Neuropsychometric correlates of efavirenz pharmacokinetics and pharmacogenetics following a single oral dose

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Although generally safe and effective, efavirenz containing regimens have been associated with high rates of central nervous system side effects, particularly in the first days or weeks of treatment. These side effects have been inconsistently associated with plasma efavirenz concentrations, as well as *CYP2B6* genetics. *CYP2B6* genetics are known to be associated with efavirenz pharmacokinetics.

WHAT THIS STUDY ADDS

- This study uniquely describes the association of efavirenz pharmacokinetics and pharmacogenomics with subjective and objective measures of neurocognitive function during the first 6 h following a single dose of efavirenz. We demonstrate a strong association between efavirenz pharmacokinetics and neurocognitive side effects as assessed by the non-dominant hand grooved pegboard test. The associations of neurocognitive testing with *CYP2B6* genotype likely are a reflection of the associations between genotype and clearance.

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AIMS

To determine pharmacokinetic and pharmacogenomic correlates of efavirenz central nervous system (CNS) side effects following a single dose.

METHODS

Thirty-four healthy HIV-negative African Americans were administered a 600 mg dose of efavirenz. Blood samples for pharmacokinetics were drawn serially from 0 to 12 h post-dose. Neuropsychometric testing with drowsiness visual analogue scale, grooved pegboard and letter digit substitution tests was done the day prior to dosing and at 1, 2, 3, 4 and 6 h post-dose. Subjective CNS symptoms were assessed at 6 h post-dose. Composite *CYP2B6* 516/983 genotype was determined.

RESULTS

Pharmacokinetic indices reflecting increased plasma efavirenz exposure were associated with slower non-dominant hand grooved pegboard task completion (C_{max} , $P_{1h} = 0.01$, $P_{2h} = 0.05$, $P_{3h} = 0.03$, $P_{4h} = 0.01$; AUC, $P_{1h} = 0.04$; clearance $P_{1h} = 0.05$, $P_{2h} = 0.02$, $P_{6h} = 0.01$). In a repeated measures model analysis that adjusted timing of neuropsychometric testing for timing of peak drug concentration, clearance ($P < 0.001$), AUC(0.312 h) ($P = 0.001$) and C_{max} ($P = 0.008$) were associated with non-dominant grooved pegboard test performance. *CYP2B6* genotype trended to correlate with non-dominant hand grooved pegboard at 4 and 6 h ($P = 0.07$ and 0.06). Decreased drowsiness at 6 h was associated with higher C_{max} ($P = 0.02$).

CONCLUSIONS

Following a single dose of efavirenz, an association between pharmacokinetics and neuropsychometric performance was discernable. A weaker association between genotype and neurocognitive test performance is likely mediated by effect of genotype on plasma clearance. Strategies that lower C_{max} during initial dosing may decrease CNS side effects.

Introduction

The non-nucleoside reverse transcriptase inhibitor efavirenz is one of the most widely prescribed antiretrovirals for treating HIV-1 infection worldwide. Although generally safe and effective, efavirenz frequently causes central nervous system (CNS) symptoms during treatment initiation, which in a small proportion of patients leads to treatment discontinuation [1–3]. Symptoms include dizziness, hangover-like sensation, impaired concentration, sleep disturbances and, rarely, psychosis or suicidal ideation. Such CNS symptoms may be of maximal intensity with the initial doses, and generally attenuate with repeated dosing over subsequent days or weeks [1, 4, 5] despite persistent plasma efavirenz exposure [6]. The mechanism(s) by which efavirenz CNS symptoms attenuate over time is not known.

Associations between higher plasma efavirenz concentrations and CNS side effects have been reported in some studies [1, 5, 7–10] but not others [11–13]. In the only double-blind, placebo-controlled study to assess specifically efavirenz CNS symptoms ($n = 290$), efavirenz was significantly associated with increased CNS symptoms within the first week following treatment initiation ($P < 0.001$). However at weeks 4, 12 and 24 there were no differences in CNS symptom scores between efavirenz and placebo recipients ($P > 0.05$) [1].

Efavirenz is metabolized primarily by hepatic cytochrome P450 (CYP) 2B6 [14]. Two *CYP2B6* loss of function variants that are frequent among individuals of African descent, *CYP2B6**6 (516 G→T) and *18 (983 T→C), are associated with decreased plasma efavirenz clearance and increased plasma efavirenz exposure [6, 9, 15–18]. A relationship between these *CYP2B6* variants and efavirenz CNS side effects and/or treatment discontinuation has been suggested in some cohorts [6, 8, 9, 16, 19] but not others [16, 17, 20].

Inconsistent results among some previous studies that assessed efavirenz pharmacokinetic–pharmacodynamic relationships may reflect the subjective nature of efavirenz CNS symptoms. To address this issue, several prior studies therefore also used objective psychometric tests to assess such relationships following repeated dosing with efavirenz [1, 4, 21, 22]. Studies in this area are also complicated by the fact that efavirenz CNS symptoms may rapidly attenuate with repeated dosing [1]. No study to date has used objective measures to quantify relationships between CNS side effects and plasma efavirenz pharmacokinetic parameters during the first few hours following the initial dose of efavirenz, before attenuation of CNS symptoms.

In a previous analysis involving 34 healthy African-American volunteers intensely studied over 13 days following a single 600 mg oral dose of efavirenz, we reported that *CYP2B6* genotype was significantly associated with efavirenz plasma area under the curve (AUC(0,312 h)

($P = 0.006$) and plasma clearance ($P = 0.02$), but not with peak plasma concentration ($C_{\max}$, $P = 0.37$) [23]. In the present analysis we use neuropsychometric data collected in that study to characterize temporal associations between neuropsychometric performance change from baseline, *CYP2B6* genotype and plasma pharmacokinetics following a first dose of efavirenz.

Methods

Pharmacokinetics and pharmacogenetics

These analyses involved a cohort previously studied for pharmacogenetics and pharmacokinetics, as described elsewhere [23]. Briefly, 34 healthy African Americans received a single 600 mg dose of efavirenz at 08.00 h. Plasma samples for efavirenz assays were obtained at 0.5, 1, 2, 4, 6, 8 and 12 h and serially thereafter up to 13 days post-dose. Plasma efavirenz concentrations were determined by liquid-liquid extraction followed by high performance liquid chromatography with UV detection at 269 nm, the details of which are given elsewhere [23]. The assay was linear from 50 to 5000 ng ml⁻¹. The mean r^2 value for calibration curves is 0.997 (range 0.991 to 0.999). The coefficient of variation from 500 to 3000 ng ml⁻¹ ranged from 4.1% to 11.8%. Each specimen was assayed once.

Genotyping for *CYP2B6* 516G→T (*CYP2B6**6, rs3745274) and 983T→C (*CYP2B6**18, rs28399499) was by TaqMan™ assays. Composite *CYP2B6* 516/983 genotypes, based on known associations with efavirenz pharmacokinetics [6, 9, 15–18], were assigned as follows: *extensive metabolizer*, no variant allele at either position 516 or 983; *intermediate metabolizer*, a single variant allele at either position 516 or 983, but not both; *slow metabolizer*, two variant alleles. Pharmacokinetic parameter estimates were determined using a non-compartmental approach (WinNonlin version 4.01, Pharsight Corp., Mountain View, CA, USA). Nomenclature for drug receptors and targets is consistent with ‘Guide to Receptors and Channels 5th Ed.’ [24].

Neuropsychometric testing

The following neuropsychometric tests were administered 1 day pre-dose and again 1, 2, 3, 4 and 6 h post-dose: (1) grooved pegboard with the dominant hand (GPB-D), (2) grooved pegboard with the non-dominant hand (GPB-ND), (3) letter digit substitution test (LDST) and (4) drowsiness visual analogue scale. A 19-item efavirenz CNS symptom questionnaire, based on the survey developed for the AIDS Clinical Trials Group (ACTG) protocol A5097s [1], was completed 6 h post-dose to capture maximal symptoms at or before 6 h, and to calculate a total CNS symptoms score based on nine CNS-targeted questions (subjective ordinal symptom score). The GPB-D, GPB-ND, and LDST are objective measures of task performance, whereas the drowsiness visual analogue scale and CNS

symptom questionnaire are subjective measures of symptoms.

The LDST (a variation of the symbol digit substitution test) is a measure of memory, complex visual processing and information processing speed [25]. A row of various letters is given and the examinee writes the corresponding digit according to a predefined key of letter-digit pairs. The score is based on number of letter-digit pairs accurately completed in 60 s. The grooved pegboard test is a measure of manual dexterity, attention and complex visual-motor coordination [26]. It involves inserting 25 pegs with a lateral protrusion into a board with 25 holes with variably oriented slots that match the protrusion. The score is based on the number of seconds required to insert the 25 pegs [27]. Grooved pegboard performances were compared with norms for African Americans with a 12th grade education of matched age and gender [28]. LDST scores were matched to norms by age and gender for individuals of an average educational level [25].

Human experimentation guidelines of the US Department of Health and Human Services were followed in the conduct of this research. All work was conducted in accordance with the Declaration of Helsinki. The Vanderbilt Institutional Review Board approved the study and all participants provided written informed consent.

Statistical methods

Continuous variables are presented as medians and inter-quartile ranges (IQR). We calculated body mass index (BMI) as weight in kg divided by the square of the height in metres. *CYP2B6* 516/983 genotype was coded as an ordered continuous variable with 1 denoting extensive, 2 intermediate and 3 slow metabolizer. To examine temporal associations of efavirenz pharmacokinetic parameters with neuropsychometric tests multivariable linear regression models were used for each time point measure separately. Adjustment for covariates were performed by including in each regression model *a priori* selected covariates that included the baseline test result, age, gender and BMI. To achieve adequate regression coefficient power, a first principal component was used to account for the variation of age, gender and BMI. Models included baseline test score and a first principal component as covariates [29].

Relationships between efavirenz plasma concentrations and neuropsychometric data (GPB-ND) were further examined at the time closest to $t_{\max}$. We also evaluated improvement in precision by utilizing all available data using repeated measures analysis adjusting for age, gender and BMI. For repeated measures analysis, we applied a multivariable generalized least squares regression model with correction for intra-subject correlation [29, 30]. Non-linear associations were tested using restricted cubic splines and were included in models when detected.

Relationships between *CYP2B6* genotypes and pharmacokinetic parameters, neuropsychometric test results and efavirenz symptom scores were assessed using the Kruskal-Wallis test. Multivariable regression analyses were performed to adjust for BMI, gender, age and baseline neuropsychometric test values as indicated above. Statistical analyses were performed using R version 2.12.1 (<http://www.r-project.org>). A two-sided significance level of 5% was required for consideration as statistical significant. Reported *P* values were not corrected for multiplicity.

Results

Characteristics of study participants

Baseline characteristics of study participants, *CYP2B6* genotypes and pharmacokinetic parameters are described in detail elsewhere [23]. Briefly, median age was 24 years, median BMI was 24 kg m⁻² and 76% of participants were female. Based on *CYP2B6* composite 516/983 genotype, seven were slow metabolizers, 17 were intermediate metabolizers and 10 were extensive metabolizers. Genotype groups did not differ significantly by age, gender or BMI. Median efavirenz $C_{\max}$ (IQR) was 1864 ng ml⁻¹ (1469, 2320 ng ml⁻¹), AUC(0,312 h) 85 mg l⁻¹ h (63–107 mg l⁻¹ h), $t_{\max}$ 2.0 h (1.0–4.0 h) and clearance was 6.22 l h⁻¹ (4.67–8.38 l h⁻¹).

Pharmacokinetics and neuropsychometric testing

Neuropsychometric test performance at baseline and serially following a single dose of efavirenz is shown in Table 1. Regarding baseline GPB-D performance, 10 participants scored mildly impaired (1.0 to 1.5 SD below matched norms), two participants scored mild-to-moderately impaired (1.5 to 2.0 SD below matched norms) and two participants scored moderately impaired (2.0 to 2.5 SD below matched norms), with no participants scoring in the moderate-to-severely impaired (2.5–3.0 SD below matched norms) or severely impaired (>3.0 SD below matched norms) ranges. Regarding the worst GPB-D performance following the efavirenz dose, two participants scored mildly impaired, six participants scored mild-moderately impaired, six scored moderate impaired and four scored severely impaired.

Regarding baseline GPB-ND performance, seven participants scored mildly impaired and five participants scored moderately impaired. Regarding the worst GPB-ND performance following the efavirenz dose, eight participants scored mildly impaired, six participants scored mild-moderately impaired, four moderately impaired and two scored severely impaired.

Regarding baseline LDST, 11 participants performed at least 1 SD below normative values, including four who were at least 2 SD below normative values. Following the efavirenz dose, 31 participants had at least one

Table 1

Neuropsychometric data before and after a single dose of efavirenz*†‡

Time point (h)	GPB-D (s)	GPB-ND (s)	Letter digit substitution (correct min ⁻¹)	Drowsiness (visual analogue scale, mm)
Baseline	97 (90–104)	105 (94–117)	37 (32–40)	15 (2–45)
1	96 (92–108)	104 (96–124)	34 (30–36)	41 (20–73)
2	95 (89–114)	107 (96–123)	30 (24–35)	49 (29–80)
3	99 (88–111)	106 (94–117)	30 (26–37)	14 (7–22)
4	94 (85–104)	102 (93–112)	34 (28–36)	25 (12–54)
6	94 (83–97)	98 (90–108)	34 (31–38)	30 (6–50)

*Abbreviations: GPB-D and GPB-ND are grooved pegboard performance with the dominant hand and non-dominant hand, respectively. †Better performance is indicated by lower scores on GPB and higher scores on the letter digit substitution test. Higher scores on visual analogue scale indicate increased drowsiness. ‡All data represent medians and interquartile ranges.

performance that was 1 SD below normative values or worse, including 23 who were at least 2 SD below normative values.

Efavirenz pharmacokinetic parameters and performance on objective neuropsychometric tests were characterized (Table 2). Of these three tests, only GPB-ND performance was significantly associated with pharmacokinetic parameters (described below). Slower performance on GPB-ND was associated with pharmacokinetic parameters that define increased plasma efavirenz exposure. Efavirenz $C_{\max}$ was associated with GPB-ND performance at 1, 2, 3 and 4 h post-dose ($P_{1h}=0.01$, $P_{2h}=0.05$, $P_{3h}=0.03$, $P_{4h}=0.01$), clearance with GPB-ND performance at 1, 2 and 6 h post-dose ($P_{1h}=0.05$, $P_{2h}=0.02$, $P_{6h}=0.01$), and AUC(0,312 h) with GPB-ND performance at 1 and 6 h post-dose ($P_{1h}=0.04$, $P_{6h}=0.04$). In addition, more rapid time to $C_{\max}$ (i.e. shorter $t_{\max}$) was associated with longer time to complete the GPB-ND task at 1 h post-dose ($P_{1h}=0.002$). In contrast, neither GPB-D nor LDST were significantly associated with efavirenz concentrations or pharmacokinetic parameters (Table 2).

There was considerable interindividual variability in efavirenz $t_{\max}$, ranging from 0.5 h to 8 h. To understand relationships further between plasma efavirenz exposure and GPB-ND, we examined GPB-ND performance at the time closest to $t_{\max}$ in each participant. In this analysis, performance on GPB-ND was again most strongly associated with $C_{\max}$ ($P=0.04$) (Table 3). To adjust further for the impact of interindividual variation in timing of peak drug concentration, we used a repeated measures model analysis that adjusted timing of neuropsychometric testing by $t_{\max}$. In this model, slower GPB-ND performance remained associated with pharmacokinetic parameters, including slower clearance ($P<0.001$), higher AUC(0,312 h) ($P=0.001$) and higher $C_{\max}$ ($P=0.008$), while other neuropsychometric tests were not found to be associated (Figure 1, Table 4).

Time dependent relationships between subjective CNS symptoms and efavirenz pharmacokinetic parameters were also examined. Greater efavirenz $C_{\max}$ was associated with decreased drowsiness based on drowsiness visual

analogue scale at 6 h post-dose ($P=0.02$), but not at earlier time points ($P_{1h}=0.64$, $P_{2h}=0.95$, $P_{3h}=0.93$, $P_{4h}=0.95$, Table 5). There was no significant association between summary score on efavirenz CNS symptom questionnaires and either $C_{\max}$, AUC(0,312 h) or clearance ($P=0.52$, $P=0.89$, $P=0.85$, Table 6).

CYP2B6 genotype and neuropsychometric testing

We previously reported that, in this cohort, *CYP2B6* 516/983 genotype was significantly associated with efavirenz clearance and AUC(0,312 h), but not with $C_{\max}$ [23]. Univariate comparisons showed no difference with GPB-D, LDST, drowsiness visual analogue scale or summary score on efavirenz CNS symptom questionnaires ($P>0.05$ for all comparisons, data not shown). There was a statistically significant difference in *CYP2B6* loss of function genotype and slower GPB-ND performance at 4 and 6 h ($P=0.04$, $P=0.02$). In multivariable analysis herein, adjusted for age, gender and BMI, there were trends towards association between *CYP2B6* loss of function genotype and slower GPB-ND performance at 4 and 6 h post-dose but not at 1, 2 or 3 h post-dose (4 h $P=0.07$, extensive vs. intermediate metabolizer effect size 8.0 [95% CI 0.43, 16], extensive vs. slow metabolizer effect size 10.2 [95% CI 0.43, 20], 6 h $P=0.06$, intermediate vs. extensive metabolizer effect size 7.46 [95% CI -1.2, 16.1], slow vs. extensive metabolizer effect size 13.6 [95% CI 2.5, 24.7]). There was no significant association between *CYP2B6* 516/983 genotype and GPB-ND at time closest to $C_{\max}$ (Table 3).

Discussion

Central nervous system side effects are frequent with initiation of efavirenz-containing regimens. This dataset from a cohort of individuals intensively studied following a single dose of efavirenz offered a unique opportunity to examine temporal relationships between *CYP2B6* genotype, pharmacokinetics, objective measures of

Table 2

Associations between objective tests of neurocognitive test performance and efavirenz pharmacokinetic parameters††

Neurocognitive test	Time point (h)	AUC(0,312 h) β (95% CI)*, P value	Clearance β (95% CI)*, P value	C _{max} β (95% CI)*, P value	t _{max} β (95% CI)*, P value
Grooved peg board (non-dominant hand)	1	8.05 (0.84, 15.27), 0.037	-4.88 (-9.50, -0.27), 0.05	9.04 (2.22, 15.86), 0.01	-13.114 (-20.815, -5.412), 0.0023
	2	7.28 (-0.89, 15.45), 0.09	-6.45 (-11.67, -1.20), 0.02	8.00 (0.18, 15.82), 0.05	-7.87 (-17.42, 1.67), 0.12
	3	7.20 (-4.82, 19.22), 0.25	-6.11 (-13.77, 1.55), 0.13	12.67 (2.03, 23.32), 0.03	-7.89 (-21.59, 5.80), 0.27
	4	4.02 (-1.26, 9.31), 0.15	-3.46 (-6.88, -0.04), 0.06	6.49 (1.70, 11.28), 0.01	-2.14 (-8.45, 4.17), 0.51
	6	6.44 (0.62, 12.27), 0.04	-5.15 (-8.82, -1.49), 0.01	4.12 (-1.79, 10.02), 0.18	-1.61 (-8.86, 5.64), 0.67
	Mean§	6.47 (0.59, 12.35), 0.04	-5.18 (-8.87, -1.49), 0.010	8.05 (2.63, 13.47), 0.01	-6.52 (-13.47, 0.428), 0.08
Grooved peg board (dominant hand)	1	0.58 (-5.41, 6.57), 0.85	-0.83 (-4.50, 2.85), 0.66	0.94 (-5.12, 7.00), 0.76	-6.62 (-13.52, 0.27), 0.067
	2	-2.63 (-11.40, 6.14), 0.56	-0.07 (-5.91, 5.78), 0.98	-3.21 (-12.19, 5.78), 0.49	-1.33 (-12.31, 9.66), 0.81
	3	0.41 (-6.51, 7.33), 0.91	-1.20 (-5.56, 3.15), 0.59	4.89 (-1.79, 11.57), 0.16	-2.97 (-11.36, 5.42), 0.49
	4	1.32 (-4.45, 7.1), 0.66	-1.26 (-5.00, 2.48), 0.52	0.33 (-5.54, 6.120), 0.91	-2.68 (-9.66, 4.31), 0.46
	6	-3.85 (-9.82, 2.13), 0.22	1.86 (-2.14, 5.86), 0.37	-1.79 (-7.96, 4.39), 0.57	1.39 (-6.06, 8.83), 0.72
	Mean§	-0.75 (-6.11, 4.60), 0.78	-0.34 (-3.81, 3.14), 0.85	0.35 (-5.07, 5.78), 0.90	-2.46 (-8.92, 4.01), 0.46
Letter digit substitution	1	-0.58 (-2.77, 1.6), 0.60	0.24 (-1.05, 1.53), 0.72	-1.17 (-3.32, 0.99), 0.30	2.15 (-0.37, 4.68), 0.10
	2	0.10 (-3.89, 4.09), 0.96	0.27 (-2.30, 2.84), 0.84	-1.08 (-5.05, 2.89), 0.60	3.68 (-0.93, 8.30), 0.13
	3	0.84 (-2.96, 4.63), 0.67	0.43 (-2.03, 2.88), 0.74	-2.3 (-5.96, 1.36), 0.23	3.15 (-1.27, 7.58), 0.17
	4	-0.83 (-3.25, 1.59), 0.51	0.82 (-0.74, 2.37), 0.31	-1.37 (-3.76, 1.03), 0.27	1.28 (-1.62, 4.18), 0.39
	6	-0.13 (-2.12, 1.87), 0.90	0.27 (-1.05, 1.59), 0.69	0.19 (-1.81, 2.18), 0.8	1.21 (-1.16, 3.57), 0.32
	Mean§	-0.11 (-2.26, 2.04), 0.92	0.40 (-0.96, 1.76), 0.57	-1.19 (-3.40, 1.02), 0.30	2.29 (-0.16, 4.75), 0.077

*Beta coefficients and 95% CI from multivariate linear regression. All analyses adjusted for baseline test performance and a principal component for BMI, age and gender. †Associations with $P < 0.05$ are shown in bold. §Means are calculated based on data from the five post-dose time points.

Table 3

Associations between pharmacokinetic parameters and GPB-ND score at time closest to C_{max} *†

	β (95% CI)	Adjusted <i>P</i> value	<i>n</i>
C_{max}	9.45 (0.85,18.05)	0.039	34
Clearance	-5.18 (-11.07,0.70)	0.095	32
AUC(0,312 h)	6.46 (-2.7, 15.7)	0.18	34
<i>CYP2B6</i> genotype	9.39 (-8.192, 26.97)	0.30	34

*Pharmacokinetic parameters and *CYP2B6* genotype were each assessed in separate analyses. Each analysis was adjusted for BMI, age, gender and baseline GPB-ND performance. †Associations with $P < 0.05$ are shown in bold.

neuropsychometric performance and subjective symptoms. A notable finding was that associations between efavirenz pharmacokinetic parameters and CNS side effects were readily demonstrated with one objective test of neuropsychometric performance (i.e. GPB-ND). Higher efavirenz C_{max} and AUC(0,312 h), and lower clearance were associated with significantly longer time to complete the GPB-ND task. When timing of the GPB-ND task was adjusted for t_{max} and all timepoints included in the analysis, the strength of association with clearance, AUC(0,312 h) and C_{max} increased compared with when the timing of GPB-ND performance scores were not adjusted, suggesting that variation in performance at a given time-point may reflect variation in t_{max} .

Figure 1

Association of pharmacokinetic parameters with psychometric test performance. Predicted psychometric test scores were generated from multivariable repeated measures regression analysis for correlated data with covariate adjustment of timing of t_{max} , age, gender and BMI.

Table 4Associations between pharmacokinetic parameters and neuropsychometric parameters, adjusted for $t_{\max}$ *†

	Clearance (χ^2 , P value)†	$C_{\max}$ (χ^2 , P value)†	AUC _{0–312h} (χ^2 , P value)†
Grooved peg board (non-dominant hand)	11.4 (<0.001)	7.0 (0.008)	13.26 (0.001)
Grooved peg board (dominant hand)	1.58 (0.45)	0.55 (0.76)	0.38 (0.82)
Letter digit substitution test	1.57 (0.46)	1.45 (0.49)	0.79 (0.67)
Subjective drowsiness (visual analog scale)	2.03 (0.36)	1.85 (0.40)	2.79 (0.25)

*Repeated measures analyses were performed. Each analysis was adjusted for baseline score, age, gender and BMI, as well as time of peak concentration. † χ^2 values are from the Wald statistic testing the regression coefficient of each association. Associations with $P < 0.05$ are shown in bold.

Table 5

Associations between subjective drowsiness (visual analogue scale) and efavirenz pharmacokinetic parameters*†

Time point (h)	AUC(0,312 h) β (95% CI), P value	Clearance β (95% CI), P value	$C_{\max}$ β (95% CI), P value	$t_{\max}$ β (95% CI), P value
1	1.32 (–8.97, 11.60), 0.80	–0.99 (–7.98, 6.00), 0.78	–2.46 (–12.73, 7.81), 0.64	–1.87 (–14.18, 10.44), 0.77
2	7 (–4.37, 18.36), 0.24	–5.47 (–12.49, 1.54), 0.137	0.35 (–11.29, 12.00), 0.95	–10.07 (–23.53, 3.38), 0.15
3	1.29 (–2.90, 5.47), 0.55	–1.10 (–3.77, 1.56), 0.43	0.18 (–3.98, 4.34), 0.93	–2.37 (–7.31, 2.58), 0.36
4	6.79 (–5.25, 18.83), 0.28	–4.89 (–12.75, 2.97), 0.23	0.42 (–11.87, 12.71), 0.95	–5.33 (–19.91, 9.26), 0.48
6	–4.88 (–17.79, 8.04), 0.47	–1.00 (–9.45, 7.45), 0.82	–14.85 (–26.76, –2.94), 0.02	14.2 (–0.56, 28.955), 0.07
Mean‡	2.72 (–4.38, 9.83), 0.46	–2.78 (–7.39, 1.84), 0.25	–3.15 (–10.24, 3.93), 0.39	–0.66 (–9.24, 7.92), 0.88

*All analyses are adjusted for baseline test performance and a principal component for BMI, gender, age. †Associations with $P < 0.05$ are shown in bold. ‡Means are calculated based on data from the five post-dose time points.

Table 6

Associations between pharmacokinetic parameters and subjective ordinal symptom score*†

	OR (95% CI)	P value	n
$t_{\max}$	0.98 (0.31, 3.05)	0.97	34
$C_{\max}$	1.35 (0.54, 3.39)	0.52	34
AUC(0,312 h)	0.93 (0.31, 2.74)	0.89	34
Clearance	0.93 (0.42, 2.05)	0.85	32

*Pharmacokinetic parameters were each assessed a separate multivariable regression analyses. Each analysis was adjusted for baseline GPB-ND performance and a first principal component for BMI, age and gender. †Associations with $P < 0.05$ are shown in bold.

Change from baseline in GPD-ND following administration of efavirenz varied considerably between individuals, with some individuals taking more than twice as long to complete the task compared with baseline. To put this in context, a previous study of individuals administered 0.8 g kg^{-1} ethanol showed an approximately 10% increase in time to complete the GPD-ND task compared with baseline [31]. Thus efavirenz appears to have substantial effect on GPD-ND performance in some individuals.

We also identified a trend towards association between *CYP2B6* loss of function genotype and slower GPB-ND performance. This likely reflects the association between these *CYP2B6* variants and decreased clearance pre-

viously described in this cohort [23]. The larger difference between slow and extensive metabolizers vs. intermediate and extensive metabolizers in GPB-ND performance is reflected in the similar associations with AUC and clearance seen previously [23, 32]. Similarly, the association between *CYP2B6* genotype and GPB-ND performance at later but not earlier times post-dose is consistent with the association between *CYP2B6* genotype, clearance and AUC(0,312 h), but not with $C_{\max}$ in this cohort.

In prior studies, associations between *CYP2B6* variants and CNS side effects have been inconsistent and found in some populations [4, 6, 8, 16] but not others [16, 17, 20], although studies that focus on the first few weeks of therapy have more consistently found associations [6, 8]. Diverse approaches to studying efavirenz CNS side effects in cohorts and clinical trials may help to explain inconsistent findings. In ACTG protocol A5097s patients randomized to efavirenz had increased CNS symptoms at week 1 compared with those who did not receive efavirenz ($P < 0.001$), but not at week 4, 12 and 24 [1]. Times earlier than 1 week of therapy were not assessed. Among efavirenz recipients, plasma efavirenz concentrations tended to be associated with CNS symptoms at week 1 ($P = 0.04$) but not at later timepoints. In contrast, summary neuropsychological test Z scores (NPZ, derived from the sum of neuropsychological performance scores on trail making tests parts A and B and digit symbol substitution test) were associated with efavirenz concentrations at week 4

($P=0.002$) and week 12 ($P=0.012$), but not at week 1. Despite the lack of change of median efavirenz concentrations from week 1 through week 24, symptoms improved over this time period. Genetic analyses of A5097s participants suggested an association between CYP2B6 516G→T and CNS symptoms at 1 week ($P=0.036$), but not at week 4 or beyond [6].

In a double-blind, randomized controlled study Gutierrez-Valencia *et al.* reported fewer neuropsychometric side effects among patients who gradually escalated to full dose efavirenz over 2 weeks compared with those who started at full dose. Statistically significant differences between groups were only found during the first week, with 66% of those in the full dose group having central nervous system adverse event vs. 46% in the dose escalation group ($P=0.04$). No associations between neuropsychiatric side effects and efavirenz concentrations were found, although drug concentrations were only measured on days 7, 14 and 30 [4]. A study from South Africa found an association between efavirenz plasma concentrations and neuropsychiatric symptoms, even though samples for drug assays were not obtained until the second week of therapy. Symptoms resolved by 1 month follow-up. The investigators reported a trend toward association between CYP2B6 516G→T genotype and neuropsychiatric side effects ($P=0.08$) [8]. The Swiss HIV Cohort Study found an association between efavirenz blood concentration and CNS symptoms, even though no sample was obtained prior to 3 months on efavirenz-containing regimens [5].

Among neuropsychometric tests used in the present study, the GPB-ND task was most sensitive to interindividual variability in efavirenz pharmacokinetic parameters. The GPB has been shown to correlate with other measures of motor execution speed, attention, perceptual speed and non-verbal reasoning, and difficulty adapting to new tasks may be more pronounced with the non-dominant hand [26]. The LDST assesses divided attention, motor speed, visual processing and memory [26]. Other studies have also used various objective neurocognitive tests to assess for efavirenz CNS side effects, including trail making tests, digit symbol substitution test [1, 21], cognitive failures questionnaire [22], Raven's matrices, digit span, spatial span, Rey's figure, double barrage, Stroop test and minimal state examination [21]. As noted above, Clifford *et al.* found associations between composite NPZ3 scores and efavirenz plasma concentrations at weeks 4 and 12 [1], while Ciccarelli *et al.* found worse performance on tests of attention and executive abilities (double barrage and Stroop tests) in patients on efavirenz-containing regimens compared with those on non-efavirenz regimens [21].

Distinct from the important role of efavirenz in HIV therapy, it has been suggested that efavirenz is also well suited to be a pharmacologic probe drug to assess hepatic CYP2B6 activity in HIV-negative individuals, given its predominant metabolism by this isoform [14]. In this regard, CYP2B6 is also expressed in the brain, but brain tissue is not

readily accessible for studies of *in vivo* CYP2B6 activity in humans. There are therefore no data regarding the relationship between brain CYP2B6 activity and susceptibility to efavirenz CNS side effects. If such a relationship exists, the present study suggests that GPB-ND may be a preferred pharmacodynamic readout for studies that use single dose efavirenz as a probe drug to study brain CYP2B6 activity.

The present study had several limitations. We only administered a single dose of efavirenz, while clinical practice involves repeated dosing over many months or years. It remains unknown why CNS side effects are typically maximal with the initial dose of efavirenz and wane thereafter, despite persistent plasma efavirenz exposure [1, 6]. It is conceivable that efavirenz induces CYP2B6 activity in the brain, leading to inactivation, as has been reported for other centrally acting drugs [33, 34]. In the present study efavirenz was administered in the morning, while in clinical practice it is administered at bedtime so that the patient may sleep through the time of maximal symptoms. As we only studied African Americans our findings may not be generalizable to other populations. Data regarding years of formal education (which might affect performance on neuropsychological tests) were not collected in this study. For the purposes of comparison with external norms we assumed individuals were of average education (for the LDST) or a 12th grade education (for the GPB), which may have falsely increased the number of impaired scores. Additionally LDST norms were only available for Dutch individuals. Repeated administration of neurocognitive tests may have improved performance over time due to a learning effect and a larger sample size may have strengthened associations.

An improved understanding of pharmacokinetic, pharmacogenetic and pharmacodynamic aspects of antiretroviral side effects will foster optimal therapy for HIV infection. The present analysis sheds new light on the CNS pharmacodynamics of efavirenz, one of the most widely prescribed antiretrovirals. Our results highlight the value of intensive, integrated analyses that utilize quantitative measures of CNS side effects.

Competing Interests

Daniel H. Johnson, Tebeb Gebretsadik, Ayumi Shintani, Gail Mayo, Edward P. Acosta and C. Michael Stein have no competing interests to declare. David W. Haas is a Principal investigator on research grants to Vanderbilt University from Boehringer-Ingelheim, Gilead Sciences and Merck.

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