Effect of CYP2B6, ABCB1, and CYP3A5 Polymorphisms on Efavirenz Pharmacokinetics and Treatment Response: An AIDS Clinical Trials Group Study

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In AIDS Clinical Trials Group protocols 384, A5095, and A5097s, we characterized relationships between 22 polymorphisms in CYP2B6, ABCB1, and CYP3A5; plasma efavirenz exposure; and/or treatment responses. A stepwise logistic regression procedure selected polymorphisms associated with reduced drug clearance adjusted for body mass index and the composite CYP2B6 516/983 genotype. Connections between selected polymorphisms and treatment responses were characterized by competing risk methodology. Association analyses involved 821 individuals (317 for pharmacokinetics and 643 for treatment response). Models that included CYP2B6 516/983 genotype best predicted pharmacokinetics. Slow-metabolizer genotypes were associated with increased central nervous system events among white participants and decreased virologic failure among black participants.

Efavirenz is widely prescribed for human immunodeficiency virus type 1 (HIV-1) infection, but some efavirenz recipients experience virologic failure [1] and/or central nervous system (CNS) symptoms [2]. Efavirenz is metabolized by cytochrome P450 (CYP) 2B6. In analyses involving 154 AIDS Clinical Trials Group (ACTG) study 5097s participants (a 24-week substudy of A5095), CYP2B6 516G→T predicted increased plasma efavirenz exposure and CNS adverse experiences [3]. Subsequent studies have replicated this pharmacokinetic association (eg, Haas et al [4]). A less frequent CYP2B6 polymorphism, 983T→C, also predicts plasma efavirenz exposure [5, 6]. Increased frequencies of 516G→T and 983T→C among black persons largely explain their greater mean plasma efavirenz concentrations. Additional CYP2B6 polymorphisms suggested to affect CYP2B6 activity have been extremely infrequent or have not predicted plasma efavirenz exposure [7, 8].

Polymorphisms in ABCB1, which encodes P glycoprotein, may predict altered pharmacokinetics of some drugs, albeit not efavirenz [3, 4]. Two studies have suggested that ABCB1 3453C→T predicts more favorable virologic responses to efavirenz-containing regimens [4, 9]. A weak association between CYP3A5 6986A→G and plasma efavirenz exposure has been suggested [3].

We evaluated whether 22 single-nucleotide polymorphisms in CYP2B6, ABCB1, and CYP3A5 predict plasma efavirenz exposure among participants in ACTG 384 and A5097s and explored associations with treatment responses in A5095.

Methods.

Treatment-naïve individuals were randomized to efavirenz-containing regimens in ACTG studies 384 [10] or...
A5095 [1] (including its neurologic substudy, A5097s [2]), with DNA obtained under protocol A5128.

The present study comprises (1) pharmacokinetic association analyses involving individuals with efavirenz assay data from ACTG 384 and A5097s and (2) treatment outcome association analyses involving efavirenz recipients from A5095. Almost all participants in the pharmacokinetic association studies were included in 2 previous studies describing the association between CYP2B6 516G→T and efavirenz exposure [3, 4]. The present study complied with the Declaration of Helsinki and was approved by institutional review boards for each site; subjects provided written informed consent.

Self-identified race/ethnicity categories—white, non-Hispanic; black, non-Hispanic; and Hispanic—are hereafter referred to as white, black, and Hispanic, respectively. Analyses are limited to white, black, and Hispanic participants, which comprised 97% of subjects.

For pharmacokinetic association analyses, genotyping was performed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Assay details have been described in part elsewhere [7, 11]. We assayed 15 nonsynonymous CYP2B6 polymorphisms and an upstream untranslated region polymorphism. We also assayed ABCB1 1236C→T, 3435C→T, and 2677G→T/A; 2 tagging intronic polymorphisms; and the CYP3A5 696A→G loss-of-function polymorphism. For treatment response analyses, genotyping of selected polymorphisms was performed with the ABI PRISM 7900 HT sequence detection system (Applied Biosystems). Customized TaqMan assay identifiers are available on request.

Plasma efavirenz concentrations were assayed by high-performance liquid chromatography at treatment weeks 1, 4, 12, and 24; sampling times were not prespecified; time of prior dose was determined by patient report. Association analyses for efavirenz pharmacokinetics were based on raw efavirenz concentration data adjusted for dosing time using simulated efavirenz concentration–time curve percentiles, generated by pharmacokinetic model simulations [12]. On the basis of dosing time, each observed concentration was assigned to a percentile. For each subject, the summary statistic for analysis was the median of observed time-adjusted percentiles over up to 4 time points for each individual. To minimize confounding by nongenetic factors and to assure steady state, we excluded (1) individual efavirenz concentrations beyond 24 h after the prior dose or before at least 2 weeks of efavirenz and (2) subjects with only 1 evaluable efavirenz value or a >30 percentile difference between any 2 concentrations. A binary outcome was defined for prediction modeling on the basis of whether a subject’s median time-adjusted percentile was at or above the 75th percentile (corresponding to an efavirenz trough concentration of ≥2350 ng/mL). Sensitivity analyses were performed using alternative percentile cutoffs and different tolerance ranges for intraindividual variability.

Response end points included time from randomization to (1) the first grade 2 or higher CNS adverse event; (2) virologic failure, defined as 2 consecutive HIV-1 RNA levels ≥200 copies/mL at or after week 16; (3) virologic failure with detectable efavirenz and/or lamivudine resistance; and (4) the first plasma HIV-1 RNA level <200 copies/mL. Scheduled A5095 study visits occurred at weeks 2, 4, 8, 12, 16, 20, and 24 and every 8 weeks thereafter.

Deviation from Hardy-Weinberg equilibrium (HWE) expectations was evaluated using exact tests. Allelic frequencies were compared among ethnic groups by the Fisher exact test.

Predictors of high efavirenz concentrations were assessed using logistic regression. All models adjusted for baseline body mass index (BMI) and then used a forward stepwise approach adding the polymorphism significant at $P < .05$ and resulting in the largest change in likelihood. The sensitivity and specificity of resulting models were estimated assuming an estimated prediction probability of >75% as a positive response. We included CYP2B6 516G→T and 983T→C in all models as a composite CYP2B6 516/983 genotype (hereafter, 516/983 genotype), on the basis of a combined number of minor allele polymorphisms (0, extensive metabolizer; 1, intermediate metabolizer; 2, slow metabolizer).

For association analyses of treatment response, time to first CNS event was truncated at 24 weeks in the absence of a prior event; other time-to-event end points were censored at study completion or discontinuation. Discontinuation of efavirenz was handled as a competing risk event; resistance-free virologic failure was an additional competing risk event for resistance-based end points. The cumulative incidence of each outcome was estimated over time by genotype and was compared using the Gray $k$-sample test. Associations between each polymorphism and a cause-specific hazard were examined using stepwise Cox regression models (with $P < .05$ used to determine whether polymorphisms enter or exit the model). With the exception of 516/983 genotype, genotype effects were introduced as a categorical covariate on 3 levels for 0, 1, or 2 polymorphic copies; 516/983 genotype was included as a binary covariate for 2 polymorphisms versus 0 or 1. Sex and BMI were included as candidate covariates as part of model selection. Analyses of virologic failure end points also included self-reported nonadherence (defined at week 12 as missing at least 1 dose during a 4-day recall) and thus included only subjects at risk at week 12.

All analyses were performed including all participants with adjustment for self-reported race/ethnicity as well as separately among race/ethnicity subgroups. Since the inception of the present study, this same clinical trial population has undergone whole genome analysis for a separate project [13]. A sensitivity
analysis that more rigorously adjusted for population stratification incorporated EIGENSTRAT values generated from whole genome data. Analyses were also repeated excluding individuals with any inconsistency in CYP2B6 or ABCB1 genotypes assayed by different methodologies.

All P values are nominal and are unadjusted for multiple comparisons. Analyses were done using SAS (version 9.1) and SPLUS (version 6.2) software.

Results. Analyses comprised 831 subjects (19% female, 48% white, 34% black, and 18% Hispanic). The mean baseline HIV-1 RNA level was 4.89 log_{10} copies/mL, and the mean baseline CD4 T cell count was 246 cells/μL. In the pharmacokinetic analysis cohort, of 16 CYP2B6 polymorphisms assayed, only 516G→T, 785A→G, 983T→C, and 1459C→T were at >5% frequency in at least 1 population (Figure 1A). There were no deviations from HWE among white or Hispanic participants in either cohort. In the treatment response cohort, there were deviations from HWE among black participants at ABCB1 positions 3435 (P = .01) and 2677 (P < .001), which are in strong linkage disequilibrium. Assay reliability at these 2 positions was confirmed among the 171 subjects with results obtained through both cohorts by both MALDI-TOF MS [11] and TaqMan, among 154 individuals with results previously obtained by a different real-time polymerase chain reaction methodology [3], and among all treatment response participants by a different mass spectroscopy–based assay [14].

The distribution of plasma efavirenz concentration–time values among 489 individuals with both efavirenz concentration and genotype data is presented in Figure 1B. Pharmacokinetic modeling analyses were limited to 317 individuals who met the inclusion criteria. Relationships between CYP2B6 polymorphisms and concentration percentiles are shown in Figure 1C. No independent associations were apparent for the other 2 frequent CYP2B6 polymorphisms, 785A→G and 1459C→T. Low allelic frequencies limited our ability to test associations with other CYP2B6 polymorphisms. One individual heterozygous for CYP2B6 136A→G (*11) with a concomitant 516/983 intermediate-metabolizer genotype had among the highest plasma efavirenz concentrations. There was no other apparent association with other CYP2B6, ABCB1, or CYP3A5 polymorphisms.

These descriptive findings were confirmed by modeling. Consistently across ethnicity groups, none of the additional polymorphisms improved prediction of high plasma efavirenz concentrations. Although the estimated model specificity including only 516/983 genotype and BMI was >90%, model sensitivity was only ~50%. This suggests that although 516/983 slow-metabolizer genotype was highly predictive of efavirenz concentrations in the top 75th percentile, the absence of this genotype could not reliably predict efavirenz concentrations below this cutoff. Results were consistent in sensitivity analyses that adjusted for ACTG protocol, concomitant nucleoside reverse-transcriptase inhibitor or nelfinavir use, cutoff points other than the 75th percentile for slow metabolizers, and alternative ranges of allowable intrapatient variability.

We next characterized relationships between genetic polymorphisms and responses to efavirenz-containing regimens among 643 participants in A5095, of whom 129 were also in the pharmacokinetic analyses. In addition to 516/983 genotype, we evaluated ABCB1 polymorphisms, because these might affect intracellular but not plasma drug concentrations.

Among white participants, there was some evidence of an increased cumulative incidence of the first CNS event associated with 516/983 slow-metabolizer genotype (P = .04). This was not apparent among black or Hispanic participants (Figure 2A–2D). Among black participants, the incidence of virologic failure over time was lower among those with 516/983 slow-metabolizer genotypes (P = .02). This association was not apparent among white or Hispanic participants (Figure 2E–2H). No other associations between CYP2B6 or ABCB1 genotype and any other treatment response end points were observed overall and among white, black, or Hispanic participants analyzed separately. No changes in these results were observed in sensitivity analyses that incorporated EIGENSTRAT values or after excluding individuals with any inconsistency in CYP2B6 or ABCB1 genotypes assayed with different methodologies.

To test for genotype-adherence interactions, we applied a stepwise selection Cox proportional hazards regression model. This analysis confirmed results of univariate analyses. In particular, regarding virologic failure among black participants, no interaction between 516/983 genotype and nonadherence was detected (P = .97).

Discussion. Numerous studies have found an association between CYP2B6 516G→T and higher plasma efavirenz concentrations. We genotyped CYP2B6 more extensively than almost every prior study, to assess whether additional nonsynonymous variants better explain interindividual variability in pharmacokinetics and treatment response. We clearly establish that CYP2B6 983T→C improves the predictive ability of CYP2B6 516G→T for efavirenz pharmacokinetics, as has been suggested by smaller studies [5, 6]. In multivariable models, the ability of 516/983 genotype to predict efavirenz exposure was not improved by additional nonsynonymous CYP2B6 polymorphisms or selected ABCB1 and CYP3A5 polymorphisms. Considerable interindividual variability remains unexplained by these 2 polymorphisms.

Regarding virologic response, we found no strong consistent associations with the polymorphisms assayed. Among black participants, the cumulative incidence of virologic failure was lowest with 516/983 slow-metabolizer genotypes. Although biologically plausible, extensive metabolizers should still have sufficient efavirenz concentrations to control HIV-1 replication.
Figure 1. Relationships between genetic polymorphisms and plasma efavirenz concentration. A, Allelic frequencies among 489 participants genotyped for the pharmacokinetic analysis cohort. B, Concentration-time relationships between plasma efavirenz concentrations and hours after last dose among all 489 participants. Some individuals contribute multiple data points to the figure. C, Median time-adjusted concentration percentile ranks, stratified for the composite CYP2B6 516/983 genotype, among the 317 pharmacokinetic modeling cohort participants, regardless of race/ethnicity. Each individual contributes a single data point to the figure. Separate displays generated for each race/ethnicity group were consistent (data not shown). EFV, efavirenz; extens., extensive metabolizers; intermed., intermediate metabolizers; slow, slow metabolizers.
We hypothesize that among slow efavirenz metabolizers, sustained high efavirenz concentrations during brief periods of nonadherence may allow continued control of HIV-1 replication. In exploratory analyses, however, we saw no interaction between CYP2B6 genotype and self-reported nonadherence. Although intriguing, this association between 516/983 slow-metabolizer genotype and virologic failure was not seen in white or Hispanic participants and thus requires replication in other cohorts.

Regarding CNS events, among white participants the cumulative incidence of a grade 2 or higher CNS event or virologic failure by genotype are displayed at the bottom. The area between the 2 curves represents the probability of remaining event free over time. P values were determined by Gray k-sample tests for comparing the cumulative incidence of competing risks. Solid lines represent extensive metabolizers (ext.); dashed lines, intermediate metabolizers (inter.); and dotted lines, slow metabolizers. Adverse events were graded using the National Institute of Allergy and Infectious Diseases Division of AIDS toxicity scale. VF, virologic failure.
Cumulative incidence of grade 2 or higher events was highest among individuals with 516/983 slow-metabolizer genotypes. This association is again plausible. It was therefore unexpected that this association was not seen among black participants, in whom slow-metabolizer genotypes are more frequent. Furthermore, none of the 16 subjects with slow-metabolizer genotypes in the Hispanic subgroup reported adverse CNS events. The original report that associated CYP2B6 516G>T with CNS adverse experiences (which included 154 individuals from the present study) was too small for subgroup analyses [3] and captured efavirenz side effects with more sensitive targeted questionnaires.

A small open-label study suggested that, among individuals receiving efavirenz-based regimens, dose reduction among individuals with slow-metabolizer genotypes may reduce self-reported CNS symptoms [15]. Our study, however, suggests that slow-metabolizer genotypes may confer some virologic benefit and raises concern that dose reduction may increase the risk of virologic failure.

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**References**


