

## ARTICLE

# Large variability in plasma efavirenz concentration in Papua New Guinea HIV/AIDS patients associated with high frequency of CYP2B6 516T allele

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## Abstract

Papua New Guinea (PNG) has a high HIV/AIDS prevalence and very high frequency of the CYP2B6 c.516G>T (rs3745274) variant. We have conducted the first investigation of the impact of c.516G>T and patient demographics on plasma efavirenz (EFV) and 8-hydroxyefavirenz (8OH-EFV) concentrations, metabolic ratio (8OH-EFV/EFV) (MR), and their association with adverse effects, in PNG patients with HIV/AIDS. For 156 PNG patients with HIV/AIDS taking EFV 600 mg/day (for 3–156 months), plasma EFV and 8OH-EFV concentrations were quantified, CYP2B6 c.516G>T genotyped, and demographic and self-reported adverse effects data recorded. Genotype differences in EFV and 8OH-EFV concentrations, MR, and percent within therapeutic range (1000–4000 ng/ml) were examined, in addition to EFV and 8OH-EFV concentration differences between patients experiencing adverse effects. CYP2B6 c.516T allele frequency was 53%. Plasma EFV ( $p < 0.0001$ ), 8OH-EFV ( $p < 0.01$ ), and MR ( $p < 0.0001$ ) differed significantly between genotypes, with genotype explaining 38%, 10%, and 50% of variability, respectively. Plasma EFV concentrations were significantly higher in T/T (median = 5168 ng/ml) than G/G (1036 ng/ml, post hoc  $p < 0.0001$ ) and G/T (1502 ng/ml,  $p < 0.0001$ ) genotypes, with all patients above therapeutic range ( $n = 23$ ) being T/T genotype ( $p < 0.0001$ ). EFV and 8OH-EFV concentrations were not significantly higher in patients experiencing adverse effects. In PNG HIV/AIDS population where the 516T frequency is very high, it explains a substantial portion of variability (38%) in EFV disposition; however, at least for the patients receiving EFV long term, this does not translate into significant side effects.

## Study Highlights

### WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

High efavirenz (EFV) concentrations are strongly associated with the development of adverse effects, particularly central nervous system (CNS) and psychiatric

toxicities. Papua New Guinea (PNG) has a high HIV/AIDS prevalence and the highest frequency of the *CYP2B6* c.516G>T decreased function variant allele of any population assessed to date.

#### WHAT QUESTION DID THIS STUDY ADDRESS?

Whether there is a gene-dose association among the c.516G>T genotype, EFV and metabolite concentrations, and adverse events.

#### WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

In PNG, the *CYP2B6* c.516T/T genotype was strongly associated with substantially higher plasma EFV concentrations and lower metabolic ratio (8-hydroxy-efavirenz/efavirenz) in a gene-dose manner. In PNG, plasma efavirenz and 8-hydroxy-efavirenz concentrations were not significantly higher in patients experiencing adverse effects after the first 3 months of treatment.

#### HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

For PNG people taking EFV, early (<3 months) pharmacokinetic, and nonpharmacokinetic, mechanisms of CNS toxicity need investigation.

## INTRODUCTION

Efavirenz (EFV) is a non-nucleoside reverse transcriptase inhibitor, frequently prescribed as a component of highly active antiretroviral therapy used for the treatment of patients infected with HIV.<sup>1</sup> EFV was introduced onto the market in 1998<sup>2</sup> and has been recommended by the World Health Organization as the first-line drug for HIV treatment for more than 20 years.<sup>3</sup> Although EFV was recently replaced by dolutegravir as the recommended first-line drug,<sup>4</sup> it remains as an alternative first-line regimen for adolescents and adults, and as a first-line therapy in a few countries in Asia and the Western Pacific regions.<sup>5</sup>

Despite its efficacy in the treatment of HIV,<sup>2,6</sup> EFV often leads to the development of adverse effects, in particular those of the central nervous system (CNS) and psychiatric toxicities.<sup>7,8</sup> The likelihood of developing these adverse effects and for achieving therapeutic efficacy has been associated with total plasma EFV concentrations within its narrow therapeutic plasma concentration range of 1000–4000 ng/ml.<sup>9–11</sup> Patients with EFV concentrations above 4000 ng/ml are at greater risk of developing toxicities, whereas patients below 1000 ng/ml are more likely to experience treatment failure.<sup>9</sup> Although the occurrence of adverse effects has been associated with drug concentrations in some studies, other studies did not find an increased risk of CNS and psychiatric toxicities in patients with high EFV plasma concentrations.<sup>12–15</sup>

EFV shows large variability between patients in terms of plasma concentrations, drug-related toxicity, and drug response.<sup>16</sup> This variability is multifactorial, and includes differences in metabolism, ethnicity, gender, body weight, drug compliance, use of comedications,

presence of concomitant diseases, as well as genetic factors.<sup>17</sup> Variations in body weight, ethnicity, and comedication affect the clearance of EFV<sup>18,19</sup> yet the association between gender and plasma EFV concentrations appears to be equivocal.<sup>20</sup> Genetic polymorphisms, especially those associated with drug metabolism, have been studied in many different populations, and the effect on plasma EFV concentrations is unequivocal.<sup>11,20,21</sup>

Cytochrome P450 *CYP2B6* is the main isoenzyme involved in hepatic EFV metabolism. About 70% of EFV is metabolized to 8-hydroxy-efavirenz (8OH-EFV), predominantly by *CYP2B6*.<sup>22</sup> *CYP2B6* is also involved in the formation of the EFV secondary metabolite, 8,14-dihydroxyefavirenz.<sup>23</sup> The gene that encodes *CYP2B6* is highly polymorphic, resulting in altered drug metabolism.<sup>21,24</sup> The single nucleotide polymorphism (SNP) most frequently studied is the c.516G>T (rs3745274) missense variant, which has been associated with decreased activity of the *CYP2B6* isoenzyme, and increased plasma EFV concentrations.<sup>11,21,23,25,26,27</sup> This SNP has also been associated with the occurrence of adverse events and EFV discontinuation.<sup>28,29</sup> The c.516T allele frequency varies significantly between different ethnic populations ranging from 16% in Finnish and Southern Han Chinese populations to up to 65% in Papua New Guinea (PNG) populations who have the highest frequency of the variant allele known to date.<sup>30,31</sup>

Even though PNG shows the highest prevalence of the c.516T variant, there are no studies evaluating the relationship among plasma EFV concentrations, adverse effects and *CYP2B6* polymorphisms.<sup>32,33</sup> In the present study, we investigated (a) the impact of *CYP2B6* c.516G>T genotypes and patient demographics such as age, gender

and body weight on plasma EFV and 8OH-EFV concentrations and the metabolic ratio (MR), and (b) the association between plasma EFV and 8OH-EFV concentrations, MR as well as 516G>T genotypes and reported side effects.

## METHODS

### Patients and sample collection

One hundred fifty-six patients receiving EFV combination antiretroviral therapy at the HIV Heduru Clinic (Port Moresby General Hospital – PNG) from October 2017 to June 2018 were enrolled in the study, after giving written informed consent. This study was approved by the Medical Research Advisory Committee of the National Department of Health of the Government of Papua New Guinea (MRAC No. 16.32) and the University of Adelaide Human Research Ethics Committee (H-2017–167). All patients were receiving EFV 600 mg once daily with tenofovir disoproxil fumarate 300 mg and lamivudine 300 mg as a single tablet (duration of therapy ranged between 3 months and 13 years). Demographic data, such as age, body weight, gender, region, comedication, and comorbidities, were recorded, in addition to the time of dosing over the previous 3 days. Self-reported CNS (tiredness, dizziness, drowsiness, insomnia, and impaired concentration) and psychiatric (agitation, depression, aggression, and euphoria) side effects were also captured with yes/no symptom questionnaires.

Venous blood samples (5 ml) were collected between 8.5 and 22 h post last dose into clot activator (CAT) and lithium heparin (LiHep) blood collection tubes (BD vacutainers, Melbourne, Australia). CAT tubes were allowed to clot at room temperature then heat treated at 60°C for 1 h and stored at –20°C. LiHep tubes were also heat treated at 60°C for 1 h in a water bath, then centrifuged (2500 rpm for 20 min) to separate the plasma, which was stored at –20°C until transportation. The samples (CAT tubes, LiHep plasma, and LiHep buffy coat/red blood cell fraction) were transported on dry ice from PNG to Adelaide (Australia) and stored at –20°C until analysis.

### EFV and 8OH-EFV plasma concentrations

Steady-state EFV and 8OH-EFV plasma concentrations were determined using a Nexera ultra-high-performance liquid chromatography (UHPLC) system coupled to a liquid chromatography mass spectrometry (LCMS)-8040 triple quadrupole mass spectrometer (Shimadzu, Tokyo, Japan).<sup>34</sup>

Plasma (100  $\mu$ l) was added into a tube containing 10  $\mu$ l of labeled internal standard (1  $\mu$ g/ml for EFV-d5, 0.5  $\mu$ g/ml for 8OH-EFV-d4) and 100  $\mu$ l of 0.5 M ammonium

hydroxide. This was vortexed and pipetted into the wells of a Strata DE 200  $\mu$ l 96-well plate placed on a 2 ml collection plate (Phenomenex, Lane Cove, NSW, Australia). Samples were then eluted using MTBE. Eluates were then dried in a vacuum centrifugal evaporator and reconstituted in mobile phase before injecting 1  $\mu$ l onto the UHPLC system.

Compounds were separated on a Kinetex C18 PS column (100  $\times$  2.1 mm, 1.7  $\mu$ m) (Phenomenex) maintained at 40°C. The mobile phase comprised of 35% 0.1% formic acid in LCMS-grade water and 65% LCMS-grade methanol, with a flow of 0.4 ml/min. The chromatogram was run for 4 min with retention times for EFV and 8OH-EFV of 3.3 and 2.7 min, respectively. Mass spectra were acquired in negative ionization mode using electrospray ionization. Mass transitions used to quantify EFV and 8OH-EFV were  $m/z$  314  $\rightarrow$  69 and  $m/z$  330  $\rightarrow$  210, respectively.

The validated calibration ranges for EFV and 8OH-EFV were 50–10000 ng/ml and 12.5–5000 ng/ml, respectively. Intra- and inter-day imprecision and inaccuracy for EFV and 8OH-EFV were all within 10%. Both analytes were shown to be stable at room temperature for 24 h, –20°C for 90 days, 60°C for 1 h, after at least three freeze/thaw cycles, and in the autosampler for at least 12 h.<sup>34</sup>

### DNA extraction and genotyping

DNA was extracted from defrosted and vortexed CAT tube and LiHep buffy coat/red blood cell samples using Maxwell 16 Blood DNA Purification kits (Promega, Sydney, Australia). Purity of the extracted DNA was tested by absorbance (260 nm:280 nm) using Synergy Mx plate reader with Take3 plate (Biotek Instruments Inc., USA). DNA was quantified by Quanti-IT dsDNA broad-range assay kit (Thermo Fisher, Adelaide, Australia) according to the manufacturer's instructions. For samples with low DNA yield (<100 ng), multiple extractions were performed, combined, and concentrated using ReliaPrep DNA Clean-up and Concentration System kits according to the manufacturer's instructions (Promega). *CYP2B6* c.516G>T (rs3745274) genotype was determined as part of a custom Agena MassArray Panel through the Australian Genome Research Facility (AGRF, Brisbane, Australia).

### Statistical analysis

Patient demographics (age, gender, and body weight) were summarized as median and range. The following statistical analyses were performed using GraphPad Prism 8.0.0 unless otherwise specified. Chi-squared analysis was used to test for genotype deviation from Hardy–Weinberg equilibrium. Median and range was applied to describe plasma

EFV and 8OH-EFV concentrations and MR, calculated as plasma 8OH-EFV concentration/EFV concentration.

Differences between c.516G>T genotypes in plasma EFV and 8OH-EFV concentrations and MR were determined using the nonparametric Kruskal-Wallis test where a *p* value of less than 0.05 was regarded as statistically significant. Dunn's multiple comparison test was performed as a post hoc test to compare differences between genotype groups. To test for "gene-dose" effects, Jonckheere-Terpstra exact (permutation) tests for ordered differences were performed in R version 1.2.5033 (RStudio, PBC) using the `jonckheere.test` function (two-sided with 200,000 permutations) of the `clinfun` package.<sup>35</sup> Chi-square test was applied to test for genotype differences in the likelihood of being below (<1000 ng/ml), within (1000–4000 ng/ml) or above (>4000 ng/ml) the EFV therapeutic range, with *p* less than 0.05 considered statistically significant.

To examine the multiple contributions of genetic and demographic variables to EFV concentrations, multiple linear regression analyses were conducted in R. Distributions of continuous variables (weight and age) were initially checked using histograms, quantile–quantile plots, and Box-Cox power transformations (R packages::functions used were: `graphics`<sup>36</sup>::`hist`, `stats`<sup>36</sup>::`qqnorm` and `qqline`, and `MASS`<sup>37</sup>::`boxcox`, respectively). Linear regression (`stats`::`lm`) analyses were performed separately for EFV, 8OH-EFV, and MR testing for main effects of genotype, age, gender, and weight (weight was not tested for MR). Models were checked for linearity, homoscedasticity, normality, and potential high leverage outliers (`stats`::`plot`). Significant explanatory variables were identified by F-test (`car`::`Anova`). Relative contributions ( $R^2$ ) of significant explanatory variables were assessed using the averaging over orderings method (`relaimpo`<sup>38</sup>::`calc.relimp`).

For the evaluation of side effects, patients were divided into four groups as follows: no side effects, CNS side effects but no psychiatric side effects, psychiatric side effects but no CNS side effects, and both CNS and psychiatric side effects. Plasma EFV and 8OH-EFV concentrations and MR were summarized as medians and range for each of the four groups. Kruskal-Wallis and Dunn's multiple comparison test were applied to evaluate the relationship between drug concentrations and the occurrence of side effects. For the association between the occurrence of side effects and genotype, the patients were divided into two groups, none and CNS and/or psychiatric side effects, and group proportions compared between genotypes by  $\chi^2$  test. Missing data were handled by pairwise exclusion within each analysis. All reported *p* values are unadjusted for multiple comparisons.

The power of the study to detect a significant difference in EFV plasma concentrations between side effect groups was estimated by performing 10,000 iterations of Kruskal-Wallis tests following random samplings from

simulated population (side effect group) distributions of plasma concentrations. Power to detect a significant difference in side effect incidence between *CYP2B6* genotype groups was estimated by performing 10,000 iterations of  $\chi^2$  tests following random samplings from simulated population (genotype group) distributions of side effects.

## RESULTS

### Participant characteristics, drug concentrations, and genotypes

Participant characteristics and genotype frequencies are summarized in Table 1. Demographic data were not available for all participants, and sufficient DNA for genotyping was obtained for 112 patients, resulting in 102 subjects with complete genotype, plasma EFV and 8OH-EFV concentration, and demographic data. *CYP2B6* c.516 G>T

**TABLE 1** Demographic data for 156 PNG HIV/AIDS patients receiving 600 mg per day EFV

		N
Age (years), median (range)	37 (14–65)	150 <sup>a</sup>
Weight (kg), median (range)	61 (36–120)	148 <sup>a</sup>
Female, n (%)	99 (66%)	151 <sup>a</sup>
Region		155
Highland, n (%)	65 (42)	
Coastal, n (%)	90 (58)	
Genotype n (%) ( <i>CYP2B6</i> c.516 G>T)		
G/G	28 (25%)	
G/T	52 (45%)	
T/T	35 (30%)	
No genotype results	41	
Comedications (n)		156
None	146	
Isoniazid	5	
Cotrimoxazole	4	
HREZ <sup>b</sup>	1	
Carbimazole	1	
Comorbidities (n)		156
None	148	
Tuberculosis	6	
Cancer	1	
Thyroid disorder	1	

Abbreviations: EFV, efavirenz; PNG, Papua New Guinea.

<sup>a</sup>Data not recorded in all case record forms.

<sup>b</sup>HREZ, fixed dose combination of isoniazid, rifampicin, ethambutol and pyrazinamide.

genotype distribution did not deviate significantly from Hardy-Weinberg equilibrium ( $p = 0.36$ ).

The median (range) plasma EFV concentrations of 153 patients was 1575 (42 to 13,212) ng/ml, with three patients below the EFV lower limit of quantification. Ninety-two (61%) patients had plasma EFV concentrations within, 33 (22%) below, and 28 (17%) above the proposed therapeutic window of 1000–4000 ng/ml. The median (range) of plasma 8OH-EFV concentration was 202 (25 to 935) ng/ml. Median (range) MR was 0.13 (0.01 to 5.0). Full de-identified data are available in Supplementary Table S1.

### CYP2B6 c.516G>T genotype differences in EFV and 8OH-EFV concentrations and MR

Univariate analysis showed that plasma EFV (Kruskal-Wallis  $p < 0.0001$ ) and 8OH-EFV concentrations ( $p < 0.01$ ), and the MR ( $p < 0.0001$ ), differed significantly between 516G>T genotypes (Figure 1). Plasma EFV concentrations in T/T genotype patients (median = 5168 ng/ml) were 58% higher than G/G (1036 ng/ml, post hoc  $p < 0.0001$ ), and 36% higher than G/T (1502 ng/ml, post hoc  $p < 0.0001$ ). The difference in plasma EFV concentrations between G/G and G/T genotypes was also statistically significant (post hoc  $p < 0.01$ ). The Jonckheere-Terpstra test confirmed a significant ( $p < 1 \times 10^{-5}$ ) “gene-dose” effect for plasma EFV. MR in T/T genotype patients (median 0.04) was 79% lower than G/G (0.19, post hoc  $p < 0.0001$ ), and 78% lower than G/T (0.18, post hoc  $p < 0.0001$ ), with no significant difference between G/G and G/T genotypes. The Jonckheere-Terpstra test confirmed a significant ( $p < 1 \times 10^{-5}$ ) “gene-dose” effect for MR. Plasma 8OH-EFV concentrations were significantly higher in G/T patients (293 ng/ml) compared to G/G (155 ng/ml) and T/T (137 ng/ml) patients (both post hoc  $p < 0.05$ ), with

no significant difference between G/G and T/T genotypes. The Jonckheere-Terpstra test indicated no significant gene-dose effect ( $p = 0.7$ ) for plasma 8OH-EFV.

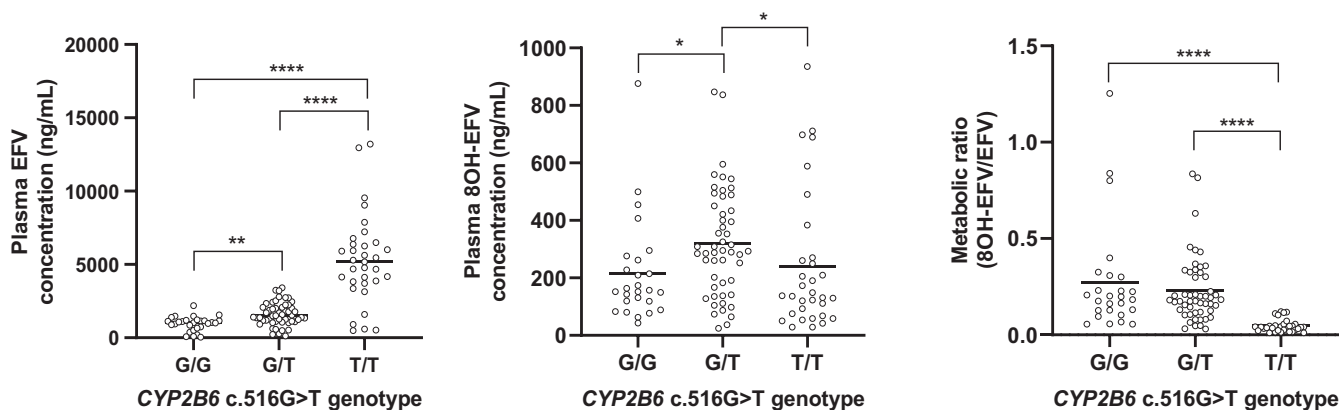
There were significant ( $p < 0.001$ ) genotype differences in the proportions of patients below, within, and above the therapeutic plasma EFV concentration range (Table 2). G/G genotype patients were the most likely to be below the therapeutic range (42% vs. 12% and 15%), and G/T patients the most likely (85%) to be within the therapeutic range, whereas all patients with EFV plasma concentrations greater than 4000 ng/ml (above EFV therapeutic range) were T/T.

### Contributions of genetic and demographic variables to EFV disposition

Initial distribution and linear regression analyses indicated a need for log transformation of plasma EFV and 8OH-EFV concentrations, MR, and weight (but not for age) to meet linear regression model assumptions. Genotype was the only significant explanatory variable for (natural log [ln]-transformed) plasma EFV concentrations (see Table 3), explaining 38% of variability. For (ln) plasma 8OH-EFV concentrations, genotype and (ln) weight variables were significant, explaining 10% and 7% of the variability, respectively (Table 4). Genotype was the most significant explanatory variable for (ln) MR followed by gender (Table 5), explaining 50% and 3% of the variability, respectively.

### Drug concentrations, genotype, and side effects

A total of 149 patients were included in the analysis of the association between drug concentrations and the



**FIGURE 1** CYP2B6 c.516G>T genotype differences in plasma EFV and 8OH-EFV concentrations and Metabolic Ratio (8OH-EFV/EFV) in 115 Papua New Guinea (PNG) patients with HIV/AIDS receiving EFV 600 mg/day. Dunn's post hoc \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\*\* $p < 0.0001$

Genotype	EFV therapeutic concentration range		
	Below (<1000 ng/ml)	Within (1000–4000 ng/ml)	Above (>4000 ng/ml)
G/G, n (%)	11 <sup>a</sup> (42)	15 (58)	0 (0)
G/T, n (%)	8 (15)	45 (85)	0 (0)
T/T, n (%)	4 (12)	6 (18)	23 (70)
Chi-square $p < 0.0001$			

Abbreviations: EFV, efavirenz; PNG, Papua New Guinea.

<sup>a</sup>n: percentage within genotype group in brackets.

**TABLE 2** *CYP2B6* c.516G>T genotype differences in the proportion of patients within the EFV therapeutic plasma concentration range among 112 PNG patients with HIV/AIDS receiving EFV 600 mg/day

**TABLE 3** Predictors of ln-transformed plasma EFV concentrations in 102 PNG patients with HIV/AIDS

Variable	Co-efficient estimate (95% CI)	p-value	Contribution <sup>a</sup> to model (R <sup>2</sup> )
Age (years)	0.0066 (−0.0089 to 0.022)	0.4	0.004
Male gender	0.19 (−0.16 to 0.55)	0.056	0.004
ln(Weight) (kg)	−0.75 (−1.5 to 0.021)	0.3	0.036
<i>CYP2B6</i> c.516G>T genotype		$3 \times 10^{-11}$	0.38
G/G	(reference genotype)		
G/T	0.61 (0.2 to 1.0)**		
T/T	1.7 (1.25 to 2.14)***,#		
(Intercept)	9.4 (6.1 to 12.6)		

Note: Co-efficient estimates are for ln-transformed plasma EFV concentrations.

Abbreviations: CI, confidence interval; EFV, efavirenz; PNG, Papua New Guinea.

<sup>a</sup>Averaging over orderings method. Post hoc \*\* $p < 0.01$  and \*\*\* $p < 0.001$  versus G/G genotype. # $p < 0.001$  versus G/T genotype.

Variable	Co-efficient estimate (95% CI)	p-value	Contribution <sup>a</sup> to model (R <sup>2</sup> )
Age (years)	−0.002 (−0.017 to 0.012)	0.72	0.003
Male gender	−0.26 (−0.6 to 0.071)	0.12	0.034
ln(Weight) (kg)	−0.97 (−1.7 to −0.25)	0.009	0.069
<i>CYP2B6</i> c.516G>T genotype		0.002	0.10
G/G	(reference genotype)		
G/T	0.44 (0.056 to 0.83)		
T/T	−0.17 (−0.6 to 0.26)**		
(Intercept)	9.3 (6.3 to 12.3)		

Note: Co-efficient estimates are for ln-transformed plasma 8OH-EFV concentrations.

Abbreviations: CI, confidence interval; EFV, efavirenz; PNG, Papua New Guinea.

<sup>a</sup>Averaging over orderings method. Post hoc \*\* $p < 0.01$  versus G/T genotype.

**TABLE 4** Predictors of ln-transformed plasma 8OH-EFV concentrations in 102 PNG patients with HIV/AIDS

occurrence of side effects; seven patients had concentrations below the lower limit of quantification for one or both analytes and were excluded in this analysis to prevent bias caused by possible medication nonadherence due to side effects. Fifty-one percent of patients reported one or more side effects. Overall, no significant ( $p > 0.05$ ) association between side effects and plasma 8OH-EFV concentrations or MR was found (Table 6).

When comparing plasma EFV concentrations between groups, a significant ( $p = 0.02$ ) difference was found between those with CNS and psychiatric side effects and those without (none). Median plasma EFV concentrations were higher in those patients that did not report any side effects (2011 ng/ml vs. 1140 ng/ml). No significant ( $p > 0.05$ ) association between side effects and treatment time was found.

**TABLE 5** Predictors of ln-transformed metabolic ratio (plasma 8OH-EFV/EFV) in 102 PNG patients with HIV/AIDS

Variable	Co-efficient estimate (95% CI)	p-value	Contribution <sup>a</sup> to model (R <sup>2</sup> )
Age (years)	−0.008 (−0.02 to 0.005)	0.2	0.01
Male gender	−0.4 (−0.7 to −0.1)	0.008	0.03
<i>CYP2B6</i> c.516G>T genotype		$2.2 \times 10^{-16}$	0.5
G/G	(reference genotype)		
G/T	−0.02 (−0.38 to 0.32)		
T/T	−1.63 (−2.02 to −1.24) <sup>***, #</sup>		
(Intercept)	−1.2 (−1.75 to −0.65)		

Note: Co-efficient estimates are for ln-transformed metabolic ratio.

Abbreviations: CI, confidence interval; EFV, efavirenz; PNG, Papua New Guinea.

<sup>a</sup>Averaging over orderings method. Post hoc <sup>\*\*\*</sup> $p < 0.001$  versus G/G genotype. <sup>#</sup> $p < 0.001$  versus G/T genotype.

**TABLE 6** Comparison of plasma EFV and 8OH-EFV concentrations and MRs between those without (none) and those with side effects in 149 PNG patients with HIV/AIDS receiving EFV 600 mg/day

Side effects	N (%)	EFV ng/ml, median (range)	8OH-EFV ng/ml, median (range)	MR, median (range)
None	73 (49)	2011 (95–13,212)	199 (42–935)	0.12 (0.01–1.25)
CNS only	53 (36)	1497 (508–7874)	214 (29–876)	0.16 (0.02–0.84)
Psychiatric only	8 (6)	1492 (1026–5293)	348 (58–837)	0.18 (0.04–0.82)
CNS and Psychiatric	15 (9)	1140 (272–12,952) <sup>a</sup>	136 (25–711)	0.1 (0.03–0.4)
Kruskal-Wallis <i>P</i>		0.02	0.06	0.3

Abbreviations: 8OH-EFV: 8-hydroxyefavirenz; CNS, central nervous system (tiredness, dizziness, drowsiness, insomnia, and impaired concentration); EFV, efavirenz; MR, metabolic ratio (8OH-EFV/EFV); PNG, Papua New Guinea.

<sup>a</sup>None versus CNS and Psychiatric post hoc  $p = 0.02$  (Dunn's multiple comparison test).

The incidence of side effects in patients with G/G, G/T, and T/T genotypes was 65% (17/26), 57% (29/51), and 41% (14/34), respectively. No statistically significant difference between genotypes per se and the occurrence of side effects was found ( $p = 0.15$ ). Kruskal-Wallis test simulations indicated that, for the expected higher concentrations in patients with side effects, the study had greater than 90% power ( $\alpha = 0.05$ ) to identify median plasma EFV concentrations 50% higher in CNS-only or psychiatric-only groups, and 100% higher in CNS and psychiatric combined group, compared to a median of 1500 ng/ml in the no side effect group. However, the study had only ~ 53% power to detect the observed side effect group differences in plasma EFV concentrations (Table 6), whereas  $\chi^2$  test simulations indicated 40% power to identify the observed genotype differences in side effect incidence as significant.

## DISCUSSION

The influence of genetic polymorphisms on EFV exposure, especially those genes encoding the enzymes involved in

EFV metabolism, has been extensively studied and documented in many different populations.<sup>8,11,21,25,26,39,40</sup> PNG has the highest incidence and prevalence of HIV infections in the Western Pacific Region.<sup>32</sup> Probably due to logistical and clinical reasons, the present study is the first to investigate the relationship between EFV pharmacogenetics and drug concentrations, as well as the influence of patient demographics and the occurrence of adverse events to drug concentrations, in a PNG HIV/AIDS population. In the present study, the frequency of the 516T variant allele was 53%, being consistent with previous findings for PNG.<sup>30,32</sup> The T allele frequency shows high variability between populations, ranging from 16 to 46% in Europeans, 16 to 33% in East Asians, and 35 to 45% in Sub-Saharan Africans.<sup>31,39</sup>

A statistically significant increase in EFV concentrations was found in homozygous carriers of the *CYP2B6* c.516G>T variant allele, with median concentrations being fivefold and approximately threefold higher than G/G and G/T, respectively. Previous studies have replicated the association between *CYP2B6* c.516G>T and plasma EFV concentrations, including studies of patients from South Africa,<sup>11</sup> Zimbabwe,<sup>40</sup> Ghana,<sup>39</sup> China,<sup>21</sup> Switzerland,<sup>13</sup>

Taiwan,<sup>41</sup> and Chile.<sup>42</sup> In our study, 23 patients had concentrations above 4000 ng/ml and all were homozygous for the variant allele, demonstrating the potential clinical relevance of the effect size of *CYP2B6* c.516G>T genotypes in PNG patients with HIV/AIDS.

Multiple linear regression analyses were conducted to examine contributions of genetic and demographic variables, such as age, gender, and body weight, to EFV concentrations. Previous studies showed contradictory results relating to the contribution of age, gender, and body weight to EFV disposition,<sup>18-20</sup> and, in our study, we could not establish a contribution of patient demographics to EFV disposition. *CYP2B6* c.516G>T genotype was the only significant explanatory variable for plasma EFV concentrations, explaining 38% of variability. The variability in EFV concentrations caused by genotypes was also reported in an AIDS Clinical Trials Group (ACTG) study, including White, Black, and Hispanic populations ( $n = 240$ ),<sup>43</sup> where the *CYP2B6* c.516G>T SNP explained 33% of variance. Part of the remaining variation in EFV concentrations in the PNG population might further be explained by other SNPs in *CYP2B6* as well as polymorphisms in other genes involved in EFV metabolism (discussed below).<sup>32,42,44</sup> Genotype was also the most significant contributor to variability in MR (50%).

Although *CYP2B6* is the main enzyme involved in the formation of 8OH-EFV, *CYP3A5*, *CYP1A2*, and *CYP2A6* also contribute to EFV metabolism, but to a lesser extent.<sup>1</sup> *CYP2B6* is also responsible for the further hydroxylation of 8OH-EFV to 8,14diOH-EFV,<sup>22</sup> possibly with other SNPs contributing to the variation in 8OH-EFV concentrations. EFV and all its three hydroxylated metabolites are subject to glucuronidation by UGT enzymes, which have SNPs associated with variation in plasma EFV concentrations.<sup>44</sup> These alternative metabolic pathways could potentially explain why the patients with T/T genotype in the present study had significantly lower MR but not lower plasma 8OH-EFV concentrations.

An important aspect to be considered in the present study is that patients were sampled at least 3 months, and up to 13 years, following initiation of treatment. EFV enhances its own metabolism by inducing the expression of *CYP2B6* through activation of the nuclear receptors NR1I3 and NR1I2.<sup>2,45</sup> Habtwold et al.<sup>46</sup> reported the long-term effect of EFV autoinduction, in which a 32% increase in median plasma 8OH-EFV concentration and a 20% decrease in MR (EFV divided by 8OH-EFV), equivalent of 20% increase in MR in our study ( $MR = 8OH-EFV/EFV$ ), was seen by week 16 of treatment compared with week 4. The same authors observed that the change in 8OH-EFV and MR over time was significant in women and in *CYP2B6\*1* and *UGT2B7\*1* carriers.<sup>46</sup> The extent of such autoinduction can also be influenced by different

sampling time-points (different sampling days) and the patient's genotype; for instance, greater EFV autoinduction in patients with *CYP2B6\*1* allele compared to \*6/\*6.<sup>47</sup>

Contrary to expectations, high plasma EFV concentrations were not associated with a higher incidence of side effects. We hypothesize that treatment duration (>3 months) could be a factor in the development of tolerance to the side effects. Two previous studies reported an association between high plasma EFV concentrations and toxicities at the beginning of treatment, but CNS symptoms did not persist beyond a few weeks.<sup>11,48</sup> Moreover, not all studies have confirmed the plasma EFV concentration and side effects association.<sup>12-15</sup> However, this does not explain why patients who reported toxicities had lower EFV concentrations, compared to the non side effects group (Table 6). This requires further investigation in particular as to whether nonadherence due to toxicities might be playing a role as adherence, including the number of doses missed in the last month, was not formally assessed in this study apart from the times of dosing for 3 days prior to blood sampling.

The relationship between *CYP2B6* c.516G>T genotypes and the occurrence of side effects has been investigated in many studies. Gallien et al.<sup>29</sup> reported the probability of the occurrence of CNS toxicity to be 16% higher in patients with c.516T/T genotype compared to G/G ( $p = 0.02$ ), although no association between EFV concentrations and side effects was found. However, in other studies, no association between *CYP2B6* c.516G>T genotype and increased risk of toxicities was established.<sup>8,40,49,50</sup> Vujkovic et al.<sup>28</sup> found an association between 516G>T genotypes and side effects, however, in their study the patients with G/G genotype reported adverse events more often than G/T or T/T ( $p = 0.041$ ). In the present study, although no statistical significance was found, the same pattern was observed. These discrepancies suggest that the relationship between EFV metabolism and side effects has more external factors than just known genetic metabolism factors.

There are several limitations to the present study. There was sufficient sample size to clearly demonstrate genotype effects on plasma EFV concentrations/MR, although the sample size for the reported psychiatric and CNS and psychiatric side effects groups was relatively small (complete information was not available for all patients, as well as insufficient sample for DNA extraction in all patients), resulting in limited power to detect significant associations between side effects and plasma concentrations or genotype.

Some of the participants (~5%) had comorbidities, including tuberculosis. In consideration of whether concomitant medications (Table 1) may interfere in data interpretation, one patient was receiving rifampicin



(HREZ combination; see Table 1), which has previously been reported to affect EFV concentrations.<sup>41</sup> However, the drug concentrations for this patient did not differ from the other patients' concentrations, and the plasma EFV concentration was within the therapeutic range. The other comedications (isoniazid, cotrimoxazole, and carbimazole) are not expected to influence EFV pharmacokinetics. For the assessment of adverse effects, a key consideration is the self-reporting of side effects, which has the potential for bias in the interpretation of these data. In addition, we only tested the 516G>T polymorphism based on it being the major SNP affecting EFV pharmacokinetics as all others studied in the PNG population<sup>32</sup> are either very rare or have no or modest effect on function.<sup>26</sup>

In conclusion, the relationship between *CYP2B6* c.516G>T genotype, drug concentrations, and adverse effects, in PNG HIV/AIDS patients was assessed for the first time. *CYP2B6* c.516G>T genotypes are a strong predictor of plasma EFV concentrations and MR, with the 516T/T genotype strongly associated with higher plasma efavirenz concentrations and lower MR. However, *CYP2B6* c.516T/T genotype effects on EFV disposition do not translate into increased incidence of side effects after the first 3 months of treatment in PNG patients with HIV/AIDS. Whether the high proportion of PNG *CYP2B6* c.516T/T genotype patients above the therapeutic range impacts adverse events in the early stages of treatment requires specific investigation.

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## CONFLICT OF INTEREST

The authors declared no competing interests for this work.

## AUTHOR CONTRIBUTIONS

N.B.A., H.K.V.S., D.B., A.S., P.P., and J.T wrote manuscript. N.B.A., A.S., H.K.V.S., P.P., J.T., and D.B. designed the research. N.B.A., J.T., P.P., and H.K.V.S. performed the research. N.B.A. and D.B. analyzed the data. J.T., P.P., and A.S. contributed new reagents/analytical tools.

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### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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