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Secondary metabolism pathway polymorphisms and plasma efavirenz concentrations in HIV-infected adults with *CYP2B6* slow metabolizer genotypes

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Objectives: Efavirenz is widely prescribed for HIV-1 infection, and *CYP2B6* polymorphisms 516G→T and 983T→C define efavirenz slow metabolizer genotypes. To identify genetic predictors of higher plasma efavirenz concentrations beyond these two common functional alleles, we characterized associations with mid-dosing interval efavirenz concentrations in 84 HIV-infected adults, all carrying two copies of these major loss-of-function *CYP2B6* alleles.

Methods: Study participants had been randomized to efavirenz-containing regimens in prospective clinical trials and had available plasma efavirenz assay data. Analyses focused on secondary metabolism pathway polymorphisms *CYP2A6* -48T→G (rs28399433), *UGT2B7* 735A→G (rs28365062) and *UGT2B7* 802T→C (rs7439366). Exploratory analyses also considered 196 polymorphisms and 8 copy number variants in 41 drug metabolism/transport genes. Mid-dosing interval efavirenz concentrations at steady-state were obtained ≥8 h but <19 h post-dose. Linear regression was used to test for associations between polymorphisms and log-transformed efavirenz concentrations.

Results: Increased efavirenz concentrations were associated with *CYP2A6* -48T→G in all subjects ($P = 3.8 \times 10^{-4}$) and in Black subjects ($P = 0.027$) and White subjects ($P = 0.0011$) analysed separately; and with *UGT2B7* 735 G/G homozygosity in all subjects ($P = 0.006$) and in Black subjects ($P = 0.046$) and White subjects ($P = 0.062$) analysed separately. In a multivariable model, *CYP2A6* -48T→G and *UGT2B7* 735 G/G homozygosity remained significant ($P < 0.05$ for each). No additional polymorphisms or copy number variants were significantly associated with efavirenz concentrations.

Conclusions: Among individuals with a *CYP2B6* slow metabolizer genotype, *CYP2A6* and possibly *UGT2B7* polymorphisms contribute to even higher efavirenz concentrations.

Keywords: pharmacogenomics, pharmacogenetics, pharmacokinetics, antiretroviral therapy, non-nucleoside reverse transcriptase inhibitor

Introduction

The once-daily non-nucleoside reverse transcriptase inhibitor efavirenz is one of the most frequently prescribed antiretrovirals worldwide. It is included among recommended first-line regimens for HIV-1 infection,[1] based on data from many prospective, randomized clinical trials.[2–7] Efavirenz is metabolized primarily by cytochrome P450 (CYP) 2B6, with minor metabolism by CYP2A6 and CYP3A4/5,[8,9] and direct *N*-glucuronidation by UDP-glucuronosyltransferase (UGT) 2B7.[10]

Three *CYP2B6* polymorphisms, 516G→T (rs3745274),[11–16] 983T→C (rs28399499)[16–19] and 15582C→T (rs4803419), have consistently been associated with increased plasma efavirenz exposure.[16] The greater frequency of *CYP2B6* 516G→T with African ancestry than with European ancestry[20] largely explains the somewhat greater mean plasma efavirenz trough concentrations

(C_{min}) with African ancestry.[21] The per allele effect of *CYP2B6* 983T→C on efavirenz concentrations is somewhat greater than that of 516G→T,[16] although 983T→C is far less frequent and appears only to be found with African ancestry.[20] The per allele effect of *CYP2B6* 15582C→T, which is frequent with European and Asian ancestry,[20] is modest compared with 516G→T.[16] These three polymorphisms stratify patients into 10 plasma trough concentration subgroups with medians that span an ~10-fold range.[16] The top three strata (i.e. *CYP2B6* slow metabolizer genotypes) are defined by either 516 T/T homozygosity, dual 516 G/T–983 C/T heterozygosity, or 983 C/C homozygosity. Because *CYP2B6* 516T, 983C and 15582T reside on mutually exclusive haplotypes, 15582T is absent in *CYP2B6* slow metabolizer genotypes. These three polymorphisms explained ~35% of overall interindividual variability in efavirenz estimated C_{min}.[16]

Additional *CYP2B6* polymorphisms suggested to affect CYP2B6 activity either have not predicted plasma efavirenz exposure[22,23] or have been extremely infrequent.[18,22] Polymorphisms in genes beyond *CYP2B6* reported to affect interindividual variability in efavirenz pharmacokinetics include *CYP2A6*,[24,25] *UGT2B7*,[25] *CYP3A5*[11] and *NR1I3*[26,27] (which encodes the constitutive androstane receptor), but findings have seemed to be inconsistent, perhaps a consequence of differences in study design. Kwara *et al*.[25,28] reported that among 94 HIV-infected Ghanaian patients, increased mid-dosing interval plasma efavirenz concentrations were associated with *CYP2A6* -48T→G (rs28399433, **9*) or **17* carrier status and *UGT2B7*1a* status, in addition to *CYP2B6* 516G→T and 983T→C. Similarly, di Iulio *et al*.[24] reported that in a predominantly Caucasian cohort of 169 HIV-infected individuals, *CYP2A6* loss-of-function alleles (primarily *CYP2A6* -48T→G) had an effect on efavirenz exposure, but only with concomitant *CYP2B6* loss-of-function genotypes. However, in a genome-wide association study (GWAS) of efavirenz estimated C_{min} values, which involved 856 ACTG protocol participants of various ancestries,[16] no independent associations were found with additional polymorphisms in or beyond *CYP2B6*, including *CYP2A6* -48T→G ($P=0.82$). Similarly, a recent report by Sarfo *et al*.[29] indicated that among 473 HIV-infected Ghanaians, *CYP2A6* -48T→G was associated with mid-dose plasma efavirenz concentrations on univariate analysis only ($P=0.002$), but not after controlling for 516G→T and 983T→C ($P=0.6$), suggesting that the *CYP2A6* -48T→G association was not independent of *CYP2B6* polymorphisms.

There were several limitations to the above GWAS. The method used to estimate efavirenz C_{min} did not allow differences between some concentrations within the top concentration strata to be discriminated; subgroup analyses were not performed solely among subjects with a *CYP2B6* slow metabolizer genotype; and previously implicated *UGT2B7* polymorphisms were not genotyped.[16] To assess whether higher plasma efavirenz concentrations are affected by polymorphisms in genes relevant to drug absorption, distribution, metabolism and elimination (ADME) beyond *CYP2B6*, the present study focused on ACTG protocol participants with a *CYP2B6* slow metabolizer genotype. Plasma efavirenz concentrations in this group should be exquisitely sensitive to loss-of-function polymorphisms in minor pathways of efavirenz metabolism, as previously suggested.[24] We only considered efavirenz concentrations from 8 to 19 h post-dose, and performed targeted genotyping of *UGT2B7* polymorphisms as well as an additional 194 polymorphisms and 8 gene copy number variants in 40 ADME genes.

Materials and methods

Study participants

Most individuals in these analyses were also included in the previously reported GWAS,[16] which involved treatment-naive individuals who were randomized to efavirenz-containing regimens in ACTG studies 384,[30] A5095 (including its neurologic substudy A5097s)[3,31] and A5202,[7] with DNA obtained under protocol A5128[32] and with available plasma efavirenz assay data. We limited the present analyses to individuals with a *CYP2B6* slow metabolizer genotype (516TT, 516T/983C or 983CC). Self-identified race/ethnicity categories 'white, non-Hispanic', 'black, non-Hispanic' and 'Hispanic' are hereafter referred to as White, Black and Hispanic, respectively. This study complied with the Helsinki Declaration, was approved by institutional review boards for each site and subjects gave written informed consent.

Genotyping

Genotypes for *CYP2B6* 516G→T, 983T→C and *CYP2A6* -48T→G were available from a custom-designed MassARRAY® iPLEX Gold assay (Sequenom Inc.), as previously described,[16] and confirmed by genotyping with the iPLEX ADME PGx panel (Sequenom Inc.). Genotypes of five *UGT2B7* polymorphisms, including 735A→G (rs28365062), 801A→T (rs7438284), 802T→C (rs7439366), 870+115G→A (rs7441750) and 870+148G→A (rs7441774) were determined using genomic PCR amplification and direct sequencing as described previously,[28] with minor modifications using Platinum® PCR SuperMix, High Fidelity (Life Technologies Inc.). The PCR reactions were denatured initially at 94°C for 2 min, then 35 cycles of 94°C for 30 s, 55°C for 30 s and 68°C for 1 min, followed by 68°C for 5 min. Four of the five *UGT2B7* polymorphisms (rs7438284, rs7439366, rs7441750, rs7441774) were in complete linkage disequilibrium in our subjects, so analyses only included 735A→G (rs28365062) and 802T→C (rs7439366). These polymorphisms were chosen for analysis since they discriminate the three most common *UGT2B7* alleles identified to date, including *UGT2B7*1a* (reference), *UGT2B7*1c* (735A→G) and *UGT2B7*2* (802T→C).[28] Furthermore, 802T→C is non-synonymous, causing a histidine to tyrosine transition at codon 268, while 735A→G was associated with altered clearance and glucuronidation of zidovudine, a specific *UGT2B7* substrate.[33] Two *NR1I3* polymorphisms previously associated with efavirenz plasma concentrations, rs2307424[26] and rs3003596,[27] were genotyped by TaqMan™ assay with ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems Inc., Foster City, CA). From iPLEX ADME PGx, we only included data from specimens with >95% genotyping efficiency and polymorphisms with >95% genotyping efficiency. Laboratory personnel with no knowledge of the clinical data performed the genotyping. Within each race/ethnicity group, each iPLEX ADME PGx polymorphism was in Hardy–Weinberg equilibrium after Bonferroni correction for multiple comparisons. All assays were run in duplicate.

Plasma efavirenz concentrations

Plasma efavirenz concentrations were assayed by HPLC at treatment weeks 1, 4, 12 and 24, as described elsewhere.[34] Sampling times were not pre-specified, and time of prior dose was by self-report. We only included mid-dosing interval efavirenz concentrations obtained ≥8 h but <19 h post-dose. Efavirenz is typically taken at bedtime to minimize CNS side effects, and mid-dose concentrations are more convenient for therapeutic drug monitoring than AUC. At steady-state, efavirenz mid-dose concentrations (~12 h post-dose) very strongly correlate with AUC values.[35] We also only included subjects with at least two efavirenz determinations within this window, and with relatively consistent values. For subjects with only two such determinations, subjects with a difference between $\log_{10}$ efavirenz concentrations ≥0.3 μg/mL were excluded. For subjects with more than two determinations, subjects with a standard

deviation of $\log_{10}$ efavirenz concentrations ≥0.2 μg/mL were excluded. These standard deviation cut-offs, chosen based on visual inspection of frequency distribution plots, only excluded a few outlier subjects with extreme inter-assay variability.

Statistical analysis

For each subject, the mean of $\log_{10}$-transformed efavirenz concentrations was used in analyses. Linear regression was used to test for association between polymorphisms and efavirenz concentrations. Primary analyses used additive genetic models. Exploratory *post hoc* analyses also considered recessive models. For *CYP2A6* -48T→G, *UGT2B7* 735A→G, 802T→C and composite *CYP2B6* 516/983 genotype, nominal two-way *P* values <0.05 were considered significant. For other polymorphisms, *P* values were Bonferroni corrected for multiple comparisons. Statistical analyses were performed with PLINK software v1.07[36] and with STATA software v13.0.[37]

Results

Study subjects

These analyses included 84 subjects, of which 73 (86.9%) were male, 44 (52.4%) Black, 24 (28.6%) White and 16 (19.1%) Hispanic. Mean age (±SD) was 38.3±9.2 years. The mean of $\log_{10}$ plasma efavirenz concentrations (of each subject's mean value) was 0.79 $\log_{10}$±0.19 $\log_{10}$ μg/mL. On a linear scale, median was 5.8 μg/mL, minimum 2.4 μg/mL, IQR 4.3–8.5 μg/mL and maximum 17.4 μg/mL. Mean time post-dose was 12.8±2.0 h (based on within-subject means).

Genetic associations with mid-dose efavirenz concentrations

All 84 subjects had a *CYP2B6* slow metabolizer genotype, including 71 (84.5%) homozygous for *CYP2B6* 516TT, 12 (14.3%) heterozygous for *CYP2B6* 516GT with 983TC and 1 (1.2%) homozygous for *CYP2B6* 983CC. There was a trend toward association between increasing *CYP2B6* 983C allele copy number and higher efavirenz concentrations in all subjects (*P*=0.12). To address possible confounding by genetic substructure, we analysed each race/ethnicity group separately. This relationship was also apparent in Black subjects analysed separately. The one Hispanic subject with a 983C allele self-identified as 'Black, Hispanic'. No White subject had a 983C allele (Figure 1).

Among all subjects, *CYP2A6* -48T→G was significantly associated with increased efavirenz concentrations by univariate analysis ($P=3.8\times10^{-4}$). This association was also apparent in Black subjects (*P*=0.027) and White subjects (*P*=0.0011) analysed separately, but not in Hispanic subjects (*P*=0.70), perhaps due to small sample size (Figure 2). Of note, the only subject with apparent homozygosity for *CYP2A6* -48 G/G was also heterozygous for deletion of the *CYP2A6* gene, and so this subject had only one *CYP2A6* -48G allele (see later regarding iPLEX ADME PGx genotypes). Among all subjects, *UGT2B7* 735A→G was not associated with increased efavirenz concentrations by univariate analysis (*P*=0.32) or in Black, White and Hispanic subjects analysed separately (*P*>0.1 for each). However, among all subjects, using a recessive genetic model, *UGT2B7* 735 G/G homozygosity was associated with higher efavirenz concentrations than the combined A/A and A/G genotypes (*P*=0.0063), although only three

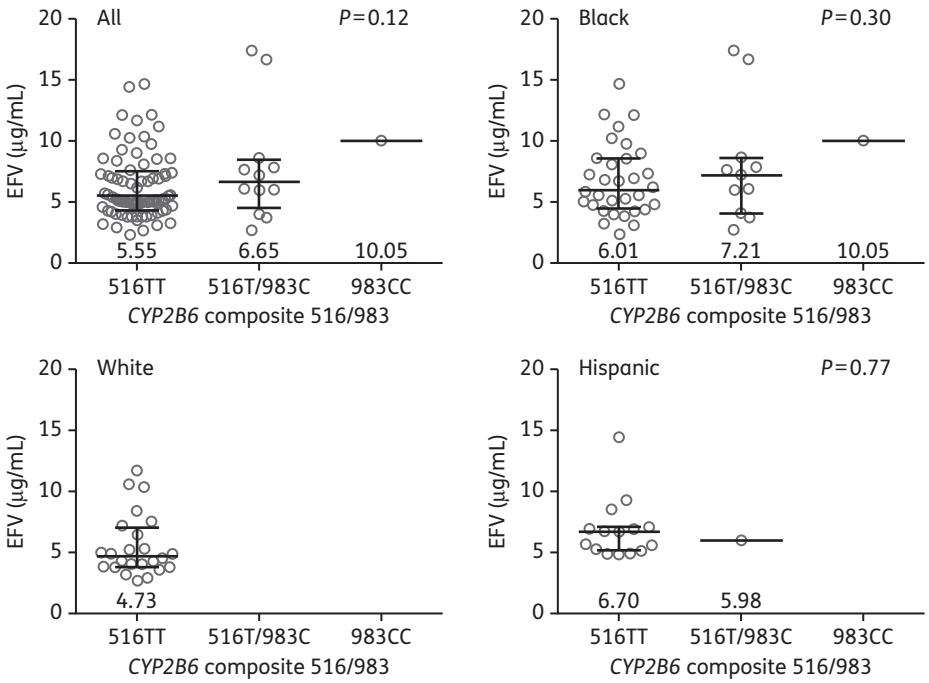

Figure 1. Plasma efavirenz (EFV) concentrations by *CYP2B6* 516/983 slow metabolizer genotype subgroup. On each graph, each marker represents a different subject, each with a *CYP2B6* slow metabolizer genotype. Panels represent all subjects (top left) and self-identified Black subjects (top right), White subjects (bottom left) and Hispanic subjects (bottom right). Each marker represents the median of at least two $\log_{10}$-transformed efavirenz determinations on plasma samples obtained 8–19 h post-dose. Horizontal bars are medians and IQRs. Median values are shown below the markers.

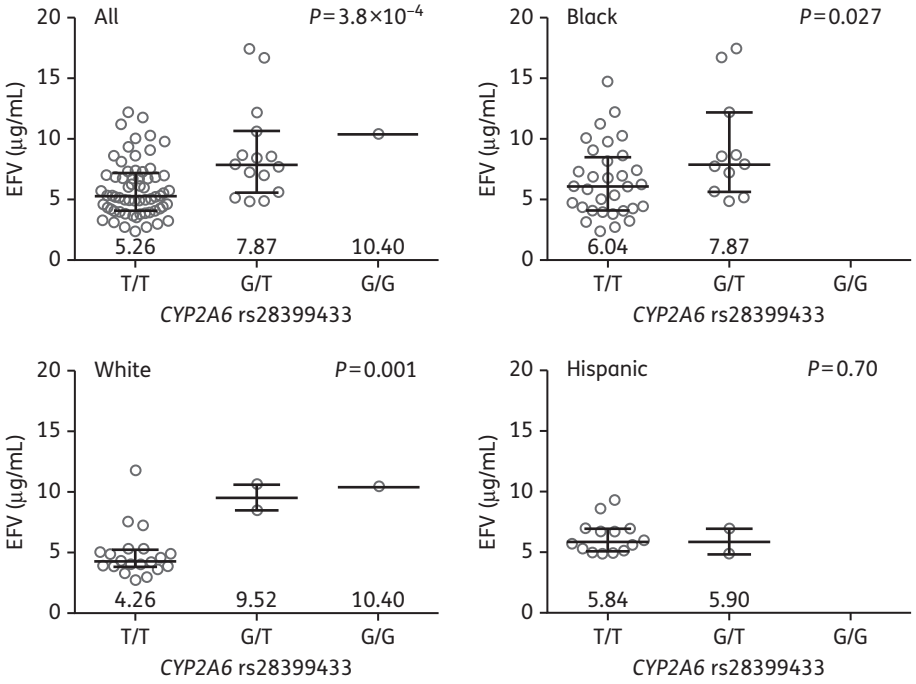

Figure 2. Plasma efavirenz (EFV) concentrations by *CYP2A6* -48T→G genotype. On each graph, each marker represents a different subject. Panels represent all subjects (top left) and self-identified Black subjects (top right), White subjects (bottom left) and Hispanic subjects (bottom right). All have a *CYP2B6* slow metabolizer genotype (i.e. 516TT, 516T/983C or 983CC). Genotypes for *CYP2A6* -48T→G (rs28399433) are shown. Each marker represents the median of at least two $\log_{10}$-transformed efavirenz determinations on plasma samples obtained 8–19 h post-dose. Horizontal bars are medians and IQRs. Median values are shown below the markers.

subjects were homozygous for G/G. This association appeared to be consistent in Black subjects ($P=0.046$) and White subjects ($P=0.062$) analysed separately (Figure 3). There was no significant association between either *UGT2B7* 802T→C, *NR1I3* rs2307424 or *NR1I3* rs3003596 and efavirenz concentrations by univariate analysis in all subjects, or in Black, White or Hispanic subjects analysed separately ($P>0.3$ for each analysis; Figures S1 to S3, available as Supplementary data at *JAC* Online).

Multivariable models were used to test for independent associations. Among all 84 subjects, in additive genetic models that controlled for *CYP2B6* slow metabolizer subgroup (516TT, 516T/983C and 983CC), efavirenz concentrations remained significantly associated with *CYP2A6* -48T→G ($P=0.0010$) but not with *UGT2B7* 735A→G ($P=0.29$), *UGT2B7* 802T→C ($P=0.89$), *NR1I3* rs2307424 ($P=0.49$) or rs3003596 ($P=0.43$); in a recessive genetic model, *UGT2B7* 735 G/G homozygosity was associated with efavirenz concentrations ($P=0.0085$). In a multivariable model that controlled for both *CYP2B6* slow metabolizer subgroup and *CYP2A6* -48T→G genotype, *CYP2A6* -48T→G ($P=0.0036$) and *UGT2B7* 735A→G ($P=0.025$, recessive model), but not *CYP2B6* 516/983 subgroup ($P=0.33$), were significantly associated with efavirenz concentrations.

In separate univariate linear regression models, *CYP2A6* -48T→G explained 15% of interindividual variance in $\log_{10}$ plasma efavirenz concentrations, *UGT2B7* 735 (G/G homozygosity) explained 9%, *CYP2B6* 983T→C explained 3% and time post-dose explained 2%. A multivariable model that included *CYP2A6* -48T→G and *UGT2B7* 735 (G/G homozygosity) explained 21% of interindividual variance in $\log_{10}$ plasma efavirenz concentrations. Inclusion of *CYP2B6* 983T→C only increased this to 22%, and time post-dose only to 24%. The final model, which included *CYP2A6* -48T→G, *UGT2B7* 735 G/G homozygosity and *CYP2B6* 983T→C, is shown in Table 1.

Associations with additional polymorphisms were explored based on iPLEX ADME PGx genotypes, which were available for 68 subjects (21 White, 39 Black and 8 Hispanic) representing 194 loci in 40 genes (of which 103 were polymorphic and 74 had minor allele frequencies >5%). This platform also assayed for copy number variants of *CYP2A6, CYP2B6*, *CYP2D6*, *GSTM1*, *GSTT1*, *GSTT2B*, *SULT1A1* and *UGT2B17*. Information regarding iPLEX ADME PGx genes, polymorphisms and minor allele frequencies are shown in Table S1 (available as Supplementary data at *JAC* Online). In univariate analyses with correction for multiple comparisons, only *CYP2A6* -48T→G in the iPLEX ADME PGx panel was significantly associated with plasma efavirenz concentrations ($P=5.8\times10^{-4}$). This association was also apparent in Whites ($P=0.0063$) and Blacks ($P=0.024$) analysed separately. Considering nominal significance (without correcting for multiple comparisons), six polymorphisms in *CYP2C9, CYP2C19, CYP3A5*, *GSTM1* and *SULT1A1* had *P* values between 0.02 and 0.05, but associations were not consistent in White and Black subjects analysed separately. These included the *CYP3A5* loss-of-function polymorphism rs776746 (*CYP3A5*3*), which had nominal significance for association in all subjects ($P=0.048$) but not in White subjects ($P=0.54$) or Black subjects ($P=0.80$) analysed separately. In a multivariable analysis that controlled for *CYP2A6* -48T→G, the lowest nominal *P* value was in *GSTM1* ($P=0.040$). None was significant after correcting for multiple comparisons.

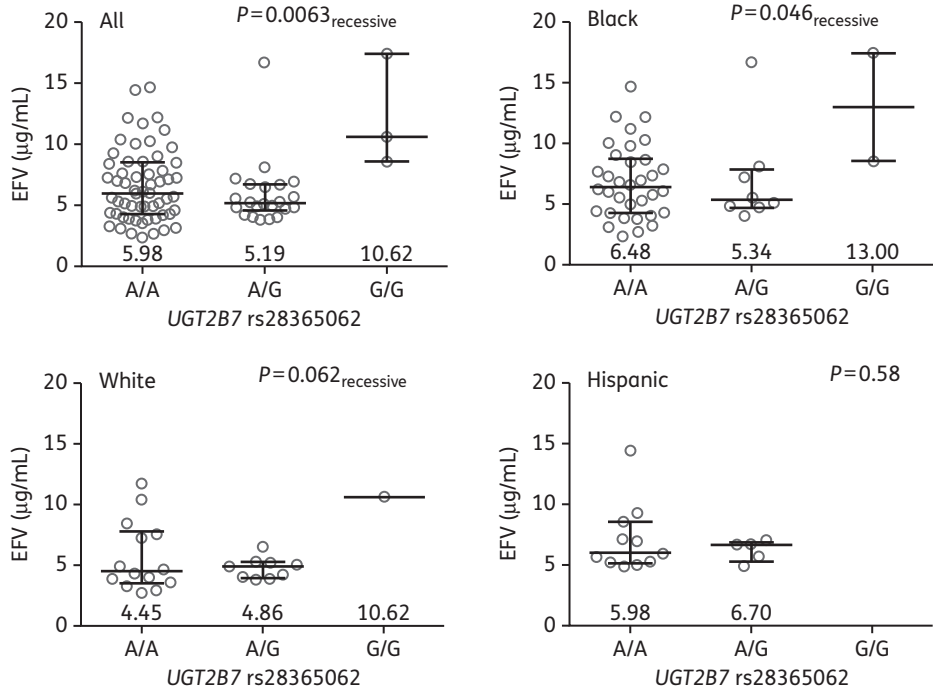

Figure 3. Plasma efavirenz (EFV) concentrations by *UGT2B7* 735A→G genotype. On each graph, each marker represents a different subject. Panels represent all subjects (top left) and self-identified Black subjects (top right), White subjects (bottom left) and Hispanic subjects (bottom right). All have a *CYP2B6* slow metabolizer genotype (i.e. 516TT, 516T/983C or 983CC). Each marker represents the median of at least two $\log_{10}$-transformed efavirenz determinations on plasma samples obtained 8–19 h post-dose. Genotypes for *UGT2B7* 735A→G (rs28365062) are shown. Horizontal bars are medians and IQRs. Median values are shown below the markers.

Table 1. Multivariate linear regression model for association between genetic polymorphisms and $\log_{10}$ plasma efavirenz concentrations

Variant	Coefficient	95% CI	Standard error	*t*	*P* value
CYP2A6 -48T→G	0.135	0.045, 0.224	0.045	3.00	0.004
UGT2B7 735 G/G[a]	0.232	0.030, 0.434	0.102	2.29	0.025
CYP2B6 983T→C	0.046	−0.047, 0.140	0.047	0.99	0.326

[a]*UGT2B7* 735A→G was analysed for recessive effect (i.e. G/G versus combined A/A and A/G).

Association analysis results for iPLEX ADME PGx genotypes and gene copy number variants are shown in Tables S2 to S6 (available as Supplementary data at *JAC* Online). Frequencies of gene copy number variants are shown in Table S7.

For three subjects heterozygous for a *CYP2A6* gene deletion (one with a *CYP2A6* -48G allele, as noted above), there was no significant association with plasma efavirenz concentrations. (This gene deletion is also known as *CYP2A6*4*).[38] The two individuals with a single *CYP2A6* -48T allele gene copy did not have particularly high plasma efavirenz concentrations (4.4 μg/mL and 9.3 μg/mL). Similarly, two subjects heterozygous for *CYP2A6* rs28399454 A/G (also known as *CYP2A6*17*)[39] did not have particularly high plasma efavirenz concentrations (4.0 μg/mL and 5.3 μg/mL). A Q–Q plot of observed versus expected $-\log_{10}$ *P* values showed that only the *CYP2A6* -48T→G *P* value was substantially less than expected by chance (Figure 4).

Sensitivity analyses assessed the effect of censoring single plasma efavirenz concentration values from selected analyses. With removal of the one subject with a single *CYP2A6* -48G allele, the univariate association with *CYP2A6* -48T→G remained significant ($P=9.4\times10^{-4}$). With removal of the one subject with the highest efavirenz value (heterozygous for *CYP2A6* -48 G/T, homozygous for *UGT2B7* 735 G/G), the association with *CYP2A6* -48T→G again remained significant ($P=0.0014$), but the association with *UGT2B7* 735A→G did not (recessive model, $P=0.11$).

Discussion

Efavirenz is one of the most frequently prescribed antiretrovirals worldwide. The present study shows that, among individuals with already increased efavirenz concentrations due to a *CYP2B6*

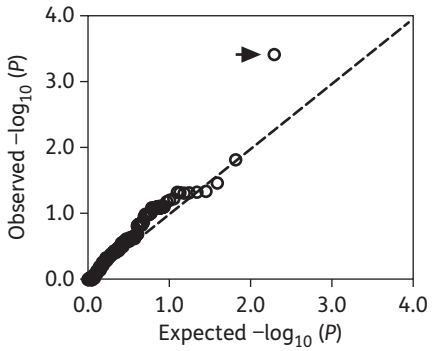

Figure 4. Q–Q plot of observed versus expected $-\log_{10} P$ values for iPLEX ADME PGx panel polymorphisms. Each marker represents a different polymorphism. The arrowhead identifies *CYP2A6* -48T→G (rs28399433).

slow metabolizer genotype, *CYP2A6* -48T→G is associated with even greater increases in plasma efavirenz concentrations. This polymorphism, which defines the *CYP2A6*9* haplotype, disrupts the TATA box and is associated with reduced CYP2A6 expression.[40,41] This association was seen in all subjects and in Black subjects and White subjects analysed separately. In multivariable analyses, the association with *CYP2A6* -48T→G was independent of *CYP2B6* slow metabolizer genotype subgroup (i.e. 516TT, 516T/983C and 983CC). The validity of this association is further supported by the Q–Q plot, which clearly shows that *CYP2A6* -48T→G stands out among the many ADME polymorphisms genotyped. A possible association between *UGT2B7* 735A→G and higher plasma efavirenz concentrations was only seen with 735 G/G homozygosity, which was independent of both *CYP2A6* -48T→G and *CYP2B6* slow metabolizer genotype subgroup in multivariable analyses. However, loss of *UGT2B7* 735A→G significance in a sensitivity analysis that censored a single subject shows the tenuousness of this association. In addition, increasing number of *CYP2B6* 983C alleles tended toward association with higher plasma efavirenz concentrations, consistent with previous reports.[16]

The present analyses only included individuals with a *CYP2B6* slow metabolizer genotype, because only in the setting of markedly reduced CYP2B6 activity are minor metabolism pathway effects most apparent, as previously suggested.[24] Although UGT2B7 can directly *N*-glucuronidate efavirenz *in vitro*,[10] our finding that an association with *UGT2B7* 735A→G was only seen with G/G homozygosity suggests that the effect (if any) of this polymorphism is weak. This is consistent with data from a study of 10 HIV-negative healthy volunteers (eight Caucasians and two African Americans), which suggested that the contribution of UGT2B7 to efavirenz metabolism *in vivo* is minimal.[42] However, there were likely no *CYP2B6* slow metabolizer genotypes in the latter study (although genotyping was not done), and the contribution of UGT2B7 might be more substantial in such individuals.

We found no additional ADME polymorphisms in or beyond *CYP2B6*, *CYP2A6* and *UGT2B7* associated with plasma efavirenz concentrations. This includes *CYP3A5*3* (rs776746), which markedly reduces *CYP3A5* expression and was very frequent in our study subjects. We conclude that CYP3A5 either does not contribute substantially to efavirenz metabolism *in vivo* or that a compensatory increase in CYP3A4 activity (or of some other enzyme) in these subjects offsets the decreased CYP3A5 activity.

We also did not replicate previously reported associations with *NR1I3* polymorphisms.[26,27] This may reflect the fact that we only studied individuals with a *CYP2B6* slow metabolizer genotype, in whom gene expression may be less influenced by nuclear receptor activity. We cannot explain the lack of association between heterozygosity for either *CYP2A6*4* or *CYP2A6*17* and plasma efavirenz concentrations, but this may reflect low statistical power, with only five subjects carrying these alleles. It is conceivable that the association between *CYP2A6* -48T→G and decreased plasma efavirenz concentrations is somehow mediated through a further reduction in CYP2B6 (rather than CYP2A6) expression and/or activity among individuals with a *CYP2B6* slow metabolizer genotype, as this polymorphism is ∼140 kb upstream of *CYP2B6* on chromosome 19. This would be consistent with the lack of association with *CYP2A6*4* and *CYP2A6*17* in the present study, but would not be consistent with the lack of association between *CYP2A6* -48T→G and plasma efavirenz concentrations in subjects with a *CYP2B6* extensive or intermediate metabolizer genotype in the previous GWAS that involved 856 subjects.[16]

This study has potential implications for efavirenz side effects. Higher plasma efavirenz concentrations have been associated with CNS side effects in some[13,29,43,44] but not all studies.[45–47] In ACTG protocol A5097s (a double-blinded, placebo-controlled study specifically designed to assess efavirenz CNS symptoms), efavirenz was significantly associated with increased CNS symptoms compared with placebo within the first week of treatment initiation but not at weeks 4, 12 and 24.[31] Changes in efavirenz-associated neurological symptoms within the first week of treatment initiation were correlated with week 1 plasma efavirenz plasma concentrations[31] and with *CYP2B6* 516G→T genotype.[11] A larger ACTG analysis suggested an association between increased CNS side effects and a *CYP2B6* slow metabolizer genotype in Whites but not in Blacks or Hispanics.[19] A subsequent Swiss HIV Cohort Study analysis involving a largely Caucasian population suggested increased risk of efavirenz discontinuation in 13 individuals with various combinations of *CYP2B6*, *CYP2A6* and *CYP3A4* polymorphisms.[48]

The present study also has potential implications for efavirenz dosing-reduction strategies, as has been proposed to reduce side effects and/or cost.[49,50] The present study suggests that among individuals with a *CYP2B6* slow metabolizer genotype and concomitant *CYP2A6* -48T→G (and possibly *UGT2B7* 735 G/G homozygosity), marked dose reduction will still maintain ample plasma efavirenz exposure to control HIV-1 replication.

This study had limitations. We only studied individuals with a *CYP2B6* slow metabolizer genotype. However, our previous GWAS showed no significant association between *CYP2A6* -48T→G and plasma efavirenz exposure among ACTG protocol participants in multivariable analyses that controlled for 516G→T, 983T→C and 15582C→T.[16] Thus, *CYP2A6* -48T→G, and possibly *UGT2B7* 735A→G, are only relevant to efavirenz exposure in individuals with markedly reduced CYP2B6 activity, as previously suggested.[24] Also, because this study included few Hispanic subjects and no Asian subjects, we cannot generalize these findings to other populations.

In summary, knowledge of *CYP2A6* -48T→G genotype improves stratification of plasma efavirenz concentrations in individuals with a *CYP2B6* slow metabolizer genotype. Stratification may also be improved by *UGT2B7* genotype, although this

association was less robust. These findings support the importance of minor metabolism pathways of efavirenz metabolism in *CYP2B6* slow metabolizers, and suggest that individuals with *CYP2A6* -48T→G, and possibly *UGT2B7* 735 G/G, are at increased risk for considerably higher plasma efavirenz exposure.

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Transparency declarations

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Supplementary data

Tables S1 to S7 and Figures S1 to S3 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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