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Exploration of CYP450 and drug transporter genotypes and correlations with nevirapine exposure in Malawians

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Ethical conduct of research
The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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Abstract

Aim—Genetic polymorphisms have the potential to influence drug metabolism and vary among ethnic groups. This study evaluated the correlation of genetic polymorphisms with nevirapine pharmacokinetics exposure in Malawians.

Materials & methods—*CYP450 2B6, 2D6, 3A4* and *3A5, ABCB1* and constitutive androstane receptor and pregnane X receptor, were analyzed for polymorphisms in 26 subjects.

Results—Allele frequencies (variant) were: *CYP2B6* 514G>T (0.31) *CYP2D6*4* (0.02); *CYP2D6*17* (0.35); *CYP3A4*1B* (0.77); *CYP3A5*3* (0.25); *ABCB1* 2677G>T (0.0), *ABCB1* 3435C>T (0.21), *NR1I3* 13711152T>C (0.02), *NR1I2* 44477T>C (0.10), *NR1I2* 63396C>T (0.33), *NR1I2* 6-bp indel (del: 0.17). *CYP2B6* 516G>T (non-wild-type/wild-type) correlated with nevirapine pharmacokinetic parameters; geometric mean ratios (95% CI): 1.75 (1.27–2.40) for area under the concentration time curve $(AUC)_{0–12\ h}$, 1.58 (1.03–2.42) for C_0, and 0.53 (0.31–0.91) for clearance. In a multivariable model, nevirapine AUC increased by 1.5% per year of age ($p < 0.0001$), *CYP2B6* 516 T allele increased AUC by 92% ($p < 0.0001$), and *CYP3A5*3* decreased AUC by 31% ($p = 0.0027$).

Conclusion—Allele frequencies were similar to other sub-Saharan African populations. The T allele for *CYP2B6* 516 was significantly associated with nevirapine exposure.

Keywords

CYP2B6; CYP450; Malawi; nevirapine; nuclear receptor; P-glycoprotein; pharmacokinetics

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Genetic polymorphisms have been identified in genes that encode drug-metabolizing enzymes and transporters such as the CYP450s (CYPs; *CYP*s) and P-glycoproteins (MDR1; *ABCB1*) [1]. These polymorphisms may explain interindividual differences in drug pharmacokinetics [1]. Patients with genotypes that produce less active enzymes in major drug metabolic pathways have reduced metabolism and therefore higher drug exposures and potentially more adverse effects. Patients with activity-enhancing genotypes clear drugs faster, which can lead to subtherapeutic concentrations and increased risk of therapy failure [1]. Specifically, *CYP* polymorphisms have been associated with interpatient variation in antiretroviral plasma concentrations, and the allele frequencies vary among ethnic groups [2,3].

HIV-infected patients on antiretroviral therapy have demonstrated altered plasma concentrations due to differing CYP450 enzyme genotypes [2,4,5]. These genotypes have

also shown differing frequencies based on ethnicity [6]. A Ugandan population has been shown to have significantly higher drug exposure compared to western populations; this can partially be explained by different frequencies of genetic polymorphisms in drug-metabolizing enzymes and transporters [7].

Antiviral therapy is expanding in developing countries using doses optimized for western populations. We recently found that steady-state nevirapine concentrations were significantly increased in a group of Malawian adults and children compared to concentrations seen in a mostly western Caucasian cohort of adults and children [8–11]. The 1.6- to 2.2-fold increase in drug exposure could not be explained by weight, age or other observable patient-specific factors. Nevirapine is non-nucleoside reverse transcriptase inhibitor used for the treatment of HIV infections in adults and children. It is metabolized by the CYP450 system, specifically CYP3A4/5, CYP2D6 and CYP2B6 [12], and data suggest that polymorphisms in the genes encoding these enzymes can alter nevirapine concentrations [2,7,13–18]. The clinical impact of the *CYP2B6* polymorphism, 516G>T, has been described in various settings. The use of single-dose nevirapine to prevent perinatal vertical transmission has been shown to have prolonged exposures in women with the *CYP2B6* 516 T allele, which greatly increases the risk of developing non-nucleoside reverse transcriptase inhibitor resistance mutations [18].

Nevirapine autoinduces its metabolism by activating *CYP3A4/5* and *CYP2B6* expression, and data show that nevirapine may do this through the activation of the nuclear receptor, constitutive androstane receptor (CAR; NR1I3) [19]. The nuclear receptor pregnane X receptor (PXR; NR1I2) regulates basal expression of CYP3A4/5 [20]. Polymorphisms in the genes coding for these nuclear receptors have been shown to influence the expression of the CYPs and therefore influence nevirapine exposure [19–21].

Frequencies of polymorphisms in CYP3A4/5, CYP2B6, ABCB1, PXR and CAR can vary between ethnic groups [22], which might contribute to the difference in drug exposure we previously observed between Malawians and western subjects [2,8,9]. However, the frequencies of the polymorphisms in the abovementioned genes have not been reported in a Malawian population.

Materials & methods

Study design

This was a nonrandomized pharmacogenetic observational study in HIV-infected Malawian children and adults who participated in pharmacokinetic studies evaluating nevirapine [8,9]. The University of North Carolina Institutional Review Board and the Malawi National Health Sciences Research Committee approved the study protocol. All research subjects provided consent or assent with guardian consent. The objective was to determine the frequencies of *CYP*, *ABCB1* and nuclear receptor polymorphisms in 30 Malawian adults and children and to explore correlations between their genotypes and nevirapine (Virammune®) exposure.

Samples & DNA extraction

Nevirapine concentrations were measured in blood plasma by a validated HPLC-UV method as previously described [8,9,23].

DNA was isolated from whole blood using the QIAamp® DNA Mini kit (Qiagen Sciences, MD, USA). Genotyping was performed using PCR and Pyrosequencing® as previously described [24]. Primers and PCR conditions for *ABCB1* 2677 (rs2032582) and 3435 (rs1045642), *CYP2B6* 516G>T (Q172H; rs3745274), *CYP2D6*4* (rs3892097), *CYP3A4*1B*

(rs2740574), and *CYP3A5*3* (rs776746) were previously described [24–27]. For *CYP2D6*17* (rs28371706), DNA was amplified using the forward primer: 5′-GAG GCG CTG GTG ACC CAC-3′ and biotinylated reverse primers: 5′-biotin-CTG TCC CCA CCG CTG CTT-3′ at an annealing temperature of 65°C. The internal primer was 5′-CGC CTG TGC CCA TCA-3′ and the sequence to analyze was C/TCCAGATCC.

NR1I2 44477T>C (rs1523130), 63396C>T (rs2472677) and the 6-bp deletion (6-bp indel; rs3842689) were genotyped by previously described methods [20]. The *trans NR1I3* polymorphism, rs10494390 (NT_004487.18:g.13711152T>C), was amplified from 50 ng of genomic DNA by the forward 5′-ACCTTCGGGGTTGAATTTT-3′ and reverse 5′-TGTAGCATTTGGGTTTTGGA-3′ primers.

Data analysis/statistics

Genotype data from the Malawi sample was compared to genotype data of Yoruban (sub-Saharan African), African–American and Caucasians of European descent obtained from the HapMap database. Fisher's Exact Test was used to determine whether the Malawian sample's genotype frequencies differed significantly from the archived data. The Cochran–Armitage Trend Test was employed to determine if a genotype trend could be detected between groups.

The pharmacokinetic parameters were generated by noncompartmental analysis using WinNonlin (v5.2, Pharsight, Inc., NC, USA). Area under the concentration time curve (AUC) $_{0–12\ h}$ was extrapolated from partial dosing interval data (adults: 0–8 h, children: 0–6 h). Weight-adjusted (kg) apparent oral clearance (CL/F) was determined to confirm AUC results of the pediatric subjects. Trough concentrations (C_0) are predose concentrations. Statistical analysis was performed using SAS (v9.1.3, SAS Institute, NC, USA). Geometric mean ratios (GMRs) of non-wild-type (WT) versus WT genotypes were calculated using linear regression of natural log transformed pharmacokinetic parameters with α significance levels of 5% and 0.625%; the latter to account for multiple statistical tests for the eight genotypes tested. Multiple linear regression was performed to develop a multiple variable model ($\alpha = 0.05$) with AUC as outcome and genotypes and demographics as covariates. WT genotypes are defined as genotypes that are homozygous for the WT allele, and non-WT genotypes are defined as genotypes that are either heterozygous or homozygous for the variant (VAR) allele. WT alleles are defined by having functional enzyme gene products, often designated as **1* for the *CYP*s, or having the highest allele frequency in this study sample.

Results

DNA was isolated from 24 Malawian subjects, of whom 11 were adults with a median (range) age of 40 (26–46) years, weight of 72 (60–80) kg, BMI of 25.4 (19.8–32.5) kg/m^2 and five were male. Thirteen were children with median (range) age of 6.7 (1.3–13.6) years, weight of 18 (9–30.5) kg, BMI z-score of 0.31 (−5.39–1.28) and six were male [8,9]. Subjects were also taking trade formulations of lamivudine (Epivir®) and stavudine (Zerit®) at the time of pharmacokinetic sampling. Nevirapine was dosed using standard dosing in adults at 200 mg twice daily. The pediatric group was dosed using standard weight-based dosing, in which children up to 8 years of age are dosed 7 mg/kg twice daily and children 8 years of age and older are dose 4 mg/kg twice daily.

Genotype frequencies of 26 Malawians are presented in Table 1; genetic sampling included two subjects without pharmacokinetic data. The genotypes could not be reliably tested for Hardy–Weinberg equilibrium due to a small sample size. The Malawian genotype frequencies did not differ from Yorubans. The Malawian's *CYP2D6*17* and *ABCB1*

2677G>T genotype data differed from that of the African–American individuals; other genotypes tested did not differ. The Malawian genotype frequencies differed from Caucasians in regards to all genotypes tested except for *CYP2B6* 516G>T (p = 0.31). The VAR allele frequencies of *CYP3A4*1B* and *CYP2D6*17* were higher and *CYP3A5*3*, *CYP2D6*4*, *ABCB1* 2677G>T, *ABCB1* 3435C>T, *NR1I3* 13711152T>C, *NR1I2* 44477T>C, 63396C>T and 6-bp indel were lower in the Malawians than Caucasians.

The results of the correlation analysis are listed in Table 2. The pharmacokinetic data were log normally distributed. With $\alpha = 0.05$, the *CYP2B6* 516 T allele was significantly associated with increased AUC, C_0 and CL/F. With $\alpha = 0.00625$ for multiple comparison correction, this significance was lost . The *NR1I2* 6-bp indel deletion allele was significantly associated with decreased $AUC_{0–12\ h}$ and increased CL/F with $\alpha = 0.05$; however, this lost significance with $\alpha = 0.00625$.

Figure 1 shows a decrease in weight-based CL/F with the number of *CYP2D6*17* alleles within the pediatric group. This was not seen in the adult subjects (Figure 1b). In order to confirm the effect of *CYP2D6*17* on CL/F in the pediatric group, the GMR for CL/F was determined separately in the pediatric and adult groups. The GMR (VAR/WT; 95% CI) for *CYP2D6*17* was 0.59 (0.36–0.98; p = 0.04) and 1.02 (0.61–1.70; p = 0.9) for children and adults, respectively, demonstrating that *CYP2D6*17* is associated with decreased clearance only in the pediatric group of our sample.

The following multiple variable model was determined for nevirapine AUC: $Ln(AUC_{0–12\ h}) = 10.67 + 0.65\ (2B6Var) – 0.37\ (3A5Var) + 0.015\ (age)$. The *CYP2B6* 516 T allele increases $AUC_{0–12\ h}$ by 92% (95% CI: 51–145%), *CYP3A5*3* allele decreases $AUC_{0–12\ h}$ by 31% (95% CI: 12–46%) and age increases $AUC_{0–12\ h}$ by 1.5% (95% CI: 0.7–2%) per year. Table 3 shows the p-values for each of the variables tested. The effect of the *PXR* 6-bp indel on nevirapine AUC was not significant in the final multiple variable model despite significance ($\alpha = 0.05$) seen in the univariate analysis. A similar model was determined for weight-adjusted CL/F (not shown).

Discussion

CYP2B6 genotype is associated with intra-population differences in Malawians. Consistent with previous studies [7], the T allele at *CYP2B6* 516 correlated with increased exposure and decreased clearance of nevirapine. Since our study and others have demonstrated association of the T allele with increased nevirapine exposures and varying frequencies of *CYP* polymorphisms among ethnic groups, antiretrovirals must be evaluated in all potential treatment populations [2,7].

Drug-metabolizing enzyme and transporter genotype data from our sample agree with other African populations as reported in the HapMap database (Luhya in Webuye, Kenya; Maasai in Kinyawa, Kenya and the Yoruban in Ibadan, Nigeria) [22]. For example, *CYP2B6* 516 T allele frequencies ranged from 0.315 to 0.42 for the African groups in the HapMap database; the Malawian frequency was 0.31. For *CYP3A5*3*, the HapMap database reports a wide range of allele frequencies: 0.128–0.486 (Malawians frequency: 0.25), which demonstrates sub-Saharan African ethnic groups could have dissimilar *CYP* allele frequencies. This may be clinically relevant to drugs metabolized by these CYP enzymes.

Following the multiple comparisons correction, *CYP2B6* 516 T allele correlated with increased AUC. *CYP2B6* and age were independently associated with nevirapine AUC, C_0 and CL/F. *CYP3A5*3* is an allele that causes decreased function of *CYP3A5* and is expected to decrease clearance of nevirapine, thereby increasing its exposure. Although there was no correlation in the univariate analysis, *CYP3A5*3* was associated with decreased AUC when

controlling for *CYP2B6* and age. *CYP3A5*3* correlating with lower nevirapine exposure may be a result of heterozygous individuals in our sample expressing more enzyme than homozygous WT individuals and/or *CYP3A5*3* being in linkage with another functional allele [28]. In addition, our sample did not include individuals who were *CYP3A5*3/*3*, which is a genotype found only in Africans based on the HapMap data, which may introduce bias in our model.

The *CYP2D6*17* allele appears to be specific to African populations and is associated with decreased CYP2D6 enzyme activity [3]. Only within the pediatric group did *CYP2D6*17* correlate with a decrease in weight-adjusted nevirapine CL/F, suggesting that CYP2D6 may play a larger role in nevirapine metabolism in children. However, this would need to be confirmed in a larger study population accounting for other *CYP2D6* SNPs and other polymorphisms in linkage disequilibrium.

CAR and PXR are functionally linked with the expression of various CYPs [19]. Nevirapine preferentially induces CYP2B6 through activation of CAR [19], and polymorphisms in *PXR* are associated with higher basal expression and reduced induction of CYP3A4 activity [20]. Polymorphisms in *NR1I3* and *NR1I2* may attenuate the induction of CYP2B6 and CYP3A5 leading to higher nevirapine exposures; however, our study did not demonstrate this. The *NR1I2* 6-bp indel, which is in linkage disequilibrium with *NR1I2* 44477T>C, was associated with lower nevirapine exposures, which may indicate increased basal expression of CYP3A4.

The multivariable model showed that nevi-rapine concentrations were influenced by *CYP2B6* and *CYP3A5* genotypes in addition to age. Currently there is limited clinical utility for this model, due to a lack of widely available genotypic testing for these enzymes in all populations. In addition, there is limited data linking genotype, concentration and safety and/or efficacy. Despite this, the clinical implications of this model include that it is possible to predict nevirapine exposures based on genotype. With more advanced modeling methods, nevi-rapine concentrations could be predicted based on genotype and other known patient factors in order to predict adverse effects such as rash due to supratherapeutic concentrations or virologic failure due to subtherapeutic concentrations. However, these models will also be limited to the groups in which they are studied. In addition, this is a potentially useful tool for research purposes in which outliers of drug concentrations exist with no obvious decreased adherence or extra dosing.

The US Department of Health and Human Services (US DHHS) currently recommends a target nevirapine trough plasma concentration of 3 μg/ml based on data demonstrating a five-fold increase in virologic failure risk with lower trough concentrations [29,30,101]. These data were developed in a Caucasian population with the same doses currently being used in African populations. The Malawian and other sub-Saharan African HIV-infected patients would benefit greatly from efficacy and safety studies focusing on their populations since there is an overwhelming amount of data demonstrating that these groups have genetic differences that affect how the drugs are metabolized and eliminated.

Factors such as nutritional status and diet can also affect drug metabolism. Low protein and high carbohydrate consumption are associated with decreased hepatic CYP metabolism, while high protein diets are associated with increased metabolism [31]. Our subjects had normal nutritional status based on BMI, except for one underweight child. Western diets are generally higher in animal protein than those in sub-Saharan Africa, while diets in Malawi are considerably lower in animal protein and include carbohydrate-rich protein sources [102]. This dietary factor may contribute to the differences observed in nevirapine exposure between Malawians and westerners.

Despite our limited sample size, our novel pilot data provide evidence that ethnic diversity of drug-metabolizing enzyme genetics may play a role in antiretroviral drug exposures. Larger studies are needed to define population allele frequencies and to evaluate the clinical impact of drug-metabolizing enzymes, transporter and nuclear receptor polymorphisms.

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Executive summary

Nevirapine pharmacokinetics in Malawians

- Previously studied HIV-infected adults and children had 1.6- to 2-fold higher exposures compared to published data in western cohorts.
- Drug exposures can be influenced by polymorphisms in genes encoding enzymes, transporters and receptors that are involved in drug metabolism and elimination.

Aim

- We sought to identify the allele frequencies of known genetic polymorphisms in drug-metabolizing enzymes, transporters and nuclear receptor genes in Malawians and evaluate the influence of these genetic polymorphisms on nevirapine pharmacokinetics.

Allele frequency & genotype correlation with nevirapine pharmacokinetics

- Malawian allele frequencies were similar to other sub-Saharan Africans, but differed from Caucasians.
- The *CYP2B6* 516 T allele was significantly correlated with increased nevirapine exposures.
- *CYP2D6*17* was associated with increased clearance only in children.
- In a multivariable model, nevirapine AUC increased by 1.5% with each year increase in age, increased by 92% with the presence of the *CYP2B6* 516 T allele and decreased by 31% with the presence of *CYP3A5*3.*

Conclusion

- Genetic polymorphisms influence nevirapine metabolism in Malawians.
- Larger studies are needed to evaluate the clinical impact of genetic variability among ethnic groups.

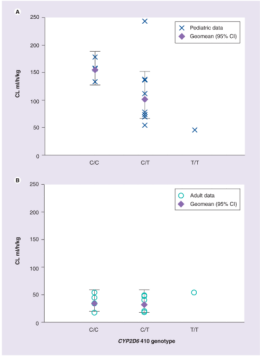

Figure 1. Weight-adjusted clearance versus *CYP2D6*17* genotype (17* = T allele) in the pediatric group and adult group**

(A) Pediatric group, **(B)** Adult group.

CL: Clearance.

Table 1

Genotype frequencies for polymorphisms in *CYPs*, *ABCB1* and nuclear receptors in Malawian subjects in comparison to sub-Saharan African, African–American and Caucasian populations using Fisher's Exact Test and Cochran–Armitage Trend Test.

Allele	Allele SNP		Genotype frequency					
	WT	VAR	Malawian†	YRI: sub-Saharan		ASW: African–American		CEU: Caucasian	
CYP2B6 516	G	T	GG: 0.54	GG: 0.34	(n = 146)	GG: 0.47	(n = 57)	GG: 0.53	(n = 113)
rs3745274			GT: 0.31	GT: 0.49	F: p = 0.14	GT: 0.46	F: p = 0.30	GT: 0.4	F: p = 0.31
			TT: 0.15	TT: 0.18		TT: 0.07		TT: 0.07	
*CYP2D6*4*	G	A	GG: 0.96	GG: 0.93	(n = 118)‡	GG: 0.82	(n = 144)§	GG: 0.58	(n = 118)‡
rs3892097			GA: 0.04	GA: 0.7	F: p = 1	GA: 0.17	F: p = 0.17	GA: 0.37	F: p < 0.001
			AA: 0.0	AA: 0.0		AA: 0.01		AA: 0.05	T: p < 0.001
*CYP2D6*17*	C	T	CC: 0.38	CC: 0.61	(n = 118)‡	CC: 0.75	(n = 118)§	CC: 1.0	(n = 120)‡
rs28371706			CT: 0.54	CT: 0.31	F: p = 0.07	CT: 0.2	F: p < 0.001	CT: 0.0	F: p < 0.001
			TT: 0.08	TT: 0.08		TT: 0.04	T: p = 0.002	TT: 0.0	T: p < 0.001
*CYP3A4*1B*	A	G	AA: 0.04	AA: 0.03	(n = 118)‡	NR		AA: 0.95	(n = 120)‡
rs2740574			AG: 0.38	AG: 0.44	F: p = 0.86			AG: 0.05	F: p < 0.001
			GG: 0.58	GG: 0.53				GG: 0.0	T: p < 0.001
*CYP3A5*3*	A	G	AA: 0.5	AA: 0.68	(n = 147)	AA: 0.37	(n = 57)	AA: 0.0	(n = 111)
rs776746			AG: 0.5	AG: 0.31	F: p = 0.22	AG: 0.54	F: p = 0.24	AG: 0.07	F: p < 0.001
			GG: 0.0	GG: 0.01		GG: 0.09		GG: 0.93	T: p < 0.001
ABCB1 2677	G	T	GG: 1.0	GG: 1.0	(n = 120)‡	GG: 0.81	(n = 57)	GG: 0.26	(n = 113)
rs2032582			GT: 0.0	GT: 0.0	F: NS	GT: 0.18	F: p = 0.04	GT: 0.55	F: p < 0.001
			TT: 0.0	TT: 0.0		TT: 0.02	T: p = 0.02	TT: 0.19	T: p < 0.001
ABCB1 3435	C	T	CC: 0.62	CC: 0.8	(n = 145)	CC: 0.63	(n = 56)	CC: 0.15	(n = 113)
rs1045642			CT: 0.35	CT: 0.19	F: p = 0.09	CT: 0.34	F: p = 1	CT: 0.56	F: p < 0.001
			TT: 0.04	TT: 0.01		TT: 0.04		TT: 0.29	T: p < 0.001
NR1I3 13711152	T	C	TT 0.96	TT: 0.92	(n = 147)	TT: 0.81	(n = 57)	TT: 0.65	(n = 113)

Allele	Allele SNP		Genotype frequency					
	WT	VAR	Malawian†	YRI: sub-Saharan		ASW: African–American		CEU: Caucasian	
rs10494390			TC 0.04	TC: 0.07	F: p = 0.74	TC: 0.19	F: p = 0.09	TC: 0.33	F: p = 0.002
			CC 0.0	CC: 0.01		CC: 0		CC: 0.03	T: p = 0.002
NR1I2 44477	T	C	TT: 0.8¶	TT: 0.85	(n = 147)	TT: 0.75	(n = 56)	TT: 0.13	(n = 112)
rs1523130			TC: 0.2¶	TC: 0.14	F: p = 0.61	TC: 0.25	F: p = 0.77	TC: 0.46	F: p < 0.001
			CC: 0.0¶	CC: 0.01		CC: 0.0		CC: 0.42	T: p < 0.001
NR1I2 63396	C	T	CC: 0.5	CC: 0.34	(n = 62)	NR		CC: 0.14	(n = 64)
rs2472677			CT: 0.35	CT: 0.6	F: p = 0.07			CT: 0.44	F: p = 0.001
			TT: 0.15	TT: 0.06				TT: 0.42	T: p < 0.001
NR1I2 6-bp indel	GAGAAG	–	+/+ 0.69	NR		NR		NR	
rs3842689			+/− 0.27						
			−/− 0.04						

WT and VAR nucleotides listed for reference. Comparison population data from International HapMap Project [12], unless otherwise indicated.

†Genotype data from n = 26 available.

‡International HapMap Project [12] data reported on [103].

§Human Genome Diversity Panel [20] data reported on [103].

¶Genotype data from n = 20 available.

ASW: African ancestry in southwest USA; CEU: Utah residents with northern and western European ancestry from the CEPH collection; F: Fisher's Exact Test; NR: Not reported; NS: Nonsignificant; T: Cochran–Armitage Trend Test; VAR: Variant allele; WT: Wild-type allele; YRI: Yoruba in Ibadan, Nigeria.

Table 2

Pharmacokinetic parameter geometric mean ratios (non-wild-type or variant versus wild-type).

Polymorphism	NVP $AUC_{0–12\ h}$	NVP $AUC_{0–6\ h}$ †	NVP C_0	CL/F‡
CYP2B6 516G>T	1.75*	1.71*	1.58**	0.53**
rs3745274	(1.27–2.40)	(1.25–2.34)	(1.03–2.42)	(0.31–0.91)
*CYP3A4*1B*§	0.86	0.87	0.87	0.96
rs2740574	(0.59–1.27)	(0.60–1.27)	(0.55–1.39)	(0.53–1.74)
*CYP3A5*3*	0.91	0.92	0.93	1.03
rs776746	(0.62–1.33)	(0.63–1.35)	(0.59–1.48)	(0.57–1.86)
*CYP2D6*17*	1.13	1.11	1.06	0.94
rs28371706	(0.76–1.68)	(0.75–1.64)	(0.65–1.70)	(0.51–1.72)
ABCB1 3435C>T	1.10	1.07	0.98	0.83
rs1045642	(0.75–1.63)	(0.73–1.58)	(0.61–1.57)	(0.46–1.50)
NR1I2 44477T>C	0.80	0.81	0.90	1.21
rs1523130	(0.50–1.28)	(0.51–1.27)	(0.51–1.59)	(0.58–2.49)
NR1I2 63396C>T	0.80	0.79	0.82	1.35
rs2472677	(0.55–1.17)	(0.55–1.14)	(0.52–1.30)	(0.76–2.42)
NR1I2 6-bp indel	0.67**	0.71	0.66	1.86**
rs3842689	(0.45–0.999)	(0.48–1.05)	(0.41–1.07)	(1.02–3.39)

The geometric mean ratios (95% CI) for nevirapine $AUC_{0–12\ h}$, $AUC_{0–6\ h}$, C_0 and CL/F comparing data from subjects (n = 24) with at least one variant allele to subjects with wild-type alleles for each SNP. ABCB1 2766G>T was not evaluated because all subjects were homozygous wild-type. CYP2D6*4 and CAR 13711152C>T were not evaluated because only one subject had the variant allele.

* $p < 0.01$;

** $p < 0.05$.

† Partial AUC from 0–6 h for verifying extrapolated $AUC_{0–12\ h}$.

‡ Weight-adjusted apparent oral clearance.

§ Correlation analysis was performed using *1B/*1B versus *1/*1B and *1/*1 as only one subject was *1/*1.

AUC: Area under the concentration time curve; CL/F: Apparent oral clearance; NVP: Nevirapine.

Table 3

Multiple variable model determination.

Covariates	p-value	Model
CYP2B6 516G>T	0.0001	Y
*CYP3A4*1B*	0.5101	N
*CYP3A5*3*	0.0128	Y
*CYP2D6*17*	0.237	N
ABCB1 3435C>T	0.6735	N
NR1I2 44477T>C	0.8535	N
NR1I2 63396C>T	0.3883	N
NR1I2 6-bp indel	0.3344	N
Age	0.0017	Y
Weight	0.7563	N
Sex	0.2216	N
Dose	0.2215	N

N: Excluded from model; Y: Included in model.