisinin and its derivatives: an important new class of antimalarial agents. Pharmacol Ther 2001;90:261–265. [PubMed: 11578659]

7. White NJ. Clinical pharmacokinetics and pharmacodynamics of artemisinin and derivatives. Trans R Soc Trop Med Hyg 1994;88:S41–S43. [PubMed: 8053024]

8. Olliaro PL, Taylor WR. Developing artemisinin based drug combinations for the treatment of drug resistant falciparum malaria: a review. J Postgrad Med 2004;50:40–44. [PubMed: 15047998]

9. Davis TM, Karunajeewa HA, Ilett KF. Artemisinin-based combination therapies for uncomplicated malaria. Med J Aust 2005;182:181–185. [PubMed: 15720175]

10. Barbier O, Turgeon D, Girard C, Green MD, Tephly TR, Hum DW, Belanger A. 3'-azido-3'-deoxythimidine (AZT) is glucuronidated by human UDP-glucuronosyltransferase 2B7 (UGT2B7). Drug Metab Dispos 2000;28:497–502. [PubMed: 10772627]

11. Mutlib AE, Chen H, Nemeth GA, Markwalder JA, Seitz SP, Gan LS, Christ DD. Identification and characterization of efavirenz metabolites by liquid chromatography/mass spectrometry and high field NMR: species differences in the metabolism of efavirenz. Drug Metab Dispos 1999;27:1319–1333. [PubMed: 10534318]

12. Riska P, Lamson M, MacGregor T, Sabo J, Hattox S, Pav J, Keirns J. Disposition and biotransformation of the antiretroviral drug nevirapine in humans. Drug Metab Dispos 1999;27:895–901. [PubMed: 10421616]

13. Gong QH, Cho JW, Huang T, Potter C, Gholami N, Basu NK, Kubota S, Carvalho S, Pennington MW, Owens IS, Popescu NC. Thirteen UDPglucuronosyltransferase genes are encoded at the human *UGT1* gene complex locus. Pharmacogenetics 2001;11:357–368. [PubMed: 11434514]

14. Villeneuve L, Girard H, Fortier LC, Gagne JF, Guillemette C. Novel functional polymorphisms in the UGT1A7 and UGT1A9 glucuronidating enzymes in Caucasian and African-American subjects and their impact on the metabolism of 7-ethyl-10-hydroxycamptothecin and flavopiridol anticancer drugs. J Pharmacol Exp Ther 2003;307:117–128. [PubMed: 12944498]

15. Jinno H, Saeki M, Saito Y, Tanaka-Kagawa T, Hanioka N, Sai K, Kaniwa N, Ando M, Shirao K, Minami H, Ohtsu A, Yoshida T, Saijo N, Ozawa S, Sawada J. Functional characterization of human UDP-glucuronosyltransferase 1A9 variant, D256N, found in Japanese cancer patients. J Pharmacol Exp Ther 2003;306:688–693. [PubMed: 12730278]

16. Saeki M, Saito Y, Jinno H, Sai K, Komamura K, Ueno K, Kamakura S, Kitakaze M, Shirao K, Minami H, Ohtsu A, Yoshida T, Saijo N, Ozawa S, Sawada J. Three novel single nucleotide polymorphisms in *UGT1A9*. Drug Metab Pharmacokinet 2003;18:146–149. [PubMed: 15618729]

17. Carrier JS, Turgeon D, Journault K, Hum DW, Belanger A. Isolation and characterization of the human *UGT2B7* gene. Biochem Biophys Res Commun 2000;272:616–621. [PubMed: 10833461]

18. Hirota T, Ieiri I, Takane H, Sano H, Kawamoto K, Aono H, Yamasaki A, Takeuchi H, Masada M, Shimizu E, Higuchi S, Otsubo K. Sequence variability and candidate gene analysis in two cancer patients with complex clinical outcomes during morphine therapy. Drug Metab Dispos 2003;31:677–680. [PubMed: 12695358]

19. Saeki M, Saito Y, Jinno H, Tanaka-Kagawa T, Ohno A, Ozawa S, Ueno K, Kamakura S, Kamatani N, Komamura K, Kitakaze M, Sawada J. Single nucleotide polymorphisms and haplotype frequencies of *UGT2B4* and *UGT2B7* in a Japanese population. Drug Metab Dispos 2004;32:1048–1054. [PubMed: 15319348]

20. Saito K, Moriya H, Sawaguchi T, Hayakawa T, Nakahara S, Goto A, Arimura Y, Imai K, Kurosawa N, Owada E, Miyamoto A. Haplotype analysis of UDP-glucuronocyltransferase 2B7 gene

(*UGT2B7*) polymorphisms in healthy Japanese subjects. Clin Biochem 2006;39:303–308. [PubMed: 16466707]

21. Bhasker CR, McKinnon W, Stone A, Lo AC, Kubota T, Ishizaki T, Miners JO. Genetic polymorphism of UDP-glucuronosyltransferase 2B7 (*UGT2B7*) at amino acid 268: ethnic diversity of alleles and potential clinical significance. Pharmacogenetics 2000;10:679–685. [PubMed: 11186130]
22. Lampe JW, Bigler J, Bush AC, Potter JD. Prevalence of polymorphisms in the human UDP-glucuronosyltransferase 2B family: *UGT2B4*(D458E), *UGT2B7*(H268Y), and *UGT2B15*(D85Y). Cancer Epidemiol Biomarkers Prev 2000;9:329–333. [PubMed: 10750673]
23. Talisuna AO, Staedke SG, D'Alessandro U. Pharmacovigilance of antimalarial treatment in Africa: is it possible? Malar J 2006;5:50. [PubMed: 16780575]
24. Zimmerman PA, Dadzie KY, De Sole G, Remme J, Alley ES, Unnasch TR. *Onchocerca volvulus* DNA probe classification correlates with epidemiologic patterns of blindness. J Infect Dis 1992;165:964–968. [PubMed: 1569351]
25. Mehlotra RK, Kasehagen LJ, Baisor M, Lorry K, Kazura JW, Bockarie MJ, Zimmerman PA. Malaria infections are randomly distributed in diverse holoendemic areas of Papua New Guinea. Am J Trop Med Hyg 2002;67:555–562. [PubMed: 12518843]
26. Zimmerman PA, Woolley I, Masinde GL, Miller SM, McNamara DT, Hazlett F, Mgone CS, Alpers MP, Genton B, Boatin BA, Kazura JW. Emergence of *FY*A*(null) in a *Plasmodium vivax*-endemic region of Papua New Guinea. Proc Natl Acad Sci USA 1999;96:13973–13977. [PubMed: 10570183]
27. Mehlotra RK, Ziats MN, Bockarie MJ, Zimmerman PA. Prevalence of CYP2B6 alleles in malaria-endemic populations of West Africa and Papua New Guinea. Eur J Clin Pharmacol 2006;62:267–275. [PubMed: 16506047]
28. Shi YY, He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res 2005;15:97–98. [PubMed: 15740637]
29. Halperin E, Eskin E. Haplotype reconstruction from genotype data using Imperfect Phylogeny. Bioinformatics 2004;20:1842–1849. [PubMed: 14988101]
30. Pacifici GM, Evangelisti L, Giuliani L, Metelli RM, Giordani R. Zidovudine glucuronidation in human liver: interindividual variability. Int J Clin Pharmacol Ther 1996;34:329–334. [PubMed: 8864794]
31. Court MH, Krishnaswamy S, Hao Q, Duan SX, Patten CJ, Von Moltke LL, Greenblatt DJ. Evaluation of 3'-azido-3'-deoxythymidine, morphine, and codeine as probe substrates for UDP-glucuronosyltransferase 2B7 (UGT2B7) in human liver microsomes: specificity and influence of the *UGT2B7*2* polymorphism. Drug Metab Dispos 2003;31:1125–1133. [PubMed: 12920168]
32. Holthe M, Klepstad P, Zahlsen K, Borchgrevink PC, Hagen L, Dale O, Kaasa S, Krokan HE, Skorpen F. Morphine glucuronide-to-morphine plasma ratios are unaffected by the *UGT2B7* H268Y and *UGT1A1*28* polymorphisms in cancer patients on chronic morphine therapy. Eur J Clin Pharmacol 2002;58:353–356. [PubMed: 12185559]
33. Holthe M, Rakvag TN, Klepstad P, Idle JR, Kaasa S, Krokan HE, Skorpen F. Sequence variations in the UDP-glucuronosyltransferase 2B7 (*UGT2B7*) gene: identification of 10 novel single nucleotide polymorphisms (SNPs) and analysis of their relevance to morphine glucuronidation in cancer patients. Pharmacogenomics J 2003;3:17–26. [PubMed: 12629580]
34. Coffman BL, Rios GR, King CD, Tephly TR. Human UGT2B7 catalyzes morphine glucuronidation. Drug Metab Dispos 1997;25:1–4. [PubMed: 9010622]
35. Coffman BL, King CD, Rios GR, Tephly TR. The glucuronidation of opioids, other xenobiotics, and androgens by human UGT2B7Y(268) and UGT2B7H(268). Drug Metab Dispos 1998;26:73–77. [PubMed: 9443856]
36. Sawyer MB, Innocenti F, Das S, Cheng C, Ramirez J, Pantle-Fisher FH, Wright C, Badner J, Pei D, Boyett JM, Cook E Jr, Ratain MJ. A pharmacogenetic study of uridine diphosphate-glucuronosyltransferase 2B7 in patients receiving morphine. Clin Pharmacol Ther 2003;73:566–574. [PubMed: 12811366]
37. Duguay Y, Baar C, Skorpen F, Guillemette C. A novel functional polymorphism in the uridine diphosphate-glucuronosyltransferase 2B7 promoter with significant impact on promoter activity. Clin Pharmacol Ther 2004;75:223–233. [PubMed: 15001974]

38. Burchell B, Lockley DJ, Staines A, Uesawa Y, Coughtrie MW. Substrate specificity of human hepatic UDP-glucuronosyltransferases. Methods Enzymol 2005;400:46–57. [PubMed: 16399342]

39. Court MH. Isoform-selective probe substrates for in vitro studies of human UDP-glucuronosyltransferases. Methods Enzymol 2005;400:104–116. [PubMed: 16399346]

40. Bernard O, Guillemette C. The main role of UGT1A9 in the hepatic metabolism of mycophenolic acid and the effects of naturally occurring variants. Drug Metab Dispos 2004;32:775–778. [PubMed: 15258099]

41. Girard H, Court MH, Bernard O, Fortier LC, Villeneuve L, Hao Q, Greenblatt DJ, von Moltke LL, Perussed L, Guillemette C. Identification of common polymorphisms in the promoter of the *UGT1A9* gene: evidence that UGT1A9 protein and activity levels are strongly genetically controlled in the liver. Pharmacogenetics 2004;14:501–515. [PubMed: 15284532]

42. Kuypers DR, Naesens M, Vermeire S, Vanrenterghem Y. The impact of uridine diphosphate-glucuronosyltransferase 1A9 (*UGT1A9*) gene promoter region single-nucleotide polymorphisms T-275A and C-2152T on early mycophenolic acid dose-interval exposure in de novo renal allograft recipients. Clin Pharmacol Ther 2005;78:351–361. [PubMed: 16198654]

43. Krishna S, Planche T, Agbenyega T, Woodrow C, Agranoff D, Bedu-Addo G, Owusu-Ofori AK, Appiah JA, Ramanathan S, Mansor SM, Navaratnam V. Bioavailability and preliminary clinical efficacy of intrarectal artesunate in Ghanaian children with moderate malaria. Antimicrob Agents Chemother 2001;45:509–516. [PubMed: 11158748]

44. Angus BJ, Thaiaporn I, Chanthapadith K, Suputtamongkol Y, White NJ. Oral artesunate dose-response relationship in acute falciparum malaria. Antimicrob Agents Chemother 2002;46:778–782. [PubMed: 11850261]

45. Karunajeewa HA, Ilett KF, Dufall K, Kemiki A, Bockarie M, Alpers MP, Barrett PH, Vicini P, Davis TM. Disposition of artesunate and dihydroartemisinin after administration of artesunate suppositories in children from Papua New Guinea with uncomplicated malaria. Antimicrob Agents Chemother 2004;48:2966–2972. [PubMed: 15273107]

46. Hien TT, Davis TM, Chuong LV, Ilett KF, Sinh DX, Phu NH, Agus C, Chiswell GM, White NJ, Farrar J. Comparative pharmacokinetics of intramuscular artesunate and artemether in patients with severe falciparum malaria. Antimicrob Agents Chemother 2004;48:4234–4239. [PubMed: 15504846]

47. Bethell DB, Teja-Isavadharm P, Cao XT, Pham TT, Ta TT, Tran TN, Nguyen TT, Pham TP, Kyle D, Day NP, White NJ. Pharmacokinetics of oral artesunate in children with moderately severe *Plasmodium falciparum* malaria. Trans R Soc Trop Med Hyg 1997;91:195–198. [PubMed: 9196768]

48. McGready R, Stepniewska K, Ward SA, Cho T, Gilveray G, Looareesuwan S, White NJ, Nosten F. Pharmacokinetics of dihydroartemisinin following oral artesunate treatment of pregnant women with acute uncomplicated falciparum malaria. Eur J Clin Pharmacol 2006;62:367–371. [PubMed: 16552504]

49. Mithwani S, Aarons L, Kokwaro GO, Majid O, Muchohi S, Edwards G, Mohamed S, Marsh K, Watkins W. Population pharmacokinetics of artemether and dihydroartemisinin following single intramuscular dosing of artemether in African children with severe falciparum malaria. Br J Clin Pharmacol 2004;57:146–152. [PubMed: 14748813]

50. Na-Bangchang K, Krudsood S, Silachamroon U, Molunto P, Tasanor O, Chalermrut K, Tangpukdee N, Matangkasombut O, Kano S, Looareesuwan S. The pharmacokinetics of oral dihydroartemisinin and artesunate in healthy Thai volunteers. Southeast Asian J Trop Med Public Health 2004;35:575–582. [PubMed: 15689069]

51. Le NH, Na-Bangchang K, Le TD, Thrinh KA, Karbwang J. Phamacokinetics of a single oral dose of dihydroartemisinin in Vietnamese healthy volunteers. Southeast Asian J Trop Med Public Health 1999;30:11–16. [PubMed: 10695779]

52. Teja-Isavadharm P, Watt G, Eamsila C, Jongsakul K, Li Q, Keeratithakul G, Sirisopana N, Luesutthiviboon L, Brewer TG, Kyle DE. Comparative pharmacokinetics and effect kinetics of orally administered artesunate in healthy volunteers and patients with uncomplicated falciparum malaria. Am J Trop Med Hyg 2001;65:717–721. [PubMed: 11791963]

53. Teja-Isavadharm P, Nosten F, Kyle DE, Luxemburger C, Ter Kuile F, Peggins JO, Brewer TG, White NJ. Comparative bioavailability of oral, rectal, and intramuscular artemether in healthy subjects: use

of simultaneous measurement by high performance liquid chromatography and bioassay. Br J Clin Pharmacol 1996;42:599–604. [PubMed: 8951191]

54. Lang T, Klein K, Fischer J, Nussler AK, Neuhaus P, Hofmann U, Eichelbaum M, Schwab M, Zanger UM. Extensive genetic polymorphism in the human *CYP2B6* gene with impact on expression and function in human liver. Pharmacogenetics 2001;11:399–415. [PubMed: 11470993]

Table 1

Mean and 95% confidence interval (CI) values of allelic ratios to determine genotypes[a]

Gene	Polymorphism	Genotype	*n*	Mean	95% CI
UGT1A9	8	GG	634	1.61	0.02
		GA	2	0.44	0.03
	98	TT	632	1.70	0.02
		TC	4	0.05	0.04
	766	GG	635	1.38	0.02
		GA	1	0.09[b]	—
UGT2B7	211	GG	618	1.69	0.02
		GT	17	0.27	0.02
		TT	1	−1.82[b]	—
	802	CC	296	0.96	0.01
		CT	262	−0.09	0.01
		TT	78	−1.00	0.02

n Number of individuals

[a] See Statistical analysis under Methods section

[b] Value for one sample only

Table 2

UGT1A9 and *UGT2B7* alleles in various populations

Gene	Allele	Frequencies					
		CA (*n*=94)	**AfA (*n*=86)**	**AsA (*n*=83)**	**HA (*n*=87)**	**WA (*n*=133)**	**PNG (*n*=153)**
UGT1A9	8G	1	1	0.99	1	1	1
	8A	0	0	0.01	0	0	0
	98T	0.99	1	0.99	0.99	1	1
	98C	0.01	0	0.01	0.01	0	0
	766G	1	1	0.99	1	1	1
	766A	0	0	0.01	0	0	0
UGT2B7	211G	1	1	0.91	0.98	1	1
	211T	0	0	0.09[a]	0.02	0	0
	802C	0.48	0.66	0.59	0.72	0.79	0.72
	802T	0.52[b]	0.34	0.41	0.28	0.21[c]	0.28
	1192G	1	1	1	1	1	1
	1192A	0	0	0	0	0	0

CA Caucasian-American; *AfA* African-American; *AsA* Asian-American; *HA* Hispanic-American; *WA* West African; *PNG* Papua New Guinean

[a] P=0.05, Fisher's exact test, compared with HA

[b] P<0.001, Fisher's exact test, compared with any other population

[c] P=0.09, Fisher's exact test, compared with PNG

Table 3

Lewontin's D' and (r^2) values in various populations

Population	*n*		*UGT1A9*			*UGT2B7*	*UGT1A9-UGT2B7*					
			8–98	**8–766**	**98–766**	**211–802**	**8–211**	**8–802**	**98–211**	**98–802**	**766–211**	**766–802**
CA	94	D'	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.00	n.d.	n.d.
		r^2								(0.012)		
AsA	83	D'	1.00	1.00	1.00	0.996	0.392	0.995	1.00	1.00	1.00	0.924
		r^2	(0)	(0)	(0)	(0.068)	(0.019)	(0.017)	(0.001)	(0.004)	(0.001)	(0.008)
HA	87	D'	n.d.	n.d.	n.d.	0.976	n.d.	n.d.	1.00	0.992	n.d.	n.d.
		r^2				(0.009)			(0)	(0.015)		

n.d. not determined