

ARTICLE

CYP2B6 Genotype-Dependent Inhibition of CYP1A2 and Induction of CYP2A6 by the Antiretroviral Drug Efavirenz in Healthy Volunteers

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We investigated the effect of efavirenz on the activities of cytochrome P450 (CYP)1A2, CYP2A6, xanthine oxidase (XO), and N-acetyltransferase 2 (NAT2), using caffeine as a probe. A single 150 mg oral dose of caffeine was administered to healthy volunteers ($n = 58$) on two separate occasions; with a single 600 mg oral dose of efavirenz and after treatment with 600 mg/day efavirenz for 17 days. Caffeine and its metabolites in plasma and urine were quantified using liquid chromatography/tandem-mass spectrometry. DNA was genotyped for *CYP2B6**4 (785A>G), *CYP2B6**9 (516G>T), and *CYP2B6**18 (983T>C) alleles using TaqMan assays. Relative to single-dose efavirenz treatment, multiple doses of efavirenz decreased CYP1A2 (by 38%) and increased CYP2A6 (by 85%) activities ($P < 0.05$); XO and NAT2 activities were unaffected. *CYP2B6**6*6 genotype was associated with lower CYP1A2 activity following both single and multiple doses of efavirenz. No similar association was noted for CYP2A6 activity. This is the first report showing that efavirenz reduces hepatic CYP1A2 and suggesting chronic efavirenz exposure likely enhances the elimination of CYP2A6 substrates. This is also the first to report the extent of efavirenz–CYP1A2 interaction may be efavirenz exposure-dependent and *CYP2B6* genotype-dependent.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Efavirenz-based human immunodeficiency virus (HIV) therapy is associated with complex and often unpredictable drug–drug interactions, potentially compromising efficacy and toxicity of coadministered drugs. Although efavirenz causes *in vivo* induction and/or inhibition of selected drug-metabolizing enzymes (e.g., cytochrome P450 (CYP)2B6, CYP2C8, CYP2C9, CYP2C19, and CYP3A), its effect on other enzymes, such as CYP1A2 and CYP2A6, is unknown.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ This work tested the effect of efavirenz on the *in vivo* hepatic activities of four enzymes: CYP1A2, CYP2A6, xanthine oxidase, and N-acetyltransferase 2. The impact of efavirenz exposure and genetic variation in *CYP2B6* expression on the magnitude of interaction was also tested. The plasma and urinary disposition of a single 150 mg oral dose of caffeine was profiled in healthy volunteers ($N = 58$)

as *in vivo* probes following a single 600 mg oral dose of efavirenz and after chronic efavirenz treatment for 17 days.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ This is the first clinical study showing that efavirenz reduces hepatic CYP1A2 and suggesting chronic efavirenz exposure likely enhances the elimination of CYP2A6 substrates. This is also the first to report the extent of efavirenz–CYP1A2 interaction is *CYP2B6* genotype-dependent.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ Concomitant use of CYP1A2 and CYP2A6 substrate drugs with efavirenz-based HIV therapy will require careful monitoring to minimize the risk of adverse drug effects or loss of efficacy. Moreover, *CYP2B6* genotypes associated with increased efavirenz exposure may be particularly at increased risk for adverse drug interactions involving CYP1A2.

The nonnucleoside human immunodeficiency virus (HIV)-1 reverse transcriptase inhibitor efavirenz, in combination with two nucleoside/nucleotide reverse transcriptase inhibitors, has been the preferred first-line regimen for over 15 years for treatment-naïve HIV-infected adults¹ and children.² Although regimens containing efavirenz are considered alternative

therapy since 2015 due to the increased effectiveness and safety of newer agents, such as integrase inhibitors,³ the World Health Organization continues to recommend efavirenz-based therapies as cost-effective and convenient first-line therapy in resource-limited countries with pandemic HIV.⁴ However, the use of efavirenz is associated with

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numerous and often unpredictable pharmacokinetic drug-drug interactions (DDIs).⁵ These DDIs are of major clinical concerns as they potentially increase the risk for loss of efficacy or adverse effects of those drugs coadministered with efavirenz. Thus, understanding the mechanisms of these DDIs is crucial to identify, predict, and manage them.

Most efavirenz-mediated DDIs can be explained by efavirenz's ability to induce drug disposition genes via activation of the constitutive androstane receptor (CAR)⁶ and pregnane X receptor (PXR),⁷ nuclear receptors that regulate the transcription of drug-metabolizing enzymes and transporters.⁸ As a result, chronic administration of efavirenz increases its own elimination ("autoinduction" of metabolism),^{6,9} and those of other drugs through induction of drug-metabolizing enzymes, including cytochrome P450 (CYP)2B6, CYP2C19, and CYP3A.^{10–12} However, not all DDIs with efavirenz are due to induction of drug disposition genes. Our *in vitro* data show that efavirenz directly inhibits certain enzymes at therapeutically relevant concentrations (e.g., CYP2B6, CYP2C8, CYP2C19, and CYP2C9),¹³ with reduction in the elimination of CYP2C8 and CYP2C9 substrates *in vivo*^{14,15} and efavirenz being a mixed inhibitor/inducer of CYP2B6¹⁶ and CYP2C19.¹¹ The potential *in vivo* effect of efavirenz on the activity of other drug metabolizing enzymes, such as CYP1A2, CYP2A6, xanthine oxidase (XO), and N-acetyltransferase 2 (NAT2), is not fully investigated. In HIV/tuberculosis-coinfected patients, model-predicted exposure of isoniazid exposure was marginally reduced by efavirenz (by 29%) in those who are fast acetylators, but no effect was observed in slow acetylators.¹⁷ However, these patients were receiving multiple medications, making it difficult to attribute the observed effect to efavirenz alone. Using human liver microsomal preparations and/or expressed enzymes, we have shown that efavirenz does not inhibit/inactivate the activities of CYP1A2 and CYP2A6.¹³ Although human liver microsomals are important *in vitro* experimental tools to estimate the estimation and prediction of inhibition DDI potential of a drug candidate, this approach would not be appropriate to test the effect of efavirenz on gene expression (e.g., due to enzyme induction). Appropriate *in vitro* models (e.g., cell lines or human hepatocytes) or *in vivo* studies using probe substrates are required to exclude the potential effect of efavirenz on CYP1A2 and CYP2A6.

Efavirenz is mainly metabolized by hepatic CYP2B6.¹⁸ Two gene variants (*CYP2B6**6 and *CYP2B6**18) produce higher efavirenz exposure after standard dosing in patients with HIV.¹⁹ The extent of efavirenz autoinduction of metabolism is influenced by *CYP2B6* genetic variants.²⁰ Recent evidence indicates that the extent of efavirenz-mediated DDIs with such CYP3A substrates as lumefantrine²¹ and levonogestrel²² also depends on the variability in *CYP2B6* gene expression, suggesting that variable efavirenz exposure due to functionally relevant *CYP2B6* genetic variants and other factors may be an important determinant of the magnitude of DDIs.

The main objectives of this study were to test whether (i) efavirenz alters the activity of CYP1A2 in healthy volunteers and (ii) the magnitude of the alteration correlated with genetic variation in *CYP2B6* expression. Caffeine is extensively metabolized in humans with at least 17 metabolites

identified in the urine following caffeine administration.^{23–25} Approximately 80–90% of the total caffeine elimination in humans is mediated via hepatic CYP1A2-mediated demethylation to paraxanthine,^{25,26} and caffeine is a well-established and validated probe drug for CYP1A2. The secondary objective of this study is to explore the effect of efavirenz on the activities of CYP2A6, XO, and NAT2. CYP2A6, XO, and NAT2 are involved in the formation of caffeine metabolites.^{24,25} Although these enzymes catalyze minor metabolic pathways, which makes it difficult to validate against oral clearance of caffeine, urinary metabolic ratios of caffeine have been widely used as convenient markers of CYP1A2, CYP2A6, XO, and NAT2 activities in population, epidemiological, and DDI studies.^{24,25,27} Activities of hepatic CYP1A2, CYP2A6, XO, and NAT2 were profiled in healthy volunteers using plasma and urinary caffeine and metabolites as an *in vivo* probes following the administration of caffeine on two occasions: with a single 600 mg oral dose of efavirenz and after chronic efavirenz (600 mg/day) treatment for 17 days.

MATERIALS AND METHODS

Study subjects

Healthy male and female volunteers (age, 18–49 years old; and body mass index, 20–32 kg/m²) were recruited to participate in this study. Potential subjects underwent a pre-enrollment screening examination (within a maximum of 6 weeks prior to enrollment into the study) for any medical abnormalities, which included physical examination, medical history, and standard clinical laboratory tests. Details of inclusion and exclusion criteria are described in our previous publications.^{11,28–30} During the screening, a blood sample (~10 mL) was obtained from each subject to extract genomic DNA for genotyping purposes. The Indiana University Institutional Review Board approved the protocol and the study was conducted at the Indiana University Clinical Research Center. All participants gave written informed consent. The study was registered at <http://www.clinicaltrials.gov> (ClinicalTrials.gov number NCT00668395).

Study design

This study used a two-session sequential design to characterize efavirenz autoinduction, pharmacogenetics, and drug interactions (**Figure S1**). To determine efavirenz-mediated changes in hepatic drug metabolizing enzyme activity, volunteers received a cocktail probe drugs orally with water on two occasions: concomitantly with a single 600 mg oral dose of efavirenz and again at the end of 17-day treatment with 600 mg efavirenz daily. On day 1, the participants were admitted to the hospital at ~7 AM, after an overnight fast. Following a predose blood sample, each participant received a single 600-mg oral dose of efavirenz (Sustiva; Bristol-Myers Squibb, Princeton, NJ) with 240 mL water on an empty stomach. One hour later, a cocktail probe drugs was administered orally with water to assess potential drug interactions: 150 mg caffeine, 250 mg tolbutamide, 20 mg omeprazole, and 1 mg of midazolam syrup. Although high doses of omeprazole can induce CYP1A2 after chronic administration, the small single dose (20 mg) used in this study is not expected to alter CYP1A2 activity. In fact, there

is no mutual pharmacokinetic interaction of caffeine and omeprazole (or among the other components of the cocktail used here), and these probe drugs (including caffeine and omeprazole) have been validated for simultaneous use without fear of interaction and, thus, widely used in several CYP phenotyping cocktails. Venous blood samples (10 mL) were collected from an indwelling catheter 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 16, and 24 hours following efavirenz administration while participants were in the hospital and at 48, 72, and 144 hours as outpatients. All urine voided during the 24 hours after efavirenz administration was collected in fractions. Plasma was separated by centrifugation for 20 minutes at 3,000 rpm within an hour of collection and two 10-mL urine aliquots were saved from each timepoint after the total urine volume was recorded. Plasma and urine samples were immediately stored at -80°C until analysis. The volunteers began chronic oral efavirenz (600 mg/day) in the evening, from day 7 to day 23. On the morning of day 24, participants were again admitted to the hospital. After predose blood collection, participants received their final dose of oral efavirenz (600 mg) and 1 hour later, a cocktail probe of drugs was administered as on day 1. All other procedures and sample processing were identical to day 1. We present here the data that relate to efavirenz interaction with caffeine metabolism as marker of CYP1A2, CYP2A6, XO, and NAT2.

DNA genotyping

The *CYP2B6* gene is highly polymorphic, with over 38 alleles reported to date (<https://www.pharmvar.org/gene/CYP2B6>). The *CYP2B6**6 allele, which consists of the *CYP2B6**4 and *CYP2B6**9 variants on the same haplotype, has functional relevance and occurs at high frequency across ethnically and geographically diverse populations.¹⁹ In addition, the *CYP2B6**18 allele, which does not express functional protein, is frequent in the black population (4–12%).¹⁹ Briefly, genomic DNA was extracted from whole blood (QIAamp DNA Mini Kit; Qiagen, Valencia, CA). Polymerase chain reaction was performed on the Quant Studio 12K Flex real-time polymerase chain reaction instrument blocks for 96-well plates. *CYP2B6**9, rs3745274 (516G>T, Q172H); *CYP2B6**4, rs2279343 (785A>G, K262R); and *CYP2B6**18, rs28399499 (983T>C, I328T) were genotyped using TaqMan Assay-Reagent Allelic Discrimination Kits (Applied Biosystems, Foster City, CA) according to the supplier's instructions. Genotype groups considered for the analysis were normal metabolizers (*1/*1 genotype, $n = 36$), intermediate metabolizers (*1/*6 genotype, $n = 15$), and slow metabolizers (*6/*6* genotype, $n = 5$ and *1/*18 genotype, $n = 1$). The additional variants in the *CYP2B6* gene and other *CYP* variants that were genotyped in these samples have been previously published.^{28,30}

Measurement of drugs and metabolites in plasma and urine

Plasma samples from single and multiple doses were assayed in the same run. Similarly, urine samples from single and multiple doses were run together. Plasma and urine samples were run separately as the number of analytes tested and the methods used were slightly different.

Chemicals

Caffeine (1,3,7 TMX), 1-methylxanthine (1 MX), 1-methyluric acid (1 MU), 1,7-dimethyluric acid (1,7 DMU), 5-acetylamin-6-formylamino-3-methyluracil (AFMU), and 1,7-dimethylxanthine (1,7 DMX) or paraxanthine and theobromine and theophylline were purchased from Sigma–Aldrich (St. Louis, MO). Efavirenz, 8-hydroxyefavirenz, 8,14-dihydroxyefavirenz, 7-hydroxyefavirenz, and β -glucuronidase were purchased from Toronto Research Chemicals (North York, Ontario, Canada). All other chemicals and solvents were high-performance liquid chromatography (HPLC) grade or the highest quality commercially available.

Plasma extraction and quantification of efavirenz, caffeine, and their respective metabolites

Plasma (250 μL) was mixed with 250 μL of 0.2 M sodium acetate (pH = 5), 12.5 μL of 600 mM sodium azide, and 25 μL of β -glucuronidase (1,000 U/mL), and incubated at 37°C for 18 hours to hydrolyze circulating glucuronide conjugates of efavirenz. Efavirenz-d4 (30 μL of 1 $\mu\text{g}/\text{mL}$) and 1 $\mu\text{g}/\text{mL}$ acetaminophen were added as internal standards and the sample vortex mixed. Hydrochloric acid (250 μL of 0.1 M) and 6 mL of ethyl acetate/hexane (50/50, v/v) was added and the sample mixture shaken for 10 minutes and then centrifuged at 3,600 rpm for 10 minutes at 0°C (Beckman Coulter, Schaumburg, IL). The organic layer was evaporated to dryness and reconstituted in 120 μL of 50% mobile phase A and 50% mobile phase B. Aliquot (25 μL) was injected onto the HPLC–tandem mass spectrometry system.

Caffeine and its metabolites were quantified on an API 3200 triple-quadrupole mass spectrometer (Applied Biosystem/MDS Sciex, Foster City, CA) equipped with a turbo ion spray source. The HPLC system consist of two LC-20AD pumps, SIL-20AHT UFLC auto-sampler, DGU-20A3 degasser, and a CBM-20A controller (Shimadzu, Columbia, MD). The chromatographic separation was achieved using a Restek C8 (250 \times 4.6 mm, 5 μm particle size) column (Bellefonte, PA). Before and after each injection, the sampling needle was washed with acetonitrile/water (75%/25%, v/v). Mobile phase A (methanol: formic acid (0.1% in water; 1/99, v/v)) and mobile phase B (methanol: formic acid (0.1% in water; 99/1, v/v)) was pumped at 0.8 mL/minute using a gradient elution described elsewhere.³⁰ The mass spectrometry optimization was achieved via adjustment of both the compound-dependent and instrument-dependent parameters for caffeine, theophylline, paraxanthine, and acetaminophen (internal standard) in positive mode. The analytes were optimized at a source temperature of 400°C in positive mode under unit resolution for quadrupole 1 and 3 and were given a dwell time of 60 ms and a setting time of 700 ms. Gas pressures for all analytes, including the internal standards, were optimized as described previously.³⁰ Multiple reaction monitoring (MRM) at mass to charge number ratio (m/z) of 195.12/138.12, 181.13/124.20, 181.13/124.2, and 152.13/110.20 for caffeine, theophylline, paraxanthine, and acetaminophen (internal standard), respectively, in positive mode was used. Theophylline and paraxanthine were chromatographically separated. Data acquisition and processing were performed using Analyst software. The lower limit of

quantification was 1 ng/mL for all analytes and was linear over a wide range of therapeutically relevant concentrations. The intraday and interday variabilities were <20%.

Efavirenz, 8-hydroxyefavirenz, 7-hydroxyefavirenz, and 8,14-dihydroxyefavirenz were quantified by liquid chromatography tandem mass spectrometry in negative mode using efavirenz-d4 as an internal standard, as detailed in our previous publication.³⁰ MRM at *m/z* of 313.97/244.01, 329.98/210.0, 329.98/257.89, 345.91/262.0, and 318.01/247.95 was used to measure first and third quadrupole (Q1/Q3) transitions for efavirenz, 8-hydroxyefavirenz, 7-hydroxyefavirenz, 8,14-dihydroxyefavirenz, and efavirenz-d4 (internal standard), respectively, in negative mode. Metabolites (8- and 7-hydroxyefavirenz) were separated chromatographically.

Urine extraction and quantification

Urine (250 μ L) was spiked with 30 μ L of internal standard (nevirapine, 500 ng/mL). Each sample was extracted by adding 300 μ L of acetic acid (0.1% in water) and 6 mL of dichloromethane: isopropyl alcohol (85:15), vortex mix for 20 seconds, centrifuged at 3,600 rpm for 5 minutes at 10°C. The aqueous layer was decanted by suction and the organic layer evaporated using speed vacuum. Residue was reconstituted in 100 μ L of 99% ammonium acetate (20 mM, pH = 4.8)/1% methanol and vortex mix for 15 seconds. An aliquot (50 μ L) was injected onto the liquid chromatography tandem mass spectrometry system described above for plasma. Analytes were separated on a Zorbax Eclipse XDB-C8 column (4.6 \times 150 mm, 3.5 μ M; Agilent Technologies, Santa Clara, CA). The gradient mobile phase was pumped at a flow rate of 0.5 mL/minute and consist of phase A (99% ammonium acetate (20 mM, pH = 3.5)/1% methanol (v/v)) and phase B (10% ammonium acetate (20 mM, pH = 3.5)/90% methanol (v/v)). MRM at *m/z* 195.12/138.12 for caffeine, 181.13/124.2 for theophylline, 181.13/124.2 for paraxanthine, 181/138 for theobromine, 166/149 for 1-methylxanthine, 183/105 1-methyluric acid, 195/180 for 1,7-dimethyluric acid, 225/197 for 5-acetyluracil (AFMU), and 152.1/110.2 for acetaminophen (internal standard) was used to measure first and third quadrupole (Q1/Q3) transitions, in positive mode. Theophylline, paraxanthine, and theobromine peaks were separated chromatographically.

Pharmacokinetic analyses

Pharmacokinetic parameters were estimated using WinNonlin software (version 5.01; Pharsight, Mountain View, CA). Area under the plasma concentration vs. time curve (AUC) from time zero to the time of the last quantifiable concentration (AUC_{0-t}) was estimated using the linear and logarithmic trapezoidal rule for the respective up and down portions of the curve. The elimination rate constant (λ_z) was determined by linear regression analysis of the terminal portion of the log concentration vs. time curve. The AUC from zero to infinity ($AUC_{0-\infty}$) was calculated as the sum of C_t/λ_z and AUC_{0-t} , where C_t was the last measured concentration. The terminal elimination half-life ($t_{1/2}$) was calculated as the quotient of 0.693 and λ_z . The maximum plasma concentration (C_{max}) and the time to C_{max} (T_{max}) were

determined by visual inspection of the respective individual concentration-time curves. Total clearance was calculated as the quotient of the administered dose and $AUC_{0-\infty}$.

Calculation of metabolic ratios

Systemic clearance, caffeine AUC, and/or plasma paraxanthine to caffeine metabolic ratios (MRs) 5–7 hours after administration of caffeine are preferred (or accepted) and validated *in vivo* metrics of CYP1A2 activity.²⁶ In addition, urinary MRs are useful markers of CYP1A2, CYP2A6, XO, and NAT2 activities. Therefore, the following plasma and urinary caffeine MRs were calculated as indices of the respective enzyme activity besides estimation of pharmacokinetic parameters of caffeine.

Plasma MRs.

$$\text{CYP1A2 activity} = \frac{\text{Paraxanthine concentrations (C5 hour + C7 hour)}}{\text{Caffeine concentrations (C5 hour + C7 hour)}}$$

where C is plasma concentrations at 5 and 7 hours post-caffeine dosing.

Urinary MRs.

$$\text{CYP1A2 activity} = (\text{AFMU} + 1 \text{ MU} + 1 \text{ MX})/1,7 \text{ DMU}$$

$$\text{CYP2A6 activity} = 1,7 \text{ DMU}/1,7 \text{ DMX}$$

$$\text{XO activity} = (1 \text{ MU})/(1 \text{ MX} + 1 \text{ MU})$$

$$\text{NAT2 activity} = \text{AFMU}/(\text{AFMU} + 1 \text{ MU} + 1 \text{ MX}),$$

where the terms are defined as follows: 1 MX, 1-methylxanthine; 1 MU, 1-methyluric acid; 1,7 DMU, 1,7-dimethyluric acid; AFMU, 5-acetyluracil; and 1,7 DMX, 1,7-dimethylxanthine (paraxanthine).

Statistical analyses

Data are expressed as mean \pm SD. Statistical analyses were performed using GraphPad Prism Software (La Jolla, CA). Comparison of pharmacokinetic parameters or enzyme activity between single and multiple doses of efavirenz were performed using the Wilcoxon-matched paired test. Testing the association among *CYP2B6* genotypes and the activities of CYP1A2 and CYP2A6 following single and multiple doses of efavirenz were evaluated by nonparametric analysis of variance (Kruskal-Wallis test) using Dunn's posttest for multiple comparison correction. Correlation analyses between efavirenz exposure and enzyme activities (CYP1A2 and CYP2A6) were determined from the Spearman's Rank-Order Correlation (r_s). Differences were judged to be due to chronic efavirenz treatment rather than single-dose efavirenz administration when $P < 0.05$.

A sample size of at least 52 subjects was calculated as sufficient to provide a 95% power to detect a 40% difference in CYP1A2 activity between a single efavirenz dose vs. multiple doses of efavirenz at a two-sided alpha level of

5%. Power calculation for efavirenz's effect on CYP2A6, XO, and NAT2 was not performed *a priori*. However, data from a relatively large sample size were analyzed in this study as compared with conventional DDIs reported in the literature, which often involves sample size < 15 subjects.

RESULTS

Human volunteers

Sixty healthy normal volunteers were recruited and completed the entire study protocol. Two participants were excluded due to missing samples from one of the efavirenz treatment phases. Caffeine metabolic ratios were calculated from 58 individuals. Plasma pharmacokinetic parameters were available from only 52 volunteers after documentation of exogenous caffeine intake by 6 individuals during the study; this was an exclusion criteria.

The demographics of the 58 participants completing the study were the following: 36 men and 22 women; median age of 28.5 years (range 18–49 years); body weight of 74.6 ± 13.7 kg; and body mass index of 24.6 ± 3.8 kg/m². Based on self-identification, 74% were white, and 19% were black/African American. The remaining ~7% belonged to other ethnic/racial groups.

The pharmacokinetics of caffeine and metabolite data are shown in **Table 1**. The caffeine $t_{1/2}$ was prolonged ($P < 0.0001$) and $AUC_{0-\infty}$ increased ($P < 0.01$) following

Table 1 Pharmacokinetic parameters of caffeine and its main metabolite paraxanthine, and activities of select enzymes calculated from plasma and urinary MRs, following co-administration of caffeine with a single dose and after multiple doses of efavirenz

	Single dose	Multiple dose	Mean percent change
Caffeine (1,3,7 TMX)			
T_{max} (hour)	1 (0.5–3)	1 (0.5–5)	
C_{max} (ng/mL)	1,825 ± 972	1,897 ± 1,050	17.4
$AUC_{0-\infty}$ (µg/mL*hour)	17.3 ± 11.6	22.7 ± 18.4**	46.2
$t_{1/2}$ (hour)	7.61 ± 8.29	8.87 ± 4.98***	32.5
Paraxanthine (1,7 MX)			
T_{max} (hour)	7 (1.5–15)	7 (1–15)	
C_{max} (ng/mL)	504 ± 236	358 ± 208***	-24.5
$AUC_{0-\infty}$ (µg/mL*hour)	10.8 ± 6.1	8.6 ± 5.8*	-8.6
$t_{1/2}$ (hour)	10.85 ± 9.21	12.22 ± 7.88**	26.3
Relative change in enzyme activity			
Plasma MR			
CYP1A2	0.52 ± 0.28	0.31 ± 0.18***	-38.1
Urinary MR			
CYP1A2	5.2 ± 2.4	3.5 ± 1.3***	-28.9
CYP2A6	0.58 ± 0.38	0.94 ± 0.59***	85.5
XO	0.37 ± 0.16	0.37 ± 0.16	1.1
NAT2	0.31 ± 0.21	0.32 ± 0.22	4.5

Data are expressed as mean ± SD except T_{max} , which was presented as median (minimum–maximum).

AUC, area under the plasma concentration-time curve; C_{max} , maximum concentration; CYP, cytochrome P450; MR, metabolic ratio; NAT2, N-acetyltransferase 2; $t_{1/2}$, terminal elimination half-life; T_{max} , time to maximum concentration; XO, xanthine oxidase.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$, Wilcoxon matched-pairs test (single dose vs. multiple doses of efavirenz).

chronic efavirenz compared with a single dose of efavirenz. Accordingly, chronic administration of efavirenz decreased the C_{max} of paraxanthine ($P < 0.0001$), the major metabolite of caffeine. Relative to the single-dose phase, chronic efavirenz treatment decreased CYP1A2 activity (plasma MRs) by 38% (**Table 1 and Figure 1a**; $P < 0.0001$) with marked interindividual variability (range -78.2% to 31.7%; **Figure 1b**). Similarly, CYP1A2 activity measured by urinary MRs decreased 28.9% ($P < 0.0001$) from 5.7 to 3.6 after chronic treatment with efavirenz (**Table 1; Figure 1c**). The percent change in urine MR also varied widely among individuals (ranging from -65.1% to 43.2%; **Figure 1d**). CYP1A2 activity measured by the plasma MRs correlated with CYP1A2 activity measured by urinary MRs in the single-dose efavirenz phase (Spearman $r_s = 0.57$; $P < 0.0001$) and after chronic efavirenz ($r_s = 0.33$; $P = 0.013$) and combination of both phases (single dose and after multiple doses of efavirenz; $r_s = 0.57$; $P < 0.0001$; **Figure S2a–c**). Plasma caffeine MRs are the preferred phenotype marker for CYP1A2 rather than urinary MRs.²⁷ Thus, only results from plasma MRs are presented as markers of CYP1A2 subsequently.

CYP2A6, XO, and NAT-2 activities were estimated from respective caffeine MRs as described in the METHODS section. Chronic efavirenz treatment significantly increased CYP2A6 activity (**Table 1; Figure 2a**) with marked inter-subject variability (range 45.4–281.4%; **Figure 2b**). Urinary metabolic ratios reflecting XO and NAT2 activities were not significantly altered by chronic efavirenz exposure (**Table 1**).

Approximately sevenfold between-subject differences in efavirenz exposure was observed following a single dose and multiple doses of efavirenz. To test whether changes in CYP1A2 and CYP2A6 activities were dependent on the exposure of efavirenz and its metabolites, correlations between efavirenz exposure and indices of CYP1A2 and CYP2A6 were examined. Greater reduction in CYP1A2 activity was observed with higher efavirenz exposure, as shown by the negatively significant correlation between CYP1A2 activity and efavirenz $AUC_{0-\infty}$ at single dose (Pearson $r_s = -0.32$; $P < 0.01$) as well as efavirenz AUC_{0-24h} after multiple doses ($r_s = -0.61$; $P < 0.001$). The percent change in CYP1A2 was negatively correlated with efavirenz AUC_{0-24h} after multiple doses ($r_s = -0.41$; $P < 0.01$; **Figure S3**). Efavirenz exposure at single dose or at steady state did not significantly correlate with urine marker of CYP2A6 activity (**Figure S4**). Exposure of efavirenz metabolites did not correlate with CYP1A2 or CYP2A6 activity.

The correlation analysis suggests that the extent of reduction of CYP1A2 activity is dependent on efavirenz exposure (the higher the efavirenz exposure the greater reduction in CYP1A2 activity). Genotype–phenotype data from the same subjects analyzed in this study and published elsewhere^{29,30} show that efavirenz exposure following a single dose of efavirenz ($AUC_{0-\infty}$) was 2.1-fold and 1.8-fold higher; and multiple doses of efavirenz (AUC_{0-24h}) was 2.5-fold and 2-fold higher in *CYP2B6*6/*6* genotypes compared with *CYP2B6*1/*1* and *CYP2B6*1/*6* genotypes, respectively. Difference in efavirenz exposure between *1/*1 and *1/*6, although statistically different, was small. To test whether the extent of efavirenz-CYP1A2 interaction is dependent on *CYP2B6* genotypes, data analyses were performed after stratifying by *CYP2B6*1/*1*, *CYP2B6*1/*6*, and *CYP2B6*6/*6*

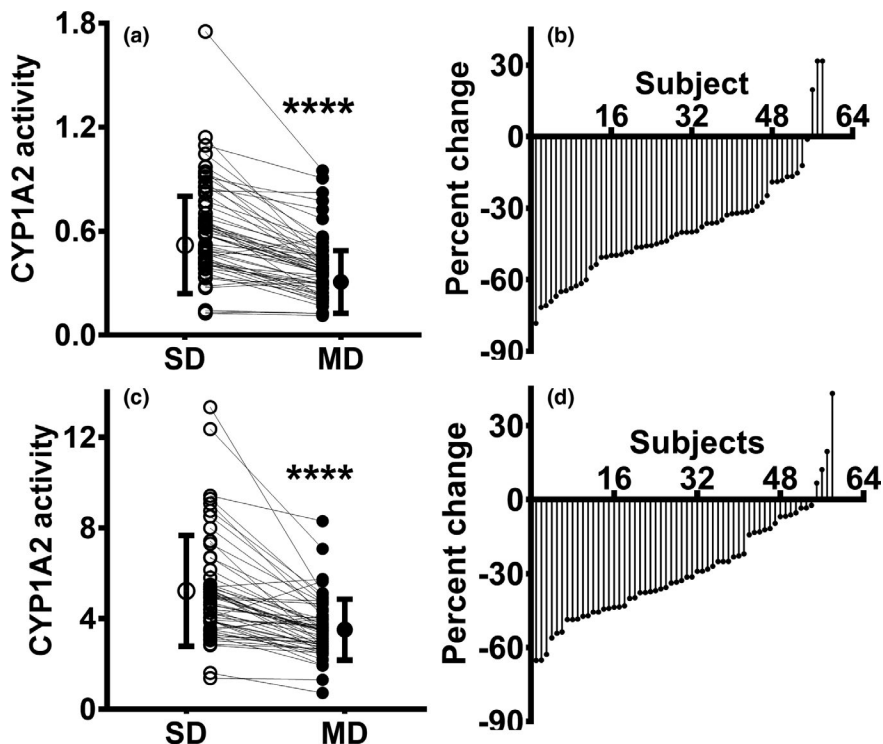


Figure 1 Relative CYP1A2 activity following single dose (SD; open circles) and after multiple doses (MDs; closed circles) of efavirenz in healthy volunteers ($N = 58$). (a) Plasma and (c) urine caffeine metabolic ratios (MRs) were calculated as the surrogate for relative changes in CYP1A2 activity. Ranked percent change in CYP1A2 activity using MRs in (b) plasma and (d) urine of 58 subjects (multiple doses vs. a single dose of efavirenz) are shown. CYP1A2 activity following SD and after MD of efavirenz was compared using Wilcoxon signed-rank test. **** $P < 0.0001$. CYP, cytochrome P450.

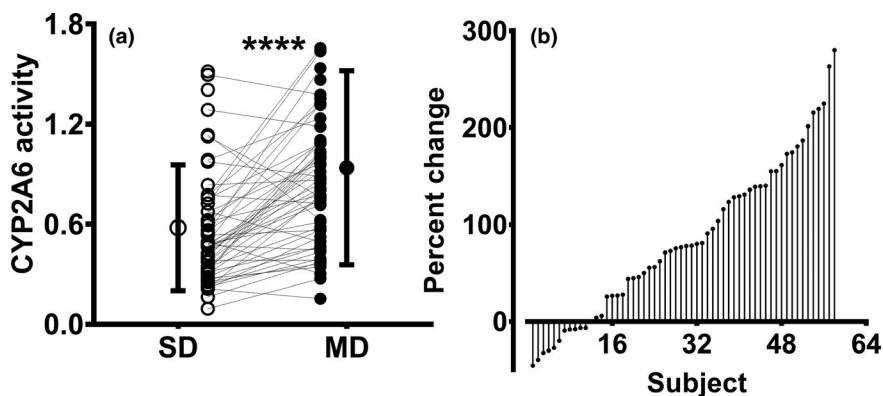


Figure 2 Relative CYP2A6 activity following single dose (SD; open circles) and after multiple doses (MDs; closed circles) of efavirenz in healthy volunteers ($N = 58$). (a) CYP2A6 activity was calculated from urinary caffeine metabolic ratios as described and (b) ranked percent change in CYP2A6 activity of 58 individuals (MD vs. SD of efavirenz). CYP2A6 activity following SD and after MD of efavirenz was compared using Wilcoxon signed-rank test. **** $P < 0.0001$ (between CYP1A2 activity following SD and after MD of efavirenz). CYP, cytochrome P450.

genotypes. One subject with $*1/*18$ genotype was grouped with $*6/*6$ as the genotype-predicted phenotype was close to a slow metabolizer. The demographics are comparable among the three genotypes (Table S1). CYP1A2 activity was lower for the CYP2B6 $*6/*6$ genotypes compared with $*1/*1$ or $*1/*6$ genotypes following single dose (Kruskal-Wallis test, $P < 0.05$; Figure 3a) and multiple doses of efavirenz ($P < 0.01$; Figure 3b). Compared with the single-dose phase,

chronic efavirenz inhibited CYP1A2 activity within each genotype ($P < 0.05$; Figure 3c). The percent change in CYP1A2 activity was greater in $*6/*6$ genotypes when compared with $*1/*1$ and $*1/*6$ genotypes (Kruskal-Wallis test, $P < 0.05$; Figure 3d). Similarly, caffeine $AUC_{0-\infty}$ was significantly higher for $*6/*6$ genotype in both efavirenz treatment groups compared with $*1/*1$ genotype (Figure S5a,b), whereas caffeine $AUC_{0-\infty}$ and CYP1A2 for the $*1/*6$ genotype were not

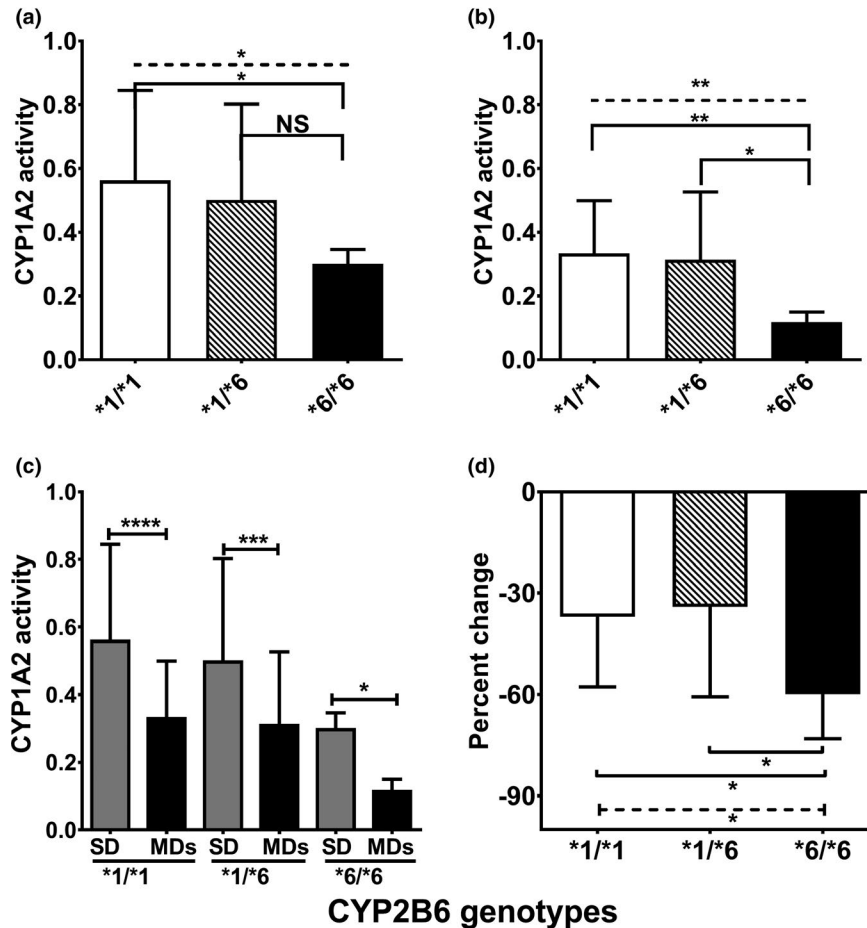


Figure 3 Relative CYP1A2 activity stratified by *CYP2B6* genotypes following a single dose (SD) and multiple doses (MDs) of efavirenz in health volunteers ($N = 58$). Plasma metabolic ratio of paraxanthine to caffeine represents relative CYP1A2 activity. (a) CYP1A2 activity following SD of efavirenz. (b) CYP1A2 activity after chronic administration of efavirenz. (c) Comparison between single and multiple efavirenz dosing within each genotype group. (d) Represents percent change in CYP1A2 activity. CYP1A2 activity was compared between an SD and MDs of efavirenz in each genotype using Wilcoxon matched-pairs test. Comparison among genotypes was tested using nonparametric analysis of variance (Kruskal–Wallis test) with *post hoc* Dunn's multiple-comparison test. **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. CYP, cytochrome P450; NS, not statistically significant ($P > 0.05$).

different from the wild type *1/*1 genotype in either treatment group.

The impact of *CYP2B6* genotypes on the extent of efavirenz-mediated induction of CYP2A6 was also tested. CYP2A6 activity was not statistically different among the *CYP2B6* genotypes either at a single dose of efavirenz (Figure 4a) or after multiple doses of efavirenz (Figure 4b). Compared with the single-dose phase, chronic efavirenz increased the urinary caffeine metabolic ratio for CYP2A6 in *1/*1 and *1/*6 genotypes but not in *6/*6 genotype (Figure 4c). Although percent change in CYP2A6 activity was lower in *6/*6 genotypes when compared with *1/*1 and *1/*6 genotypes, this did not reach a statistically significant level (Figure 4d).

DISCUSSION

In the present study, chronic administration of efavirenz significantly reduced and increased the *in vivo* activity of hepatic CYP1A2 and CYP2A6, respectively. The effect of

efavirenz on CYP1A2 activity was more pronounced in individuals with higher efavirenz exposure and those expressing *CYP2B6**6/*6 genotype, whereas the increase in CYP2A6 activity was neither efavirenz exposure-dependent nor *CYP2B6* genotype-dependent. Efavirenz did not significantly alter the activity of XO or NAT-2. These data suggest that there is an increased risk for DDIs in individuals receiving efavirenz-based HIV therapy who are comedicated with CYP1A2 or CYP2A6 substrates. Moreover, *CYP2B6* genotypes associated with reduced efavirenz clearance may particularly alter the risk for adverse drug interactions involving CYP1A2.

Efavirenz activates two nuclear receptors that regulate a range of genes involved in drug disposition, namely the CAR (*NR1I3*) and PXR (*NR1I2*).^{6,7} Accordingly, efavirenz treatment enhances the elimination of numerous drugs metabolized by the cytochromes P450 (e.g., CYP3A, CYP2B6, and CYP2C19^{10–12}). This together with the fact that CYP2A6 is also transcriptionally regulated via PXR³¹ and CAR³² may explain our observed increase in CYP2A6 activity following

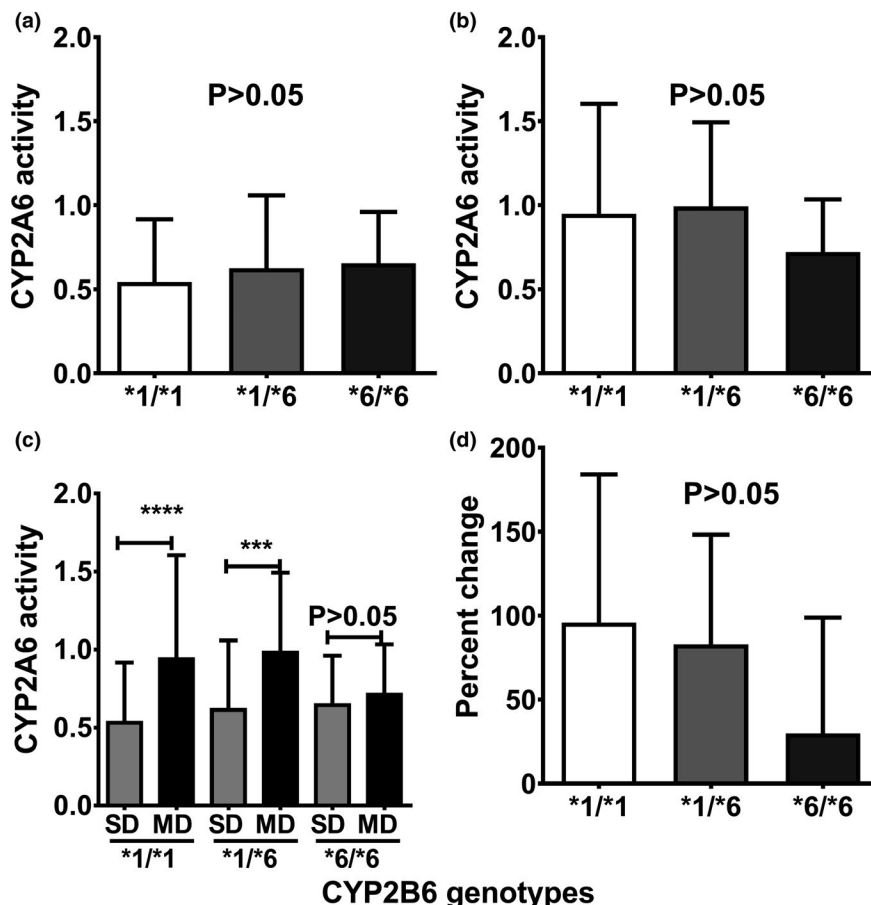


Figure 4 Relative CYP2A6 activity stratified by *CYP2B6* genotypes (*1/*1, $n = 37$; *1/*6, $n = 15$; and *6/*6, $n = 6$) following a single dose (SD) and multiple doses (MDs) of efavirenz in health volunteers ($N = 58$). (a) Relative CYP2A6 activity following SD of efavirenz. (b) Relative CYP2A6 activity after MDs of efavirenz. (c) Compares CYP2A6 activity between single and multiple dosing within each genotype group. (d) Represents percent change in CYP2A6 activity (MDs vs. SD of efavirenz). CYP2A6 activity was compared between an SD and MDs of efavirenz in each genotype using Wilcoxon matched pairs test. Comparison among genotypes was tested using nonparametric analysis of variance (Kruskal–Wallis test) with *post hoc* Dunn's multiple comparison test. **** $P < 0.0001$, *** $P < 0.001$. CYP, cytochrome P450; NS, not statistically significant ($P > 0.05$).

efavirenz treatment. CYP2A6 catalyzes, fully or partially, the metabolism of several clinically used drugs (e.g., letrozole, metronidazole, efavirenz, and tegafur)³³ and, based on our findings, it is anticipated that efavirenz would alter the disposition of these drugs. CYP2A6 is also the main metabolic pathway for nicotine elimination and contributes to the biotransformation of several nicotine-derived carcinogens (N'-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone).³³ Hepatic CYP2A6 activity has been associated with the number of cigarettes smoked per day, smoking topography (puff volume, duration, and velocity), and smoking cessation.³³ Thus, it is conceivable that cigarette smokers would be at increased risk for tobacco-related illnesses when coprescribed efavirenz-based HIV therapy. Our data show that neither efavirenz exposure nor the presence of the *CYP2B6* polymorphisms altered the magnitude of the increase in CYP2A6 activity suggesting that maximum nuclear receptor activation occurs at relatively low efavirenz concentrations.

In contrast to the induction effects of efavirenz on gene expression, drug interactions also occur as a result of direct

enzymatic inhibition by efavirenz.^{15,34} Our findings clearly indicate that chronic doses of efavirenz significantly reduced CYP1A2 activity *in vivo* compared with CYP1A2 activity determined when caffeine was administered with a single dose of efavirenz. The mechanism by which efavirenz reduces CYP1A2 activity remains unknown. *In vitro* studies indicate that efavirenz is neither a reversible inhibitor nor a mechanism-based (i.e., time-dependent) inactivator of CYP1A2 activity.¹³ It is unlikely that efavirenz metabolites produce CYP1A2 inhibition because the major oxidative metabolite, 8-hydroxyefavirenz, had no effect on CYP1A2 activity *in vitro*.¹³ Efavirenz-hydroxylated metabolites undergo extensive conjugation mainly by glucuronosyltransferases. With few exceptions (e.g., glucuronides of gemfibrozil³⁵ and clopidogrel³⁶), glucuronide conjugates are relatively hydrophilic and do not seem to produce clinically significant drug interactions. The possibility that efavirenz reduces CYP1A2 activity *in vivo* via mechanisms other than direct enzymatic inhibition/inactivation cannot be excluded. However, a limitation of these findings is that the study design in which probe substrates, including caffeine, were administered

on two occasions (with a single dose of efavirenz and after multiple doses of efavirenz) without inclusion of the caffeine-alone arm (absence of efavirenz) to determine baseline CYP1A2 did not allow accurate determination of the true magnitude of efavirenz–CYP1A2 interaction. Initially, the study was designed to address potential induction of drug metabolism by efavirenz.

Of note, the CYP1A2 probe, caffeine, has low intrinsic clearance with no significant presystemic metabolism. The magnitude of the efavirenz–CYP1A2 interaction reported here is likely an underestimate for any CYP1A2 drugs known to exhibit significant presystemic (i.e., first-pass) metabolism and low bioavailability (e.g., tizanidine).³⁷ Thus, efavirenz coadministration with CYP1A2 substrates possessing narrow therapeutic ranges (e.g., clozapine, olanzapine, duloxetine, remolten, and tizanidine), would likely produce clinically significant drug interactions. For example, ciprofloxacin and fluvoxamine (potent inhibitors of CYP1A2) increase the plasma exposure of tizanidine up to 30-fold, resulting in concomitant increased toxicity.^{37,38} Tizanidine is an α_2 -adrenergic agonist approved for therapy of muscle spasm and other conditions. It is frequently prescribed, off-label, for suppression of the symptoms associated with opiate withdrawal during detoxification.^{39,40} Because some individuals with HIV infection may also suffer from and be treated for opioid abuse, the likelihood that tizanidine and efavirenz may be coadministered is high.

Drug interactions with efavirenz are difficult to predict because of the marked interindividual variability in its plasma exposure.⁴¹ Efavirenz is mainly eliminated by metabolism via CYP2B6, with minor contributions from CYP2A6, CYP3A, CYP1A2, and UGT2B7.^{18,42,43} Patients with variants in the CYP2B6 gene (e.g., *CYP2B6*6* and *CYP2B6*18*) demonstrate higher efavirenz plasma exposure after administration of single³⁰ and multiple doses¹⁹ of efavirenz. Consistent with the literature, genotype-phenotype data from the same subjects analyzed in this study and published elsewhere^{29,30} show that efavirenz exposure is over twofold higher in *CYP2B6*6*6* genotypes compared with *CYP2B6*1*1* genotype. Evidence exist that genetic variants in the *CYP2B6* gene that contributes to variable efavirenz exposure may dictate the magnitude of certain induction drug interactions caused by efavirenz. For example, efavirenz reduced lumefantrine concentrations in patients coinfecting with HIV and malaria, resulting in poor malaria treatment response, and the decrease in lumefantrine concentrations was greater in those with higher efavirenz exposure and in CYP2B6 slow metabolizer.²¹ Efavirenz-based HIV therapy has been associated with lower levonorgestrel concentrations in women with the contraceptive subdermal implant,²² resulting in a risk for unintended pregnancy⁴⁴ particularly in CYP2B6 slow metabolizer. *CYP2B6* genotypes are also associated with differential efavirenz autoinduction of metabolism.²⁰ However, this is the first report to suggest greater reduction of CYP1A2 in carriers of the *CYP2B6*6*6* genotype compared with **1*1* and **1*6* genotypes. These data along with the correlation analysis showing efavirenz exposure-dependent reduction in CYP1A2 activity suggest that factors that increase efavirenz exposure would increase the risk for efavirenz–CYP1A2 interaction. The genotype-dependent effect

may have particularly important clinical consequences in certain populations (e.g., this likely poses unique risks in African or African American populations) as the allele frequencies of relevant CYP2B6 genotypes (e.g., **6* and **18*) are higher in these populations compared with other ethnic and racial groups.⁴⁵ Taken together, although the association of *CYP2B6*6*6* genotype on the extent of efavirenz–CYP1A2 interaction seems plausible and anticipated, our sample size for the *CYP2B6*6*6* was small. Thus, a well-designed (including a caffeine-alone arm) and more detailed study in adequate sample size of *CYP2B6* genotype subgroups may be warranted to validate our findings and to assess the true extent of efavirenz–CYP1A2 interaction.

In summary, we have demonstrated that efavirenz is an inhibitor of CYP1A2 activity and an inducer of CYP2A6 activity and is likely to alter the disposition and efficacy of drugs metabolized by these enzymes. The results further suggest that genetic and nongenetic factors will increase the risk for efavirenz-mediated drug interactions. Finally, specific populations expressing higher frequencies of *CYP2B6* variants (e.g., black and African American) may be at increased risk for efavirenz drug interactions. Concomitant use of CYP1A2 and CYP2A6 substrate drugs with efavirenz-based HIV therapy will require careful monitoring to minimize the risk of adverse drug effects.

Supporting Information. Supplementary information accompanies this paper on the *Clinical and Translational Science* website (www.cts-journal.com).

Supplemental Tables and Figures.

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Conflict of Interest. The authors declared no competing interests for this work.

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