

# Pharmacogenetics of Plasma Drug Exposure and Treatment Outcomes with Efavirenz-containing Regimens: An ACTG Study

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## ABSTRACT

**Objective:** EFV is metabolized by cytochrome P450 (CYP) 2B6. Single nucleotide polymorphisms (SNPs) in *CYP2B6* delay efavirenz clearance, whereas *ABCB1* SNPs have been associated with treatment response. We characterized relationships between SNPs, pharmacokinetics (PK), and response to EFV-containing regimens.

**Methods:** Associations between EFV PK and 22 SNPs in *CYP2B6*, *ABCB1* and *CYP3A5* were investigated among subjects with PK data from ACTG protocol A5097s (PK substudy of A5095) and ACTG 384. Among subjects randomized to EFV in A5095 we assayed *CYP2B6* 516G>T, 983T>C and 5 SNPs in *ABCB1*. We defined *CYP2B6* 516/983 metabolizer genotype as rapid (516G+983T), intermediate (516GT or 983TC), slow (516TT, 983CC, or [516TT+983TC]). Stepwise selection models investigated genotype associations with time to: (a) EFV discontinuation (d/c), (b) first CNS adverse event (AE), and (c) virologic failure (VF). Analyses adjusted for BMI, and self-reported adherence at week 12 (VF only). Genotyping was by MALDI-TOF (A5097s, ACTG 384) and TaqMan (A5095).

**Results:** Of 22 SNPs assayed in A5097s (n=123) and ACTG 384 (n=174), PK associations were dominated by *CYP2B6* 516G>T and 983T>C. Other alleles were infrequent (<2%) or had little independent association with PK. Of 765 subjects randomized to EFV-containing regimens in A5095, 287 whites, 225 blacks, and 131 Hispanics were genotyped. Among whites, slow metabolizer *CYP2B6* genotype (compared to rapid) was associated with an increased hazard of CNS AE (HR=2.40; 95% CI 1.07-5.37; P=0.034) and EFV d/c (2.18; 1.03-4.63, P=0.04), but not VF (1.16; 0.27-4.94, P=0.85). Among blacks with slow metabolizer genotype, there was some evidence of a lower hazard of VF (0.34; 0.12-1.00, P=0.050), but not CNS AE (1.18; 0.51-2.71; P=0.69) or EFV d/c (0.69; 0.38-1.26; P=0.23). Among Hispanics, significant associations with slow metabolizer genotype were not seen for VF (1.0; P=0.99), CNS AE (0.98; 0.37-2.62; P=0.97), or EFV d/c (0.90; 0.19-4.32; P=0.89). A previously reported association between *ABCB1* 3435C>T and decreased hazard of VF was not seen.

**Conclusions:** Associations with EFV PK were dominated by *CYP2B6* 516G>T and 983T>C. Relationships between SNPs in *CYP2B6* and *ABCB1*, CNS AEs, EFV discontinuation, and VF were not consistently identified among populations. A unifying model that relates pharmacogenetics to EFV treatment response remains elusive, and may be affected by additional genetic and/or non-genetic factors.

## SPECIFIC AIMS

- To determine whether SNPs in addition to *CYP2B6* 516G>T predict plasma EFV exposure among participants in clinical trials ACTG 384 and A5097s.
- To determine whether selected SNPs in *CYP2B6* and *ABCB1* predict responses to EFV-containing regimens in A5095.

## BACKGROUND

- Efavirenz (EFV) is metabolized primarily by cytochrome P450 (CYP) 2B6.
- Analyses involving 154 subjects in ACTG study A5097s first showed that *CYP2B6* 516G>T predicted increased plasma EFV exposure [1].
- A less frequent *CYP2B6* SNP (983T>C) also predicts increased EFV levels [2,3].
- CYP2B6* 516G>T and 983T>C are more frequent in blacks than in whites.
- The role of P-glycoprotein (encoded by *ABCB1*) in EFV disposition is uncertain.
- Two studies suggested that *ABCB1* 3435C>T may predict more favorable virologic responses to EFV-containing regimens [4,5].
- One study suggested an association between *CYP3A5* 6986A>G and EFV exposure [1].

## ACTG A5095/A5097S

- Antiretroviral therapy (ART) naive individuals were randomized to EFV (600 mg) QD, abacavir (ABV) BID, or both, with zidovudine (AZT) BID + 3TC BID. EFV and ABV were double-blinded with matching placebos.
- Of 1,147 A5095 participants, 765 were randomized to EFV-based regimens.
- The A5097s substudy of A5095 characterized evaluated neurological outcomes and association to EFV exposure over the first 24 weeks of treatment in all three study arms
- EFV was quantified by HPLC at weeks 1, 4, 12 and 24.

## ACTG 384

- Individuals with <7 days of prior ART were randomized to EFV (600 mg) QD, nelfinavir (NFV) BID, or both, with ddI/d4T BID or AZT/3TC BID. EFV and NFV were double-blinded with matching placebos.
- EFV was quantified by HPLC at weeks 1, 4, 12 and 24.

## METHODS

### Study Participants

- PK analyses involved subjects randomized to EFV in either ACTG 384 or A5097s/A5095 and with EFV assay data available.
- Self-identified categories "white, non-Hispanic", "black, non-Hispanic", and "Hispanic" are referred to as white, black, and Hispanic, respectively.

### Genotyping

- Human DNA was obtained under ACTG protocol A5128.
- For relationships between SNPs and EFV PK in ACTG 384/A5097s, genotyping was by MALDI-TOF mass spectrometry in the lab of Dr. M. Schwab [3,6]. This included 16 *CYP2B6* coding and promoter SNPs selected based on frequency and possible functional effects, plus 5 *ABCB1* and 1 *CYP3A5* SNPs (Table 1).
- For relationships between SNPs and treatment responses in A5095, genotyping was TaqMan at the Vanderbilt DNA Resources Core. These included 2 *CYP2B6* SNPs (*CYP2B6* 526G>T and 983T>C) and 5 *ABCB1* SNPs.

### Modeling genetic predictors of EFV concentrations

- To minimize confounding we studied 297 subjects with consistent EFV values ( $\geq 2$  values between 6 and 24-hours post-dose, with <2-fold variability).
- EFV concentration data were modeled in a linear model using generalized estimating equations to account for multiple observations measured for each patient.
- Time to next dose (in hours), baseline BMI (centered around 20 kg/m<sup>2</sup>), age (centered around 40 years), sex, and *CYP2B6* 516 genotype were included in the basic model.
- Under this parameterization, the intercept term of the model reflects the expected trough log concentration for a 40 year old man with a BMI of 20 kg/m<sup>2</sup>.

### Treatment responses

- Endpoints of interest included: 1) time to grade  $\geq 2$  CNS adverse event; 2) time to EFV discontinuation; and 3) time to virologic failure (confirmed HIV RNA  $\geq 200$  copies/mL at or after week 16).
- Failure time distributions were estimated using the method of Kaplan-Meier and compared among ordered genotypes for each locus utilizing Tarone's exact tests.
- Cox regression models examined associations between each SNP and each endpoint. To determine whether effects enter or exit the model, a stepwise procedure with a P<0.05 level of statistical significance was used for the entire study cohort, and P<0.10 for each race/ethnicity group.
- No adjustments were made for multiple comparisons; p-values should be interpreted cautiously.

## RESULTS - Pharmacokinetics in ACTG 384/A5097s

- Among 489 participants from ACTG 384/A5097s, allelic frequencies of the 22 SNPs are shown in Table 1.
- Most *CYP2B6* SNPs were infrequent; *CYP2B6* 516G>T and 785A>G were in very strong linkage disequilibrium.
- In multivariable models involving 297 individuals with relatively consistent EFV concentrations, *CYP2B6* 516G>T predicted EFV concentrations in each population (Table 2).
- Additional information was contributed by *CYP2B6* 785A>T in whites, *CYP2B6* 983T>C in blacks, and *CYP3A5* 6986A>G in Hispanics (Table 2A-C, respectively).

Table 2. Predictive models for EFV concentration

| Covariate                             | Estimate (Empirical standard error) | 95% confidence interval |       | P-value |       |        |
|---------------------------------------|-------------------------------------|-------------------------|-------|---------|-------|--------|
|                                       |                                     | Lower                   | Upper |         |       |        |
| Intercept                             | 0.35                                | 0.12                    | 0.13  | 0.58    | 0.002 |        |
| Time to next dose (per hour)          | -0.02                               | 0.01                    | -0.04 | -0.00   | 0.015 |        |
| Gender                                | Female                              | 0.10                    | 0.14  | -0.17   | 0.38  | 0.46   |
| Baseline BMI (per kg/m <sup>2</sup> ) |                                     | -0.11                   | 0.02  | -0.16   | -0.07 | <0.001 |
| Age (per 10 years)                    |                                     | -0.02                   | 0.03  | -0.08   | 0.04  | 0.49   |
| <i>CYP2B6</i> 785 A>G                 |                                     | -0.14                   | 0.14  | -0.40   | 0.13  | 0.32   |
|                                       | **                                  | -0.88                   | 0.15  | -1.17   | -0.59 | <0.001 |
| 516 G>T                               |                                     | 0.47                    | 0.14  | 0.20    | 0.74  | <0.001 |
|                                       | **                                  | 2.05                    | 0.18  | 1.69    | 2.41  | <0.001 |

### B. Blacks

| Covariate                             | Estimate (Empirical standard error) | 95% confidence interval |       | P-value |       |        |        |
|---------------------------------------|-------------------------------------|-------------------------|-------|---------|-------|--------|--------|
|                                       |                                     | Lower                   | Upper |         |       |        |        |
| Intercept                             | 0.13                                | 0.21                    | -0.28 | 0.54    | 0.53  |        |        |
| Time to next dose (per hour)          | -0.03                               | 0.02                    | -0.08 | 0.01    | 0.011 |        |        |
| Gender                                | Female                              | 0.08                    | 0.11  | -0.14   | 0.30  | 0.48   |        |
| Baseline BMI (per kg/m <sup>2</sup> ) |                                     | -0.09                   | 0.04  | -0.18   | -0.01 | 0.036  |        |
| Age (per 10 years)                    |                                     | -0.00                   | 0.05  | -0.09   | 0.08  | 0.92   |        |
| <i>CYP2B6</i> 983 T>C                 |                                     | **                      | 0.10  | 0.15    | 0.40  | 1.00   | <0.001 |
| 516 G>T                               |                                     | **                      | 0.49  | 0.12    | 0.25  | 0.73   | <0.001 |
|                                       | **                                  | 1.15                    | 0.12  | 0.92    | 1.39  | <0.001 |        |

### C. Hispanics

| Covariate                             | Estimate (Empirical standard error) | 95% confidence interval |       | P-value |        |        |        |
|---------------------------------------|-------------------------------------|-------------------------|-------|---------|--------|--------|--------|
|                                       |                                     | Lower                   | Upper |         |        |        |        |
| Intercept                             | 1.20                                | 0.26                    | 0.70  | 1.70    | <0.001 |        |        |
| Time to next dose (per hour)          | -0.03                               | 0.02                    | -0.06 | 0.01    | 0.15   |        |        |
| Gender                                | Female                              | 0.02                    | 0.12  | -0.21   | 0.25   | 0.87   |        |
| Baseline BMI (per kg/m <sup>2</sup> ) |                                     | -0.07                   | 0.07  | -0.21   | 0.06   | 0.009  |        |
| Age (per 10 years)                    |                                     | -0.15                   | 0.06  | -0.27   | -0.03  | 0.013  |        |
| <i>CYP2B6</i> 516 G>T                 |                                     | **                      | 0.20  | 0.10    | -0.00  | 0.40   | 0.055  |
|                                       | **                                  | 0.33                    | 0.17  | 0.00    | 0.65   | 0.050  |        |
| <i>CYP3A5</i> 6986 A>G                |                                     | **                      | -0.82 | 0.16    | -1.14  | -0.51  | <0.001 |
|                                       | **                                  | -0.70                   | 0.13  | -0.95   | -0.44  | <0.001 |        |

Table 1. Allelic frequencies

| SNP           | n       | White |       |                        | Black |       |        | Hispanic |      |        |      |
|---------------|---------|-------|-------|------------------------|-------|-------|--------|----------|------|--------|------|
|               |         | n     | %     | 95% CI                 | n     | %     | 95% CI | n        | %    | 95% CI |      |
| <i>CYP2B6</i> | -82 T>C | 0.8%  | 1.9%  | 2.7%                   | 0.0%  | 0.0%  | 0.0%   | 0.0%     | 0.0% | 0.0%   |      |
|               | 86 G>C  | 0%    | 2.8%  | 0%                     | 0%    | 0%    | 0%     | 0%       | 0%   | 0%     |      |
|               | 136 A>G | 0.2%  | 0.3%  | 0%                     | 0%    | 0%    | 0%     | 0%       | 0%   | 0%     |      |
|               | 296 G>A | 0%    | 0%    | 0%                     | 0%    | 0%    | 0%     | 0%       | 0%   | 0%     |      |
|               | 415 A>G | 0.5%  | 0.3%  | 1.4%                   | 0%    | 0%    | 0%     | 0%       | 0%   | 0%     |      |
|               | 419 G>A | 0.2%  | 0%    | 0.7%                   | 0%    | 0%    | 0%     | 0%       | 0%   | 0%     |      |
| 516 G>T       | 24.5%   | 34.2% | 32.2% | 54.7 G>A               | 0.4%  | 0%    | 0%     | 0%       | 0%   | 0%     |      |
| 547 G>A       | 0%      | 0%    | 0%    | 593 T>C                | 0%    | 0.3%  | 0%     | 769 G>A  | 0%   | 1.2%   | 0%   |
| 769 G>A       | 0%      | 1.2%  | 0%    | 785 A>G                | 27.3% | 34.8% | 34.7%  | 983 T>C  | 0.2% | 7.5%   | 1.4% |
| 785 A>G       | 27.3%   | 34.8% | 34.7% | 1006 C>T               | 0%    | 0.6%  | 0%     | 1172 T>A | 1.2% | 0%     | 0%   |
| 983 T>C       | 0.2%    | 7.5%  | 1.4%  | 1172 T>A               | 1.2%  | 0%    | 0%     | 1262 C>A | 0%   | 0%     | 0%   |
| 1006 C>T      | 0%      | 0.6%  | 0%    | 1459 C>T               | 12.0% | 3.7%  | 7.5%   |          |      |        |      |
| 1172 T>A      | 1.2%    | 0%    | 0%    | <i>ABCB1</i>           |       |       |        |          |      |        |      |
| 1262 C>A      | 0%      | 0%    | 0%    | 3435 C>T               | 54.9% | 17.4% | 41.1%  |          |      |        |      |
| 1459 C>T      | 12.0%   | 3.7%  | 7.5%  | 2677 G>T               | 40.7% | 8.7%  | 34.9%  |          |      |        |      |
|               |         |       |       | 2677 G>A               | 2.2%  | 0.3%  | 3.4%   |          |      |        |      |
|               |         |       |       | Intron 16 G>A          | 43.3% | 23.3% | 39.7%  |          |      |        |      |
|               |         |       |       | Intron 3 C>T           | 49.5% | 47.8% | 55.6%  |          |      |        |      |
|               |         |       |       | <i>CYP3A5</i> 6986 A>G | 91.1% | 29.5% | 76.0%  |          |      |        |      |

## RESULTS - Treatment Responses in A5095

- Among 655 EFV recipients in A5095 included in this genetic analysis, there were inconsistent associations across populations between individual *CYP2B6* or *ABCB1* SNPs and treatment responses by univariate analysis.
- To further characterize associations we categorized *CYP2B6* metabolizer status (positions 516 & 983) as rapid = 0 SNPs at either 516 or 983; intermediate = 1 SNP at 516 or 983, but not both; slow = 2 SNPs (516 T/T, 983 C/C, or [516 G/T with 983 T/C]).
- Among whites, *CYP2B6* slow metabolizer genotype predicted increased CNS adverse event and EFV discontinuation (Figure, Table 3).
- Among blacks, *CYP2B6* slow metabolizer genotype predicted decreased virologic failure (Figure, Table 3)
- There was no association between *ABCB1* 3435C>T and risk of virologic failure.

Figure. Time to CNS event, virologic failure, and EFV discontinuation (Univariate analyses)

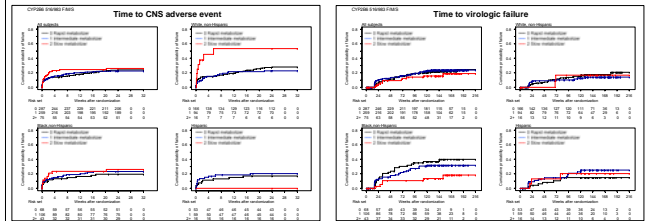


Table 3. *CYP2B6* 516/983 genotype and response\* (Multivariable analyses)

| Endpoint                          | Covariate                  | HR [95% CI]       | P-value |
|-----------------------------------|----------------------------|-------------------|---------|
| Time to Virologic Failure (Black) | <i>CYP2B6</i> Intermediate | 0.92 [0.91, 1.25] | 0.75    |
|                                   | <i>CYP2B6</i> slow         | 0.84 [0.72, 1.00] | 0.03    |
|                                   | Intra-P1 score (wk-12)     | 2.30 [1.27, 4.16] | 0.005   |
| Time to CNS event (White)         | <i>CYP2B6</i> Intermediate | 0.71 [0.41, 1.24] | 0.23    |
|                                   | <i>CYP2B6</i> slow         | 2.40 [1.47, 3.97] | 0.001   |
|                                   | Intra-P1 score (wk-12)     | 0.96 [0.81, 1.07] | 0.60    |
| Time to EFV d/c (Hispanic)        | <i>CYP2B6</i> Intermediate | 0.97 [0.81, 1.05] | 0.60    |
|                                   | <i>CYP2B6</i> slow         | 2.18 [1.43, 3.40] | 0.001   |
|                                   | <i>ABCB1</i> Intron 16 GT  | 0.80 [0.37, 0.95] | 0.032   |
|                                   | <i>ABCB1</i> Intron 16 TT  | 0.57 [0.28, 1.11] | 0.10    |

\*Cox proportional hazard models adjusted for *ABCB1* SNPs, BMI, and self-reported adherence [virologic failure only]

## CONCLUSIONS

### Regarding EFV PK:

- Associations between SNPs and EFV concentrations were dominated by *CYP2B6* 516G>T.
- Additional predictive information for EFV concentrations was provided by additional SNPs, especially *CYP2B6* 983T>C in blacks, but also *CYP2B6* 785A>T in whites, and *CYP3A5* 6986A>G in Hispanics.
- Other SNPs were either very infrequent and/or not significantly associated with EFV concentrations.

### Regarding treatment responses:

- Relationships between SNPs in *CYP2B6* and *ABCB1*, CNS adverse events, EFV discontinuation, and virologic failure were not consistently identified among populations.
- Results suggested possible associations between *CYP2B6* slow metabolizer status and increased CNS adverse events in whites, and decreased virologic failure in blacks.
- Associations do not hold up to the rigorous adjustment for multiple comparisons.

## REFERENCES

- Haas DW, Ribado HJ, et al. Pharmacogenetics of efavirenz and central nervous system side effects: an Adult AIDS Clinical Trials Group study. *AIDS* 2004;18:2391-400.
- Wang J et al. Identification of a novel specific *CYP2B6* allele in Africans causing impaired metabolism of the HIV drug efavirenz. *Pharmacogenetics Genomics* 2006;16:191-8.
- Rolger M et al. Predictive value of known and novel alleles of *CYP2B6* for efavirenz plasma concentrations in HIV-infected individuals. *Clin Pharmacol Ther* 2007;81:557-66.
- Fellay J et al. Response to antiretroviral treatment in HIV-1-infected individuals with allelic variants of the multidrug resistance transporter 1: a pharmacogenetics study. *Lancet* 2002;359:30-6.
- Haas DW 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;192:1831-42.
- Blevierich JK, Schaeffeler E et al. MALDI-TOF mass spectrometry for multiplex genotyping of *CYP2B6* single-nucleotide polymorphisms. *Clin Chem* 2007;53:24-33



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