Published in final edited form as:

Pharmacogenet Genomics. 2012 December ; 22(12): 868–876. doi:10.1097/FPC.0b013e32835a5af2.

Multiple genetic variants predict steady-state nevirapine clearance in HIV-infected Cambodians

Julie Bertrand^{a,d,e}, Monidarin Chou^f, Danielle M. Richardson^g, Céline Verstuyft^{b,d}, Paul D. Leger^{g,h}, France Mentré^a, Anne-Marie Taburet^{c,d}, David W. Haas^{g,h,i}, and the ANRS 12154 Study Group

^aUMR 738, French National Institute of Health and Medical Research, Paris Diderot University, Paris

^bDepartments of Molecular Genetics, Pharmacogenetics, and Hormonology

^cDepartment of Clinical Pharmacy, Assistance Publique-Hôpitaux de Paris, Hôpital Bicêtre

^dUniversity of Paris-Sud, Le Kremlin-Bicêtre, France

^eInstitute of Genetics, University College of London, London, UK

^fFaculty of Pharmacy, University of Health Sciences, Phnom Penh, Cambodia

^gCenter for Human Genetics Research, Vanderbilt University School of Medicine, Nashville, Tennessee, USA

^hDepartment of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee, USA

ⁱDepartment of Pharmacology, Pathology, Microbiology & Immunology, Vanderbilt University School of Medicine, Nashville, Tennessee, USA

Abstract

Objective—In a previous analysis involving protocol ANRS 12154, interindividual variability in steady-state nevirapine clearance among HIV-infected Cambodians was partially explained by *CYP2B6* 516G→T (*CYP2B6**6). Here, we examine whether additional genetic variants predict nevirapine clearance in this cohort.

Methods—Analyses included Phnom Penh ESTHER (Ensemble pour une Solidarité Thérapeutique Hospitalière en Réseau) cohort participants who had consented for genetic testing. All participants were receiving nevirapine plus two nucleoside analogs. The mean individual nevirapine clearance estimates were derived from a population model developed on nevirapine concentrations at 18 and 36 months of therapy. Polymorphisms were assayed in *ABCB1*, *CYP2A6*, *CYP2B6*, *CYP2C19*, *CYP3A4*, *CYP3A5*, and *NR1I2*.

Results—Of 198 assayed loci, 130 were polymorphic. Among 129 individuals with evaluable genetic data, nevirapine clearance ranged from 1.06 to 5.00 l/h in 128 individuals and was 7.81 l/h in one individual. In bivariate linear regression, *CYP2B6* 516G→T (*CYP2B6**6) was associated

© 2012 Wolters Kluwer Health | Lippincott Williams & Wilkins

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Website (www.pharmacogeneticsandgenomics.com).

Conflicts of interest France Mentré has received research grants from Novartis Pharma, Servier, Sanofi, and Roche, and has been consultant for Novartis Pharma, Servier, and Sanofi. Anne-Marie Taburet has received research grants from Bristol-Myers Squibb and has received lecture support from Gilead. David W. Haas has received research grants from Boehringer-Ingelheim, Bristol-Myers Squibb, Gilead Sciences, and Merck, and has been a consultant to Boehringer-Ingelheim. For the remaining authors there are no conflicts of interest.

with lower nevirapine clearances ($P = 3.5 \times 10^{-6}$). In a multivariate linear regression model conditioned on *CYP2B6* 516G→T, independent associations were identified with *CYP2B6* rs7251950, *CYP2B6* rs2279343, and *CYP3A4* rs2687116. The *CYP3A4* association disappeared after censoring the outlier clearance value. A model that included *CYP2B6* 516G→T ($P = 1.0 \times 10^{-9}$), rs7251950 ($P = 4.8 \times 10^{-5}$), and rs2279343 ($P = 7.1 \times 10^{-5}$) explained 11% of interindividual variability in nevirapine clearance.

Conclusion—Among HIV-infected Cambodians, several *CYP2B6* polymorphisms were associated independently with steady-state nevirapine clearance. The prediction of nevirapine clearance was improved by considering several polymorphisms in combination.

Keywords

Cambodia; *CYP2B6*; nevirapine pharmacokinetics; pharmacogenetics; population approach

Introduction

The non-nucleoside reverse transcriptase inhibitor nevirapine is very extensively prescribed worldwide in first-line regimens for HIV-1 infection. In resource-limited countries, nevirapine has been a preferred agent in part because of its availability coformulated with two nucleoside reverse transcriptase inhibitors in generic fixed-dose tablets [1,2]. Although nevirapine is generally safe and effective, there is considerable interindividual variability in its efficacy, toxicity, and pharmacokinetics.

Nevirapine induces its own metabolism (i.e. autoinduction) such that plasma nevirapine clearance increases with repeated dosing [3]. Nevirapine undergoes oxidative metabolism primarily by *CYP2B6* and *CYP3A4*, with minor metabolism by *CYP2D6* [4]. Four hydroxyl metabolites have been identified *in vivo* at positions 2, 3, 8, and 12. A 4-carboxyl derivative is formed from the 12-hydroxyl metabolite. In-vitro data suggest that *CYP2B6* and *CYP3A4* catalyze 3-hydroxyl and 2-hydroxyl formation, respectively [5]. Several CYPs are involved in the other pathways [5,6]. One study suggests that nevirapine is a weak substrate for the efflux transporter P-glycoprotein (renamed ABCB1) [7]. Metabolites are eliminated in urine largely as glucuronide conjugates. The pregnane X receptor (renamed NR1I2) induces many drug metabolism and transport genes including *CYP2B6*, *CYP3A*, and *ABCB1* [8].

The *CYP2B6* 516G→T variant (rs3745274) was first associated with increased steady-state plasma exposure of the non-nucleotide reverse transcriptase inhibitor efavirenz in a retrospective analysis of data and specimens from AIDS Clinical Trials Group (ACTG) protocol A5097s [9]. Many studies subsequently associated rs3745274 with higher steady-state plasma nevirapine exposure in HIV-infected patients, including studies in Europe [10–12], Uganda [13], the USA [14], India [15], and Thailand [16]. With single-dose nevirapine, associations between rs3745274 and pharmacokinetics have not been as readily apparent in analyses involving healthy, HIV-negative African Americans [17] and pregnant, HIV-infected Thai women [18].

In a previous report, Chou *et al.* [19] described a significant association between rs3745274 and interindividual variability in apparent steady-state clearance of nevirapine (on the basis of nonlinear mixed-effect models) among 170 HIV-infected Cambodians who had participated in protocol ANRS 12154. The present study explores whether additional polymorphisms contributed to variable nevirapine clearance in this cohort.

Methods

Study participants and design

Beginning in 2003, HIV-infected Cambodians were enrolled into an open-label multiple-dose pharmacokinetic study at the Calmette Hospital in Phnom Penh, as part of the ESTHER (Ensemble pour une Solidarité Thérapeutique Hospitalière en Réseau) cohort. Antiretroviral therapy was provided as the standard of care. Patients who provided written informed consent had additional blood drawn at year 3 for pharmacogenetics. Patients were prescribed nevirapine 200 mg once daily for the first two weeks, and then 200 mg twice daily thereafter. All patients received twice-daily stavudine (30 mg) and lamivudine (150 mg). After 18 months, most patients replaced stavudine with zidovudine 300 mg twice daily. There were monthly clinic visits for medical consultation and drug refills. All participants completed at least three adherence consultations. Hepatic transaminase, renal function tests, and CD4 T-cell counts were assayed every 6 months. At 18 and 36 months, morning plasma samples for nevirapine assays were obtained at trough 12 ± 2 h after the evening dose and assayed by HPLC as described previously [19]. Plasma HIV-1 RNA was also quantified at these visits. The National Ethics Committee of Cambodia approved the additional pharmacokinetic and pharmacogenetic analyses.

Characterization of human genetic variants

DNA was extracted from whole blood using the QIAamp DNA Mini Kit according to the manufacturer's protocol (Qiagen, Valencia, California, USA). A total of 198 polymorphisms (47 in *CYP2B6*, 1 in *CYP2A6*, 1 in *CYP2C19*, 63 in *ABCB1*, 36 in *CYP3A4*, 1 in *CYP3A5*, and 49 in *NR1I2*) were successfully assayed in the Vanderbilt DNA Resources Core using MassARRAY iPLEX Gold (Sequenom Inc., San Diego, California, USA). Our strategy for genotyping was as follows: for *CYP2B6*, *ABCB1*, and *NR1I2*, we tagged each entire gene using SeattleSNPs [20], using a cosmopolitan strategy across populations (Yoruba, Asian, African-American, European-American, and Hispanic) with a 5% allelic frequency cut-off, a 0.80 threshold for r^2 , 85% data convergence for tagging polymorphisms, and 70% data convergence for clustering. For *CYP2B6*, we included 5 kb in each 5' and 3' untranslated region (UTR), and for *ABCB1* and *NR1I2* 20 kb in each UTR. For *CYP2B6*, additional polymorphisms of interest (but that were not extremely infrequent) were added on the basis of a previous report [21], as were polymorphisms with at least a 5% allelic frequency in 20 kb of the 5' UTR identified using Ensembl Genome Browser [22], and upstream polymorphisms possibly associated with *CYP2B6* expression [23]. The *CYP2B6* assay included two polymorphisms in nearby *CYP2A* (rs28399454 and rs28399433). We also included *ABCB1* 3435C→T (rs1045642) and 2677G/T/A (rs2032582), *CYP2C19* 681G→A (rs4244285), and *CYP3A5* 6986A→G (rs776746). The final Sequenom assay design is available upon request. Genotypes were confirmed by visual inspection of plots. Laboratory personnel with no knowledge of clinical data carried out genotyping. Ample duplicate and blank assays were included to ensure validity. An additional *CYP3A4* polymorphism (rs35599367, *CYP3A4**22) was assayed post-hoc by TaqMan. Hereafter, all 'rs' numbers indicate *CYP2B6* polymorphisms unless indicated otherwise.

Statistical methods

Nevirapine concentration data from 170 study participants were analyzed using a nonlinear mixed-effect model approach with MONOLIX software version 2.4 (<http://software.monolix.org>). The model-building process is detailed elsewhere [19]. A one-compartment model with first-order absorption (k_a) and elimination adequately described nevirapine concentrations. The mean apparent clearance of nevirapine in the population was estimated to be 2.67 l/h, with 28% interpatient variability and 17% intraindividual variability. The mean absorption constant and the apparent volume of distribution were 1.64/

h and 213 l, respectively. Using this population model, empirical Bayes estimates of individual nevirapine clearances at months 18 and 36 were derived for the 129 patients included in genetic analyses. We calculated the average over the two (three for the 10 patients also with a complete pharmacokinetic profile) estimates to be used as the phenotype for genetic association analyses.

Associations between nevirapine clearance and genetic polymorphisms were first analyzed by bivariate regression using the Wald test on effect coefficients. The triallelic *ABCB1* rs2032582 was analyzed as three polymorphisms (i.e. A vs. not A, G vs. not G, and T vs. not T). Because of the strong association between rs3745274 and nevirapine clearance [19], we also carried out analyses adjusting for rs3745274. All tests used a 5% two-sided significance level and were carried out with PLINK version 1.07 (<http://pngu.mgh.harvard.edu/~purcell/plink/>) and R version 2.12 (<http://www.r-project.org>). To account for multiple testing, we used a correction of Bonferroni [24]. However, with *a priori* rationale (e.g. replication in an independent data set), the statistical significance threshold to carry forward a SNP into the next linear regression model was increased to 0.01. In the previous population pharmacokinetic analysis of this cohort, alanine aminotransferase was not significantly associated with nevirapine clearance, and creatinine clearance explained less than 0.5% of variability [19]. Therefore, neither alanine aminotransferase nor creatinine clearance was included as a covariate.

Haplotypic blocks were defined using the D' confidence intervals method in the Haploview software [25] and haplotype phases were inferred using the SEM algorithm in the PLINK software [26]. Linkage disequilibrium (LD) plots and values were generated using Haploview (<http://www.broad.mit.edu/mpg/haploview/>).

Results

Patient characteristics

Of 170 ESTHER cohort patients included in a previous report [19], 129 (75.9%) who were successfully genotyped are also included in the present analyses. All analyses hereafter are based on these 129 patients unless otherwise stated. At baseline, the median age was 35 years (range 20 to 58 years), the median weight was 55 kg (range 38.5 to 80.5 kg), and 56 (43%) patients were women. Plasma HIV-1 RNA was less than 400 copies/ml in 95 and 96% at 18 and 36 months, respectively, and self-reported adherence was high on the basis of a visual analog scale [27]. The demographic and clinical characteristics of the study participants at months 18 and 36 are presented in Table 1.

Nevirapine clearance estimates

On the basis of data at months 18 and 36 (Table 1), the median plasma nevirapine clearance was 2.63 l/h (range 1.06 to 7.81 l/h, interquartile range 2.14 to 3.1 l/h). The greatest clearance value represented one outlier (8.28 l/h at month 18, 7.34 l/h at month 36), the next greatest being 5.00 l/h. There was no apparent clinical explanation for this outlying value.

Genetic variants

A total of 198 genetic variants were successfully assayed. Among 129 evaluable patients, the average genotyping success for these 198 variants was 99.1% and all were 94.0% or more. Of the 198 polymorphisms, the average genotyping success was 99.1% and all were 94.5% or more. No minor alleles were detected in 67 of 198 loci (i.e. were monomorphic). Each of the remaining 131 variants was in Hardy–Weinberg equilibrium on the basis of a Bonferroni-adjusted *P* value threshold of 0.0004. Three variants had unadjusted *P* values of less than 0.05 (*CYP2A6*rs28399433, *P* = 0.0014; *CYP2B6*rs1552222, *P* = 0.0032; and

ABCB1 rs1978095, $P = 0.045$). The 131 variants are included in subsequent analyses. In a post-hoc assay for *CYP3A4* rs35599367 (*CYP3A4**22), only one patient was heterozygous for this allele. Allele frequencies for all 198 variants are presented in Supplementary digital content 1 (<http://links.lww.com/FPC/A524>).

Genetic associations with nevirapine clearance

Of the 131 polymorphisms, 19 were associated with nevirapine clearance at P value less than 0.01, including 18 in *CYP2B6* and one in *CYP3A4*. As reported previously in this cohort [19], rs3745274 was associated with lower nevirapine clearances ($\beta = -0.56$, $P = 3.5 \times 10^{-6}$). Seven of the other 18 polymorphisms were in LD with rs3745274 at $r^2 > 0.6$ (Table 2, Model 1). The minor allele frequency of rs3745274 was 0.34, and predicted metabolizer status for nevirapine solely on the basis of rs3745274 was extensive (GG) in 51 (40.2%), intermediate (GT) in 65 (51.2%), and slow (TT) in 11 (8.7%).

To identify polymorphisms associated independently with nevirapine clearance, we first carried out multivariate linear regression analysis adjusted for rs3745274 (Table 2, Model 2). By this analysis, four polymorphisms in addition to rs3745274 were associated with nevirapine clearance at P value less than 0.01, the lowest P value being for *CYP3A4* rs2687116, an intronic polymorphism ($\beta = 1.76$, $P = 1.6 \times 10^{-4}$) as shown in Table 2, Model 3. In a multivariate linear regression analysis adjusted for both rs3745274 and *CYP3A4* rs2687116, seven *CYP2B6* polymorphisms in addition to rs3745274 remained associated with nevirapine clearance at P value less than 0.01, the lowest P value being for rs7251950 ($\beta = 0.40$, $P = 7.1 \times 10^{-4}$) as shown in Table 2, Model 4. Four of the other six polymorphisms were in LD with rs7251950 at $r^2 > 0.6$. In an analysis adjusted for rs3745274, *CYP3A4* rs2687116, and *CYP2B6* rs7251950, the only additional polymorphism associated with nevirapine clearance at $P < 0.01$ was rs2279343 ($\beta = 0.90$, $P = 7.1 \times 10^{-5}$). In a final analysis adjusted for rs3745274, *CYP3A4* rs2687116, rs7251950, and rs2279343, no additional polymorphism was associated with nevirapine clearance at $P < 0.01$. The next lowest P value was for the A allele of triallelic *ABCB1* rs2032582 ($\beta = 0.38$, $P = 0.014$).

In the above analyses, the *CYP3A4* rs2687116 association seemed attributable to one heterozygous individual with an outlier nevirapine clearance value. This could reflect a true functional genetic association or could be spurious because of nongenetic factors (e.g. missed nevirapine doses at months 18 and 36 despite self-reported adherence). In a sensitivity analysis, we repeated the above step-wise approach, but excluding the individual with outlier nevirapine data. By this analysis, after controlling for rs3745274, rs7251950, and rs2279343, there was no longer an association with *CYP3A4* rs2687116 ($\beta = 0.25$, $P = 0.565$), whereas that with the *ABCB1* rs2032582 A allele became more statistically significant ($\beta = 0.46$, $P = 4.73 \times 10^{-4}$).

A previous report suggested a possible association between nevirapine clearance and a polymorphism in LD with the *CYP2C19**2 loss-of-function variant (rs4244285, 681G→A). In the present cohort, the minor allele frequency for *CYP2C19* rs4244285 was 27.4%, and 12 individuals were homozygous for A/A. In the last analysis described above (excluding the outlier individual, and controlling for rs3745274, rs7251950, and rs2279343), there was no association between *CYP2C19* rs4244285 and nevirapine clearance ($\beta = 0.03$, $P = 0.68$).

Final multivariate linear regression models, with and without the outlier individual, are presented in Table 3. The three *CYP2B6* variants were very common, with minor allele frequencies of 0.34, 0.37, and 0.40 for rs3745274, rs7251950, and rs2279343, respectively. The minor allele frequency for *ABCB1* rs2032582A was only 0.09.

A recent genome-wide association study of another CYP2B6 substrate, efavirenz, showed that rs4803419 (which is in LD with rs7251950) was independently associated with plasma efavirenz estimated $C_{\min}$ values (reported elsewhere in this journal issue [28]). To explore the relationship with rs4803419 in this Cambodian cohort, we therefore carried out an additional post-hoc analysis, again excluding the outlier individual, but this time conditioning on both rs3745274 and rs4803419. This showed associations with rs3745274 ($\beta = -1.39$, $P = 1.7 \times 10^{-6}$), rs4803419 T ($\beta = -0.35$, $P = 8.9 \times 10^{-4}$), and rs2279343 G ($\beta = 0.72$, $P = 2.7 \times 10^{-4}$).

On the basis of the above analyses, three *CYP2B6* polymorphisms (rs3745274, rs7251950, and rs2279343) were independently associated with steady-state nevirapine clearance. These relationships between the various genotype combinations and nevirapine clearance are presented in Fig. 1. As shown, there are associations between nevirapine clearance and both rs7251950 and rs2279343 among individuals who are homozygous for rs3745274 GG (i.e. extensive metabolizers), but not among other individuals. Nevirapine clearance was most rapid among individuals who were homozygous for rs3745274 GG who also carried both rs2279343 G and rs7251950 T (excluding the outlier individual).

In the aforementioned genome-wide association study of efavirenz, homozygosity for rs3745274 T/T appeared to preclude the presence of rs4803419T, suggesting that these alleles reside on mutually exclusive haplotypes [28]. This also appeared to be the case in the present cohort. Of 11 individuals homozygous for rs3745274 T/T none carried rs4803419T, and of 11 individuals homozygous for rs4803419 T/T, none carried 516T.

Estimated *CYP2B6* haplotypes on the basis of the 129 individuals are presented in Fig. 2. Combinations of the two variants rs3745274 and rs2279343 uniquely defined two haplotypes in the LD block of 24 polymorphisms: *CYP2B6**6 and *CYP2B6**4. The frequency of *CYP2B6**6 (rs2279343 with rs3745274, uppermost haplotype in Fig. 2) was 0.34, whereas the frequency of *CYP2B6**4 (rs2279343 without rs3745274, fifth haplotype from top) was 0.05. In contrast, the rs7251950 T allele appeared to be on at least two haplotypes in the LD block of three polymorphisms. To assess whether a particular haplotype was driving the association between rs7251950 and nevirapine clearance, we carried out association analyses focused on these haplotypes. In analyses that conditioned on both rs3745274 and rs2279343, and considering the two haplotypes that contain rs7251950 in the 3-polymorphism LD block (Fig. 2), the G–A–T haplotype (third from top) was significantly associated with nevirapine clearance ($\beta = -0.41$, $P = 0.006$), whereas G–C–T (fourth from top) was not ($\beta = 0.23$, $P = 0.158$). The results were consistent in analyses that censored the outlier individual (data not shown). To further explore the basis for association with rs7251950, we considered rs4803419. The polymorphism in incomplete LD with rs7251950 ($r^2 = 0.65$) and similarly associated with nevirapine clearance in the above multivariate analyses is on three haplotypes in the 24-polymorphism LD block (one of which is *CYP2B6**4, Fig. 2) and within 49 bases of rs3745274 on chromosome 19. In analyses that conditioned on both rs3745274 and rs2279343, no single haplotype containing rs4803419 in the 24-polymorphism LD block was significantly associated with nevirapine clearance (data not shown).

In the final multivariate model, each rs3745274 T allele was associated with a 30% decrease in nevirapine clearance, each rs7251950 T allele with a 9% decrease in nevirapine clearance, and each rs2279343 A allele with a 16% decrease in nevirapine clearance. A total of 11% of interparticipant variability in nevirapine clearance in our study population was explained by these polymorphisms (8.5% by 516G→T, 2.3% by rs7251950, and 0.2% by rs2279343).

Discussion

Nevirapine is one of the most extensively prescribed medications worldwide for HIV-1 infection, and multiple previous studies have associated *CYP2B6* 516G→T (rs3745274) with decreased steady-state plasma clearance of nevirapine [10,13–16]. The most important finding of this study is that *CYP2B6* polymorphisms in addition to rs3745274 are independently associated with nevirapine plasma clearance, which, in combination, improves the ability to predict plasma nevirapine plasma clearance. The rs3745274 polymorphism explained 8.5% of interindividual variability in nevirapine clearance, which increased to 11% by considering two additional *CYP2B6* polymorphisms.

An association between rs7251950 and nevirapine pharmacokinetics has not been reported previously. The validity of this association is strongly supported by a recent genome-wide association study of another *CYP2B6* substrate, efavirenz, as reported elsewhere in this journal issue [28]. That study involved individuals of European descent, African descent, and Hispanics, but not Asians, and showed that rs4803419 (which is in LD with rs7251950) was independently associated with plasma efavirenz estimated $C_{\min}$ values ($P = 4.4 \times 10^{-15}$) after adjusting for rs3745274 and rs28399499 (983T→C) [28]. The association between *CYP2B6**4 (rs2279343 without rs3745274) and nevirapine pharmacokinetics has also not been reported previously, but the validity of this association is less certain. It is supported by the report by Kirchheiner *et al.* [29], which identified a similar association between *CYP2B6**4 and increased clearance of the *CYP2B6* substrate bupropion. However, the recent genome-wide association study of efavirenz pharmacokinetics found no independent association with rs2279343. We are similarly skeptical in terms of the association between *CYP3A4* rs2687116 and increased nevirapine clearance, which was driven by an outlier with an apparent rapid clearance of nevirapine, and was eliminated in a sensitivity analyses that excluded this individual. In addition, putative functional variants of *CYP3A4* have typically failed to replicate in independent studies. Additional analyses of this individual are warranted. The previously suggested association between the *CYP2C19**2 loss-of-function variant and nevirapine clearance [30] did not replicate in the present cohort, despite a high frequency of *CYP2C19**2 in this Cambodian cohort. The high frequency of *CYP2C19**2 is consistent with what has been reported for other Asian populations [31]. The *CYP3A4**22 variant, which has been associated with pharmacokinetics and/or response to statin drugs [32] and tacrolimus [33], was too infrequent to evaluate in this cohort.

In our initial multivariate linear regression model that did not control for *CYP2B6* polymorphisms, the strong association with rs3745274 obscured associations with the other two polymorphisms (in Model 1 unadjusted for rs3745274, $P = 0.61$ for rs7251950, whereas rs2279343A was associated with decreased nevirapine clearance because of its presence on *CYP2B6**6). Only after adjusting for rs3745274 was rs7251950 significantly associated with decreased nevirapine clearance and was rs2279343G associated with increased nevirapine clearance. This exemplifies how the ability to show genotype–phenotype associations is strengthened if one can adjust for other factors (in this case rs3745274) that are strongly and independently associated with the phenotype under study.

The clinical implications of functional genetic variants depend on allelic frequencies in various populations. Frequencies of *CYP2B6* variants beyond rs3745274 have not been described previously in Cambodians. Among the 129 patients in this analysis, the minor allele frequency of rs3745274 was 0.34, with 11 (8.6%) homozygous for TT. The minor allele frequency of the rs7251950 T allele was 0.38 and the frequency of rs2279343G without rs3745274 (*CYP2B6**4) was 0.048. The *CYP2B6* 983T→C (rs28399499) polymorphism that predicts decreased plasma clearance of efavirenz [11,34–36] is found

almost exclusively among individuals of African descent, and was absent from the present cohort.

There are several implications of the present findings. Although nevirapine is generally safe, well tolerated, and effective, some nevirapine recipients experience virologic failure, and there is a pharmacokinetic–pharmacodynamic relationship between exposure and response for nevirapine. It is possible that the genetic variants identified herein that are associated with increased clearance of nevirapine may in some situations increase the risk of virologic failure. Severe hypersensitivity reactions to nevirapine may occur among treatment-naïve individuals who initiate therapy at higher CD4 T-cell counts (> 250 cells/mm for women and >400 cells/mm for men), and particular HLA types have been shown to confer an increased risk (e.g. *HLA-B*35* and *HLA-Cw*04* alleles for skin events [37,38], *HLA-DRB*01* alleles for hepatic events [39]). In a recent retrospective analysis involving 175 cutaneous adverse events, 101 hepatic adverse events, and 587 controls, rs3745274 was also associated with an increased risk of cutaneous adverse events, but not with hepatic adverse events [40]. This is consistent with analyses involving individuals who initiated nevirapine in a prospective AIDS Clinical Trials Group study in Africa (A5208), in which the likelihood of rash was 50% higher for every 20% decrease in plasma nevirapine clearance [41]. It is possible that the additional *CYP2B6* variants associated with nevirapine clearance herein further modulate the risk for severe nevirapine-associated skin adverse events.

The population analysis approach using nonlinear mixed-effect modeling techniques is useful for the analysis of pharmacogenetic data as it can handle sparse and/or unbalanced data from clinical studies, to account for interactions between genetic markers and other continuous and categorical covariates, and the final model can consider different dosing schemes. In a previous analysis that simulated plasma nevirapine pharmacokinetic profiles on the basis of data from 275 HIV-infected patients, greater body weight was associated with an increased risk of subtherapeutic trough plasma concentrations with once-daily than with twice-daily nevirapine [42].

Nevirapine is metabolized primarily by CYP2B6 and CYP3A4, with minor metabolism by other enzymes [4]. One study suggests that nevirapine is a weak substrate for P-glycoprotein (ABCB1) [7]. The pregnane X receptor (renamed NR1I2) induces many drug metabolism and transport genes including *CYP2B6*, *CYP3A*, and *ABCB1* [8]. In the present study, consistent associations were not identified between any variant in these genes and steady-state nevirapine clearance.

The present study had several limitations. Because of the study design, individual nevirapine clearance values were estimated on the basis of a population pharmacokinetic model in which between-participant variability in the volume of distribution (V/F) and absorption rate constant (k_a) could not be estimated. Indeed, most patients had only one trough concentration measured at each evaluation, providing information mostly on apparent clearance (CL/F). As a consequence, the observed associations between genetic polymorphisms and nevirapine clearance can be directly interpreted in terms of trough concentration. Only patients who had tolerated nevirapine for at least 18 months underwent genetic analyses. Thus, patients who discontinued therapy because of nevirapine toxicity (e.g. severe rash or liver toxicity) or virologic failure may be underrepresented in this pharmacogenetic analysis. A candidate gene approach was used on the basis of a priori knowledge. More extensive, high-throughput genotyping might identify additional novel associations. Finally, medication doses were by self-report rather than directly observed.

In summary, among HIV-infected Cambodians receiving nevirapine-containing antiretroviral regimens, steady-state plasma nevirapine clearance was best described by a

combination of *CYP2B6* polymorphisms. Further research should address whether these variants influence nevirapine efficacy and/or toxicity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by a grant from ANRS (J.B.) and NIH grants AI-077505, AI-054999, and TR-000011 (D.W.H.).

References

1. Calmy A, Pinoges L, Szumilin E, Zachariah R, Ford N, Ferradini L. Generic fixed-dose combination antiretroviral treatment in resource-poor settings: multicentric observational cohort. *AIDS*. 2006; 20:1163–1169. [PubMed: 16691068]
2. Laurent C, Kouanfack C, Koulla-Shiro S, Nkoue N, Bourgeois A, Calmy A, et al. Effectiveness and safety of a generic fixed-dose combination of nevirapine, stavudine, and lamivudine in HIV-1-infected adults in Cameroon: open-label multicentre trial. *Lancet*. 2004; 364:29–34. [PubMed: 15234853]
3. Riska P, Lamson M, MacGregor T, Sabo J, Hattox S, Pav J, et al. Disposition and biotransformation of the antiretroviral drug nevirapine in humans. *Drug Metab Dispos*. 1999; 27:895–901. [PubMed: 10421616]
4. Erickson DA, Mather G, Trager WF, Levy RH, Keirns JJ. Characterization of the in vitro biotransformation of the HIV-1 reverse transcriptase inhibitor nevirapine by human hepatic cytochromes P-450. *Drug Metab Dispos*. 1999; 27:1488–1495. [PubMed: 10570031]
5. Wen B, Chen Y, Fitch WL. Metabolic activation of nevirapine in human liver microsomes: dehydrogenation and inactivation of cytochrome P450 3A4. *Drug Metab Dispos*. 2009; 37:1557–1562. [PubMed: 19364830]
6. Wang H, Tompkins LM. CYP2B6: new insights into a historically overlooked cytochrome P450 isozyme. *Curr Drug Metab*. 2008; 9:598–610. [PubMed: 18781911]
7. Almond LM, Edirisinghe D, Dalton M, Bonington A, Back DJ, Khoo SH. Intracellular and plasma pharmacokinetics of nevirapine in human immunodeficiency virus-infected individuals. *Clin Pharmacol Ther*. 2005; 78:132–142. [PubMed: 16084848]
8. Kojima K, Nagata K, Matsubara T, Yamazoe Y. Broad but distinct role of pregnane X receptor on the expression of individual cytochrome p450s in human hepatocytes. *Drug Metab Pharmacokinet*. 2007; 22:276–286. [PubMed: 17827782]
9. Haas DW, Ribaldo HJ, Kim RB, Tierney C, Wilkinson GR, Gulick RM, et al. Pharmacogenetics of efavirenz and central nervous system side effects: an adult AIDS Clinical Trials Group study. *AIDS*. 2004; 18:2391–2400. [PubMed: 15622315]
10. Rotger M, Colombo S, Furrer H, Bleiber G, Buclin T, Lee BL, et al. Influence of CYP2B6 polymorphism on plasma and intracellular concentrations and toxicity of efavirenz and nevirapine in HIV-infected patients. *Pharmacogenet Genomics*. 2005; 15:1–5. [PubMed: 15864119]
11. Wyen C, Hendra H, Vogel M, Hoffmann C, Knechten H, Brockmeyer NH, et al. Impact of CYP2B6 983T > C polymorphism on non-nucleoside reverse transcriptase inhibitor plasma concentrations in HIV-infected patients. *J Antimicrob Chemother*. 2008; 61:914–918. [PubMed: 18281305]
12. Mahungu T, Smith C, Turner F, Egan D, Youle M, Johnson M, et al. Cytochrome P450 2B6 516G→T is associated with plasma concentrations of nevirapine at both 200 mg twice daily and 400 mg once daily in an ethnically diverse population. *HIV Med*. 2009; 10:310–317. [PubMed: 19228205]
13. Penzak SR, Kabuye G, Mugenyi P, Mbamanya F, Natarajan V, Alfaro RM, et al. Cytochrome P450 2B6 (CYP2B6) G516T influences nevirapine plasma concentrations in HIV-infected patients in Uganda. *HIV Med*. 2007; 8:86–91. [PubMed: 17352764]

14. Saitoh A, Sarles E, Capparelli E, Aweeka F, Kovacs A, Burchett SK, et al. CYP2B6 genetic variants are associated with nevirapine pharmacokinetics and clinical response in HIV-1-infected children. *AIDS*. 2007; 21:2191–2199. [PubMed: 18090046]
15. Ramachandran G, Ramesh K, Hemanth Kumar AK, Jagan I, Vasantha M, Padmapriyadarsini C, et al. Association of high T allele frequency of CYP2B6 G516T polymorphism among ethnic south Indian HIV-infected patients with elevated plasma efavirenz and nevirapine. *J Antimicrob Chemother*. 2009; 63:841–843. [PubMed: 19218571]
16. Uttayamakul S, Likanonsakul S, Manosuthi W, Wichukchinda N, Kalambaheti T, Nakayama EE, et al. Effects of CYP2B6 G516T polymorphisms on plasma efavirenz and nevirapine levels when co-administered with rifampicin in HIV/TB co-infected Thai adults. *AIDS Res Ther*. 2010; 7:8. [PubMed: 20338069]
17. Haas DW, Gebretsadik T, Mayo G, Menon UN, Acosta EP, Shintani A, et al. Associations between CYP2B6 polymorphisms and pharmacokinetics after a single dose of nevirapine or efavirenz in African Americans. *J Infect Dis*. 2009; 199:872–880. [PubMed: 19239339]
18. Chantarangsu S, Cressey TR, Mahasirimongkol S, Capparelli E, Tawon Y, Ngo-Giang-Huong N, et al. Influence of CYP2B6 polymorphisms on the persistence of plasma nevirapine concentrations following a single intra-partum dose for the prevention of mother to child transmission in HIV-infected Thai women. *J Antimicrob Chemother*. 2009; 64:1265–1273. [PubMed: 19812066]
19. Chou M, Bertrand J, Segéral O, Verstuyt C, Borand L, Comets E, et al. Population pharmacokinetic-pharmacogenetic study of nevirapine in HIV-infected Cambodian patients. *Antimicrob Agents Chemother*. 2010; 54:4432–4439. [PubMed: 20696882]
20. [1 May 2012] SeattleSNPs Variation Discovery Resource. Available at: <http://pga.gs.washington.edu/>
21. Rotger M, Tegude H, Colombo S, Cavassini M, Furrer H, Decosterd L, et al. Predictive value of known and novel alleles of CYP2B6 for efavirenz plasma concentrations in HIV-infected individuals. *Clin Pharmacol Ther*. 2007; 81:557–566. [PubMed: 17235330]
22. [1 May 2012] Ensembl. Available at: <http://useast.ensembl.org/index.html>
23. Lamba V, Lamba J, Yasuda K, Strom S, Davila JC, Hancock M, et al. Hepatic CYP2B6 expression: gender and ethnic differences and relationship to CYP2B6 genotype and CAR expression. *J Pharmacol Exp Ther*. 2003; 307:906–922. [PubMed: 14551287]
24. Miller RG Jr. Simultaneous statistical inference. 2nd ed. New York: Springer-Verlag. 1981
25. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2005; 21:263–265. [PubMed: 15297300]
26. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007; 81:559–575. [PubMed: 17701901]
27. Amico KR, Fisher WA, Cornman DH, Shuper PA, Redding CG, Konkle-Parker DJ, et al. Visual analog scale of ART adherence: association with 3-day self-report and adherence barriers. *J Acquir Immune Defic Syndr*. 2006; 42:455–459. [PubMed: 16810111]
28. Holzinger ER, Grady B, Ritchie MD, Ribaud H, Acosta EP, Morse G, et al. Genome-wide association study of plasma efavirenz pharmacokinetics in AIDS Clinical Trials Group protocols. *Pharmacogenet Genomics*. 2012 [In press].
29. Kirchheiner J, Klein C, Meineke I, Sasse J, Zanger UM, Mordt TE, et al. Bupropion and 4-OH-bupropion pharmacokinetics in relation to genetic polymorphisms in CYP2B6. *Pharmacogenetics*. 2003; 13:619–626. [PubMed: 14515060]
30. Lehr T, Yuan J, Hall D, Zimdahl-Gelling H, Schaefer HG, Staab A, et al. Integration of absorption, distribution, metabolism, and elimination genotyping data into a population pharmacokinetic analysis of nevirapine. *Pharmacogenet Genomics*. 2011; 21:721–730. [PubMed: 21860339]
31. [1 May 2012] dbSNP – short genetic variations. Available at: <http://www.ncbi.nlm.nih.gov/projects/SNP/>
32. Wang D, Guo Y, Wrighton SA, Cooke GE, Sadee W. Intronic polymorphism in CYP3A4 affects hepatic expression and response to statin drugs. *Pharmacogenom J*. 2011; 11:274–286.

33. Elens L, Bouamar R, Hesselink DA, Haufroid V, van der Heiden IP, van Gelder T, et al. A new functional CYP3A4 intron 6 polymorphism significantly affects tacrolimus pharmacokinetics in kidney transplant recipients. *Clin Chem*. 2011; 57:1574–1583. [PubMed: 21903774]
34. Wang J, Sonnerborg A, Rane A, Josephson F, Lundgren S, Stahle L, et al. Identification of a novel specific CYP2B6 allele in Africans causing impaired metabolism of the HIV drug efavirenz. *Pharmacogenet Genomics*. 2006; 16:191–198. [PubMed: 16495778]
35. Ribaud HJ, Liu H, Schwab M, Schaeffeler E, Eichelbaum M, Motsinger-Reif AA, et al. Effect of CYP2B6, ABCB1, and CYP3A5 polymorphisms on efavirenz pharmacokinetics and treatment response: an AIDS Clinical Trials Group study. *J Infect Dis*. 2010; 202:717–722. [PubMed: 20662624]
36. Heil SG, van der Ende ME, Schenk PW, van der Heiden I, Lindemans J, Burger D, et al. Associations between ABCB1, CYP2A6, CYP2B6, CYP2D6, and CYP3A5 alleles in relation to efavirenz and nevirapine pharmacokinetics in HIV-infected individuals. *Ther Drug Monit*. 2012; 34:153–159. [PubMed: 22354160]
37. Chantarangsu S, Mushiroda T, Mahasirimongkol S, Kiertiburanakul S, Sungkanuparph S, Manosuthi W, et al. HLA-B*3505 allele is a strong predictor for nevirapine-induced skin adverse drug reactions in HIV-infected Thai patients. *Pharmacogenet Genomics*. 2009; 19:139–146. [PubMed: 19104471]
38. Likanonsakul S, Rattanatham T, Feangvad S, Uttayamakul S, Prasithsirikul W, Tunthanathip P, et al. HLA-Cw*04 allele associated with nevirapine-induced rash in HIV-infected Thai patients. *AIDS Res Ther*. 2009; 6:22. [PubMed: 19845952]
39. Martin AM, Nolan D, James I, Cameron P, Keller J, Moore C, et al. Predisposition to nevirapine hypersensitivity associated with HLA-DRB1*0101 and abrogated by low CD4 T-cell counts. *AIDS*. 2005; 19:97–99. [PubMed: 15627041]
40. Yuan J, Guo S, Hall D, Cammett AM, Jayadev S, Distel M, et al. Toxicogenomics of nevirapine-associated cutaneous and hepatic adverse events among populations of African, Asian, and European descent. *AIDS*. 2011; 25:1271–1280. [PubMed: 21505298]
41. Dong BJ, Zheng Y, Hughes MD, Frymoyer A, Verotta D, Lizak P, et al. Nevirapine (NVP) pharmacokinetics (PK) and risk of rash and hepatitis among HIV-infected Sub-Saharan African Women. *AIDS*. 2012; 26:833–841. [PubMed: 22301417]
42. Schipani A, Wyen C, Mahungu T, Hendra H, Egan D, Siccardi M, et al. Integration of population pharmacokinetics and pharmacogenetics: an aid to optimal nevirapine dose selection in HIV-infected individuals. *J Antimicrob Chemother*. 2011; 66:1332–1339. [PubMed: 21441248]

Fig. 1. Steady-state nevirapine clearance estimates stratified by *CYP2B6* polymorphisms in HIV-infected Cambodians. The y-axes represent estimated steady-state clearance values for nevirapine. The x-axes represent genotypes for three *CYP2B6* polymorphisms independently associated with nevirapine clearance by multivariate analysis. (a) Relationships with *CYP2B6* 516G→T; (b) relationships with *CYP2B6* rs2279343 stratified by *CYP2B6* 516G→T genotype; (c) relationships with *CYP2B6* rs7251950 stratified by *CYP2B6* rs3745274 and *CYP2B6* rs2279343 genotypes. Gray shaded panels represent *CYP2B6* rs3745274 intermediate metabolizer genotypes (i.e. GT heterozygosity). NVP, nevirapine.

Fig. 2.

Estimated haplotypes of *CYP2B6*. Two haplotypic blocks comprising 3 and 25 polymorphisms were estimated for *CYP2B6* using Haploview software. The rs numbers are shown above. Haplotype frequencies in our study participants are shown to the right. Numbers 1 to 10 identify each haplotype, and weights of connecting lines reflect the frequencies of haplotype pairings (i.e. the most frequent pairings are 1 with 5, 2 with 6, and 4 with 8). The multiallelic D' of 0.91 is shown (a measure of recombination between the two blocks). Arrows and rectangles represent polymorphisms and alleles of interest, as discussed in the text.

Table 1

Demographic and clinical characteristics at 18 and 36 months among 129 pharmacogenetic study participants in Cambodia

	Month 18	<i>n</i>	Month 36	<i>n</i>
Nevirapine clearance (l/h) ^a	2.74 (1.19–8.28)	114	2.70 (1.05–7.34)	124
Age (years) ^a	36 (19–57)	114	37 (21–59)	124
Weight (kg) ^a	54 (25–79)	112	56 (39–82)	122
Female sex ^b	51 (45)	114	51 (41)	124
ALT (IU/ml) ^a	31 (7–291)	105	30 (11–212)	124
Bilirubin (μmol/ml) ^a	7 (5–32)	101	7 (5–37)	124
Creatinine clearance (ml/min) ^a	90 (47–162)	102	85 (48–144)	121
CD4 T cells (cells/mm ³) ^a	211 (27–2306)	114	299 (75–819)	124
HIV-1 RNA 400 copies/ml ^b	104 (95)	110	114 (95)	120
Stavudine use ^b	96 (92)	104	8 (6)	124
Zidovudine use ^b	8 (8)	104	116 (94)	124
Adherence visual analog scale > 8 ^b	100 (98)	102	118 (96)	124

ALT, alanine aminotransferase.

^aMedians are shown (ranges in parentheses).

^bNumbers of patients are shown (percentages in parentheses).

Table 2
Linear regression analyses for the association between genetic variants and nevirapine clearance estimate

Conditioning variants						
1	2	3	Test variant	LD r^2 values ^c	β	P value
Model 1						
–	–	–	rs7246456 ^a	0.94	–0.58	2.39×10^{-6}
–	–	–	rs3745274	–	–0.56	3.53×10^{-6}
–	–	–	rs10853744 ^a	0.95	–0.53	1.10×10^{-5}
–	–	–	rs4803417	0.24	0.49	3.04×10^{-5}
–	–	–	rs2279344	0.79	0.52	3.18×10^{-5}
–	–	–	rs7250873 ^a	0.98	–0.51	3.41×10^{-5}
–	–	–	rs11083595 ^a	0.94	–0.52	3.45×10^{-5}
–	–	–	rs10418990	0.21	0.49	4.03×10^{-5}
–	–	–	rs1987236	0.24	0.49	4.15×10^{-5}
–	–	–	rs2279345	0.79	0.51	4.44×10^{-5}
–	–	–	rs6508966	0	0.49	6.59×10^{-5}
–	–	–	rs4802101	0.23	0.47	6.93×10^{-5}
–	–	–	rs11671243	0	0.49	1.06×10^{-4}
–	–	–	rs2687116	–	1.95	1.19×10^{-4}
–	–	–	rs2054675 ^a	0.92	–0.47	1.26×10^{-4}
–	–	–	rs2099361	0	0.43	6.16×10^{-4}
–	–	–	rs7246465	0	0.41	6.17×10^{-4}
–	–	–	rs8109818 ^a	0.60	–0.37	2.37×10^{-3}
–	–	–	rs2279343 ^a	0.79	–0.33	7.71×10^{-3}
Model 2						
rs3745274	–	–	rs2687116	–	1.76	1.56×10^{-4}
rs3745274	–	–	rs7251950	–	–0.39	1.78×10^{-3}
rs3745274	–	–	rs4803419 ^b	0.65	–0.34	6.51×10^{-3}

Conditioning variants				
1	2	3	Test variant	LD r^2 values ^c β P value
rs3745274	–	–	rs2279343	0 0.67 7.00×10^{-3}
Model 3				
rs3745274	rs2687116	–	rs7251950	– –0.40 7.12×10^{-4}
rs3745274	rs2687116	–	rs2279343	0 0.74 1.51×10^{-3}
rs3745274	rs2687116	–	rs4803419 ^b	0.65 –0.37 1.89×10^{-3}
rs3745274	rs2687116	–	rs7254579 ^b	0.71 –0.32 6.71×10^{-3}
rs3745274	rs2687116	–	rs892216 ^b	0.71 –0.32 7.18×10^{-3}
rs3745274	rs2687116	–	rs8100458 ^b	0.71 –0.32 7.18×10^{-3}
rs3745274	rs2687116	–	rs4802100	0 –0.42 8.60×10^{-3}
Model 4 ^d				
rs3745274	rs2687116	rs7251950	rs2279343	0 0.90 7.07×10^{-5}

LD, linkage disequilibrium.

^aPolymorphisms in LD with rs3745274 at $r^2 > 0.6$.

^bPolymorphisms in LD with rs7251950 at $r^2 > 0.6$.

^cLD r^2 value of the test variant with rs3745274 in Model 1 and with rs7251950 in Models 2, 3, and 4.

^dEach variable in Table 1 was explored as a covariate in the final model, except for categorical covariates with a substantial change over time (HIV-1 RNA <400 copies/ml, stavudine use, zidovudine use). None was significant at $P < 0.01$, and thus were not included in the final model.

Table 3

Multivariate linear regression for association between genetic variants and nevirapine clearance estimate

Variants	Gene	β	Statistic	P value
<i>n</i> = 129 (all participants)				
rs3745274	<i>CYP2B6</i>	-1.606	-6.66	1.01×10^{-9}
rs2687116	<i>CYP3A4</i>	-1.913	-4.68	7.95×10^{-6}
rs2279343	<i>CYP2B6</i>	0.904	4.13	7.07×10^{-5}
rs7251950	<i>CYP2B6</i>	-0.499	-4.23	4.77×10^{-5}
<i>n</i> = 128 (without outlier) ^a				
rs3745274	<i>CYP2B6</i>	-1.568	-7.66	6.78×10^{-12}
rs2279343	<i>CYP2B6</i>	0.835	4.50	1.66×10^{-5}
rs7251950	<i>CYP2B6</i>	-0.404	-4.01	1.09×10^{-4}
rs2032582A	<i>ABCB1</i>	0.456	3.60	4.73×10^{-4}

^aData from one individual outlier (extremely high) nevirapine clearance value were censored from this analysis.