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PXR Variants and Artemisinin Use in Vietnamese Subjects: Frequency Distribution and Impact on the Interindividual Variability of CYP3A Induction by Artemisinin

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Artemisinins induce drug metabolism through the activation of the pregnane X receptor (PXR) *in vitro*. Here, we report the resequencing and genotyping of *PXR* variants in 75 Vietnamese individuals previously characterized for CYP3A enzyme activity after artemisinin exposure. We identified a total of 31 *PXR* variants, including 5 novel single nucleotide polymorphisms (SNPs), and we identified significantly different allele frequencies relative to other ethnic groups. A trend of significance was observed between the level of CYP3A4 induction by artemisinin and two *PXR* variants, the 8118C→T (Y328Y) and 10719A→G variants.

Artemisinin combination therapy (ACT) is an integral part of the global management of malaria (7). In this treatment strategy, an artemisinin-related compound with a short half-life ($t_{1/2}$; ~0.25 to 4 h) is combined with a more slowly eliminated antimalarial to reduce recrudescence and to slow the development of resistance (24). Currently, several ACT formulations, including artesunate-mefloquine, artemether-lumefantrine, and artesunate-amodiaquine, are used (27), and a second generation of ACTs is being scheduled for global launch. These ACTs include dihydroartemisinin-piperaquine (5) and artesunate-pyronaridine (30).

In vitro studies indicate that artemisinin, arteether, and artemether are effective ligands of the pregnane X receptor (PXR) (4), a nuclear receptor and a key player in the regulation of the expression of proteins involved in drug metabolism (e.g., cytochrome P450s [CYP450s]) and transport (e.g., ABC transporters) (6). Variability in the expression and function of these proteins may lead to alterations in the pharmacokinetics of artemisinin derivatives, possibly resulting in pharmacodynamic changes and subsequent clinical consequences such as side effects (14).

A previously performed *in vivo* study including 75 Vietnamese subjects showed a significant interindividual variation in the degree of artemisinin-driven induction of several CYP450 enzymes, including CYP3As, the genes which are canonical targets of PXR (1). In the present work, we built upon this study by hypothesizing that specific single nucleotide polymorphisms (SNPs) in *PXR* might explain the observed interindividual variability in the level of CYP3A induction. For this purpose, the *PXR* gene was fully resequenced in all individuals who participated in the study mentioned above, with a focus on the open reading frame (ORF) (mutations in which could lead to proteins with altered activities), intron-exon boundaries (mutations in which could lead to disturbances in the well-documented alternative splicing of PXR), and the proximal promoter (mutations in which could modulate basal expression). Additionally, known variants with putative functional consequences located in introns and other regions (e.g., the 3′ region) were genotyped by using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) technology. Primers and amplification conditions are listed in Table S1 in the supplemental material.

In this extensive analysis, a total of 79 polymorphic sites were scrutinized, and we identified 31 SNPs, 5 of which, to the best of our knowledge, are documented for the first time: −24910G→A and −23925C→T in the promoter region of PXR, 8582T→G in intron 8, and 10098C→T and 10976G→A in the 3′ untranslated region (UTR) (Fig. 1). Only three SNPs in the *PXR* ORF were observed, and these were the synonymous SNP 8118C→T (Y328Y), with a minor allele frequency of 0.26, and two rare nonsynonymous variants, 9683A→G and 9932C→G (I403V and Q426V). The rarity of SNPs in the ORF supports the view that the stability of the protein sequence is essential for PXR function, and thus, there has been sufficient selection pressure to reduce genetic variability during evolution (11, 34).

With the exception of *PXR* 8055C→T and 10976G→A, all SNPs were in Hardy-Weinberg equilibrium (HWE) (Fisher's exact test; GraphPad Prism V.4 software [GraphPad, La Jolla, CA]). Because we excluded genotyping errors by regenotyping using an independent method, the deviation from HWE may be explained by the small sample size of our study population or population admixture, a well-known phenomenon discussed elsewhere (32).

While data on the prevalence of *PXR* variants in Caucasian and Japanese individuals are well established, limited data for other ethnic groups are available. Thus, our study is the first analysis of the full *PXR* sequence in a population from a region where malaria is endemic. Generally, the prevalence data for Vietnamese individ-

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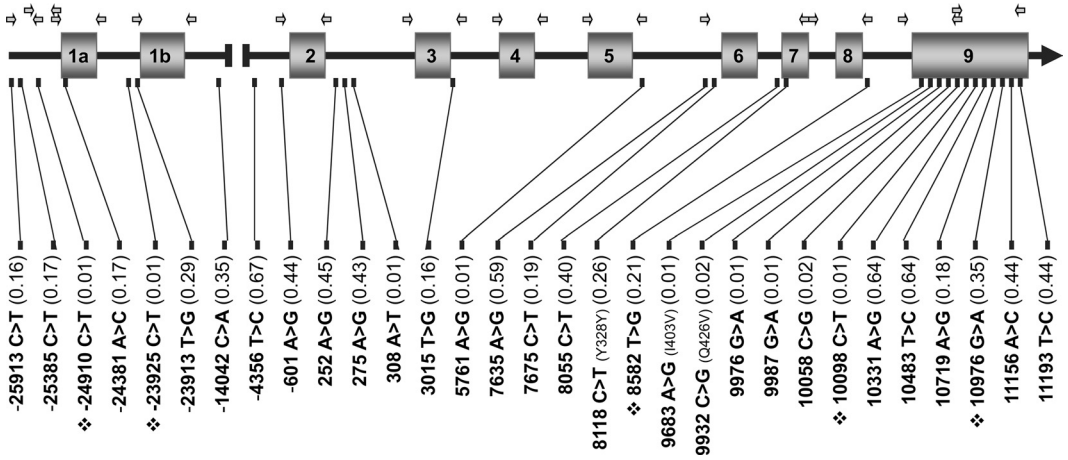

FIG 1 Genomic structure of the *PXR* gene. All single nucleotide polymorphisms identified in this study and their minor allelic frequencies are annotated. The arrows denote the position of the sequencing primers, and the symbol ❖ denotes the newly identified SNPs.

uals are similar to the data on *PXR* variants in Indians and Asian-Americans, consistent with the roots of these populations in the southern Chinese and Thai-Indonesian populations (Fig. 2) (12, 25). However, of note, the prevalence of the *PXR* variant 10331A→G among Vietnamese individuals is significantly different from that among Chinese ($P = 0.004$) and Malay ($P = 0.001$) populations (25). Moreover, the frequency distribution of the 10331A→G variant is substantially different from that in Caucasian populations ($P = 0.0001$ to 0.062) (3, 21). The prevalence of the −4356T→C variant in our cohort was not different from the frequency in a Caucasian population ($P = 0.881$) (19). In contrast, the prevalence of the 7635A→G variant in Vietnamese individuals was significantly different from the prevalences in all previously described populations ($P = 0.0001$ to 0.01), except for Asian-Americans, Indians, and, despite geographic distance, Scandinavians (13, 16, 25). The same observation was made for the 10483T→C SNP, for which there were significantly different frequency distributions for all other described populations ($P =$

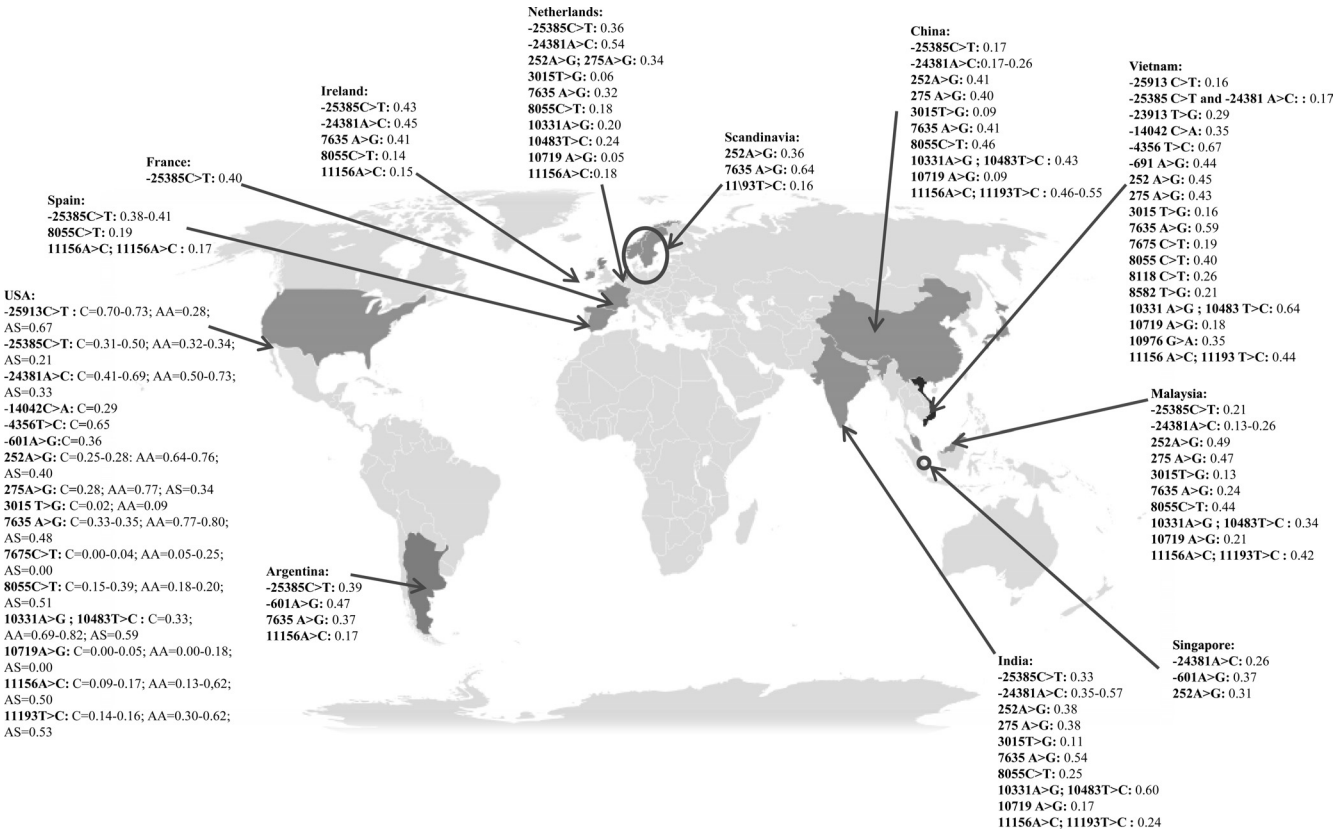

FIG 2 World distribution of PXR SNPs with a frequency of >0.10 in the Vietnamese population (2, 3, 8, 10, 13, 16, 18–23, 25, 26, 28, 29, 33, 34).

TABLE 1 Genotyping data of PXR in Vietnamese individuals treated with 500 mg artemisinin orally and associations with CYP3A activity induction

Mutation(s) by position[a]	n[b]	Genotype	Genotypic frequency (95% CI)[c]	Mean (±SD) CYP3A activity induction[d]	Fold change (95% CI)[e]	Unadjusted P (Holm-adjusted P)[f]
−25913C→T, −25385C→T, −24381A→C	14	wt/wt	0.57 (0.32–0.79)	2.76 (0.83)	1.1 (0.81–1.51)	0.543 (1)
		wt/mut	0.36 (0.16–0.61)	2.82 (1.32)		
		mut/mut	0.07 (0.00–0.33)	3.74		
−23913T→G	10	T/T	0.50 (0.24–0.76)	2.56 (1.13)	1.14 (0.8–1.61)	0.471 (1)
		T/G	0.40 (0.17–0.69)	3.06 (0.54)		
		G/G	0.10 (0.00–0.43)	2.62		
−14042C→A	14	C/C	0.29 (0.11–0.55)	2.87 (0.62)	0.94 (0.69–1.28)	0.678 (1)
		C/A	0.57 (0.32–0.79)	2.77 (0.74)		
		A/A	0.14 (0.03–0.41)	3.13 (2.74)		
−4356T→C	14	T/T	0.14 (0.03–0.41)	3.13 (2.74)	1 (0.74–1.34)	0.989 (1)
		T/C	0.50 (0.27–0.73)	2.90 (0.70)		
		C/C	0.36 (0.16–0.61)	2.67 (0.70)		
−601A→G	14	A/A	0.29 (0.11–0.55)	2.77 (0.77)	0.97 (0.71–1.32)	0.845 (1)
		A/G	0.57 (0.32–0.79)	2.82 (0.68)		
		G/G	0.14 (0.03–0.41)	3.13 (2.74)		
252A→G	14	A/A	0.21 (0.07–0.48)	2.34 (1.18)	1.16 (0.87–1.56)	0.312 (1)
		A/G	0.58 (0.32–0.79)	3.01 (1.07)		
		G/G	0.21 (0.07–0.48)	2.93 (0.70)		
275A→G	14	A/A	0.29 (0.11–0.55)	3.03 (1.67)	1.04 (0.78–1.38)	0.788 (1)
		A/G	0.50 (0.27–0.73)	2.71 (0.72)		
		G/G	0.21 (0.07–0.48)	2.93 (0.70)		
3015T→G	13	T/T	0.54 (0.29–0.77)	2.88 (1.31)	1.08 (0.82–1.43)	0.577 (1)
		T/G	0.31 (0.12–0.58)	2.94 (0.55)		
		G/G	0.15 (0.03–0.43)	3.05 (0.60)		
7635A→G	14	A/A	0.07 (0.00–0.33)	2.28	1.09 (0.77–1.56)	0.615 (1)
		A/G	0.64 (0.39–0.84)	2.98 (0.67)		
		G/G	0.29 (0.11–0.55)	2.69 (1.69)		
7675C→T	13	C/C	0.46 (0.23–0.71)	2.91 (1.40)	1.04 (0.68–1.6)	0.848 (1)
		C/T	0.54 (0.29–0.77)	2.80 (0.69)		
		T/T	0 (0.00–0.27)			
8055C→T	14	C/C	0.58 (0.32–0.79)	2.76 (0.67)	0.94 (0.74–1.2)	0.626 (1)
		C/T	0.21 (0.07–0.48)	3.22 (0.52)		
		T/T	0.21 (0.07–0.48)	2.71 (2.07)		
8118C→T	13	C/C	0.54 (0.29–0.77)	3.29 (0.99)	0.69 (0.48–1.01)	0.057 (1)
		C/T	0.46 (0.23–0.71)	2.34 (0.88)		
		T/T	0 (0.00–0.27)			
8582T→G	13	T/T	0.54 (0.29–0.77)	2.85 (1.26)	1.1 (0.72–1.68)	0.666 (1)
		T/G	0.46 (0.23–0.71)	2.95 (0.74)		
		G/G	0 (0.00–0.27)			
10058C→G	14	C/C	0.93 (0.67–1.00)	2.88 (1.02)	0.89 (0.37–2.15)	1 (1)
		C/G	0.07 (0.00–0.34)	2.45		
		G/G	0.00 (0.00–0.25)			
10331A→G	14	A/A	0.14 (0.03–0.41)	2.08 (0.29)	1 (0.72–1.39)	0.995 (1)
		A/G	0.29 (0.11–0.55)	3.06 (0.54)		
		G/G	0.57 (0.33–0.79)	2.94 (1.21)		

(Continued on following page)

TABLE 1 (Continued)

Mutation(s) by position[a]	n^b	Genotype	Genotypic frequency (95% CI)[c]	Mean (±SD) CYP3A activity induction[d]	Fold change (95% CI)[e]	Unadjusted *P* (Holm-adjusted *P*)[f]
10483T→C	14	T/T	0.14 (0.03–0.41)	2.08 (0.29)	1.01 (0.76–1.33)	0.958 (1)
		T/C	0.29 (0.11–0.55)	3.06 (0.54)		
		C/C	0.57 (0.33–0.79)	2.94 (1.21)		
10719A→G	13	A/A	0.62 (0.35–0.82)	2.74 (1.26)	1.3 (1.01–1.66)	0.040 (0.767)
		A/G	0.23 (0.07–0.51)	2.61 (0.16)		
		G/G	0.15 (0.03–0.44)	3.28 (0.62)		
10976G→A	14	G/G	0.29 (0.11–0.55)	2.31 (1.01)	1.38 (0.92–2.06)	0.117 (1)
		G/A	0.71 (0.45–0.89)	3.07 (0.94)		
		A/A	0 (0.00–0.25)			
11156A→C, 11193T→C	14	wt/wt	0.50 (0.27–0.73)	2.62 (0.58)	0.97 (0.76–1.25)	0.840 (1)
		wt/mut	0.29 (0.11–0.55)	3.35 (0.50)		
		mut/mut	0.21 (0.07–0.48)	2.71 (2.07)		

[a] Position of SNP in GenBank sequence AF364606.1, with +1 being the first nucleotide of the start codon (CTG) in exon 2 (nucleotide 70390).
[b] Number of individuals analyzed (numbers less than 14 are due to missing values).
[c] Values in parentheses are the 95% confidence intervals (CIs) determined by the modified Wald method using GraphPad Quickcalcs software.
[d] Mean induction of CYP3A activity. Values in parentheses are the standard deviations determined using GraphPad Prism software.
[e] Fold changes (representing mean multiplicative increase/decrease per minor allele) and 95% CIs from association analysis with CYP3A4 activity induction, assuming an additive genetic model.
[f] Corresponding unadjusted *P* values and Holm-adjusted *P* values for additive genetic models.

<0.001 to 0.0258) (16). Because the 7635A→G, 10331A→G, and 10483T→C variants have been shown to alter CYP3A4 expression and function (21, 34), the significantly different prevalences of these variants in Vietnamese individuals compared with those for individuals of other ethnicities may have consequences for the metabolism of CYP3A4 substrates.

In addition, we investigated the impact of individual *PXR* variants in an artemisinin-exposed subgroup of 14 subjects who are characterized by higher CYP3A induction levels (Table 1). Although we observed a trend approaching significance between the 10719A (unadjusted $P = 0.04$) and 8118T alleles (unadjusted $P = 0.057$) and lower CYP3A induction according to a log-additive model, these effects did not persist after adjustment for multiple testing (adjusted $P = 1$). The use of codominant, dominant, and recessive models did not result in statistically significant unadjusted *P* values (SNPassoc 1.6-0 in R-2.13.0 [www.r-project.org]). We are aware that the validity of our data is limited by the small size of our study cohort. Nevertheless, because it has been recently shown that a synonymous mutation (*ABCB1* 3435C→T) can result in functional consequences, a similar mechanism for the 8118C→T variant cannot be excluded (15, 17). The 10719A→G SNP is located in the 3′ UTR region of the *PXR* gene, an area rich in microRNA (miRNA) binding sites. However, the initial *in silico* analysis did not support the hypothesis that this variant alters a specific miRNA binding site.

In conclusion, our study results indicate that the frequency distribution of particular *PXR* variants in Vietnam, a region where malaria is endemic, is different from those in other ethnic populations. Moreover, the associative trend between the *PXR* 8118C→T and 10719A→G variants and the induction of CYP3A activity via artemisinin warrants further studies. Although only a mild increase in CYP3A activity in response to artemisinin derivatives has been shown *in vivo*, the parent drug, artemisinin, leads to strong induction (approximately 3-fold) and therefore increases the risk of clinically significant drug-drug interaction (1). This may be of importance, as artemisinin, despite its pharmacological shortcomings, has been proposed as a valuable component for future ACT formulations (e.g., in combination with naphthoquine and piperaquine) (9, 31).

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