Functional CYP2B6 variants and virologic response to an efavirenz-containing regimen in Port-au-Prince, Haiti

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Objectives: Efavirenz is widely prescribed for HIV-1 infection. Three polymorphisms in CYP2B6 define plasma efavirenz trough concentration strata that vary across an ~10-fold range. We characterized associations between human genetic polymorphisms and virologic response among participants who received efavirenz-containing regimens in a prospective clinical trial.

Methods: We genotyped 76 polymorphisms in CYP2B6 (including those that define efavirenz concentration strata), CYP2A6, CYP3A4, CYP3A5 and ABCB1 and week 48 virologic responses in 360 Haitians who initiated efavirenz-containing regimens in protocol HT 001. Associations were characterized by logistic regression analysis and Fisher’s exact test.

Results: Proportions with HIV-1 RNA <50 or <200 copies/mL did not differ across 10 CYP2B6 metabolizer strata. In analyses that combined strata into three metabolizer levels (extensive, intermediate and slow), the respective proportions were 0.79, 0.79 and 0.81 (<50 copies/mL cut-off) and 0.84, 0.86 and 0.87 (<200 copies/mL cut-off).

Genetic associations were not identified after controlling for baseline variables or with other polymorphisms after adjusting for multiple comparisons.

Conclusions: Virologic failures in HT 001 were not explained by genetic polymorphisms known to define the lowest plasma efavirenz concentration stratum.

Keywords: HIV, AIDS, pharmacogenomics, antiretroviral therapy

Introduction

Efavirenz is widely prescribed for HIV-1 infection. Three CYP2B6 polymorphisms predict increased plasma efavirenz exposure: G516T (rs3745274), T983C (rs28399499) and C15582T (rs4803419). Plasma Cmin concentration strata defined by these polymorphisms vary across an ~10-fold range. Homozygosity for CYP2B6 15582 CC, 516 GG and 983 TT predict the lowest stratum, but C15582T does not contribute to the highest stratum, which are largely explained by G516T and T983C.

Previous reports suggest that CYP2B6 polymorphisms may affect treatment response. In AIDS Clinical Trials Group (ACTG) protocols, an association was suggested between the CYP2B6 composite 516/983 extensive metabolizer genotype and increased virologic failure among black subjects, but not among white or Hispanic subjects. A subsequent Swiss HIV Cohort Study report (~80% Caucasians) suggested that CYP2B6 polymorphisms did not predict efavirenz discontinuation, although the risk may have been increased with various combinations of CYP2B6, CYP2A6 and CYP3A4 polymorphisms.

Until 2012, WHO guidelines had recommended initiating antiretroviral therapy at ≤200 CD4 cells/mm3 or with clinical AIDS. Randomized clinical trial HT 001, performed in Port-au-Prince, Haiti, demonstrated that earlier therapy initiation decreased rates of death and incident tuberculosis, which affected treatment guidelines. The present study determined whether polymorphisms in CYP2B6 and/or other genes predicted virologic failure in HT 001.

Patients and methods

Protocol HT 001 (ClinicalTrials.gov NCT00120510) was a randomized, open-label, controlled trial of early versus standard timing for initiation of antiretroviral therapy among adults with between 200 and 350 CD4 cells/mm3. The study was conducted at the centre of the Haitian Group for the Study of Kaposi’s Sarcoma and Opportunistic Infections (GHESKIO). Participants were randomized 1:1 to early or standard timing for treatment initiation with fixed-dose zidovudine/lamivudine (every 12 h) plus efavirenz (600 mg every 24 h, at bedtime). Informed consent was required from participants before study entry. The early group...
began treatment within 2 weeks after enrolment. For the first 2 months, a field worker observed the morning dose. Participants in the standard group started therapy for any CD4 count ≤200 cells/mm\(^3\) or for any AIDS-defining illness. A total of 816 participants (408 per arm) were followed for a median of 21 months.

Laboratory results included CD4 counts at baseline and at least every 6 months. Complete blood counts and serum chemistries were obtained every 3 months for participants receiving antiretroviral drugs. Plasma specimens obtained at enrolment and every 48 weeks were cryopreserved until HT 001 was completed and were then assayed for HIV-1 RNA using NucliSens EasyQ\(^\oplus\) (bioMérieux, Paris, France). Participants were given the option to consent for pharmacogenomic analyses. The present analyses are based on the clinical data available when the dataset was closed for primary analysis.\(^7\)

Genomic DNA was extracted from saliva. A total of 76 polymorphisms (49 in CYP2B6, 22 in CYP2A6, 1 in CYP2A4, 1 in CYP3A4 and 3 in ABCB1) were assayed using MassARRAY\(^\oplus\) iPLEX Gold (Sequenom). The assay design is described elsewhere.\(^3\) Polymorphisms with missingness >5% or minor allele frequencies ≤5% were excluded, as were samples with genotyping failure >2%. Each polymorphism was in Hardy-Weinberg equilibrium. Associations with each of 10 CYP2B6 metabolizer strata were assessed with Fisher’s exact test. Other associations were assessed by logistic regression. Associations with CYP2B6 polymorphisms known to predict efavirenz plasma concentrations were not corrected for multiple comparisons, because their pharmacokinetic associations are so well established. This use of DNA was approved by institutional review boards at Weill Cornell Medical College, GHESKIO and Vanderbilt. The former two boards approved HT 001 and its informed consent document.

Results

Of 816 subjects in HT 001, 387 initiated antiretroviral therapy and had ≥12 months of follow-up (327 in the early group and 60 in the standard group)\(^7\) and 636 had evaluable genotype data. Pharmacogenomic association analyses are based on 360 individuals who initiated 600 mg of efavirenz once daily, had no change in their antiretroviral therapy regimen before week 48 and had HIV-1 RNA data available at weeks 0 and 48. Among these subjects, 211 (58.6%) were female. At week 0, the median age was 40.6 years (IQR 32.9 – 47.0 years) and the median CD4 count was 260 cells/mm\(^3\) (IQR 214 – 301 cells/mm\(^3\)), closely reflecting the larger HT 001 cohort.\(^7\) Minor allele frequencies of the 76 polymorphisms and combined strata based on these polymorphisms are described elsewhere.\(^9\) Polymorphisms with missingness >5% or minor allele frequencies ≤5% were excluded, as were samples with genotyping failure >2%. Each polymorphism was in Hardy-Weinberg equilibrium. Associations with each of 10 CYP2B6 metabolizer strata were assessed with Fisher’s exact test. Other associations were assessed by logistic regression. Associations with CYP2B6 polymorphisms known to predict efavirenz plasma concentrations were not corrected for multiple comparisons, because their pharmacokinetic associations are so well established. This use of DNA was approved by institutional review boards at Weill Cornell Medical College, GHESKIO and Vanderbilt. The former two boards approved HT 001 and its informed consent document.

### Figure 1

Figure 1. Proportions of efavirenz recipients with plasma HIV-1 RNA below limits of quantification at 48 weeks. Graphs represent subjects who initiated 600 mg of efavirenz once daily, had no change in their antiretroviral therapy regimen before week 48 and had HIV-1 RNA data available at both week 0 and week 48. (a) Week 48 HIV-1 RNA <50 copies/mL for each of 10 CYP2B6 metabolizer strata, defined by C15582T, G516T and T983C. (b) Week 48 HIV-1 RNA <200 copies/mL for each of 10 CYP2B6 metabolizer strata, defined by C15582T, G516T and T983C. Predicted median efavirenz C\(_{\text{min}}\) concentrations are from the study by Holzinger et al.\(^5\) (c, left half) Week 48 HIV-1 RNA <50 copies/mL for each of three combined CYP2B6 metabolizer strata. (c, right half) Week 48 HIV-1 RNA <200 copies/mL for each of three combined CYP2B6 metabolizer strata. Combined CYP2B6 metabolizer strata are defined as follows: EXT. (extensive) = left two strata in (a) and (b); Figure 1; INT. (intermediate) = middle five strata in (a) and (b); and SLO. (slow) = right three strata in (a) and (b). Error bars represent 95% CI by the modified Wald method. BLQ, below the limit of quantification.
the likelihood of week 48 HIV-1 RNA <50 or <200 copies/mL (Figure 1c, P>0.05 for each comparison).

Genetic associations with virologic response were further tested in multivariable analyses that controlled for baseline log10 HIV-1 RNA, sex, age and baseline CD4 count. There was again no significant association between combined CYP2B6 strata and the likelihood of week 48 HIV-1 RNA <50 or <200 copies/mL (Table 1).

Additional analyses explored associations beyond CYP2B6 C15582T, G516T and T983C. By univariate logistic regression analysis, there was no association between any of the 76 polymorphisms and the likelihood of week 48 HIV-1 RNA <50 or <200 copies/mL, at a Bonferroni-corrected P value threshold of P=0.0007 (data not shown). The lowest P value was for CYP3A4 rs2740574 for <50 copies/mL (OR=0.53, P=0.0016) and CYP2A6 rs28399433 for <200 copies/mL (OR=0.42, P=0.0045). The results for all 76 polymorphisms are shown in Tables S1 and S2 (available as Supplementary data at JAC Online). The results did not differ in analyses that controlled for combined CYP2B6 metabolizer strata.

**Discussion**

A previous analysis involving 217 black, non-Hispanic individuals who initiated efavirenz-containing regimens in ACTG trials suggested an association between CYP2B6 extensive metabolizer genotypes and virologic failure.6 We found no association between functional CYP2B6 polymorphisms and virologic response among patients in Haiti who initiated an efavirenz-containing regimen and continued the regimen for ≥48 weeks. This is remarkable considering that median plasma efavirenz C\text{min} values vary ~10-fold across CYP2B6 strata.5 Additional polymorphisms in CYP2B6, CYP2A6, CYP3A4, CYP3A5 and ABCB1 were not significantly associated with the likelihood of virologic response. We must consider polymorphisms with the lowest P values (CYP3A4 rs2740574 and CYP2A6 rs28399433) spurious unless replicated in other cohorts.

This study has several implications. With CYP2B6 extensive metabolizer genotypes, virologic responses would likely not improve with increasing the efavirenz dose (i.e. to move into a higher C\text{min} stratum). Conversely, these results suggest that with CYP2B6 intermediate or slow extensive metabolizer genotypes, the efavirenz dose could be decreased from 600 mg once daily (perhaps to 400 mg in intermediate metabolizers and to 200 mg in slow metabolizers) without reducing virologic responses (i.e. to move into a lower C\text{min} stratum). Such dose reduction might reduce CNS side effects. These results complement the ENCORE1 study, which showed no difference in virologic response among 630 subjects randomized to initiate once-daily efavirenz at 600 versus 400 mg, with once-daily tenofovir/emtricitabine, regardless of the CYP2B6 genotype.10 Because 600 mg of efavirenz is often prescribed in fixed-dose combination tablets, efavirenz dose reduction may require an increased number of pills, which could reduce adherence.

This analysis had particular strengths. By considering CYP2B6 C15582T, rather than only CYP2B6 G516T and T983C, we could assign subjects to the very lowest efavirenz C\text{min} stratum. Genotyping for CYP2B6 C15582T reassigned 33% of subjects out of this lowest stratum, who were otherwise considered extensive metabolizers based solely on CYP2B6 G516T and T983C. Possible confounding by non-adherence and other non-genetic factors was reduced by the use of modified direct observation in HT 001 and by focusing on individuals with no change in their antiretroviral therapy regimen before week 48 and with HIV-1 RNA data available at both week 0 and week 48.

There were limitations to this study. Associations between CYP2B6 extensive metabolizer genotypes and virologic failure might be identified in individuals at increased likelihood of virologic failure if concomitant non-genetic factors (e.g. other medications) were to further lower plasma efavirenz exposure. While the present analysis was not designed to address possible side effects associated with higher plasma efavirenz concentrations, frequencies of CYP2B6 slow metabolizer genotypes did not differ significantly between individuals included in this analysis and the other subjects with available genotype data.

This study enhances understanding of the influence of human genetic variants on antiretroviral pharmacodynamics and encourages continued efforts to use this knowledge to optimize antiretroviral safety and efficacy.

<table>
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<tr>
<th>Variable</th>
<th>OR (95% CI)</th>
<th>P value</th>
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<tbody>
<tr>
<td>Week 48 HIV-1 RNA &lt;50 copies/mL</td>
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<tr>
<td>age (per year)</td>
<td>1.046 (1.015–1.078)</td>
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<td>week 0 HIV RNA (per log_{10} copies/mL)</td>
<td>0.81 (0.57–1.16)</td>
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<td>baseline CD4 T cells (per cell/mm³)</td>
<td>1.001 (0.998–1.005)</td>
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<td>CYP2B6 genotype (per metabolizer stratum)a</td>
<td>1.13 (0.77–1.65)</td>
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<tr>
<td>sex (female)</td>
<td>0.87 (0.50–1.49)</td>
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<td>Week 48 HIV-1 RNA &lt;200 copies/mL</td>
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<td>age (per year)</td>
<td>1.039 (1.004–1.075)</td>
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<td>week 0 HIV RNA (per log_{10} copies/mL)</td>
<td>0.81 (0.54–1.22)</td>
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<tr>
<td>baseline CD4 T cells (per cell/mm³)</td>
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<td>sex (female)</td>
<td>0.75 (0.40–1.41)</td>
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aThis OR refers to each step up, from extensive metabolizer to intermediate metabolizer to slow metabolizer.
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Transparency declarations
D. W. H. has been principal investigator on research grants to Vanderbilt University from Boehringer Ingelheim, Merck and Gilead Sciences, and has been a consultant to Merck. The remaining authors have none to declare.

Supplementary data
Tables S1 and S2 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References
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