# CYP2B6 (c.516G→T) and CYP2A6 (*9B and/or *17) polymorphisms are independent predictors of efavirenz plasma concentrations in HIV-infected patients

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## WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Cytochrome P450 (CYP) 2B6 polymorphisms, particularly c.516G→T, are strongly associated with plasma efavirenz concentrations, but do not entirely explain interindividual variability in efavirenz exposure.
- In vitro data suggest that CYP2A6 is involved in the metabolism of efavirenz.
- Rifampicin can induce the function and activity of the main metabolizing for efavirenz and causes small (22–26%) reductions in efavirenz area under the curve during co-administration, although with wide interindividual variability.

## WHAT THIS STUDY ADDS

- Identifies CYP2B6 516G→T polymorphism and carriers of CYP2A6*9B and/or *17 variants as independent predictors of efavirenz mid-dose concentration in human immunodeficiency virus-infected patients.
- Factors such as concurrent therapy with rifampicin-containing tuberculosis regimen, gender and body mass index had no a significant influence on efavirenz mid-dose concentration.
- Provides in vivo evidence that CYP2A6 is likely to be involved in the metabolism of efavirenz.

## AIMS

Interindividual variability in efavirenz pharmacokinetics is not entirely explained by the well-recognized CYP2B6 516G→T single nucleotide polymorphism. The aim of this study was to determine whether polymorphisms in the CYP2A6 gene can be used to enhance the predictability of efavirenz concentrations in human immunodeficiency virus (HIV)-infected native African patients.

## METHODS

Mid-dose efavirenz plasma concentrations were determined at 4 and 8 weeks following initiation of antiretroviral therapy in 65 HIV-infected Ghanaian patients. Selected CYP2B6 and CYP2A6 genotypes were determined by commercial 5′-nuclease assays. Relationships between averaged 4- and 8-week mid-dose efavirenz concentrations, demographic variables and genotypes were evaluated by univariate and multivariate statistical approaches including gene–gene interactions.

## RESULTS

CYP2B6 c.516G→T, CYP2B6 c.983T→C, CYP2A6*9B and CYP2A6*17 allele frequencies were 45, 4, 5 and 12%, respectively. Rifampicin therapy, gender, age and body mass index had no significant influence on efavirenz mid-dose concentrations. Median efavirenz concentrations were more than five times higher ($P < 0.001$) in patients with CYP2B6 c.516TT genotype compared with GG and GT genotypes. Although none of the CYP2A6 genotypes was associated with altered efavirenz concentrations individually, CYP2A6*9B and/or CYP2A6*17 carriers showed a 1.8 times higher median efavirenz concentration ($P = 0.017$) compared with noncarriers. Multiple linear regression analysis indicated that the CYP2B6 c.516G→T polymorphism and CYP2A6 slow-metabolizing variants accounted for as much as 36 and 12% of the total variance in efavirenz concentrations, respectively.

## CONCLUSIONS

Our findings support previous work showing efavirenz oxidation by CYP2A6, and suggest that both CYP2A6 and CYP2B6 genotyping may be useful for predicting efavirenz plasma concentrations.

# Introduction

Efavirenz is an essential component of the preferred non-nucleoside reverse transcriptase regimen for the initial treatment of human immunodeficiency virus (HIV) infection [1, 2]. Despite the potency and favourable tolerability of efavirenz-based regimens, some patients develop treatment-limiting toxicity or fail to achieve durable viral load suppression [3, 4]. Efavirenz plasma concentrations >4 μg/ml have been associated with an increased risk of adverse central nervous system effects, whereas concentrations <1 μg/ml have been associated with virological failure [5, 6]. However, other studies, including the large 'double non-nucleoside (2NN)' study, have failed to find any significant relationships between efavirenz plasma concentrations and virological failure [7, 8]. The fixed daily dose of 600 mg efavirenz for adults results in significant interindividual variability in plasma concentrations and clinical effects [5, 6, 9]. Consequently, it is of substantial clinical importance to identify factors that contribute to interindividual variability in efavirenz disposition, as efavirenz-based therapy is the preferred regimen in patients with tuberculosis (TB)/HIV co-infection receiving rifampicin-containing therapy in settings where rifabutin is not available [10, 11].

The main enzyme that metabolizes efavirenz is cytochrome P450 2B6 (CYP2B6) [12]. The CYP2B6 gene is highly polymorphic, and genotyping for functional single nucleotide polymorphisms (SNPs) has proven to be useful in the prediction of efavirenz pharmacokinetics [13, 14]. In particular, the CYP2B6 c.516G→T is a common polymorphism (21–38% allele frequency),[15] that has been consistently associated with reduced enzyme activity and higher efavirenz exposure in studies of different populations with varied racial and ethnic backgrounds [13, 15–18]. The more recently described CYP2B6 c.983T→C variant with up to 10% allele frequency is also associated with lower enzyme activity and higher efavirenz concentrations, but appears to be exclusively found in populations of African descent [14, 19, 20]. Other CYP2B6 polymorphisms that have been identified either have minimal impact on efavirenz metabolism, or are relatively rare (i.e. <5% allele frequency) [15].

Not all interindividual variability in efavirenz pharmacokinetics appears to be explained by CYP2B6 genetic variants [15], suggesting that there may be polymorphisms in other genes that influence efavirenz disposition. In addition to CYP2B6, several other CYPs, including CYP1A2, CYP2A6, CYP2C9, CYP3A4 and CYP3A5, may contribute to efavirenz metabolism [12, 16]. Several other studies have shown that polymorphisms in the CYP3A4 and CYP3A5 genes do not influence efavirenz plasma concentrations [21–23]. Polymorphisms in CYP1A2 and CYP2C9 either have no established association with phenotype [24], or, if associated, the variants are relatively rare in Black African populations [25]. However, several CYP2A6 genetic variants associated with reduced enzyme activity are relatively common in Black populations [26–28]. Specifically, the CYP2A6*9B allele with a mutation in the TATA box (T–48G) and CYP2A6*17 with the amino acid substitution (V365M) are associated with substantially decreased enzyme activity with reported allele frequencies of 5.7 and 9.4%, respectively, in Black populations [26–28]. The influence of these polymorphisms on efavirenz plasma concentrations in HIV-infected patients has yet to be reported. Consequently, in the present study we determined whether assaying for slow metabolizer CYP2A6 genetic variants can enhance the predictability of efavirenz plasma concentrations over that of CYP2B6 genotype alone in a cohort of HIV-infected Ghanaian patients receiving efavirenz-based therapy.

# Methods

## Study population and treatment regimens

Seventy-four HIV-infected patients with CD4 count ≤250 cells μl$^{-1}$ were enrolled between January 2005 and December 2007 in a pilot trial of a simplified once-daily antiretroviral therapy. Thirty-four (46%) of the patients also had TB co-infection. Enrolled patients were naive to antiretroviral therapy, aged ≥18 years and had no other opportunistic conditions. The once-daily antiretroviral regimen consisted of didanosine-buffered tablets 400 mg (body weight >60 kg) or 300 mg (body weight <60 kg), lamivudine 300 mg, and efavirenz 600 mg. Adherence, assessed monthly by pill count and patient self-report, was found to be good in all patients except for one (not included in the final analysis), who had undetectable antiretroviral drug concentrations.

In the patients with TB, antiretroviral therapy was initiated 2–8 weeks after the start of rifampicin-containing TB therapy. Tuberculosis therapy consisted of daily self-administration of isoniazid 5 mg kg$^{-1}$ (maximum 300 mg) daily, rifampicin 10 mg day$^{-1}$ (maximum 750 mg) daily, pyrazinamide 20–25 mg kg$^{-1}$ (maximum 2000 mg) daily and ethambutol 15–20 mg kg$^{-1}$ (maximum 1600 mg) daily for 2 months in the induction phase. Patients enrolled before 1 July 2007 received daily isoniazid and ethambutol for 6 months in the continuation phase of therapy and those enrolled after that date received daily isoniazid and rifampicin for 4 months in accordance with the Ghana National TB treatment guidelines at the time of the study. Also, after 1 July 2007, all patients received a fixed dose combination tablet of isoniazid 75 mg, rifampicin 150 mg, pyrazinamide 400 mg and ethambutol 275 mg, isoniazid 75 mg and rifampicin 150 mg in the induction and continuation phases, respectively. The study was reviewed and approved by the Institutional Review Board of the Nogouchi Memorial Institute for Medical Research (Legon, Ghana). Informed written consent was obtained from all patients.

## Study procedures

Clinical assessments including a medical history, physical examination and baseline CD4 cell counts and HIV RNA

were performed at study entry and at scheduled follow-up visits. Antiretroviral therapy was self-administered at night and a mid-dose blood sampling was obtained at weeks 4 and 8 of therapy. Patients were asked to record the time of the evening dose before pharmacokinetic sampling, and samples were drawn at approximately 12 h after the efavirenz dose was taken. Plasma samples were stored at −70°C until testing. Mid-dose sampling (i.e. sampling done between doses, usually between 8–20 h postdose) is frequently used in clinical studies of efavirenz disposition for patient convenience since the drug is invariably taken at bedtime to minimize central nervous system side-effects during the day [5, 29]. We have recently reported that mid-dose efavirenz concentrations are highly correlated with efavirenz area under the curve (AUC) values when measured at steady state, which is consistent with the relatively prolonged half-life of this drug [18]. In HIV-infected patients, efavirenz steady-state concentrations are reached in 6–10 days [30]. Patients who had pharmacokinetic sampling performed during concurrent administration with rifampicin-containing TB therapy or for up to 1 week after stopping this therapy (two patients) were classified as receiving rifampicin-containing TB therapy. This was based on the known effects of rifampicin on increasing CYP activity, which are estimated to occur with 6 days of therapy initiation and to dissipate by 2 weeks after discontinuation [31].

## Drug concentration assays

Efavirenz concentrations in plasma were measured using a validated high-performance liquid chromatography/ultraviolet method [32]. This method was validated over a range of 15–10 000 ng ml$^{-1}$ and is accurate (90.4–110.5%) with intraday and interday precision of 2.3–8.3%. All analytical work was performed by the University of North Carolina Center for AIDS Research Clinical Pharmacology and Analytical Chemistry Core, which is Clinical Laboratory Improvement Amendments certified and participates in quarterly national and international external proficiency testing [33, 34].

## Genotyping and copy number assays

Subjects were genotyped for CYP2B6 c.516G→T (Q172H, rs3745274), c.983T→C (I328T, rs28399499), CYP2A6 *9B, (g.1836G→T, rs8192726, which is in linkage disequilibrium with the *9B defining TATA box mutation g.-48T→G, rs28399433), and CYP2A6 *17 (g.5065G→A, c.1093G→A, M365V, rs28399454) using the fluorometric 5′ nuclease genotyping assay (TaqMan Assays; Applied Biosystems, Foster City, CA, USA). CYP2A6 gene copy number variation was also performed using the gene copy number assay (TaqMan®; Applied Biosystems) according to the manufacturer's instructions. All reactions were performed in an ABI PRISM 7300 HT system (Applied Biosystems), and results were verified by two investigators (A.K.,

M.H.C.). We performed direct sequencing to confirm the genotyping results in one patient with CYP2B6 516TT genotype, who had a relatively low efavirenz concentration, and in all the six patients with CYP2B6 983TC genotype.

## Statistical analyses

Statistical analyses were performed using Sigmplot 11 software, which incorporates a comprehensive statistical analysis module (Systat, San Jose, CA, USA). Unless otherwise indicated, $P < 0.05$ was considered to be statistically significant. It was necessary to rank transform efavirenz concentration data to achieve data normality and equal variance. The relationship between efavirenz concentrations determined at 4 weeks and at 8 weeks of therapy was evaluated by Spearman rank order correlation analysis and compared by Mann–Whitney rank sum test. Univariate analyses of effects of patient demographics and genotypes on mean efavirenz mid-dose concentrations were assessed by Mann–Whitney rank sum test (gender, rifampin use, alcohol use, and for two genotype groups), analysis of variance (ANOVA) (for three genotype groups) or by linear regression [for age, body weight and body mass index (BMI)]. Two-way ANOVA, which incorporates an interaction model, was also used to investigate effects of CYP2B6 516G→T genotype and CYP2A6 variants. In instances where the ANOVA (one-way or two-way) indicated a significant effect ($P < 0.05$), post hoc multiple pair-wise comparison testing was performed using the Student–Newman–Keuls method to identify groups that were significantly different from each other. Finally, multiple linear regression analysis was used to construct a predictive model using patient demographic factors and genotypes as independent variables and efavirenz concentrations as the dependent variable. Dichotomous variables, including gender, rifampin use, alcohol use, CYP2A6 variant, and CYP2B6 983T→C genotype were coded as 0 or 1, while age, body weight and BMI were included as continuous variables. Two variables were used to describe CYP2B6 516G→T genotype, coded as GG/GT = 0 and TT = 1 for the recessive genetic model and also as GG = 0 and GT/TT = 1 for the dominant genetic model. This also allowed for the additive genetic model if both CYP2B6 516G→T variables were found to be significant. Using an automated method implemented in Sigmaplot 11, a minimal multiple linear regression model was then identified by stepwise removal of nonsignificant variables until all remaining independent variables had coefficients with $P$-values $< 0.05$. The percentage of variance explained by the model was based on the coefficient of determination ($R^2$). The contribution of each independent variable (expressed as a percentage) to the total explained variance was derived from the standardized regression coefficients, which had been normalized to the total explained variance.

## Results

### Study population

Seventy-four HIV-infected patients treated with efavirenz-containing highly active antiretroviral therapy were initially enrolled in the study. Of these, nine were excluded from the final genotype–phenotype analysis either because they had died before study completion ($n = 5$), had discontinued the study for other reasons prior to pharmacokinetic sampling ($n = 2$), or had undetectable concentrations of all antiretroviral drugs or incomplete data ($n = 2$).

### Patient demographics and efavirenz plasma concentrations

Of the 65 patients included in final analysis, the mean ($\pm$SD) age was $38 \pm 8$ years, baseline body weight was $54 \pm 12$ kg, and BMI was $19.5 \pm 4.1$. Thirty-six patients (55%) were female, nine (14%) reported alcohol use, and 19 (29%) were receiving concurrent rifampicin-containing TB therapy at the time of efavirenz sampling. The mean time of pharmacokinetic sampling was $14.1 \pm 1.1$ h postdose, median 13.9 h (range 12–18 h) postdose.

As shown in Figure 1, efavirenz concentrations in plasma samples collected at 4 weeks and 8 weeks following initiation of antiretroviral therapy were well correlated (Spearman correlation coefficient $Rs = 0.68$; $P < 0.001$). Values were also equally clustered around the line of identity consistent with steady-state pharmacokinetics being reached by 4 weeks and maintained until the 8 weeks
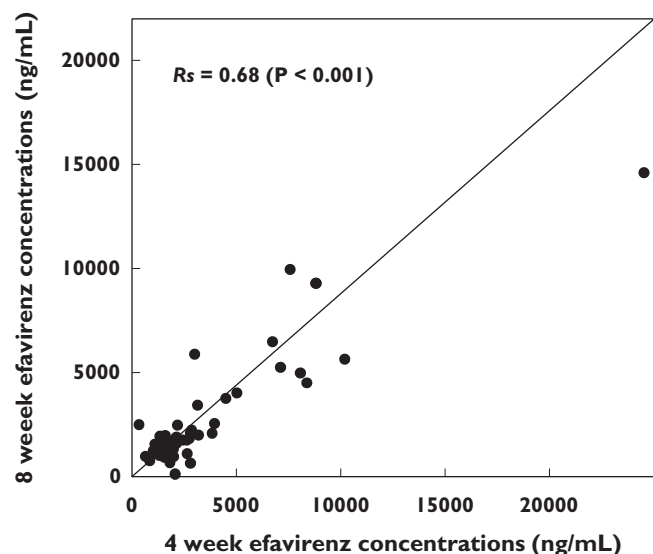


**Figure 1**

Correlation of mid-dose efavirenz concentrations in plasma from 65 human immunodeficiency virus-infected Ghanaian patients measured 4 weeks (abscissa) and 8 weeks (ordinate) after commencing treatment. Shown are the Spearman correlation coefficient ($Rs$) and associated $P$-value for the correlation analysis, and the line of identity representing perfect correlation

sampling time. The median [interquartile range (IQR)] mid-dose efavirenz plasma concentrations at 4 weeks and 8 weeks were 1763 (1251–2795) ng ml$^{-1}$ and 1609 (1244–2465) ng ml$^{-1}$, respectively, and were statistically indistinguishable ($P = 0.40$; Mann–Whitney rank sum test). Consequently, to simplify the presentation of results of further analyses, 4-week and 8-week values were averaged for each subject resulting in median (IQR) mid-dose efavirenz plasma concentrations of 1597 (1279–2803) ng ml$^{-1}$ for all 65 subjects. This approach was also supported by preliminary analyses, which showed essentially identical results for the results reported below regardless of whether 4-week, 8-week, or averaged results were used.

None of the patient demographic variables examined including age ($P = 0.47$; linear regression), BMI ($P = 0.068$; linear regression), gender ($P = 0.98$; Mann–Whitney rank sum test) or alcohol use ($P = 0.42$; Mann–Whitney rank sum test) was associated with altered mid-dose efavirenz concentrations (data not shown). Furthermore [median (IQR)] efavirenz concentrations in patients receiving rifampicin-containing TB therapy [1628 (1224–2315) ng ml$^{-1}$] were not different from levels in patients that were not receiving this additional therapy [1582 (1360–3020) ng ml$^{-1}$] ($P = 0.86$; Mann–Whitney rank sum test).

### CYP2B6 and CYP2A6 polymorphisms

Variant allele frequencies for the 65 study patients were 45, 4, 5 and 12% for *CYP2B6* c.516G→T, *CYP2B6* c.983T→C, *CYP2A6*\*9B and *CYP2A6*\*17, respectively. The numbers of subjects with each individual genotype are given in Table 1. No subjects were identified who were homozygous variant for *CYP2B6* c.983T→C. We also determined that all subjects had two copies of the *CYP2A6* gene based on a *CYP2A6* gene copy number variation assay.

### CYP2B6 and CYP2A6 genotype effects on efavirenz exposure

*Univariate analyses* Figure 2 shows the distribution of efavirenz plasma levels stratified by CYP2B6 and CYP2A6 genotypes, while the results of univariate statistical analyses of these genotypes are given in Table 1. As expected, *CYP2B6* 516G→T genotype was significantly associated with decreased efavirenz concentration ($P < 0.001$, ANOVA). Median efavirenz concentrations for *CYP2B6* 516TT genotype was at least five times higher than both the *CYP2B6* 516GT ($P < 0.001$) and 516GG ($P < 0.001$) genotype groups. The median efavirenz concentration for *CYP2B6* 516GT genotype was about 1.2 times higher than values for the 516GG genotype ($P > 0.027$). None of the other individual genotypes evaluated, including *CYP2B6* c.983T→C ($P = 0.61$), *CYP2A6*\*9B ($P = 0.07$) and *CYP2A6*\*17 ($P = 0.15$), was individually associated with altered efavirenz concentrations. However, when both *CYP2A6* slow metabolizer variants were considered together, the median efavirenz concentrations among *CYP2A6* variant carriers (\*9 or \*17)

## Table 1

Impact of CYP2B6 and CYP2A6 polymorphisms on mid-dose efavirenz concentrations* in 65 human immunodeficiency virus-infected patients

| Variable | n (%) | Median (IQR) efavirenz mid-dose concentration* | P-value |
|---|---|---|---|
| **CYP2B6 c.516G→T** | | | <0.001 |
| GG | 22 (30%) | 1 282 (1089–1901)† | |
| GT | 38 (51%) | 1 560 (1379–2205)† | |
| TT | 14 (19%) | 8 320 (6085–10 673)† | |
| **CYP2B6 c.983T→C** | | | 0.88 |
| TT | 59 (91%) | 1 567 (1274–2649) | |
| TC | 6 (9%) | 1 832 (1334–3102) | |
| **CYP2A6*9B (g.1836G→T)** | | | 0.07 |
| TT | 59 (91%) | 1 562 (1265–2356) | |
| TG | 5 (8%) | 3 102 (1568–4712) | |
| GG | 1 (1%) | 10 726 | |
| **CYP2A6*17 (c.1093G→A)** | | | 0.15 |
| GG | 51 (78%) | 1 557 (1274–2334) | |
| GA | 12 (19%) | 1 994 (1325–4063) | |
| AA | 2 (3%) | 6 374 (4220–8529) | |
| **CYP2A6 *9B or *17** | | | 0.017 |
| Reference | 47 (72%) | 1 516 (1242–2084) | |
| Variant carrier | 18 (28%) | 2 694 (1597–5943) | |

*Values are the average of efavirenz concentrations determined at 4 and 8 weeks in each patient; note that four patients had missing efavirenz concentration data at week 4, and seven patients had missing concentration data at week 8. †P < 0.001 for 516TT vs. GT, P < 0.001 for 516TT vs. GG, P = 0.027 for 516GG vs. GT groups determined by Student–Newman–Keuls test.

was 1.8 times higher than among individuals without either of these variants ($P = 0.017$).

*Bivariate analysis* Two-way ANOVA was then used to control for the *CYP2B6* 516G→T genotype effect and evaluate whether there was any additional influence from the *CYP2A6* slow metabolizer variants, including evaluation of possible gene–gene interactions. As shown in Figure 3, *CYP2A6* slow metabolizer variants were independently associated with higher efavirenz concentrations regardless of *CYP2B6* 516G→T genotype ($P < 0.001$ for *CYP2B6* 516G→T genotype; $P = 0.028$ for *CYP2A6* variants; $P = 0.55$ for interaction). Post hoc analysis of the *CYP2A6* variant effect within each of the *CYP2B6* 516G→T genotype groups using the Student–Newman–Keuls method showed that patients with *CYP2B6* 516GT genotype who also had *CYP2A6* variants had significantly higher median efavirenz concentration than noncarriers (2694 *vs.* 1448; $P = 0.01$) (Figure 3). Trends for higher median efavirenz concentrations in *CYP2A6* variant carriers compared with noncarriers were also observed in the two other *CYP2B6* 516G→T genotype groups, although the differences did not achieve statistical significance ($P > 0.05$).

Of note in this analysis were three patients (indicated in Figure 3) that did not have the slow metabolizer *CYP2B6* 516TT genotype and yet had relatively high efavirenz concentrations (>4000 ng ml⁻¹). Of these, one individual (patient 159 with *CYP2B6* 516GG genotype) was homozy-

gous for the *CYP2A6*17* variant, another individual (patient 158 with *CYP2B6* 516GT genotype) carried both the *CYP2A6*9B* and *17* alleles, and the other individual (patient 13 with *CYP2B6* 516GT genotype) was heterozygous for both the *CYP2A6*9* and *CYP2B6* 983T→C variants.

*Multiple linear regression analysis* Stepwise backward multiple linear regression analysis was then performed to identify the minimum set of independent variables that are predictive of efavirenz plasma concentrations, and also determine the relative contribution of these variables to overall variability in efavirenz concentrations. Variables entered into the initial model included sex, age, weight, BMI, alcohol use, rifampin treatment, *CYP2B6* 516G→T genotypes, 983T→C genotype, as well as CYP2A6 slow metabolizer variant carrier status. Only three independent variables remained in the final model with $P < 0.05$, including *CYP2B6* 516TT genotype (standardized regression coefficient = 0.51; $P < 0.001$), *CYP2B6* 516GG genotype (standardized regression coefficient = 0.24; $P = 0.017$), and *CYP2A6* variants (standardized regression coefficient = 0.25; $P = 0.008$). The coefficient of determination ($R^2$) for the regression was 0.48, indicating that 48% of the total variance in efavirenz concentrations was explained by the model. A comparison of the standardized regression coefficients indicated that *CYP2B6* 516TT, *CYP2B6* 516GG and *CYP2A6* variant genotypes accounted for 24, 12 and 12% (respectively) of the total variance in efavirenz plasma concentrations. Inclusion of both *CYP2B6* 516TT and *CYP2B6* 516GG genotypes in the final model is consistent with an additive genetic model with all *CYP2B6* c.516G→T genotypes accounting for 36% of the total variance. We found no significant association ($P > 0.05$) between efavirenz plasma concentrations and patient age, gender, weight, BMI, alcohol use, or rifampicin-containing TB therapy.

## Discussion

In this study, we have identified *CYP2A6* slow metabolizer variants (*9B* and/or *17*) as independent predictors of efavirenz plasma concentration beyond that provided by the well-known *CYP2B6* 516G→T polymorphism. This finding suggests that *CYP2A6* genotyping may be useful in enhancing the accuracy of prediction of efavirenz concentrations in HIV-infected patients over that of *CYP2B6* 516G→T alone. Our published data have been confirmed by a recent report (unpublished conference abstract) demonstrating an association between slow metabolizer *CYP2A6* alleles and increased efavirenz concentrations in 169 patients in the Swiss HIV Cohort [35]. However, in that study the effect of the *CYP2A6*7*, *9* and/or *17* alleles was observed only in patients with *CYP2B6* slow metabolizer genotypes [35], which contrasts with our study, which showed an effect regardless of *CYP2B6* genotype as reflected by a lack of significant gene–gene interaction in
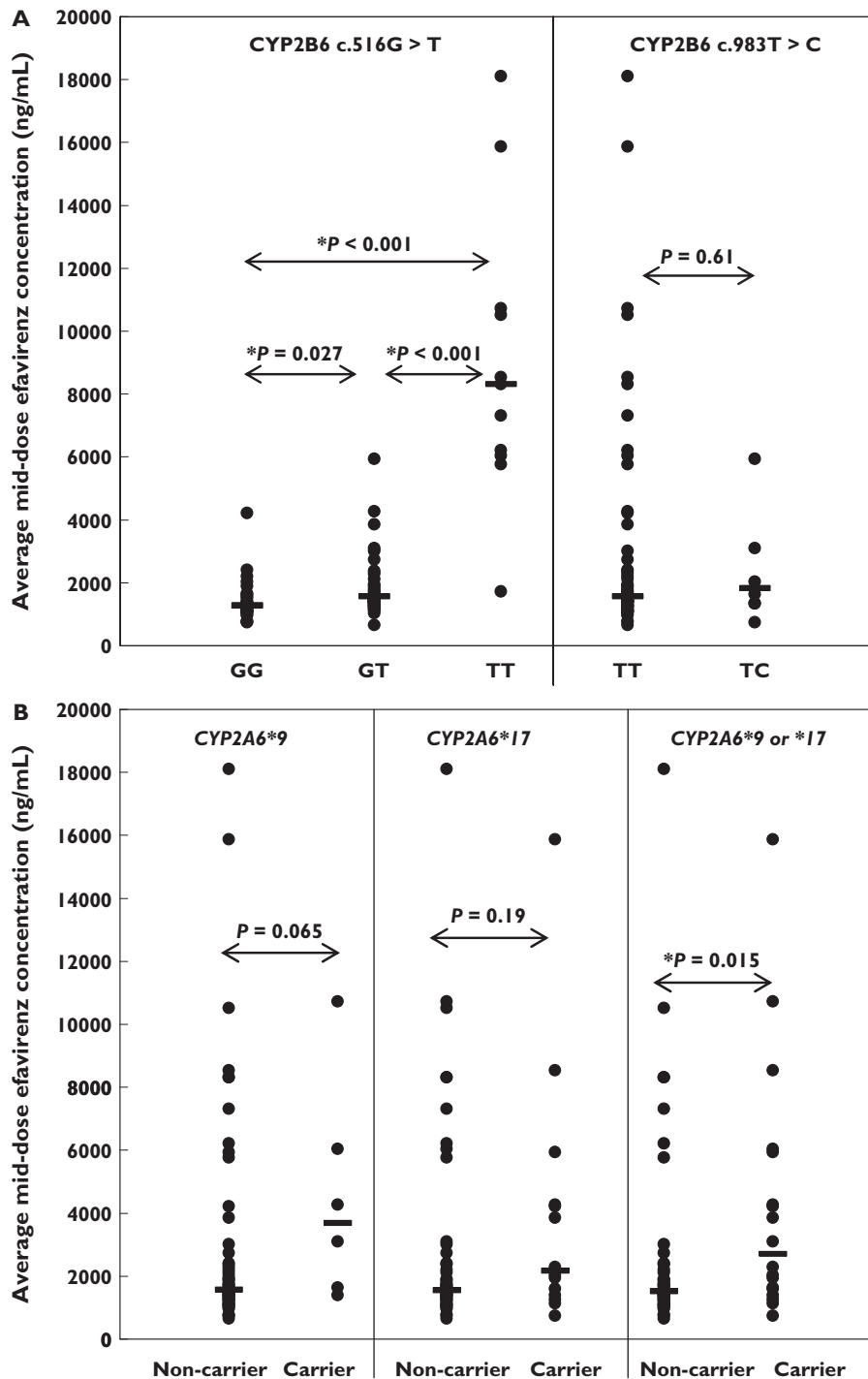
## Figure 2

Influence of *CYP2B6* c.516G→T and *CYP2B6* c.983T→C polymorphisms (A) and of *CYP2A6*9* and *CYP2A6*17* polymorphisms (B) on efavirenz mid-dose concentrations (average of 4-week and 8-week samples) in human immunodeficiency virus (HIV) and HIV/tuberculosis co-infected patients in Ghana. Differences between groups were examined by Mann–Whitney rank sum test (two genotype groups) or by ANOVA (three genotype groups) with post hoc Student–Newman–Keuls pair-wise multiple comparisons testing

the two-way ANOVA analysis. However, we did observe the greatest CYP2A6 variant effect in patients with *CYP2B6* 516GT genotype (see Figure 2). The reasons for the differences between these two studies are not immediately

apparent but could be related to differences in populations, as our study enrolled only native Africans, whereas the other study was primarily of Whites. There were also differences in use of concurrent medications, as 19 of our
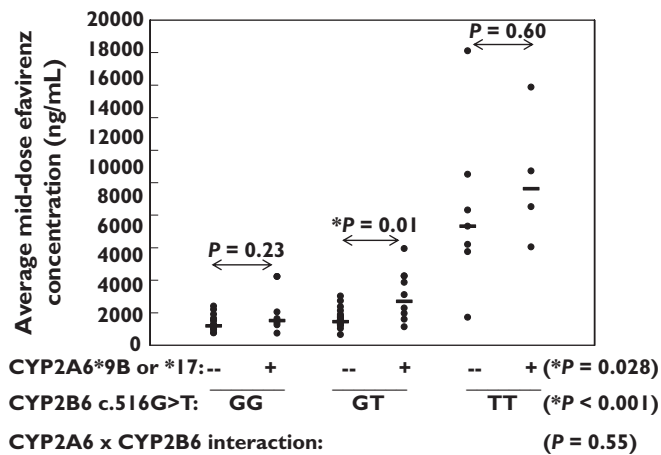
**Figure 3**

Influence of *CYP2A6* slow metabolizer genotypes (*9B* and/or *17*) and *CYP2B6* c.516G→T genotypes on efavirenz mid-dose concentrations (average of 4-week and 8-week samples). Shown are the *P*-values from the two-way ANOVA (including analysis of gene–gene interactions) and post hoc multiple pair-wise comparisons (Student–Newman–Keuls test) within genotype groups

patients with HIV/TB co-infection also received anti-TB drugs, which may have altered CYP gene expression.

*In vitro* studies have demonstrated that recombinant CYP2A6 is capable of hydroxylating efavirenz at both the 8- and 14- positions, although the activity of CYP2A6 compared with CYP2B6 in these reactions is admittedly low [12]. Furthermore, the rate of efavirenz 7-hydroxylation measured in a human liver bank was most highly correlated with CYP2A6 immunoquantified protein and coumarin hydroxylation, a CYP2A6-selective enzyme activity [16]. Unfortunately, as yet there are no reports of the quantitative contribution of CYP2A6 to efavirenz metabolism in human liver, such as through use of isoform-specific CYP2A6 chemical or antibody inhibitors. Nevertheless, the results of the present study showing higher efavirenz concentrations in patients with *CYP2A6* slow metabolizer alleles support a role for CYP2A6 in efavirenz metabolism in patients.

We also examined the effect of the *CYP2B6* 983TC genotype on efavirenz exposure but found no significant relationship, possibly because of the small size of our population, which contained no homozygous variant individuals. Recent clinical studies suggest that high efavirenz concentrations are more likely to occur in patients who are *CYP2B6* 983T→C homozygous variant [14] or in patients who are heterozygous for *CYP2B6* 983T→C and are heterozygous for *CYP2B6* 516G→T [15]. The *CYP2B6* 983T→C polymorphism results in a I328T amino acid substitution and has been considered a null allele since recombinant expression in COS-1 cells results in complete lack of CYP2B6 protein and activity [19]. However, *CYP2B6* 983T→C has also been reported to occur as an allele (*CYP2B6*16*) with *CYP2B6* c.785A→G (results in amino acid substitution

K262R), and expression of CYP2B6*16 cDNA constructs with both these mutations results in somewhat decreased (but not absent) enzyme protein and activity [36].

Our data are consistent with previous reports of a high frequency of *CYP2B6* 516G→T variant alleles in native Africans [18, 37], but a somewhat lower frequency of *CYP2B6* 983T→C genetic variation [20]. The frequency of the *CYP2A6*17* variant allele of 11% in our population was similar to that among Black subjects recruited in the USA and Asia [26, 28]. *CYP2A6*9B* variation in our population was also similar to that reported among healthy Ghanaian subjects [27]. Overall, nearly one-third of our patients had *CYP2A6*9B* and/or *CYP2A6*17* variants, and if the association between *CYP2A6* genetic variation and efavirenz disposition is confirmed in other studies, genotyping for *CYP2A6* variation would help improve pharmacokinetic prediction afforded by *CYP2B6* 516G→T SNP screening alone.

As a CYP2B6 enzyme inducer, rifampicin co-administration would be expected to enhance efavirenz clearance and reduce plasma concentrations. However, in this study we found no difference in efavirenz concentrations between patients receiving rifampicin-containing TB therapy and those not receiving this therapy. These data are reassuring, as HIV/TB co-infection is common in sub-Saharan Africa, and efavirenz-based antiretroviral therapy is often needed in patients receiving rifampicin-containing TB treatment. It is important to recognize that this was not a crossover study and so results could have been confounded by population differences. However, available published studies in which efavirenz concentrations were evaluated with and without rifampicin-containing treatment at different times in the same patients have also reported no significant differences in efavirenz concentrations [38, 39]. In the South African study, the geometric mean efavirenz trough concentration on rifampicin was 1730 ng ml$^{-1}$ (range 345–27 179) and 1377 ng ml$^{-1}$ (range 572–3975) off rifampicin (*P* = 0.55) [38]. The study by López-Cortés *et al.*, in which they evaluated the effect of rifampicin on efavirenz pharmacokinetic parameters in a subgroup, also did not reach statistical significance (*P* > 0.05) [39]. Although the mean efavirenz AUC declined by 22% during co-administration with rifampicin (*P* > 0.05) in the aforementioned study, the change in efavirenz AUC ranged from a decrease of 65% to an increase of 37%, suggesting wide interpatient variability in the rifampicin effect [39]. Since efavirenz itself induces *CYP2B6* (i.e. auto-induction), it is possible that rifampicin cannot increase CYP2B6 expression beyond that resulting from chronic efavirenz exposure. However, it is also important to point out that other drugs were administered to our patients for TB therapy in addition to rifampicin that may have confounded any effect of rifampicin. For example, it is also possible that the use of isoniazid with rifampicin in the TB co-infected patients could have minimized the induction effect of rifampicin on *CYP2B6*, as isoniazid has been

shown to be an inhibitor of cytochrome P450 *in vitro* [40–42]

We recognize that our study has several limitations. First, the size of the study population was small, as there were six or fewer patients each with *CYP2B6* 983T→C or *CYP2A6*9B* variants, limiting our ability to detect small treatment and genotype effects. Second, we were unable to assess efavirenz concentrations before or after rifampicin treatment to exclude the effect of differences in population on the analysis. Third, patients self-administered the evening dose and so the exact timing of the mid-dose pharmacokinetic sample (relative to dose) could have differed between patients.

Despite the above limitations, our findings demonstrate that in addition to the strong predictive value of CYP2B6 516G→T polymorphism, *CYP2A6* genetic variation is an independent determinant of efavirenz plasma concentrations. If confirmed in larger studies, CYP2A6 genotyping could be a useful adjunct to *CYP2B6* genotyping for the prediction of efavirenz concentrations in HIV-infected patients.

## Competing interests

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