



Published in final edited form as:

*Pharmacogenomics J.* 2018 September ; 18(5): 678–688. doi:10.1038/s41397-018-0028-2.

## Polymorphisms in Cytochrome P450 are associated with Extensive Efavirenz Pharmacokinetics and CNS Toxicities in an HIV Cohort in Botswana

Marijana Vujkovic, PhD MSCE<sup>1</sup>, Scarlett L. Bellamy, ScD<sup>2</sup>, Athena F. Zuppa, MD MSCE<sup>3</sup>, Marc R. Gastonguay, PhD<sup>4</sup>, Ganesh S Moorthy, PhD<sup>3</sup>, Bakgaki Ratshaa, RN<sup>5</sup>, Xiaoyan Han, MS<sup>1</sup>, Andrew P. Steenhoff, MBBCh<sup>3</sup>, Mosepele Mosepele, MBBCh<sup>7</sup>, Brian L. Strom, MD MPH<sup>8</sup>, Gregory P. Bisson, MD MSCE<sup>1,9</sup>, Richard Aplenc, MD PhD<sup>10</sup>, and Robert Gross, MD MSCE<sup>1,9</sup>

<sup>1</sup>Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA

<sup>2</sup>Department of Epidemiology and Biostatistics, Dornsife School of Public Health, Drexel University, Philadelphia, PA

<sup>3</sup>Department of Anesthesiology and Critical Care Medicine, The Children's Hospital of Philadelphia, Philadelphia, PA

<sup>4</sup>Metrum Research Group, Tariffville, CT

<sup>5</sup>Botswana UPenn Partnership, Gaborone, Botswana

<sup>6</sup>Division of Infectious Diseases, The Children's Hospital of Philadelphia, Philadelphia, PA

<sup>7</sup>Faculty of Medicine, University of Botswana, Gaborone, Botswana

<sup>8</sup>Rutgers Biomedical and Health Sciences, Newark, NJ

<sup>9</sup>Department of Medicine (Infectious Diseases), University of Pennsylvania Perelman School of Medicine, Philadelphia, PA

<sup>10</sup>Division of Oncology, The Children's Hospital of Philadelphia, Philadelphia, PA

### Abstract

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Corresponding Author: Robert Gross MD MSCE, Associate Professor of Medicine (ID) and Epidemiology, University of Pennsylvania Perelman School of Medicine. Address: 804 Blockley Hall, 423 Guardian Drive, Philadelphia, PA 19104-6021. Tel: 215-898-2437, Fax: 215-573-5315, [grossr@upenn.edu](mailto:grossr@upenn.edu).

#### Conflict of Interest

All authors had full access to study data and agreed to submit for publication. The corresponding author had final responsibility for the decision to submit for publication. No medical writers were used and no agency made any payments for writing. Neither the funding agency nor pharmaceutical companies supporting the trial played any role in collection, analysis, interpretation, or reporting of these data.

#### Author Contributions

M.V. wrote the article; R.G., G.P.B., M.V, B.L.S., and M.M. designed the research; M.V., S.L.B, A.Z, M.G., B.R., X.H., A.P.S., M.M., B.L.S., G.P.B., A.R. and R.G. performed the research; M.V. analyzed the data. The final manuscript reviewed and approved by all authors.

Inter-individual variability in efavirenz (EFV) pharmacokinetics and dynamics are dominantly driven by the polymorphism in cytochrome P450 (CYP) isoenzyme 2B6 516G>T. We hypothesized that additional CYP polymorphisms mediate the relationship between CYP2B6 516G>T, EFV metabolism, and clinical events. We investigated 21 SNPs in 814 HIV-infected adults initiating EFV-based therapy in Botswana for population pharmacokinetics, CNS toxicities, and treatment outcomes. Two SNPs (rs28399499 and rs28399433) showed reduced apparent oral EFV clearance. Four SNPs (rs2279345, rs4803417, rs4802101, and rs61663607) showed extensive clearance. Composite CYP2B-mediated EFV metabolism was significantly associated with CNS toxicity ( $p=0.04$ ), with extensive metabolizers reporting more and slow and very slow metabolizers reporting less toxicity after one month compared to intermediate metabolizers. Composite CYP2B6 metabolism was not associated with composite early treatment failure. In conclusion, our data suggest that CNS-related toxicities might not be solely the result of super-therapeutic parent EFV concentrations in HIV-infected individuals in patients of African ancestry.

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## Introduction

The number of people with human immunodeficiency virus (HIV) type 1 initiating antiretroviral therapy (ART) in sub-Saharan Africa has dramatically increased over the past decade [1]. Combination therapy containing efavirenz (EFV), a non-nucleoside reverse transcriptase inhibitor (NNRTI), and two nucleoside reverse transcriptase inhibitors (emtricitabine and tenofovir disoproxil fumarate) has been the preferred management strategy in this region. EFV has a high efficacy, low cost, and long elimination half-life with once daily dosing fixed at 600 mg [2]. Plasma EFV concentrations  $<1 \mu\text{g/ml}$  are associated with virologic failure whereas super-therapeutic levels of  $>4 \mu\text{g/ml}$  have been associated with central nervous system (CNS) adverse effects including vivid dreams, headaches, dizziness, insomnia, and fatigue, that frequently lead to therapy discontinuation [3–6]. Recently, US treatment guidelines have relegated EFV to an alternative choice for first line therapy, primarily due to concerns about tolerability [7]. Yet, EFV remains a mainstay of treatment in resource-limited settings, in part due to the higher cost associated with drugs thought to be better tolerated.

EFV is primarily metabolized by cytochrome P450 (CYP) 2B6 and alternatively by CYP 2A6, 3A4, and 3A5 isoenzymes [8, 9]. Pharmacokinetic (PK) variability in EFV is mainly attributed to single nucleotide polymorphisms (SNPs) present in these highly polymorphic isoenzymes responsible for its metabolism and clearance [10, 11]. Among these variants, CYP2B6 516G>T has a high frequency and functional relevance, as confirmed in several settings including Botswana [12–16]. The presence of the minor slow-metabolizing allele has been implicated with CNS toxicities, but results are controversial. For example, Haas et al found the T-allele to be associated with CNS symptoms after 1 week of treatment and Rotger et al. reported a positive association between CYP2B6 genotype and sleep disorders and fatigue [17]. Studies in African populations showed that the relationship between CYP2B6 and CNS toxicity is not as clearly apparent or non-existent as compared to people of European ancestry [15, 18–21], and there have been reports in European populations as well that did not find this association [22–27]. In light of these differential findings for CYP2B6 genotype among African patients, we aimed to establish robust PK estimates at

steady-state for 21 SNPs in CYP 2B6, 2A6, 3A4, and 3A5 in HIV infected individuals of African origin initiating EFV-based therapy. We hypothesized that the presence of additional CYP polymorphisms mediate the association between CYP2B6 516G>T, EFV metabolism, CNS toxicities, and treatment outcomes.

## Subjects and Methods

### Study Design

Data were used from a cohort study of HIV-infected patients newly initiating their first ART regimen at clinics in and around Gaborone, Botswana. Participants were approached for enrollment in the clinics on the day they were scheduled to initiate antiretroviral therapy from June of 2009 to November of 2013. The inclusion criteria were (i) confirmed HIV infection, (ii) black African origin, (iii) no pregnancy or pregnancy intention, (iv) age 21 years or older, and (v) initiation of a standard three-drug regimen including EFV at 600 mg/day plus two NRTIs. Follow-up visits occurred at month 1 and month 6 and patients were included if they had at least one EFV plasma concentration available for analysis and pharmacokinetics modeling. The study was approved by the Ethics Board of the Health Research and Development Committee of the Botswana Ministry of Health and by the Committee on Human Subjects Research of the University of Pennsylvania, and each study participant provided written informed consent in English or Setswana.

### Drug assay

Midpoint plasma samples for measuring drug concentrations were collected at steady-state at 1 month after initiation of therapy and 6 months after initiation. EFV steady-state plasma concentrations are generally reached within a 6–10 day time frame [28]. Dates and times of blood draw and last ingestion were recorded. Samples were prepared using protein precipitation followed by one to one dilution with water. EFV was separated from plasma by high performance liquid chromatography, and concentrations measured with tandem mass spectrometry in negative ionization mode with multiple reaction monitoring having a lower limit of quantification of 1 ng/mL, as described previously [29].

### Genotyping

In order to characterize the genetic polymorphisms of CYP2B6, CYP2A6, CYP3A4, and CYP3A5, genomic DNA was extracted from whole blood samples obtained at the baseline visit. A total of 21 SNPs besides CYP2B6 516 G>T were selected in CYP2B6, CYP2A6, CYP3A4, and CYP3A5. SNPs were chosen based on results from previous studies, allele frequencies in people of African ancestry, and the availability of the SNP template on the Taqman OpenArray platform. All SNPs genotyped in the study were identified in the International HapMap project [30]. Allele-specific fluorescent discrimination was performed using Taqman Genotyper software, version 1.3 (Life Technology). All genotype-specific MAFs were compared against populations of African ancestry from the International HapMap project reference panel.

## Medication Adherence

EFV adherence was calculated from pharmacy data over the first month and last 3-months before end of follow-up. Refill dates were used to calculate medication possession ratio according to the formula: (number of doses dispensed minus the number of doses returned) divided by (number of days between the initial fill and the fill closest to the target date) [31].

## Population PK Model Development

Population pharmacokinetic modeling was performed using nonlinear mixed-effects modeling in NONMEM VII (ICON Development Solutions, Ellicott City, MD, USA). The ADVAN2 subroutine (one compartment linear model with first order absorption) was selected with the first-order conditional estimation (FOCE-I) method with interaction to estimate the typical model parameters. Random inter-individual and inter-occasional variability were estimated and the final model was minimized with successful execution of the covariance step. The baseline EFV PK model parameters were adopted from a previous study in the same cohort, where clearance (CL/F) was the only parameter to be estimated while holding the parameters of volume of distribution (Vd) and absorption rate constant (Ka) constant at 150 L and 0.18 h<sup>-1</sup> respectively [13]. We used an exponential variance model to describe the unexplained random variability of parameters across individuals.

To test whether baseline characteristics influence EFV PK, a structural baseline population PK covariate model was established. Models were explored using various inter-individual random effect covariance structures. Inter-individual variability was initially estimated for CL/F, and then subsequently evaluated for the remaining pharmacokinetic parameters. A composite additive and proportional error model was used to describe random residual variability. Covariates included subject weight, gender, EFV adherence, and suspected or confirmed tuberculosis co-occurrence. Weight was included in an allometric model and fixed at 0.75 CL/F and normalized to a reference weight of 70 kg. All baseline covariates were included with power terms into the model. Subsequently, SNPs in CYP2B6, CYP2A6, CYP3A4, and CYP3A5 were tested for model improvement in a univariate fashion.

The final model was identified using stepwise forward selection and backward elimination of covariates using NONMEM. Goodness of fit diagnostic plots were evaluated in R (version 3.2.3). The covariate that resulted in the greatest statistically significant decrease in the Minimum Objective Function Value (MOFV) was kept in the model and remaining significant covariates were added in the same fashion. The MOFV following a Chi-Square distribution with a change of 3.84 (df=1) or 5.99 (df=2) was considered statistically significant at  $\alpha=0.05$ . A p-value of 0.20 was used for the backward elimination procedure. SNPs were considered extensive or slow metabolizers if they respectively increased or decreased CL by 20%, and were not in LD with other SNPs with a similar effect on clearance (e.g. multi-collinearity).

## Pharmacodynamics

In subsequent pharmacodynamics (PD) analyses patients were classified into composite CYP2B6-mediated metabolizer groups. Patients are classified into 'Very Extensive', 'Extensive', 'Intermediate', 'Slow', and 'Very Slow' composite metabolizer group based on

their CYP2B6 516G>T genotype, 983T>C genotype and presence of an extensive metabolizer allele in 18492T>C, 15817A>C, or -745A>G. The 35-item Subject Experience Questionnaire (SEQ) [22] questionnaire was completed at months 1 and 6 after initiation of therapy [32]. We limited our analysis to the 21 items related to CNS-related adverse effects: difficulty sleeping, abnormal dreams, fatigue, dizziness, nervousness, mood change, irritability, memory loss and impaired concentration. Patients were classified into ordinal categories according to their CNS scores into: none, mild (1-3), moderate (4-7), severe (8-12), and very severe (>12). A proportional odds model for ordinal regression using VGAM R-package was performed to quantify the relationship between CNS symptoms classes and composite CYP2B6 metabolizer class. We also ran the ordinal logistic regression model with CYP2B6 as categorical variable. Treatment outcome measures included a composite treatment endpoint defined as death, detectable viral load (HIV RNA >25 copies/ml at month 6), or loss to follow-up at 6 months. Composite treatment endpoint was modeled using multivariate logistic regression with composite CYP2B6 group where the intermediate composite metabolizer group was considered the reference. Two additional logistic regression analyses were performed to assess whether the associations were more or less strong for the endpoints of detectable viral load or loss to follow-up [33].

### Code availability

Programming code for population PK and PD analyses using NONMEM and R are available upon request.

## Results

### Population Pharmacokinetics

A total of 941 individuals were initially enrolled in the cohort study with a scheduled follow-up of 6 months, in which 814 were successfully genotyped for CYP2B6 516G>T (rs3745274). 742 genotyped participants had at least one midpoint steady-state plasma EFV specimen available at month 1 for drug quantification, of whom 562 also had an available blood sample at month 6 (Table 1). Median plasma EFV concentration dropped from 2.17 µg/ml after one month of therapy initiation to 2.05 µg/ml after six months ( $p < 0.01$ ). The general characteristics as shown in Table 1 indicate patients with lower CD4 counts were more likely to have dropped out of the study at month 6 (Table 1). The structural baseline population PK model without covariates showed a MOFV of 2314. Inter-occasion variability resulted in 3% higher apparent clearance at month 6 compared to month 1 measurements (7.3 vs. 7.6 L/hr/70kg for month 1 and month 6, respectively, MOFV = 2304). CYP2B6 516G>T genotypes demonstrated an impact on EFV clearance for normal (GG, 9.439 L/hr/70kg), slow (GT, 7.233 L/hr/70kg), and very slow (TT, 4.033 L/hr/70kg) genotypes respectively, with a MOFV of 2085. History of tuberculosis, gender, and drug adherence did not significantly improve MOFV[13]. Therefore, the final baseline covariate model before inclusion of the additional SNPs under investigation included CYP2B6 516G>T genotype, allometrically scaled weight, and visit as covariates (MOFV = 2075) with an inter-individual variability ( $\omega^2$  CL) of 40.5% coefficient variation, proportional residual variability of 0.0831% coefficient variation, and residual additive variability of 0.0699 SD.

## SNPs and Pharmacokinetics

The effects of 21 individual SNPs from CYP isoenzymes on apparent oral EFV clearance are shown in Table 2. In particular, two SNPs were associated with decreased apparent EFV clearance, which were CYP2B6 983T>C (rs28399499, MOFV = 1862) with a MAF of 11% and CYP2A6 -48T>G (rs28399433, MOFV = 2016) with a MAF of 8%. Four SNPs were associated with apparent extensive EFV clearance, namely 1) rs2279345 (CYP2B6 18492T>C, MOFV = 1995), an intronic variant in CYP2B6 with a MAF of 19%, 2) rs4803417 (CYP2B6 15817A>C, MOFV = 2016) located in the UTR-5 region of CYP2B6 with a MAF of 12%, 3) CYP2B6, -745A>G (rs4802101, MOFV = 2037) with a MAF of 4% and 4) finally, a SNP in the 5 prime flanking region of CYP2A6 (-750T>C, rs61663607, MOFV = 2021) were associated with extensive apparent clearance (Table 2).

SNPs that were not considered for evaluation due to their high linkage disequilibrium with index SNPs were rs707265 ( $r^2 = 0.44$ ,  $D' = 1$  with rs28399499), rs8192726 ( $r^2 = 0.99$ ,  $D' = 1$  with rs28399433), rs2279343 ( $r^2 = 0.30$ ,  $D' = 1$  with rs3745274), and rs8192719 ( $r^2 = 0.82$ ,  $D' = 1$  with rs3745274). The composite variable of CYP2B6 metabolism was created based on these PK findings, and subsequently tested for associations with CNS toxicities and treatment endpoints.

## Pharmacodynamics

Patients reported an average of 1.67 CNS-related symptoms at Month 1 (median 1, IQR 0-2). Among the patients that remained on study, the average number of CNS-related symptoms was 0.79 at Month 6 (median 0, IQR 0-1). However, the majority of the participants ( $n = 372$ ) reported no CNS adverse experiences. Figure 2 depicts levels of reported CNS toxicities between different CYP2B6 metabolizer categories. Patients with very extensive or extensive CYP2B6 metabolism more frequently reported severe CNS adverse experiences compared to intermediate, slow metabolizers and very slow metabolizers reported less toxicity despite their higher plasma EFV concentrations ( $p = 0.041$ ). The association between composite metabolizer group and CNS toxicities disappeared at the six month time point ( $p=0.425$ , figure not shown). We did not observe an association between composite CYP2B6 metabolism and composite failure (Table 4). However, there was a tendency of loss to follow-up occurring more frequently in both the very extensive and slow/very slow metabolizer groups compared to intermediate metabolizers, OR 1.42 (0.84-2.36) and 1.50 (0.93-2.42) respectively. Similarly a mild protective effect was observed for the outcome of detectable viral load for both the extensive and slow/very slow metabolizer groups, although none of these associations met the traditional thresholds for statistical significance (Table 4).

## Discussion

The present study confirms the influence of two well-described cytochrome P450 polymorphisms, CYP2B6 983T>C and CYP2A6 -48T>G, which were both associated with increased plasma EFV exposure [10, 11, 34]. We additionally identified 4 cytochrome P450 variants (CYP2B6 -745A>G, 15817A>C, 18492T>C, and CYP2A6 -750T>C) associated with decreased plasma EFV exposure in HIV-infected individuals initiating ART treatment

in Botswana. We observed an association between composite CYP2B6-mediated EFV metabolism and CNS toxicities, with extensive metabolizers reporting more and very slow metabolizers reporting less toxicities. These data suggest that CNS-related toxicities might not be a result of super-therapeutic EFV levels in this HIV-infected population of African ancestry.

While prior research has found EFV levels exceeding 4 mg/mL to be associated with neurotoxicity [8], Ribaud et al. found the relation between EFV concentrations and CNS adverse experiences to be apparent among Caucasian, but not among African-American patients [18]. This discrepancy may have been due to a more complex relation between EFV metabolism and symptoms than previously recognized. Recent *in vitro* studies suggest a major neurotoxic role for the major phase I metabolite of EFV, 8-hydroxyefavirenz (8-OH-EFV) due to its ability to generate reactive oxygen species [18]. Efavirenz metabolites may contribute to drug effectiveness but also to toxicity and interactions. Thus, there is a need for extensive EFV metabolic profiling. In extensive metabolizers, even though parent EFV is low, 8-OH metabolite concentrations higher. But in slow metabolizers, despite high EFV concentrations, the concentration of 8-OH-EFV would be expected to be lower due to upregulated hydrolysis by the CYP2A6 enzyme into 7-OH-EFV.

P-glycoprotein, an efflux membrane transporter encoded by ABCB1 is predominantly found at the blood brain barrier that limits entry into the CNS for a large number of drugs. Several studies assessed EFV substrate specificity for the main ATP-dependent drug efflux transporters suggesting that EFV does not interact with ABCB1 [35–39] and is therefore unlikely to explain our findings. These data are in line with the observations of Winston *et al.* who showed central spine fluid (CSFG) 8-OH-EFV not to be differential between CYP2B6 516G>T genotypes [40]. They postulated an EFV dose-dependent up regulation of efflux transporters specifically affecting CNS 8-OH-EFV removal [41]. Similarly, Aouri *et al.* recently measured both phase I and II metabolites of EFV in CSF and found that 8-OH-EFV did not differ between CYP2B6 genotypes [42]. They did not observe a direct association between EFV or any metabolite and CNS toxicity, however they might have been underpowered for an association analysis with only 71 patients. While we did not measure plasma or CSF concentrations of 8-OH-EFV, our data are consistent with the hypothesis that EFV-related CNS toxicity might mediated via a neurotoxic 8-OH-EFV metabolite [43]. However, additional studies are essential to explain this phenomenon.

Several CYP2B6 polymorphisms evaluated other than G516T have been implicated in the inter-individual variability in plasma EFV levels previously. The current investigation validates several previously repeatedly identified SNPs in a cohort of African HIV patients treated with EFV in a clinical setting. Our population PK analyses on apparent EFV clearance support the previously observed association of the missense CYP2B6 983T>C (rs28399499) [9–11, 21, 34, 44–48] and intronic CYP2A6 -48T>G (rs28399433) SNPs with poor EFV clearance. We additionally identified 4 SNPs with substantially reduced MOFV and altered EFV clearance estimates by >20%, namely CYP2B6 -745A>G, 15817A>C, 18492T>C, and CYP2A6 -750T>C.

CYP2B6 18492T>C (rs2279345) has been previously associated with increased metabolism of EFV, resulting in lower EFV plasma levels as compared to patients with the TT genotype [49–53]. Similarly, rs4802101 (CYP2B6 -750T>C) is a promoter SNP part of CYP2B6 \*28 and located within a transcription factor binding site. It has been associated with increased exposure to EFV as compared to CYP2B6 \*1 haplotype in people with HIV [19]. It should be noted that since these polymorphisms are relatively frequent in African populations, a substantial proportion of the patients might be at the risk of having sub-therapeutic EFV plasma concentrations and consequently higher rates of resistance when failing EFV [8].

To our knowledge, we identified two SNPs not described to date in relation to EFV pharmacokinetics. First, rs4803417 (CYP2B6 15817A>C) located in the UTR-5 region of CYP2B6 and has been associated with lowering circulating concentrations of a major brominated flame retardant, BDE-47 [54]. Since there is evidence that in Botswana atmospheric polybrominated diphenyl ethers (PBDEs) levels have been steadily increasing [55, 56], it is intriguing to consider that PBDE-induced CYP activation might be at play [57]. Second, rs61663607 (-745A>G) is a polymorphism located in the 5 prime flanking region of CYP2A6 that disrupts the core region of a CCAAT box to which the regulatory element NF-Y binds [58, 59]. Genetic polymorphisms in the promoter region of CYP2A6 have been implicated for decreased promoter activity and subsequent transcriptional activity [58, 60]. The epigenomic signature of the SNP obtained from CHIP-seq data of the Roadmap Epigenomics Consortium reveals the presence of both a genetic enhancer and confirms the presence of poised (e.g. inactive) promoter [61]. Our study found that patients heterozygous for the minor allele have increased apparent EFV clearance, suggesting increased CYP2A6 activity. Therefore, the CYP2A6 -745A>G potential mechanisms of switching between promoter, enhancer, and transcriptional transition states of CYP2A6 chromatin and the effect on EFV pharmacokinetics requires further investigation.

Major strengths of our study include 1) a large sample size, 2) a racially homogenous group, and 3) the routine care setting. Several limitations apply. Although the association between genotype and CNS toxicities was substantial, the reporting of EFV-based toxicities in Africa was lower than in Western countries [22]. In our study in Botswana, 66.3% of patients reported at least one EFV-based adverse event, whereas prevalences of 9.4%, 30.9%, 42.7%, and 83.3% were reported in Ghana, Uganda, Zimbabwe, and South Africa respectively [15, 20, 21, 62]. These observations highlight the need for an adverse events assessment method tailored for and validated in African HIV-infected individuals. Furthermore, we lacked a precise measure of adherence, such as microelectronic monitors [63], and so the accuracy of adherence measures might be sub-optimal. Detectable viral load – one of the study endpoints – was defined at the low level of >25 copies/ml and therefore may not be associated with clinical failure or virologic transmission if individuals engage in unprotected sex [64]. Data on whether resistance emerged in these failures was unavailable. Moreover, a substantial part of the patient population was lost to care within the 6 month time frame, where others have demonstrated these individuals are more likely to have poor outcomes [33]. Also, the current study is restricted to an average follow-up time of 6 months, and so questions regarding the impact of these polymorphisms in the response to therapeutic drugs and the long-term virologic and immunological response in this population remain to be answered. However, in a follow-up study in Botswana we observed that the CYP2B6



516G>T polymorphism showed a protective association with regards to the maintenance of HIV suppression [14]. Finally, in this exploratory analysis evaluating a total of 21 SNPs we cannot exclude that these findings were not due to chance and therefore future replication is essential to establish the validity of these findings.

In conclusion, extensive SNPs in cytochrome P450 enzymes appear to contribute to variability of EFV pharmacokinetic parameters in HIV patients and appear to be associated with greater CNS toxicity. Additional investigation is required to replicate our seemingly paradoxical observation with regard to EFV-induced CNS toxicity, identify potential underlying causal variants, and characterize their biological mechanistic importance.

## Acknowledgments

We thank the medical staff at the Bontleng, BH3, Broadhurst Traditional Area, Morwa, Nkoyaphiri, Phase II, and Village Infectious Diseases Care Clinics for their assistance with carrying out this study. We also thank the Ministry of Health of Botswana for supporting the project and the patients who participated.

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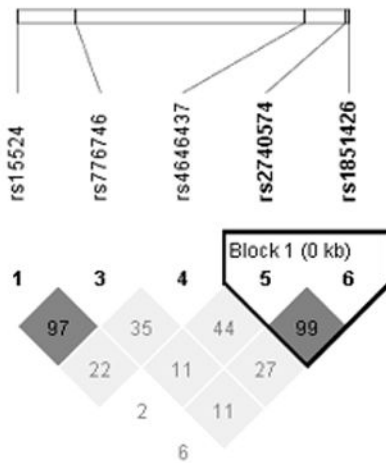
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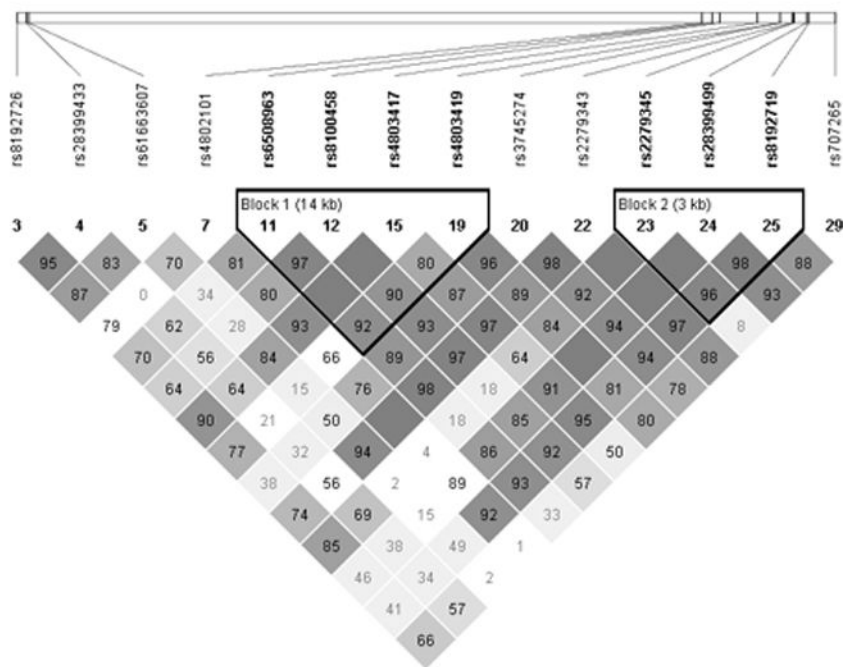
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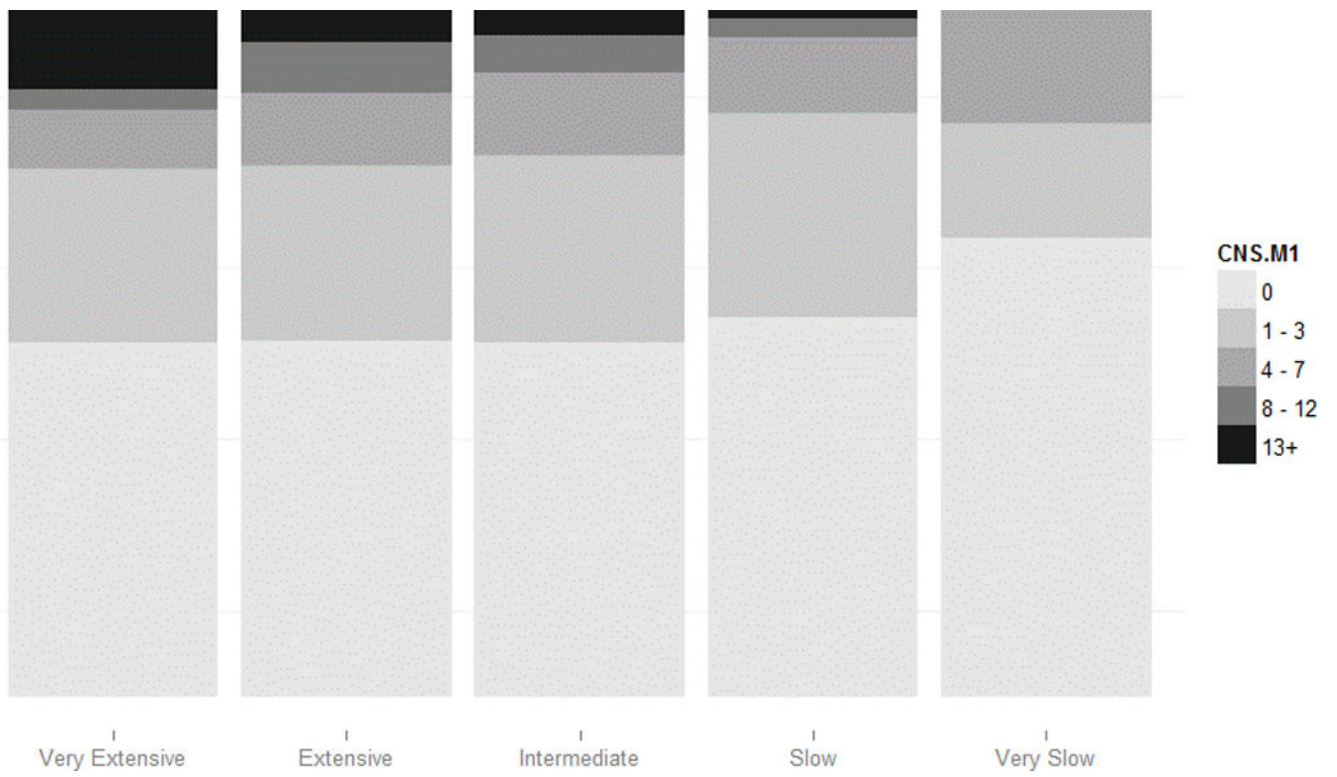
**A. Chromosome 7**



**b. Chromosome 10**



**Figure 1.**  
LD Blocks and Allele Frequencies for SNPs



**Figure 2.**  
CNS toxicities by composite CYP P450 metabolizer group

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**Table 1**

Baseline Characteristics Stratified by Availability of Treatment Outcome Data at Month 6.

Characteristic	Included Patients	Treatment Outcome data available at Month 6		P
		Yes	No	
Total *, n	941	712	229	
Genotyped at baseline, n	814	624	190	
Genotyped and provided one EFV sample, n	742	567	175	
Genotyped and provided two EFV samples, n	562	525	37	
Plasma EFV at month 1 (µg/ml), median (IQR)	2.17 (1.62 – 3.88)	2.14 (1.60 – 3.72)	2.34 (1.71 – 4.68)	0.085
Age in years, median (IQR)	37 (33 – 44)	37 (32 – 44)	38 (34 – 44)	0.14
Male gender, n (%)	399 (49.0%)	280 (44.9%)	98 (51.5%)	0.15
Body Mass Index, median (IQR)	22.0 (19.8 – 25.1)	22.0 (19.8 – 25.1)	21.7 (20.0 – 25.1)	>0.5
CD4 count (cells/mm <sup>3</sup> ), median (IQR)	196 (112 – 256)	204 (118 – 271)	184 (117 – 235)	<0.02
Plasma viral load (log <sub>10</sub> copies/ml), median (IQR)	4.9 (4.2 – 5.4)	4.9 (4.2 – 5.4)	5.0 (4.3 – 5.4)	>0.5
History of tuberculosis, n (%)	31 (3.8%)	23 (3.7%)	8 (4.2%)	>0.5

\* Study cohort included patients were successfully genotyped for CYP2B6 516G>T polymorphism. P-values were calculated with Wilcoxon rank sum test for continuous variables, and chi-square test for categorical covariates and represent statistical differences between patients that have outcome data at Month 6 available versus who do not.



**Table 2**

Apparent EFV Clearance for Genotyped SNPs

CYP	SNP	MAF	LD	No. Participants			Clearance (L/hr/70kg)			MOFV
				Block	Wt	Het	Hom	Wt	Het	
3A5	rs15524	0.37	-	287	353	95	7.302	7.250	7.270	2027
	rs41303343	0.09	-	614	113	10	7.539	7.217	7.157	2009
	rs10264272	0.21	-	462	247	29	7.387	7.388	7.852	2032
	rs776746	0.18	-	494	208	26	7.421	7.219	7.355	2011
3A4	rs4646437	0.14	-	554	174	12	7.423	7.425	7.686	2052
	rs2740574	0.24	A	418	281	37	7.466	7.319	7.316	2042
	rs1851426	0.20	A	470	243	26	7.439	7.306	7.321	2038
2A6	rs143731390	0.06	-	631	90	0	7.481	7.788	-	2001
	rs8192726	0.07	B	643	87	9	7.636	5.737	4.671	2004
2B6	rs28399433	0.08	B	635	95	8	7.598	5.926	4.939	2016
	rs61663607	0.14	-	543	171	16	7.225	7.172	8.678	2021
	rs4802101	0.04	-	687	54	0	7.251	8.640	-	2037
2A6	rs6508963	0.21	C	461	250	29	6.734	7.238	7.537	2044
	rs8100458	0.16	C	528	190	23	6.848	7.633	7.738	2041
2A6	rs4803417	0.12	C	572	151	14	6.819	8.303	9.377	2016
	rs4803419	0.07	C	640	92	7	7.225	7.431	6.739	2046
2A6	rs2279343	0.06	-	645	96	0	8.139	5.091	-	2041
	rs2279345	0.19	-	485	224	27	6.539	8.005	8.349	1995
2A6	rs28399499	0.11	D	586	149	5	7.984	4.691	1.440	1862
	rs8192719	0.37	D	283	349	99	9.546	7.377	5.600	2017
2A6	rs707265	0.19	D	490	223	26	7.719	5.614	4.729	1933

LD blocks are denoted by capital letters for SNPs that are in linkage disequilibrium. MOFV of the model prior to evaluating SNPs was 2075, which includes the covariates of CYP2B6 516G>T, allometrically scaled weight, and inter-occasion variability. Clearance estimates are corrected for the covariates: CYP2B6 G516T genotype (rs3745274), allometrically scaled weight, and inter-occasion variability between visits. MAF=minor allele frequency. Wt=wild type, het=heterozygote, hom-homozygote variant

**Table 3**

Clearance-Based Classification of CYP2B6 SNPs into a Composite Metabolizer Group.

516G>T	983T>C	Extensive SNP	N	Composite Metabolizer
0	0	0	69	Extensive
0	0	1	141	Very Extensive
0	1	0	57	Intermediate
0	1	1	36	Intermediate
0	2	0	6	Very Slow
0	2	1	0	Slow
1	0	0	179	Intermediate
1	0	1	127	Extensive
1	1	0	66	Slow
1	1	1	3	Intermediate
1	2	0	0	Very Slow
1	2	1	0	Slow
2	0	0	112	Slow
2	0	1	2	Intermediate
2	1	0	1	Very Slow
2	1	1	0	Slow
2	2	0	0	Very Slow
2	2	1	0	Very Slow

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**Table 4**

## Association Between Composite CYP2B6 Metabolism and Treatment Endpoints

	Failure		Detectable Viral Load		Loss to Follow-up	
	OR	(95%CI)	P	OR	(95%CI)	P
Composite Metabolizer						
Very Extensive	0.99	(0.65-1.50)	0.68	(0.34-1.29)	1.42	(0.84-2.36)
Extensive	0.84	(0.57-1.23)	0.58	(0.31-1.04)	1.00	(0.61-1.64)
Intermediate	1.00	(Ref)	1.00	(Ref)	1.00	(Ref)
Slow/Very Slow	1.18	(0.80-1.72)	0.74	(0.40-1.33)	1.50	(0.93-2.42)

\* Composite virologic endpoint was defined as death, HIV RNA >25 copies/ml, or loss to follow-up at 6 months