Contribution of *CYP2A6*, *UGT2B7*, and other non-*CYP2B6* Polymorphisms to Plasma Efavirenz Exposure

David W. Haas[1], Awewura Kwara[2], Danielle M. Richardson[1], Paxton Baker[1], Ioannis Papageorgiou[3], Edward P. Acosta[4], Gene D. Morse[5], Michael H. Court[3], the AIDS Clinical Trials Group (ACTG).

[1]Vanderbilt University, Nashville, TN, [2]Brown University, Providence, RI, [3]Washington State University, Pullman, WA,
[4]University of Alabama at Birmingham, Birmingham, AL, [5]University at Buffalo, SUNY, Buffalo, NY.

ABSTRACT

Background: Two CYP2B6 variants, 516G→T and 983C→T, define high efavirenz (EFV) concentration strata (slow metabolizers). Contributions by *CYP2A6*, *UGT2B7*, *CYP3A4* and *CYP3A5* variants have been reported, but have been inconsistent. We characterized associations between EFV concentrations and genetic variants in 84 AIDS Clinical Trials Group (ACTG) study participants, all with *CYP2B6* 516/983 slow metabolizer genotypes.

Methodology: Several randomized ACTG treatment trials (esp. A5202, A5095/A5097s, and ACTG 384) included EFV-containing arms, and plasma sampling for EFV at multiple study weeks. We included subjects with minimal intraindividual variability between "2 EFV determinations at 8-19 hours post-dose. Slow metabolizer *CYP2B6* genotypes (*CYP2B6* 516TT, 516T/983C, and 983CC) were available from previous analyses. Genotyping of *UGT2B7* was by sequencing, and 198 additional loci (107 polymorphic) in 40 drug metabolism/ transport genes by iPLEX ADME PGx platform. Race/ethnicity was by self-report. Associations with the mean of log10-transformed EFV concentrations were assessed by linear regression.

Results: Analyses included Black (56%), White (29%), and Hispanic (15%) subjects. *CYP2B6* genotypes were 516TT in 71 subjects, 516T/983C in 12, and 983CC in 1. EFV concentrations were somewhat higher with 983C alleles (P=0.12). In univariate analyses (additive models), *CYP2A6* -48T→G (rs28399433) was significantly associated with increased EFV concentrations in all subjects (P=3.8x10E-4, Figure, top); this was consistent in Black and White groups analyzed separately. *UGT2B7* 735A→G (rs28365062, *1c) and 801T→A (rs7438284, *2) were not associated with EFV concentrations by univariate analysis (p=0.34 and P=0.63), and after controlling for *CYP2A6* -48T→G (p=0.42 and P=0.67, respectively). Homozygosity for *UGT2B7* 735GG (n=3), but not heterozygosity, was associated with higher EFV concentrations (P=0.006, Figure, bottom). With correction for multiple comparisons, no iPLEX ADME PGx variant other than *CYP2A6* -48T→G was associated with EFV concentrations, including 22 in *CYP2A6* and *3A4/5*.

Conclusions: In *CYP2B6* slow metabolizers, CYP2A6 -48T→G is associated with higher EFV concentrations. An effect of *UGT2B7* 735A→G on EFV concentrations, if true, is much less. No other significant associations in these or other genes were found. These findings support a contribution of *CYP2A6*, and possibly *UGT2B7* variants to even higher EFV concentrations in *CYP2B6* slow metabolizers.

Introduction

- Efavirenz (EFV)-containing regimens are widely prescribed as initial therapy for HIV-1 infection.
- EFV is generally safe and effective, but some patients experience virologic failure, central nervous system side effects, or drug-drug interactions.
- EFV is metabolized primarily by hepatic cytochrome P450 (CYP) 2B6.
- *CYP2B6* single nucleotide polymorphisms (SNPs) 516G→T, 983T→C, and 15582C→T predict decreased plasma EFV clearance and increased plasma EFV exposure.
- Genetic predictors of EFV concentrations beyond these three SNPs have been either extremely infrequent or inconsistent.

Objectives

- To assess whether higher plasma EFV concentrations are affected by polymorphisms in drug absorption, distribution, metabolism, and elimination (ADME) genes beyond *CYP2B6.*

Eligibility Criteria

- Treatment-naïve individuals randomized to EFV-containing regimens in ACTG studies 384, A5095/A5097s, and A5202.
- We limited analyses to individuals with *CYP2B6* slow metabolizer genotypes (516TT, 516T/983C, or 983CC).
- Available DNA obtained under ACTG protocol A5128.
- Available plasma EFV assay data.

NOTES:

- Many participants were in prior reports describing associations between *CYP2B6* 516G→T, 983T→C, and 15582C→T and EFV exposure [1-4].
- Self-identified race/ethnicity categories "white, non-Hispanic", "black, non-Hispanic", and "Hispanic" are hereafter referred to as White, Black, and Hispanic, respectively.

Pharmacokinetics

- Plasma EFV was assayed by HPLC at treatment weeks 1, 4, 12, and 24.
- Sampling times were not pre-specified, and time of prior dose was by self-report.
- Mid-dosing interval EFV concentrations were ≥8 hours but <19 hours post dose.
- We included subjects with ≥2 EFV determinations within this window, and with relatively consistent values.
- For subjects with only 2 determinations, those with a between assay difference ≥0.3 µg/mL were excluded. For subjects with >2 determinations, subjects with standard deviation $\log_{10}$ EFV concentrations ≥0.2 µg/mL were excluded.

Statistical Analyses

- Means of $\log_{10}$-transformed EFV concentrations was used in analyses.
- Linear regression tested for associations with EFV concentrations.
- Primary analyses used additive genetic models, while exploratory post hoc analyses 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.
- Analyses were performed with PLINK (v1.07) and STATA software (v13.0).

Genotyping

- Genotypes for *CYP2B6* 516G→T, 983T→C, and *CYP2A6* -48T→G (rs28399433) were available from a custom MassARRAY® iPLEX Gold assay, and confirmed by iPLEX ADME PGx panel (Sequenom, Inc.).
- Genotypes of five *UGT2B7* SNPs, including 735A→G (rs28365062), 801A→T (rs7438284), 802T→C (rs7439366), 870+115G→A (rs7441750), and 870+148G→A (rs7441774) were by PCR amplification and direct sequencing.
- Four *UGT2B7* polymorphisms (rs7438284, rs7439366, rs7441750, rs7441774) were in complete linkage, so analyses only included 735A→G and 802T→C.
- From iPLEX ADME PGx, we only included specimens with >95% genotyping efficiency, and polymorphisms with >95% genotyping efficiency.
- Within each race/ethnicity group, each iPLEX ADME PGx polymorphism was in Hardy Weinberg equilibrium after Bonferroni correction for multiple comparisons.

RESULTS

Study subjects

- Analyses included 84 subjects (73 males, 44 Black, 24 White, and 16 Hispanic).
- Mean age (± standard deviation) was 38.3 years ± 9.2 years.
- Mean $\log_{10}$ plasma EFV concentration (of each subject's mean value) was 0.79 $\log_{10}$ µg/mL ± 0.19 $\log_{10}$ µg/mL.
- On a linear scale, median was 5.8 µg/mL, minimum 2.4 µg/mL, interquartile range 4.3 µg/mL to 8.5 µg/mL, and maximum 17.4 µg/mL.
- Mean time post dose was 12.8 hours ± 2.0 hours (based on within-subject means).

Associations with EFV concentrations

***CYP2B6* 983T→C**

- All 84 subjects had *CYP2B6* slow metabolizer genotypes, including 71 (84.5%) with 516TT, 12 (14.3%) with 516GT with 983TC, and 1 (1.2%) with 983CC.
- There was a trend toward association between *CYP2B6* 983 C and higher EFV concentrations in all subjects (P = 0.12).
- This relationship was apparent in Black subjects. The one Hispanic subject with 983 C self identified as "Black, Hispanic". No White subject had 983 C (**Figure 1**).

***CYP2A6* -48T→G**

- Among all subjects, *CYP2A6* -48T→G was associated with increased EFV concentrations by univariate analysis (P = 3.8 x 10^{-4}).
- This was apparent in Black subjects (P = 0.027) and Whites subjects (P = 0.0011), but not in Hispanic subjects (P = 0.70, **Figure 2**).
- The subject with apparent *CYP2A6* -48 G/G was heterozygous for deletion of the *CYP2A6* gene, so had only one *CYP2A6* -48G allele.

***UGT2B7* 735A→G and 802T→C**

- Among all subjects, *UGT2B7* 735A→G was not associated with increased EFV concentrations by univariate analysis (P = 0.32), or in Black, White, and Hispanic subjects analyzed separately (P > 0.1 for each).
- Among all subjects, using a recessive genetic model, *UGT2B7* 735 G/G was associated with higher EFV concentrations than combined A/A and A/G (P=0.0063); Only 3 subjects were homozygous for G/G.
- This appeared to be consistent in Black subjects (P = 0.046) and White subjects (P = 0.062, **Figure 3**).
- There was no association between *UGT2B7* 802T→C and EFV concentrations (P>0.5, data not shown).

iPLEX ADME PGx genotypes

- Genotypes were available for 68 subjects, representing 194 loci in 40 genes, and copy number variants (CNV) of *CYP2A6*, *CYP2B6*, *CYP2D6*, *GSTM1*, *GSTT1*, *GSTT2B*, *SULT1A1* and *UGT2B17*.
- In univariate analyses, *CYP2A6* -48T→G was associated with EFV concentrations (P = 5.8 x 10^{-4}). This was apparent in Whites (P = 0.0063) and Blacks (P = 0.024).
- Considering nominal significance, six SNPs in *CYP2C9*, *CYP2C19*, *CYP3A5*, *GSTM1* and *SULT1A1* had p-values between 0.02 and 0.05, but were not consistent in White and Black subjects.
- After controlling for *CYP2A6* -48T→G, the lowest p-value was in *GSTM1* (P=0.040).
- For three subjects heterozygous for *CYP2A6* gene deletion (one with a *CYP2A6* -48G allele), there was no association with plasma EFV concentrations.
- Only the *CYP2A6* -48T→G p-value was substantially less than was expected by chance (**Figure 4**).

Acknowledgments

We are grateful to the HIV-infected individuals who volunteered for these studies. Staff and investigators at the participating ACTG Clinical Research Sites. Funding included NIH grants AI-068636, AI-077505, TR-000445.

Figure 1. Associations between *CYP2B6* 983T→C and EFV concentrations in *CYP2B6* slow metabolizers.

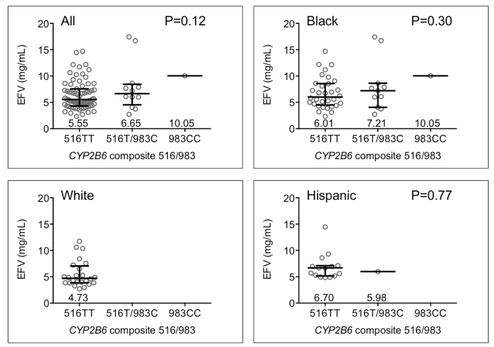


Figure 2. Associations between *CYP2A6* -48T→G and EFV concentrations in *CYP2B6* slow metabolizers.

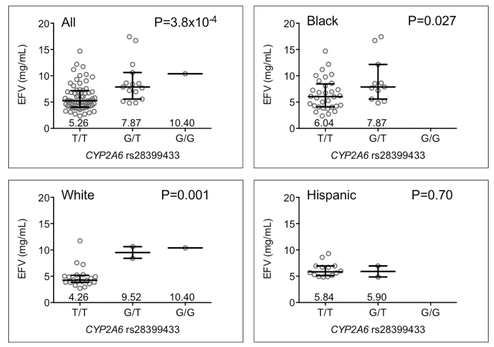


Figure 3. Associations between *UGT2B7* 735A→G and EFV concentrations in *CYP2B6* slow metabolizers.

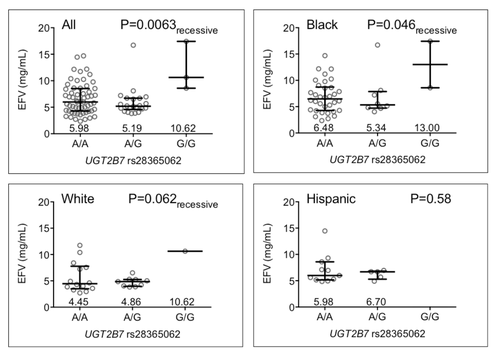
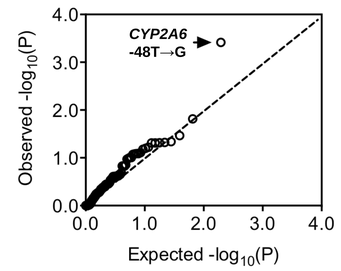


Table. Multivariate linear regression model for association between genetic polymorphisms and $\log_{10}$ EFV concentrations

Variant	Coefficient	95% C.I.	Std. Err.	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 analyzed for recessive effect (i.e. G/G versus combined A/A and A/G).

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.



Associations with EFV concentrations

Multivariable models

- Among all subjects, in additive models that controlled for *CYP2B6* sub-group (516TT, 516T/983C, and 983CC), EFV concentration was associated with *CYP2A6* -48T→G (0.0010), but not *UGT2B7* 735A→G (P = 0.29) or UGT2B7 802T→C (P = 0.89).
- In a recessive model, *UGT2B7* 735 G/G was associated with EFV concentrations (P = 0.0085). In a multivariable model that controlled for *CYP2B6* sub-group, *CYP2A6* -48T→G (P = 0.0036) and *UGT2B7* 735A→G (P = 0.025, recessive model), but not *CYP2B6* 516/983 (P = 0.33), were associated with EFV concentrations.
- In separate univariate linear regression models, *CYP2A6* -48T→G explained 15% of variance in $\log_{10}$ EFV concentrations, *UGT2B7* 735 (G/G homozygosity) 9%, *CYP2B6* 983T→C 3%, and hours post dose 2%.
- A multivariable model that included *CYP2A6* -48T→G and *UGT2B7* 735 (G/G homozygosity) explained 21% of interindividual variance in $\log_{10}$ EFV concentrations. Inclusion of *CYP2B6* 983T→C increased this to 22%, and hours post dose to 24%.
- The final model that included *CYP2A6* -48T→G, *UGT2B7* 735 G/G homozygosity, and *CYP2B6* 983T→C is shown in the **Table**.

Conclusions

- Among individuals with already increased EFV concentrations due to *CYP2B6* slow metabolizer genotypes, *CYP2A6* -48T→G is associated with even greater increases in plasma EFV concentrations.
- This association was seen in all subjects, and in Black subjects and White subjects analyzed separately.
- In multivariable analyses, the association with *CYP2A6* -48T→G was independent of *CYP2B6* slow metabolizer genotype sub-group (i.e. 516 TT, 516T/983C, and 983CC).
- Validity is further supported by the Q-Q plot, which clearly shows that *CYP2A6* -48T→G stands out among the many ADME polymorphisms genotyped.
- The *CYP2A6* -48T→G association replicates studies by di Iulio *et al* [6] and Kwara *et al* [7].
- A possible association with *UGT2B7* 735A→G was only seen with 735 G/G homozygosity, which was independent of *CYP2A6* -48T→G and *CYP2B6* 983C.
- The *UGT2B7* 735A→G association supports the study by Kwara *et al* [8].
- Increasing number of CYP2B6 983C alleles tended toward association with higher plasma EFV concentrations.
- No additional ADME polymorphisms in or beyond *CYP2B6*, *CYP2A6*, and *UGT2B7* were associated with plasma EFV concentrations.
- This study has potential implications for EFV side effects and EFV dosing-reduction strategies.

References

1) Haas DW, Ribaudo HJ, Kim RB, et al. Pharmacogenetics of efavirenz and central nervous system side effects: an Adult AIDS Clinical Trials Group study. *AIDS* 2004;18:2391-400.
2) Haas DW, Smeaton LM, Shafer RW, et al. Pharmacogenetics of Long-Term Responses to Antiretroviral Regimens Containing Efavirenz and/or Nelfinavir: An Adult AIDS Clinical Trials Group Study. *J Infect Dis* 2005;192:1931-42.
3) Ribaudo HJ, Liu H, Schwab M, et al. Effect of CYP2B6, ABCB1, and CYP3A5 polymorphisms on efavirenz pharmacokinetics and treatment response: an AIDS Clinical Trials Group study. *J Infect Dis* 2010;202:717-22.
4) Holzinger ER, Grady B, Ritchie MD, Ribaudo HJ, Acosta EP, Morse GD, Gulick RM, Robbins GK, Clifford DB, Daar ES, McLaren P, Haas DW (2012) Genome-Wide Association Study of Plasma Efavirenz Pharmacokinetics in AIDS Clinical Trials Group Protocols. *Pharmacogenet Genom* 2012; 22: 858-67.
5) Pereyra F, Jia X, McLaren PJ, et al. The major genetic determinants of HIV-1 control affect HLA class I peptide presentation. *Science* 2010;330:1551-7.
6) di Iulio J, Fayet A, Arab-Alameddine M et al. In vivo analysis of efavirenz metabolism in individuals with impaired CYP2A6 function. *Pharmacogenet Genom* 2009; 19: 300-9.
7) Kwara A, Lartey M, Sagoe KW et al. *CYP2B6* (c.516G-->T) and *CYP2A6* (*9B and/or *17) polymorphisms are independent predictors of efavirenz plasma concentrations in HIV-infected patients. *Br J Clin Pharmacol* 2009; 67: 427-36.
8) Kwara A, Lartey M, Sagoe KW et al. *CYP2B6*, *CYP2A6* and *UGT2B7* genetic polymorphisms are predictors of efavirenz mid-dose concentration in HIV-infected patients. *AIDS* 2009; 23: 2101-6.