CYP2B6 Variants and Plasma Efavirenz Concentrations during Antiretroviral Therapy in Port-au-Prince, Haiti

Paul Leger, Rebecca Dillingham, Carole Anne Beauharnais, Angela D. M. Kashuba, Naser L. Rezk, Daniel W. Fitzgerald, Jean William Pape, and David W. Haas

1Groupe Haïtien d’Étude du Sarcome de Kaposi et des Infections Opportunistes, Port-au-Prince, Haiti; 2Department of Medicine, Division of Infectious Disease and International Health and Public Health Sciences, University of Virginia, Charlottesville, Virginia; 3Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; 4Department of Medicine, Weill Medical College of Cornell University, New York, New York; Departments of 5Medicine and 6Microbiology & Immunology, Vanderbilt University School of Medicine, Nashville, Tennessee

Background. Polymorphisms in CYP2B6 are known to predict increased steady-state plasma concentrations of efavirenz. We characterized relationships between genetic polymorphisms and plasma efavirenz concentrations among 45 Haitians who initiated antiretroviral therapy in Port-au-Prince.

Methods. An observational study characterized relationships between clinical factors, pharmacokinetics, and treatment response among antiretroviral-naive patients initiating once-daily treatment with efavirenz plus twice-daily treatment with zidovudine and lamivudine. Plasma drug concentrations were determined at weeks 2 and 4. Drug doses were directly observed by field workers or designated family members. We retrospectively characterized relationships between efavirenz concentrations and 50 single-nucleotide polymorphisms in CYP2B6 and several polymorphisms in CYP2A6, CYP3A4, CYP3A5, and ABCB1.

Results. Plasma specimens for efavirenz analysis were obtained from study participants a mean (± standard deviation) of 13.9 ± 1.6 h after they received the dose. As expected, CYP2B6 516G→T was associated with increased plasma efavirenz concentrations (Spearman ρ = 0.71; P < .001), as were 10 polymorphisms in linkage disequilibrium with 516G→T. Distinct CYP2B6 polymorphisms were associated with decreased plasma efavirenz concentrations (greatest absolute ρ = 0.48; P < .001). Associations were replicated by results from a recent pharmacokinetic study involving 34 healthy, human immunodeficiency virus–negative African Americans.

Conclusions. Relatively frequent CYP2B6 polymorphisms may predict decreased plasma efavirenz exposure in patients of African descent. If replicated in other cohorts, the implications of these novel associations for treatment response warrant further study.

Initial therapy for human immunodeficiency virus type 1 (HIV-1) infection with the nonnucleoside reverse transcriptase inhibitor (NNRTI) efavirenz plus 2 nucleoside reverse transcriptase inhibitors (NRTIs) typically provides sustained virologic and immunologic benefits, but some patients experience virologic failure [1–6]. Efavirenz is metabolized primarily by cytochrome P450 (CYP) 2B6 [7]. A frequent nonsynonymous polymorphism in CYP2B6 exon 4 (516G→T; rs3745274) predicts decreased plasma efavirenz clearance and increased plasma efavirenz exposure at steady state [8–10], as does a less frequent nonsynonymous CYP2B6 exon 9 polymorphism, 983T→C (rs28399499) [11–15]. Increased frequencies of both CYP2B6 516T and 983C among individuals of African ancestry [8–16]
largely explain their greater mean plasma efavirenz concentrations as compared with white individuals [17–13]. Additional CYP2B6 polymorphisms have been suggested to affect CYP2B6 activity [19], but they have either been extremely infrequent or have not predicted plasma efavirenz exposure in vivo. In 2 studies involving 169 and 489 individuals and in which 15 nonsynonymous exonic CYP2B6 polymorphisms were assayed, only 516G→T and 983T→C appeared to predict substantial differences in efavirenz exposure [12, 15].

Most previous pharmacogenomics studies of efavirenz have focused on coding, nonsynonymous CYP2B6 polymorphisms [8, 15]. However, many additional CYP2B6 polymorphisms in 5′ and 3′ untranslated regions (UTRs) and introns could potentially affect CYP2B6 expression or activity [20, 21]. In this regard, a recent study explored associations between 50 CYP2B6 polymorphisms and pharmacokinetics of single-dose efavirenz among 34 healthy, HIV-negative African Americans [22]. In addition to the expected pharmacokinetic association with the so-called composite CYP2B6 516/983 genotype, associations were suggested between an additional 13 CYP2B6 polymorphisms and efavirenz pharmacokinetics, pending replication in other studies. These polymorphisms were frequent and in non-coding regions of CYP2B6, and some were not in strong linkage disequilibrium with either CYP2B6 516G→T or 983T→C.

The present study was designed to characterize relationships between CYP2B6 polymorphisms and steady-state plasma efavirenz concentrations among 45 HIV-positive, antiretroviral-naive Haitians who initiated an efavirenz-containing regimen in Port-au-Prince, Haiti. Our findings replicate novel associations previously suggested between relatively frequent non-coding CYP2B6 polymorphisms and interindividual variability in plasma efavirenz exposure, and they suggest that at least 1 CYP2B6 variant may be associated with an increased likelihood of subtherapeutic plasma efavirenz concentrations.

SUBJECTS, MATERIALS, AND METHODS

Study subjects and design. This pharmacogenomic study involved 45 Haitians of African descent. An additional individual with undetectable plasma efavirenz, zidovudine (AZT), and lamivudine (3TC) concentrations was excluded from analyses for presumed nonadherence. Eligible participants were HIV-1 seropositive, had a CD4+ T cell count of >200 cells/mm³, a hemoglobin level of >7.5 mg/dL, and normal results of kidney and liver function tests. Patients with prior antiretroviral exposure or requiring medications known or predicted to interact with antiretrovirals were excluded. All participants initiated therapy with efavirenz (600 mg every 24 h in the evening) plus fixed doses of AZT and 3TC (300 mg and 100 mg, respectively, every 12 h). To enhance medication adherence, during the 4 weeks of therapy the study medications were administered under direct observation (morning doses were observed by study personnel [field workers] at the participant’s residence, and evening doses were observed by a designated family member [accompagnateur]). All doses were documented in a medication diary.

Plasma drug assays. Plasma samples for drug assays were obtained at week 2 and week 4 of therapy, before the morning dose of AZT and 3TC. Times since the prior doses of efavirenz, AZT, and 3TC were documented. Plasma was separated by centrifugation at 4°C and stored at −70°C. Efavirenz, AZT, and 3TC were quantified using 2 validated methods in the University of North Carolina Center for AIDS Research Clinical Pharmacology and Analytical Chemistry Core [23, 24]. The dynamic range for efavirenz was 25–10,000 ng/mL, with an intraday and interday precision of 4.8%–5.5% and an accuracy of 100.4%–101.7%. The dynamic range for AZT and 3TC was 10–10,000 ng/mL, with an intraday and interday precision of <7% and an accuracy of ≥90% for all concentrations.

Statistical methods. Pharmacokinetic parameters are presented as median and interquartile ranges (IQRs). Body mass index was calculated as the weight in kilograms divided by the square of the height in meters. For each participant a single plasma efavirenz concentration value derived based on the geometric mean of week 2 and week 4 values was used for statistical analyses. In 1 individual without week 2 efavirenz data, the value from week 4 was used. The geometric mean concentration for each individual was then used to determine medians, IQRs, and correlations among groups of individuals. The Spearman correlation coefficient (ρ) was used to assess for dose-response trends in bivariate relationships between continuous variables and genotype and to determine the directionality of such relationships. The same analyses repeated using Jonckheere-Terpstra test for trend if 3 genotypes, or the Wilcoxon rank sum test if 2 genotypes, yielded remarkably similar P values (data not shown). For CYP2B6 516/983, genotype was coded as an ordered continuous variable, with 1 denoting an extensive metabolizer, 2 denoting an intermediate metabolizer, and 3 denoting a slow metabolizer. For other exploratory polymorphism analyses, 1 denoted one homozygous genotype, 2 denoted the heterozygous genotype, and 3 denoted the other homozygous genotype, ordered as A < C < G < T (eg, for 983T→C, C/C = 1, G/T = 2, and T/T = 3). For univariate correlations between plasma efavirenz concentrations and genetic polymorphisms, P values that remained significant after Bonferroni correction for multiple tests are indicated in the tables. All analyses used a 5% two-sided significance level and were performed with Stata/IC, version 10.0 (Stata). Linkage disequilibrium plots and r² values were generated using Haplovie [25]. Estimated haplotypes for each individual were generated using Powermarker [26], excluding 2 individuals with <85% haplotype probability. Hardy-Weinberg equilibrium was assessed using exact tests [27].
Characterization of human genetic variants. Genomic DNA was extracted from saliva collected with Oragene kits (DNA Genotek). A total of 56 single nucleotide polymorphisms (50 in CYP2B6, 1 in CYP2A6, 1 in CYP3A4, 1 in CYP3A5, and 3 in ABCB1) were assayed in the Vanderbilt DNA Resources Core, using MassARRAY iPLEX Gold (Sequenom). Our strategy for the CYP2B6 Sequenom assay design was as follows. We tagged the entire CYP2B6 gene by use of SeattleSNPs [21], including 5 kb in each 5′ and 3′ UTR, using a cosmopolitan strategy across populations (Yoruba, Asian, African American, European-American, and Hispanic) with a 5% allelic frequency cutoff, a 0.80 threshold for r², 85% data convergence for tagging polymorphisms, and 70% data convergence for clustering. Additional polymorphisms of interest (but that were not extremely infrequent) were added on the basis of previous reports [12, 15]. We also added polymorphisms with at least 5% allelic frequency in 20 kb of the 5′UTR, using a cosmopolitan strategy identified using the Ensembl genome browser [28], as well as upstream polymorphisms possibly associated with CYP2B6 expression, on the basis of a previous report [29]; 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 performed genotyping. All assays were run in duplicate and were in complete agreement.

Composite CYP2B6 516/983 genotypes, based on reported associations between CYP2B6 516G→T and 983T→C and steady-state efavirenz pharmacokinetics [11–15], were assigned as follows, as described elsewhere [22]: extensive metabolizer, no variant allele at either position 516 or 983; intermediate metabolizer, a single variant allele at either position 516 or 983 but not both; and slow metabolizer, 2 variant alleles (either 516 T/T, 983 C/C, or 516 G/T with 983 T/C). Relative CYP2B6 gene copy number was assessed by allelotyping at 17 polymorphic sites, using the Sequenom platform.

Protection of human subjects. Study volunteers were enrolled and followed at the GHESKIO Centers clinic in Port-au-Prince. Human experimentation guidelines of the US Department of Health and Human Services were followed in the conduct of this research. All work was conducted in accordance with the Declaration of Helsinki. The study was approved by the Institutional Review Board of GHESKIO, Cornell University, University of Virginia, and Vanderbilt University, and all participants provided written informed consent.

RESULTS

Participant characteristics. Forty-five Haitians of African descent were included in this pharmacogenomic association study. Median baseline age was 36 years (IQR, 32-43 years), the median weight was 56 kg (IQR, 48–59 kg), the median body mass index was 20.8 kg/m² (IQR, 19.0–22.9 kg/m²), and 27 (60%) were female. At baseline, the median plasma HIV-1 RNA load was 5.3 log₁₀ copies/mL (IQR, 4.9–5.6 log₁₀ copies/mL), and the CD4⁺ T cell count was 54 cells/mm³ (IQR 25–138 cells/mm³).

Efavirenz plasma concentrations. The 45 participants initiated 3-drug therapy with efavirenz, AZT, and 3TC. Plasma specimens for efavirenz analysis were obtained a mean (±SD) of 14.1 ± 1.8 h after dosing at week 2 and 13.8 ± 1.4 h after dosing at week 4. Mean intraindividual differences (±SD) between week 2 and week 4 efavirenz concentrations were 18.6% ± 15.5%. One individual had extremely low plasma efavirenz concentrations of 241 ng/mL and 126 ng/mL. Paired efavirenz concentration values were used to calculate geometric mean concentrations for each individual (used in all analyses hereafter). There was an ~100-fold interindividual range in plasma efavirenz concentrations. The median plasma efavirenz concentration was 3295 ng/mL (IQR, 2435–6473 ng/mL; range, 174–15,380 ng/mL). There was no apparent association between either body mass index or sex and plasma efavirenz concentrations (P >.05 for each comparison).

Genetic polymorphisms. Minor allele frequencies for all 56 polymorphisms are presented in table 1. All were in Hardy-Weinberg equilibrium (P >.05). On the basis of composite CYP2B6 516/983 genotype (as defined in Subjects, Materials, and Methods), 10 individuals were predicted to be slow metabolizers, 21 intermediate metabolizers, and 14 extensive metabolizers. A linkage disequilibrium plot for CYP2B6 polymorphisms is provided in figure 1.

Genetic predictors of plasma efavirenz concentrations. Of the 56 polymorphisms assayed, 20 were associated with efavirenz plasma concentrations at a p of greater than 0.3 or less than ~0.3 (without correcting for multiple comparisons) as shown in table 2. Associations for all 56 polymorphisms are provided in figure 2 and table 1. There was, as expected, a strong correlation between composite CYP2B6 516/983 genotype and higher plasma efavirenz concentrations (ρ = 0.76; P <.001) (figure 3A). In analyses involving CYP2B6 516G→T and 983T→C separately there was also a significant association between CYP2B6 516G→T and efavirenz plasma concentrations (ρ = 0.72; P <.001). Of the other 19 polymorphisms associated with plasma efavirenz concentrations, 10 were in linkage disequilibrium with CYP2B6 516G→T at r² > 0.5 (figure 1 and table 2). These included three 5′UTRs, 6 intronic polymorphisms, and 1 exonuc polymorphism. On the basis of ρ values, no polymorphism had a stronger association than CYP2B6 516G→T with plasma efavirenz concentrations. Among 10 in-

Table 1. Positions, Frequencies, and Associations between Efavirenz (EfV) Concentrations and Each Polymorphism Assayed in CYP2A6, CYP2B6, ABCB1, CYP3A5, and CYP3A4

This table is available in its entirety in the online version of The Journal of Infectious Diseases.
Figure 1. Linkage disequilibrium (LD) plot of CYP2B6 polymorphisms. Data from the 45 participants are included. Black, $r^2 = 1$; shades of grey, $0 < r^2 < 1$; white, $r^2 = 0$. The plot was generated using Haploview (25). Solid arrows indicate the CYP2B6 516G→T (rs3745274) polymorphism. Open arrows indicate rs36118214. The CYP2A6 promoter polymorphism (rs28399433) is also included at far left. Four LD blocks are shown.

Individuals with slow metabolizer composite CYP2B6 516/983 genotypes, plasma efavirenz concentrations did not differ in 2 individuals heterozygous for the CYP2A6 promoter polymorphism (rs28399433), as compared to the 8 individuals lacking this polymorphism (data not shown).

Nine polymorphisms that were not in strong linkage disequilibrium with CYP2B6 516G→T ($r^2 < 0.5$) were also associated with plasma efavirenz concentrations at a $\rho$ of greater than 0.3 or less than −0.3 (figure 1 and table 2). These included three 5′ UTRs and 5 intronic polymorphisms in CYP2B6, as well as the CYP2A6 promoter polymorphism. Of these 9 polymorphisms, an intron 8 polymorphism (rs36118214) was most strongly associated with plasma efavirenz concentrations ($\rho = 0.48$; $P = 0.001$) (figure 3B). In contrast to both CYP2B6 516G→T and 983T→C, the less frequent rs36118214 A allele appeared to predict lower plasma efavirenz concentrations. Two of the other polymorphisms associated with plasma efavirenz concentrations were in linkage disequilibrium with rs36118214 at $r^2 > 0.5$ (rs1872121 at $r^2 = 0.941$ and rs12721649 at $r^2 = 0.668$) (figure 3C and 3D).

To assess whether associations were spurious, we compared $\rho$ values from the present study with those from a recent study that characterized genetic variants and plasma efavirenz pharmacokinetics among 34 healthy, HIV-negative African Americans who received a single 200-mg dose of nevirapine, followed several weeks later by a single 600-mg dose of efavirenz [22]. A total of 49 polymorphisms assayed in the present study were also evaluated in the previous study. Three polymorphisms associated with plasma efavirenz concentrations in the present study (rs28399433, rs8109818, and rs10853744) were not assayed in the previous study. Associations replicated remarkably well between studies, including polymorphisms in linkage disequilibrium with CYP2B6 516G→T and those in linkage disequilibrium with rs36118214 (figure 4). This supports the validity of these associations.

As shown in figure 3B, 4 individuals were homozygous for rs36118214, of whom 1 had extremely low plasma efavirenz concentrations (241 ng/mL and 126 ng/mL at weeks 2 and 4, respectively). This individual was also homozygous for rs1872121 and heterozygous for rs12721649. To further assess associations with lower plasma efavirenz concentrations, subgroup analyses were performed on the 14 individuals with ex-
<table>
<thead>
<tr>
<th>Gene, polymorphism</th>
<th>Linkage disequilibrium, r²</th>
<th>First homozygous genotype</th>
<th>Heterozygous genotype</th>
<th>Second homozygous genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With 516G&gt;T</td>
<td>With rs36118214</td>
<td>Alleles</td>
<td>Efav level, ng/mL, median (IQR)</td>
</tr>
<tr>
<td>CYP2A6</td>
<td></td>
<td>rs28399433</td>
<td>0.124</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>rs8109818</td>
<td>0.266</td>
<td>0.443</td>
<td>A/A</td>
</tr>
<tr>
<td></td>
<td>rs8105382</td>
<td>0.32</td>
<td>0.115</td>
<td>C/C</td>
</tr>
<tr>
<td></td>
<td>rs11063995</td>
<td>0.786</td>
<td>0.183</td>
<td>C/C</td>
</tr>
<tr>
<td></td>
<td>rs892216</td>
<td>0.71</td>
<td>0.276</td>
<td>C/C</td>
</tr>
<tr>
<td></td>
<td>rs12721652</td>
<td>0.372</td>
<td>0.24</td>
<td>C/C</td>
</tr>
<tr>
<td></td>
<td>rs2054675</td>
<td>0.831</td>
<td>0.194</td>
<td>C/C</td>
</tr>
<tr>
<td></td>
<td>rs8739581</td>
<td>0.38</td>
<td>0.257</td>
<td>A/A</td>
</tr>
<tr>
<td></td>
<td>rs7250873</td>
<td>0.656</td>
<td>0.086</td>
<td>C/A</td>
</tr>
<tr>
<td></td>
<td>rs3786547</td>
<td>0.831</td>
<td>0.194</td>
<td>C/C</td>
</tr>
<tr>
<td></td>
<td>rs35490259</td>
<td>0.174</td>
<td>0.5</td>
<td>C/C</td>
</tr>
<tr>
<td></td>
<td>rs1872121</td>
<td>0.228</td>
<td>0.941</td>
<td>A/A</td>
</tr>
<tr>
<td></td>
<td>rs3745274</td>
<td>...</td>
<td>0.242</td>
<td>G/G</td>
</tr>
<tr>
<td></td>
<td>rs10401737</td>
<td>0.592</td>
<td>0.264</td>
<td>C/C</td>
</tr>
<tr>
<td></td>
<td>rs2279343</td>
<td>0.957</td>
<td>0.231</td>
<td>A/A</td>
</tr>
<tr>
<td></td>
<td>rs12721649</td>
<td>0.165</td>
<td>0.668</td>
<td>A/A</td>
</tr>
<tr>
<td></td>
<td>rs7246456</td>
<td>0.957</td>
<td>0.231</td>
<td>C/C</td>
</tr>
<tr>
<td></td>
<td>rs8109217</td>
<td>0.956</td>
<td>0.236</td>
<td>C/C</td>
</tr>
<tr>
<td></td>
<td>rs36118214</td>
<td>0.242</td>
<td>0.321</td>
<td>A/A</td>
</tr>
<tr>
<td></td>
<td>rs10853744</td>
<td>0.228</td>
<td>0.941</td>
<td>A/A</td>
</tr>
<tr>
<td>Composite 516/983c</td>
<td>...</td>
<td>0.242</td>
<td>0.321</td>
<td>A/A</td>
</tr>
</tbody>
</table>

**NOTE.** IQR, interquartile range.

a Data indicate the r² measure of linkage disequilibrium between the chromosome 19 polymorphisms rs3745274 (516G>T) and rs36118214.

b Spearman rank correlation coefficient assesses monotonically increasing or decreasing trend by genotype as an ordered continuous variable. The signs (positive or negative) for r are determined by assigning numbers to each base as follows: A p 1, C p 2, G p 3, T p 4.

P value corresponding to the Spearman rank coefficient test. Only polymorphisms with a r of greater than 0.3 or less than –0.3 are shown.

Significant after Bonferroni correction for multiple comparisons.

c Composite CYP2B6 genotypes were as follows: extensive metabolizer (EXT), no variant allele at either position 516 or 983; intermediate metabolizer (INT), a single variant allele at either position 516 or 983, but not both; and slow metabolizer (SLO), 2 variant alleles (ie, either 516 T/T, 983 C/C, or 516 G/T with 983 T/C).
Figure 2. Relationships between each polymorphism and plasma efavirenz (EfV) concentrations.

did not ($\rho = 0.526; P = .065$). Although clinical relevance is uncertain with so few individuals, the patient in the present study with extremely low plasma efavirenz concentrations experienced virologic failure at week 24. No study participant discontinued efavirenz because of medication-associated adverse events.

In the previous study of 34 HIV-negative African Americans [22], the only individual homozygous for rs36118214 was also the only individual with extremely rapid plasma clearance of efavirenz. That individual had a 24-h efavirenz concentration of 89 ng/mL, considerably less than the median value of 582 ng/mL among other participants, and was homozygous for rs1872121 and homozygous for rs12721649. The association with rs36118214 in that study was also apparent in the subgroup of 10 individuals with extensive metabolizer composite 516/983

Figure 3. Relationships between selected polymorphisms and plasma efavirenz (EfV) concentrations. A, Relationship between composite CYP2B6 516/983 genotype and plasma efavirenz concentrations among all study participants. B, Relationship between rs36118214 and plasma efavirenz concentrations among all study participants. C, Relationship between rs1872121 and plasma efavirenz concentrations among all study participants. D, Relationship between rs12721649 and plasma efavirenz concentrations among all study participants. E, Relationship between rs36118214 and plasma efavirenz concentrations 24 h after dosing among 10 participants with composite CYP2B6 516/983 extensive metabolizer genotypes. F, Data from a previous study in which 34 healthy, HIV-negative African Americans received a single 600 mg dose of efavirenz, showing the relationship between rs36118214 and plasma efavirenz concentrations 24 h after dosing among 10 participants with composite CYP2B6 516/983 extensive metabolizer genotypes [22]. Horizontal lines represent medians. Each marker in A–E represents the geometric mean of paired efavirenz concentrations from each participant.
haplotype was unique to the individual with very low efavirenz concentrations.

Lower plasma efavirenz concentrations could be a consequence of increased CYP2B6 gene copy number. By allelotyping at 17 single nucleotide polymorphisms across CYP2B6, 37 participants (including the individual with very low plasma efavirenz concentrations) could be studied for gene copy number by allelotyping. None had >2 copies of CYP2B6 (data not shown).

Although the present study included a directly observed strategy to enhance adherence, and medication diaries documented complete adherence at weeks 2 and 4, we cannot exclude nonadherence. In fact, 1 individual with no detectable efavirenz, AZT, or 3TC in plasma was excluded from analyses for presumed nonadherence, despite medication diaries indicating adherence. If nonadherence covaried with genotype, one might expect this to also affect AZT and 3TC. However, there was no correlation between plasma concentrations of efavirenz and either AZT (\( \rho = 0.0187; \ P = .9 \)) or 3TC (\( \rho = -0.0057; \ P = .97 \)), and there was no association between any of the 56 polymorphisms and plasma concentrations of either AZT or 3TC (data not shown). This suggests that associations between polymorphisms and efavirenz concentrations were not explained by gross differences in adherence.

**DISCUSSION**

Efavirenz is a preferred component of first-line regimens for HIV-1 infection [1, 2] because of its superior performance in randomized clinical trials [3–6]. It is one of the most widely prescribed antiretroviral drugs in both affluent and resource-limited countries. The most important finding from the present study is that at least 1 CYP2B6 genetic polymorphism appears to be associated with decreased plasma efavirenz exposure. Replication of associations between the present study, involving HIV-infected Haitians who initiated antiretroviral therapy in Port-au-Prince, and a previous study involving healthy African Americans in Nashville, Tennessee [22], support the validity of this finding. This does not, however, preclude the need for further replication in larger cohorts.

Many genetic polymorphisms vary in frequency among populations [14]. The polymorphism most consistently associated with lower plasma efavirenz concentrations in the present report, rs36118214, was relatively frequent in both this study and in the previous study (minor allele frequency, 0.24 and 0.22, respectively) [22]. This polymorphism appears to vary markedly in frequency depending on geographic region of ancestry. According to the National Center for Biotechnology Information Web–based resource, dbSNP [16], the frequency of the A allele (based on analysis of relatively few genotypes) was 0.42 among sub-Saharan Africans and 0.21 among African Americans but was absent among Europeans and Asians.
polymorphisms that differ in frequency among populations might help explain, at least in part, disparate responses to therapy. In this regard, it is noteworthy that in AIDS Clinical Trials Group protocol A5095, in which most participants received efavirenz-containing regimens, virologic failure was considerably more frequent among black participants than among white participants, particularly in analyses that adjusted for self-reported nonadherence [30, 31].

Efavirenz is primarily metabolized by CYP2B6 [7]. A number of previous studies have examined associations between genetic polymorphisms and efavirenz pharmacokinetics and/or treatment response [8–15, 32]. Associations between increased plasma efavirenz exposure, CYP2B6 516G→T [8–15], and 983T→C [11–15] have been consistent across multiple studies and populations. Although the 516G→T polymorphism alters amino acid sequence, its effect appears to be through aberrant splicing that reduces functional mRNA and protein [33]. Additional CYP2B6 polymorphisms have been suggested to affect CYP2B6 activity [19], but they are either extremely infrequent or have not predicted plasma efavirenz exposure in vivo [12, 15]. In analyses designed to identify CYP2B6 copy number variants among 226 individuals, including 138 white individuals and 77 black individuals, only 1 individual heterozygous for a copy number variant was identified [34]. This was a crossover between CYP2B6 and the pseudogene CYP2B7 that was associated with increased plasma efavirenz concentra-

Figure 5. Relationships between CYP2B6 diplotype and plasma efavirenz (Efv) concentrations. A, Estimated haplotypes in region spanning CYP2B6 rs3745274 (516G→T) to rs10853744. Haplotypes are numbered from 1 to 14 in order of decreasing frequency. Shaded positions represent minor alleles for rs3745274 (516G→T), rs28399499 (983T→C), and rs36118214. Estimated haplotypes were generated using Powermarker [26] for the region spanning rs3745274 to rs10853744. B, Relationships between CYP2B6 diplotypes and plasma efavirenz concentrations. Horizontal lines represent medians. Diplotypes are ordered from lowest to highest median efavirenz concentrations. Each marker represents the geometric mean of paired efavirenz concentrations from each participant.
tions. CYP2A6, which is located ~141 kb upstream of CYP2B6 on chromosome 19, catalyzes a secondary metabolism pathway for efavirenz. A recent study suggests that loss of function polymorphisms in CYP2A6 (including the promoter polymorphism studied herein) when combined with CYP2B6 slow metabolism genotypes may predict particularly high efavirenz concentrations [35]. Our inability to show this association with a CYP2A6 promoter polymorphism (rs28399433) among composite 516/983 slow metabolizers may reflect the absence of individuals homozygous for the G allele.

The mechanism(s) by which the polymorphisms identified herein would be associated with decreased efavirenz exposure are unknown. We found no evidence of increased CYP2B6 copy number. Furthermore, the 3 implicated CYP2B6 polymorphisms are noncoding (rs36118214 and rs12721649 in intron 8 and rs1872121 in intron 3). There are several possibilities. These polymorphisms may tag as yet unidentified chromosome 19 variants that are directly causative. Alternatively, at least 1 of these polymorphisms may in some way be involved in regulating CYP2B6 transcription. Or these may cause aberrant splicing of CYP2B6 that in some way increases enzymatic activity. Extensive genotyping of large cohorts may identify more precise genetic predictors of low plasma efavirenz concentrations.

There were limitations to the present study. Because the sample size was relatively small, only 4 individuals were homozygous for the polymorphism most strongly associated with decreased plasma efavirenz concentrations. Larger cohorts would also strengthen haplotype analyses. Despite associations being consistent between 2 studies [22], genomic association studies are plagued by false discovery [36]. It is therefore critical that these putative predictors of decreased plasma efavirenz exposure be replicated in other cohorts. In addition, because genetic predictors of efavirenz pharmacokinetics may differ depending on geographic region of ancestry, associations may differ in other populations. Associations with virologic response in large cohorts should be assessed to understand potential implications for clinical care. Despite the use of directly observed drug administration to enhance adherence, we cannot absolutely exclude nonadherence. We used self-identified race rather than population-informative genetic markers, which could identify unrecognized population stratification.

These findings, if replicated in additional cohorts, may have implications for the HIV-1 pandemic. A lower therapeutic cutoff of 1000 ng/mL has been suggested for efavirenz [37]. Virologic failure with efavirenz plus 2 NRTIs typically leads to emergence of resistance to NNRTIs and NRTIs [38]. With continued selective pressure, progressive cross-resistance to multiple drugs, including newer NNRTIs such as etravirine, can develop [39]. This may result in HIV-1 disease progression, fewer future treatment options, and transmission of multidrug-resistant virus. Antiretroviral prescribing strategies could be improved by understanding whether certain individuals are genetically predisposed to virologic failure with efavirenz.

Acknowledgments

We are grateful to the persons who volunteered for this study, and we thank Guylaine Pierre-Louis and Guerline Antoine for performing research study visits at GHESKIO; Hailing Jin, Danielle Richardson, Cara Sutcliffe, and Ping Mayo of the Vanderbilt University DNA Resources Core for providing technical assistance for genetic assays; and Tebed Gebretsadik, Gail Mayo, Usha Menon, Edward P. Acosta, Ayumi Shintani, Michael Floyd, C. Michael Stein, and Grant R. Wilkinson for performing the previously published pharmacogenomics study [22].

References
