Pharmacogenomics of , , , , , and in Vietnam

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PHARMACOGENETICS

Pharmacogenomics of *CYP2A6*, *CYP2B6*, *CYP2C19*, *CYP2D6*, *CYP3A4*, *CYP3A5* and *MDR1* in Vietnam

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Abstract
Aim The aim of this study was to obtain pharmacogenetic data in a Vietnamese population on genes coding for proteins involved in the elimination of drugs currently used for the treatment of malaria and human immunodeficiency virus/acquired immunodeficiency syndrome.
Method The main polymorphisms on the cytochrome P450 (CYP) genes, *CYP2A6*, *CYP2B6*, *CYP2C19*, *CYP2D6*, *CYP3A4* and *CYP3A5*, and the multi-drug resistance 1 gene (*MDR1*) were genotyped in 78 healthy Vietnamese subjects. Pharmacokinetic metrics were available for CYP2A6 (coumarin), CYP2C19 (mephenytoin), CYP2D6 (metoprolol) and CYP3As (midazolam), allowing correlations with the determined genotype.
Results In the *CYP2* family, we detected alleles *CYP2A6*4* (12%) and **5* (15%); *CYP2B6*4* (8%), **6* (27%); *CYP2C19*2* (31%) and **3* (6%); *CYP2D6*4*, **5*, **10* (1, 8 and 44%, respectively). In the *CYP3A* family, *CYP3A4*1B* was detected at a low frequency (2%), whereas *CYP3A5 *3* was detected at a frequency of 67%. The *MDR1* 3435T allele was present with a prevalence of 40%. Allele proportions in our cohort were compared with those reported for other Asian populations. CYP2C19 genotypes were associated to the S-4′-OH-mephenytoin/S-mephenytoin ratio quantified in plasma 4 h after intake of 100 mg mephenytoin. While CYP2D6 genotypes were partially reflected by the α-OH-metroprolol/metoprolol ratio in plasma 4 h after dosing, no correlation existed between midazolam plasma concentrations 4 h post-dose and CYP3A genotypes.
Conclusions The Vietnamese subjects of our study cohort presented allele prevalences in drug-metabolising enzymes that were generally comparable with those reported in other Asian populations. Deviations were found for *CYP2A6*4* compared to a Chinese population (12 vs. 5%, respectively; P=0.023), *CYP2A6*5* compared with a Korean population (15 vs. <1%, respectively; P<0.0001), a Malaysian population (1%; P<0.0001) and a Chinese population (1%; P< 0.0001); *CYP2B6*6* compared with a Korean population (27 vs. 12%; P=0.002) and a Japanese population (16%; P= 0.021). Pharmacokinetic metrics versus genotype analysis reinforces the view that the predictive value of certain globally common variants (e.g. *CYP2D6* single nucleotide polymorphisms) should be evaluated in a population-specific manner.

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Introduction

Despite the large efforts of projects such as the HapMap Consortium (www.hapmap.org), detailed pharmacogenetic information in ethnically specific regions is still lacking, particularly in the developing world. This is true for Southeastern Asian populations, where the main body of available data is limited to Thai populations [1–3].

One main aim of therapeutic drug administration in Vietnam, in agreement with the general sentiment throughout the developing world, is the treatment/prevention of transmittable diseases, which represent major national public health issues. The lack of data on intrinsically relevant factors, such as polymorphisms in the main drug-metabolising enzymes and transporters, may lead to misinterpretations of the metabolic capacity of drugs or confounding results in terms of their use. Many of the anti-infective drugs currently being used in Vietnam—namely those against malaria and human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) – are metabolised by the cytochrome P450s (CYPs). We have therefore analysed the major polymorphisms in the genes coding the CYPs, *CYP2A6*, *CYP2B6*, *CYP2C19*, *CYP2D6*, *CYP3A4* and *CYP3A5*, in a group of Vietnamese subjects, and compared the frequencies of these polymorphisms with those reported in other Asian populations. The main single nucleotide polymorphism (SNP) in the multi-drug resistance 1 (*MDR1*) transporter gene was added to the study, due to the importance of the P-glycoprotein (Pgp) in the oral bioavailability of a range of therapeutic drugs.

The Vietnamese individuals studied herein were also subject to a panel of specific CYP drug probe substrates—coumarin (CYP2A6), mephenytoin (CYP2C19), metoprolol (CYP2D6) and midazolam (CYP3As)—allowing the attentive association of functional gene polymorphisms with determined pharmacokinetic metrics.

Materials and methods

Subjects

Seventy-eight healthy subjects were included in our study (54 men and 24 women; average age 29 years, range 18–45 years). The majority of the subjects had participated in a previous study to determine which principal human CYP enzymes are affected by artemisinin and its derivatives [4]. Thirty-six of the subjects were smokers of no more than ten cigarettes per day.

With the exception of three individuals of Thai origin, all subjects in this study were reported to be of Kinh origin, the most prevalent ethnic group in Vietnam. Twelve subjects were found to be related. One volunteer in each pair of related participants was randomly excluded from the allele frequency analysis, but all participants were included in the correlation of genotype to pharmacokinetic metric (Fig. 1).

Venous blood samples for genotyping were obtained from each subject at the National Institute of Malariology, Parasitology and Entomology, Hanoi, Vietnam. Pharmacokinetic metrics were available from all except for four subjects (Fig. 1). All participants provided written informed consent prior to enrolment. Study protocols were approved by the Ministry of Health, Hanoi, Vietnam, the Swedish Medical Products Agency, Uppsala, Sweden and the Ethics

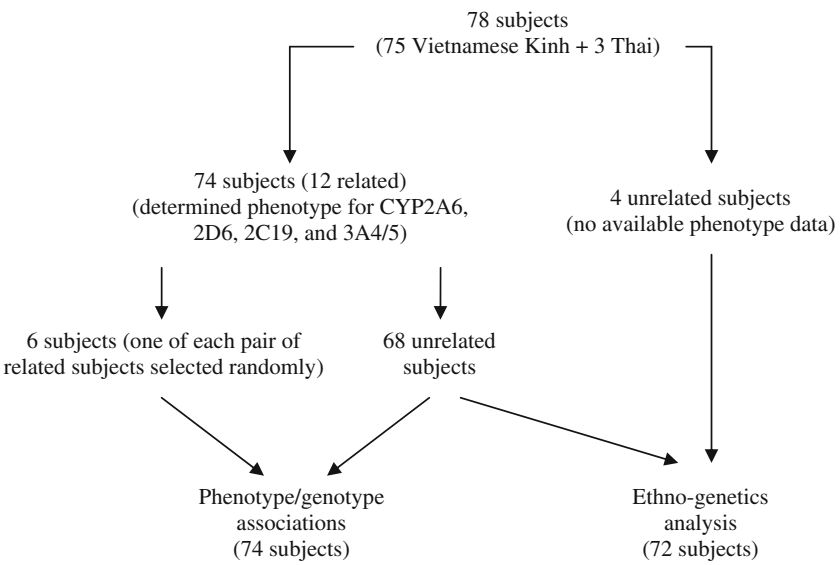

Fig. 1 Flow chart of outcome subjects analysed for pharmacokinetic metrics and/or genotyping. *CYP* Cytochrome P450

Committee of University of Gothenburg, Göteborg, Sweden. The studies were performed according to the principles of the Declaration of Helsinki.

Molecular analysis

Genomic DNA was extracted from peripheral blood of the 78 subjects using the BloodPrep Chemistry protocol together with an ABI 6100 Nucleic Acid PrepStation (Applied Biosystems, Fresno, CA).

The genotype of the CYP2A6 deletion was performed as described elsewhere [5], and gene conversion in the 3′ flanking region 1436G>T was accomplished by a novel pyrosequencing based method. Briefly, PCR analysis was performed in a total volume of 50 μL with primers 5′-ATTGACGTGTCCCCCAAAC-3′ and 5′-biotinylated-GGCAGGAAGCTCATGGTGTAG-3′. The PCR programme was 94°C for 5 min, followed by 45 cycles of 94°C for 30 s, 53°C for 30 s and 72°C for 20 s, with a final extension step of 72°C for 5 min. To create single-stranded biotinylated PCR products, we mixed streptavidin sepharose beads with binding buffer, followed by purification in a pyrosequencing vacuum prep tool. An annealing buffer and 0.3 m*M* of sequencing primer 5′-CCCCCAAACACGTGG-3′ were added to the purified product and analysed in a PSQTM 96MA instrument (Biotage AB, Uppsala, Sweden) with a dispensation nucleotide order of TGGG/TCTTT.

CYP2B6 516G>T, 785A>G and 1459C>T were analysed by PCR–restriction fragment length polymorphism (RFLP) as described elsewhere [6]. The CYP2C19 splicing defect 681G>A was analysed using Taqman assays (Applied Biosystems) and PCR–RFLP to the stop codon 636G>A [7].

The CYP2D6 total gene deletion analysis was performed as described by Hersberger and collaborators [8], the splicing defect 1846G>A as reported by Spurr et al. [9] and 100C>T through a novel pyrosequencing-based method. The pyrosequencing protocol was identical to the one described for the CYP2A6 1436G>T analysis with exception of the two PCR amplification primers (5′-CCCCTGGCCGTGATAGTG-3′ and 5′-biotinylated-CACCTGGTCGAAGCAGTATG-3′), the PCR programme (annealing temperature of 58°C), the sequencing primer (5′-CTGGGCTGCACGCTA-3′) and the order of the dispensation nucleotides (CC/TCACCAG).

The *CYP3A4* -392A>G SNP was analysed as described by Cavaco and collaborators [10]. Analysis of *CYP3A5* splice defect-associated 6986A>G mutation was performed in Taqman assays and newly designed PCR–RFLPs to the *CYP3A5* frameshift 27131–32insT and 3699C>T SNP. The 14690G>A allele analysis was detected as described by Garsa et al. [11]. The membrane transporter MDR1 3435C>T synonymous SNPs were genotyped based on results from the Taqman assays.

Novel pyrosequencing protocols were designed with the aid of the Pyrosequencing Assay Design software, ver. 1.0 and analysis on PSQTM 96MA (Biotage AB). Taqman assays were performed with the recommended protocol in an ABI PRISM 7000 Sequence Detection System (Applied Biosystems). Negative controls were added in all assays.

Oligonuclotide primers were purchased from the Thermo Electron Corporation (Ulm, Germany), restriction endonucleases from New England Biolabs (Beverly, MA) and Taq DNA polymerase, $MgCl_2$ and dNTPs were obtained from Promega (Madison, WI).

Determination of metabolic capacity of CYP2A6, CYP2C19, CYP2D6 and CYP3A

Information on drug-metabolising capacity was available for 74 of the 78 volunteers. These same individuals had also participated in a study previously reported involving artemisinin antimalarials [4]. The subjects were randomised to receive therapeutic doses of artemisinin or one of its derivatives for 5 days (days 1–5). Six drugs—caffeine (100 mg), coumarin (5 mg), mephenytoin (100 mg), metoprolol (100 mg), midazolam (7.5 mg) and chlorzoxazone (250 mg)—were given orally and simultaneously at baseline as a cocktail 1 week before (day 6) and on the first and last day of endoperoxide drug administration. None of the participants were taking any other drugs (pharmaceutical or traditional), grapefruit juice or caffeine-containing drinks during these days. Four-hour plasma concentrations of the parent drugs and corresponding metabolites and 7-hydroxycoumarin urine concentrations were quantified by liquid chromatography/tandem mass spectrometry (LC/MS/MS). In the present study, we used baseline data (day 6) and focused on the activities of CYP2A6 [amount of 7-OH-coumarin (mg) excreted in urine collected 0–8 h after coumarin intake], CYP2C19 (S-4'-OH- mephenytoin/S-mephenytoin 4 h plasma ratio), CYP2D6 (α-OH-metoprolol/metoprolol 4 h plasma ratio) and CYP3A (midazolam 4 hr plasma concentrations).

Statistical analysis

Allelic and genotypic proportions are presented together with 95% confidence intervals. The Fisher's Exact Test two-tail analysis was performed in order to be able to compare our data with allele proportions reported from previous population trials. Pharmacokinetic metrics are presented as arithmetic means, and differences in levels between genotypes were assessed using analysis of variance (ANOVA) and Bonferroni adjusted post-hoc tests. The ANOVA results were reported for the original scale, but ANOVA was also performed for the logarithmic scale and using the corresponding non-parametric Kruskal–Wallis

test. Using the pre-specified significance level of 0.05, no irregularities between the three tests were found. Hardy–Weinberg equilibrium testing for the analysed SNPs was performed with the GenePop software (http://wbiomed.curtin.edu.au/genepop/)

Results

The SNP genotype and allele frequencies of the different *loci* analysed are shown in Table 1. The wild-type (**1*) alleles were defined as not harbouring the alternative alleles of the SNPs under study.

The results of the genotypic analysis of our Vietnamese population follow, in general, the trends reported for other Asian populations (Table 2). Some SNPs were not detected, including *CYP2B6* 1459C>T (determinant for the **5* and **7* alleles) as well as the *CYP3A5* splicing defect, 14690G>A, frameshift 27131–32insT and the 3699C>T (determinant for the **6*, **7* and **8* alleles). The frequency of *CYP2B6*6*, however, was significantly higher than that reported for the Korean population ($P<0.01$) (Table 2). Two SNPs were found not to be in Hardy–Weinberg equilibrium: *MDR1* 3435C>T ($P<0.05$) and *CYP2D6* 100C>T ($P<0.01$).

The relationships between the genotypes and the pharmacokinetic metrics of CYP2A6, CYP3As, CYP2C19 and CYP2D6 are statistically presented in Table 3; those for CYP2C19 and CYP2D6 are graphically depicted in Fig. 2a and b, respectively. The subjects are ranked according to their metabolic capacity and plotted as per descending pharmacokinetic metric against a background of the distribution of *CYP* genotype. For the *CYP2C19* and *CYP2D6* loci (Fig. 2a and b), the genotypes were associated to metabolic capacity (ANOVA $P<0.001$ and $P<0.001$, respectively), which was distributed in a suggested trimodal mode (poor, intermediate and extensive metabolisers). No meaningful association was observed between the CYP2A6 genotypes and the amount of 7-OH coumarin excreted in urine 0–8 h after dosing, although the ANOVA yielded a significant result. *CYP3A* alleles *3A4*1B* and *3A5*3* showed no significant association ($P=0.218$) with 4-h plasma concentrations of midazolam.

Discussion

We report comprehensive pharmacogenetic information on a Vietnamese population that is directly relevant to drug elimination capacity. Statistically significant differences ($P<0.05$) were noted for a number of allele frequencies, compared with those reported for other Asian populations (Table 2). The frequency of *CYP2A6*4* was higher in our Vietnamese population (12%) than that reported for a Chinese population (5%), while the frequency of *CYP2A6*5* was generally several-fold more frequent than that reported in all other Asian populations studied to date. *CYP2B6*6* was about twofold more frequent (27%) in our sample of Vietnamese subjects than that found in Korean (12%) and Japanese (16%) populations.

The frequencies of the main SNPs in *CYP2B6* have not been previously described in Southeast Asia, despite this enzyme being of importance for the metabolic clearance of several important drugs, including antimalarial and anti-HIV compounds. The observed relatively high frequency of *CYP2B6*6* among our Vietnamese population (Table 2) may be of interest. The 516G>T SNP present in this allele has been associated to greater efavirenz plasma exposure and to more frequent central nervous system side effects [12]. Our data therefore indicate that a significant number of AIDS patients in Vietnam (near 10%) may be expected to have an elevated exposure to efavirenz.

Two exceptions to the Hardy–Weinberg equilibrium were found, namely the *CYP2D6* 100C>T SNP and the *MDR1* 3435C>T SNP. However, their frequencies were not significantly different from those observed in other Asian populations (Table 2). As our Vietnamese subjects were unrelated, the best explanation for these observations is that it reflects a certain degree of ethnic admixture, as this study was conducted in a highly populated central large metropolis (Hanoi). A similar deviation from the Hardy–Weinberg equilibrium for *MDR1* 3435C>T was previously found by Lee et al. [13], who also emphasised ethnic admixture as a possible explanation.

The primary aim of this study was to obtain pharmacogenetic information on principal CYP enzymes in a Vietnamese population. As a secondary aim we also attempted to explore any association between available pharmacokinetic metrics and the determined genotypes. It should be noted, however, that neither the used combination of probe compounds nor the metrics represent best practice when seeking to relate individuals' metabolic capacity with their genotype, and these results should be considered with caution. One limitation of the cocktail approach is the risk of interactions between the co-administered probe drugs. Chlorzoxazone has been reported to inhibit the metabolism of midazolam when the two drugs are given in the same cocktail [14]. This has been suggested to result from chlorzoxazone inhibiting the first-pass metabolism of midazolam by CYP3A in the gut. The midazolam concentrations used in our study could have been increased due to such an inhibition. Further interactions between the other probe compounds can not be excluded, since co-administration of this particular combination of drugs has not been reported earlier. Thus, any attempt to relate our pharmacokinetic metrics with determined genotypes has clear limitations. Despite the use of an unvalidated approach,

Table 1 Single nucleotide polymorphic allele and genotype frequencies among Kinh Vietnamese[a]

Gene	Amino acid change[b]	Single nucleotide polymorphism[b]	Allele frequency[c]	wt[c]/wt	wt/mut	mut/mut[d]
CYP2A6	Gene deleted		F(wt) = 0.882 (0.829–0.935)	55/72	17/72	0/72
			F(del) = 0.118 (0.065–0.171)	0.764 (0.649–0.856)	0.236 (0.144–0.351)	0 (0–0.050)
	G479V	1436G>T[f]	F(G) = 0.854 (0.797–0.912)	51/72	21/72	0/72
			F(T) = 0.146 (0.088–0.203)	0.708 (0.589–0.810)	0.292 (0.190–0.411)	0 (0–0.050)
CYP2B6	Q172H	516G>T[f]	F(G) = 0.729 (0.657–0.802)	38/72	29/72	5/72
			F(T) = 0.271 (0.198–0.343)	0.528 (0.407–0.647)	0.403 (0.289–0.525)	0.069 (0.023–0.155)
	K262R	785A>G[f]	F(A) = 0.646 (0.568–0.724)	32/72	29/72	11/72
			F(G) = 0.354 (0.276–0.432)	0.444 (0.327–0.566)	0.403 (0.289–0.525)	0.153 (0.079–0.257)
	R487C	1459C>T[f]	F(C) = 1 (0.975–1)	72/72	0/72	0/72
			F(T) = 0 (0–0.025)	1 (0.950–1)	0 (0–0.050)	0 (0–0.050)
CYP2C19	Splicing defect	681G>A[f]	F(G) = 0.694 (0.619–0.770)	33/72	34/72	5/72
			F(A) = 0.306 (0.230–0.381)	0.458 (0.340–0.580)	0.472 (0.353–0.593)	0.069 (0.023–0.155)
	W212X	636G>A[f]	F(G) = 0.938 (0.885–0.971)	63/72	9/72	0/72
			F(A) = 0.062 (0.029–0.115)	0.875 (0.776–0.941)	0.125 (0.059–0.224)	0 (0–0.050)
CYP2D6	Gene deleted		F(wt) = 0.923 (0.866–0.961)	61/71	9/71	1/71
			F(del) = 0.077 (0.039–0.134)	0.859 (0.756–0.930)	0.127 (0.060–0.227)	0.014 (0.0003–0.076)
	P34S	100C>T[f]	F(C) = 0.549 (0.467–0.631)	17/71	44/71	10/71
			F(T) = 0.451 (0.369–0.533)	0.239 (0.146–0.355)	0.620 (0.497–0.732)	0.141 (0.070–0.244)
	Splicing defect	1846G>A[g]	F(G) = 0.986 (0.949–0.998)	67/69	2/69	0/69
			F(A) = 0.014 (0.002–0.051)	0.971 (0.899–0.997)	0.029 (0.003–0.101)	0 (0–0.052)
CYP3A4	Promoter region	-392A>G[g]	F(A) = 0.979 (0.940–0.996)	69/72	3/72	0/72
			F(G) = 0.021 (0.004–0.060)	0.958 (0.883–0.991)	0.042 (0.009–0.117)	0 (0–0.050)
CYP3A5	Splicing defect	6986A>G[g]	F(A) = 0.333 (0.256–0.410)	7/72	34/72	31/72
			F(G) = 0.667 (0.590–0.744)	0.097 (0.040–0.190)	0.472 (0.353–0.593)	0.431 (0.314–0.553)
	Splicing defect	14690G>A[g]	F(G) = 1 (0.975–1)	72/72	0/72	0/72
			F(A) = 0 (0–0.025)	1 (0.950–1)	0 (0–0.050)	0 (0–0.050)
	346Frameshift	27131_27132. insT[g]	F(T) = 1 (0.975–1)	72/72	0/72	0/72
			F(TT) = 0 (0–0.025)	1 (0.950–1)	0 (0–0.050)	0 (0–0.050)
	R28C	3699C>T[g]	F(C) = 1 (0.975–1)	72/72	0/72	0/72
			F(T) = 0 (0–0.025)	1 (0.950–1)	0 (0–0.050)	0 (0–0.050)
MDR1	Syn[e]	3435C>T[f]	F(C) = 0.597 (0.517–0.677)	23/72	40/72	9/72
			F(T) = 0.403 (0.323–0.483)	0.319 (0.214–0.440)	0.556 (0.434–0.673)	0.125 (0.059–0.224)

CYP, Cytochrome P450; MDR1, multi-drug resistance 1 gene. Value in parenthesis is the 95% confidence interval (95% CI)

[a] Three of the analysed subjects were of Thai origin— their inclusion in the study did not change any of its conclusions.

[b] Description as represented by the cytochrome P450 (CYP) International Allele Nomenclature Committee (http://www.cypalleles.ki.se/)

[c] Wild-type, defined as the sequence of reference, representing the group of all the remaining alleles not detected by the employed methods

[d] Mutant, defined as the alternative sequence

[e] Synonymous (Ile1145Ile)

[f] cDNA position

[g] gDNA position

Table 2 Allele frequencies of 72 Vietnamese healthy subjects[a]. Comparison with documented data in Southeast and Far East Asian populations

Gene	Haplotype[b]	Vietnam	*n* [ref] [c]	Korea	*P* value	*n* [ref] [c]	Malaysia	*P* value	*n* [ref] [c]	China	*P* value	*n* [ref] [c]	Thailand	*P* value	*n* [ref][c]	Japan	*P* value
CYP2A6	**1*	0.736	209 [24]	0.885		540 [25]	0.916		344 [25]	0.937		198 [1]	0.922		[26]	0.78–0.83	
	**4* (gene deletion)	0.118		0.110	0.866		0.074	0.133		0.051	0.023**		0.078	0.264		0.20–0.31	No data
	**5* (1436G>T)	0.146		0.005	<0.0001**		0.010	<0.0001**		0.012	<0.0001**					0.0	
CYP2B6	**1*	0.646	316						1014	0.655					530	0.732	
	**4* (785A>G)	0.083	[27]	0.050	0.214				[28]						[29]	0.093	0.871
	**5* (1459C>T)	0														0.011	
	**6*(516G>T+785A>G)	0.271		0.120	0.002**					0.345	0.474					0.164	0.021**
CYP2C19	**1*	0.632	200	0.67		54	0.720		200	0.668		107	0.710		200	0.565	
	**2* (681G>A)	0.306	[30]	0.25	0.412	[31]	0.230	0.490	[30]	0.297	0.910	[32]	0.270	0.688	[30]	0.345	0.660
	**3* (636G>A)	0.063		0.08	0.676		0.050	0.680		0.035	0.305		0.020	0.129		0.090	0.424
CYP2D6	**1*	0.471	200	0.415		138	0.538		223	0.413					200	0.490	
	**4* (100C>T+1846G>A)	0.014	[30]	0.005	0.570	[33]	0.04	0.447	[34]	0.002	0.561				[30]	0.005	0.570
	**5* (gene deletion)	0.080		0.075	0.642		0.02	0.053		0.072	0.839					0.070	0.834
	**10* (100C>T)	0.435		0.505	0.494		0.402	0.740		0.513	0.445					0.435	0.540
CYP3A4	**1*A	0.979	186	1					200	1		320	0.991		160	1	
	**1*B (-392A>G)	0.021	[30]	0					[30]	0		[35]	0.009	0.383	[30]	0	
CYP3A5	**1*	0.333	486	0.221					200	0.277		320	0.331		530	0.260	
	**3* (6986A>G)	0.667	[36]	0.780	0.303				[30]	0.723	0.670	[35]	0.669	0.525	[29]	0.740	0.509
MDR1	3435C	0.597	632	0.607		92	0.630		100	0.545					160	0.556	
	3435T	0.403	[13]	0.393	0.863	[37]	0.370	0.801	[38]	0.455	0.635				[39]	0.444	0.674

**Significant differences ($P<0.05$) between the Vietnamese and the other Far East Asian populations

[a] Three of the analysed subjects were of Thai origin—their inclusion in the study did not change any of the conclusions

[b] Note that the allele frequencies presented in Table 2 represent the frequencies of CYP alleles defined in several cases as haplotypes of SNPs

[c] Number of studied subjects

Table 3 Genotype association to pharmacokinetic metrics in 74 healthy Vietnamese subjects

Gene	Genotype	*n*	Pharmacokinetic metrics[a]	Standard deviation	Standard error	Significance (One-way original scale)	
						Pairwise significant from genotype	ANOVA
CYP2A6 [b]	Coumarin						
	**1*1*	40	2.32 (2.02–2.63)	0.95	0.15	**1*4*	0.011
	**1*4*	11	1.21 (0.65–1.77)	0.83	0.25	**1*5*	
	**1*5*	16	2.29 (1.78–2.79)	0.95	0.24		
	**4D*5*	7	1.93 (0.71–3.15)	1.32	0.50		
CYP2C19	Mephenytoin						
	**1*1*	26	3.85 (2.87–4.83)	2.43	0.48	**1*2.*2*2.*2*3*	<0.001
	**1*2*	33	1.27 (0.95–1.59)	0.90	0.16		
	**1*3*	6	1.77 (0.71–2.84)	1.01	0.41		
	**2*2*	6	0.00	0.00	0.00		
	**2*3*	3	0.00	0.00	0.00		
CYP2D6[c]	Metoprolol						
	**1*1*	12	1.11 (0.81–1.41)	0.47	0.14	**1*10.*5*10. *10*10*	<0.001
	**1*4*	2	0.07 (-0.86–1.01)	0.10	0.07	**1*5*	
	**1*5*	3	2.06 (-4.40–8.52)	2.60	1.50	**1*10. *5*10. *10*10*	
	**1*10*	37	0.53 (0.41–0.64)	0.34	0.06		
	**5*5*	1	.	.	.	Not tested	
	**5*10*	6	0.25 (-0.03–0.53)	0.27	0.11		
	**10*10*	10	0.15 (0.12–0.17)	0.04	0.01		
CYP3As	Midazolam						
	**1A*1A/*1*1*	7	25.16 (19.56–30.77)	6.06	2.29		0.218
	**1A*1A/*3*3*	31	30.58 (25.90–35.25)	12.74	2.29		
	**1A*1A/*1*3*	33	25.59 (22.20–28.98)	9.56	1.66		
	**1A*1B/*3*3*	3	22.63 (10.43–34.83)	4.91	2.83		

Results are from a one-way analysis of variance (ANOVA; original scale). Pairwise significant differences between genotypes are given (Bonferroni adjusted post-hoc tests)

[a] Presented as the arithmetic mean, with the 95% confidence interval (95% CI) given in parenthesis. The amount of urinary excreted 7-OH-coumarin (mg) 0–8 h after oral intake of 5 mg coumarin (CYP2A6), S-4'-OH-mephenytoin/S-mephenytoin 4-h plasma concentration ratio after oral intake of 100 mg mephenytoin (CYP2C19), α-OH-metoprolol/metoprolol 4-h plasma concentration ratio after oral intake of 100 mg metoprolol (CYP2D6), midazolam 4-h plasma concentration (ng/mL) after oral intake of 7.5 mg midazolam (CYP3A)

[b] The genotype/pharmacokinetic metric association concerning CYP2A6 were found to be probably spurious. The two SNPs analysed result in no enzyme activity. The haplotype *1*5 and *4D*5 does not follow the expected trend.

[c] The genotype was only available for 71 subjects.

the pharmacokinetic results are presented as a complement to genetic studies in order to provide some illustration of how the metrics at hand vary with genotype in our study group of Vietnamese subjects. Any conclusions based on the results from this part of the study should, however, be drawn bearing these limitations in mind.

CYP2D6 genotypes were significantly associated with the α-OH-metoprolol/metoprolol concentration ratio ($P<0.001$) (Table 3). The predictive values of some of the analysed SNPs were found to be debatable in this population, particularly those associated with the *CYP2D6*10* allele, with some individuals heterozygous for this allele exhibiting metabolic ratios similar to those observed for subjects with the wild-type homozygous genotype. One explanation for this result may be the presence of *CYP2D6* duplications in one of the chromosomes. It should also be noted that the discriminative SNP 100C>T used for identifying *CYP2D6*10* (amino acid change: P34S) is also present on other *CYP2D6* rare alleles not analysed in our study (**36*, **37*, **47*, **49*, **52*, **54*) and whose effects on metabolic activity are not well defined.

The strong association ($P<0.001$) between genotype and S-4'-OH-mephenytoin/S-mephenytoin ratio found here supports the validity of **2* and **3* allele analysis for the prediction of the activity of CYP2C19 in the studied population.

The results of the CYP2A6 genotype/pharmacokinetic metric analysis are most likely spurious, with the amounts of excreted 7-OH-coumarin not coherent with the mutations known to severely affect the coded enzyme. Various factors, such as variations in the urine collection, interactions of coumarin with the other probe compounds in the cocktail or the fact that smokers were included in the study, could have confounded the urinary excretion of 7-OH-

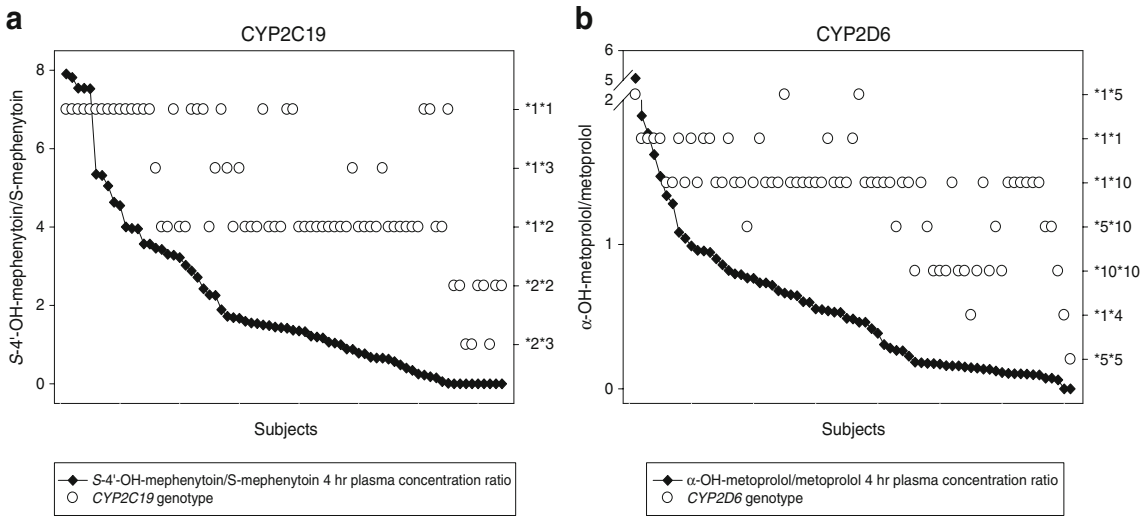

Fig. 2 Pharmacokinetic metrics and respective distribution of analysed genotypes in healthy Vietnamese subjects. **a** S-4'-OH-mephenytoin/S-mephenytoin 4-h plasma concentration ratio after oral intake of 100 mg mephenytoin vs. *CYP2C19* alleles in 74 subjects. **b** α-OH-metoprolol/metoprolol 4-h plasma concentration ratio after oral intake of 100 mg vs. *CYP2D6* alleles in 71 subjects (genotype only available for 71 subjects). Note that pharmacokinetic metrics employed were based on feasibility and may not fully reflect enzyme activity

coumarin as a metric for CYP2A6 activity. In addition, total urinary excretion of a metabolite is an indirect measure of enzyme activity—and not a specific metric to reflect intrinsic clearance of a drug [15]. Urinary excretion of 7-OH-coumarin may, therefore, be a questionable pharmacokinetic metric to relate to *CYP2A6* genotype.

The *CYP3A4* promoter SNP (-392A>G) [16], was not associated (P=0.218) to midazolam 4-h plasma concentrations, thereby confirming reports, where no association with the metabolism of this probe drug has been demonstrated [17, 18]. On the other hand, limitations of using single point measurements of midazolam to predict CYP3A activity have been reported [19], and clearance or the area under the plasma concentration versus time curve of midazolam have been suggested as preferred CYP3A metrics.

The only *CYP3A5* SNP that we found (6986A>G, *CYP3A5*3*) results in aberrant mRNA splicing and a pronounced reduction in protein synthesis with unequivocal effects on the gene product. However, this mutation did not predict the midazolam 4-h plasma concentrations, as has been confirmed by others [17, 20], questioning the use of this SNP as a reliable marker for CYP3A activity.

The results from previous studies have suggested that the populations of Southeast Asia, although carrying typical pharmacogenetic patterns of Asian populations, have specific characteristics [21]. We found the pharmacogenetic pattern in our study group of the Vietnamese population to be in general agreement with that reported for other Asian populations, as previously observed among Vietnamese for the *MDR1* and *CYP2C9* genes [13, 22]. However, some diverging trends were detected (*CYP2A6*4*, *CYP2A6*5* and *CYP2B6*6*), pointing to a non-negligible degree of diversity between these populations. This observation follows our recent investigations documenting an unusual prevalence of the N-acetyltransferase 2 (*NAT2*) allele in this population [23].

In conclusion, our study reinforces the viewpoint that, in parallel with the recent massive whole genome approaches, limited but population-specific studies are fundamental for our understanding of global pharmacogenetics.

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