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Pharmacogenetic associations with plasma efavirenz concentrations and clinical correlates in a retrospective cohort of Ghanaian HIV-infected patients

Fred S. Sarfo[1], Yuan Zhang[2], Deirdre Egan[2], Lambert A. Tetteh[1], Richard Phillips[1,3], George Bedu-Addo[1,3], Maame Anima Sarfo[1], Saye Khoo[2], Andrew Owen[2] and David R. Chadwick[4]*

[1]*Department of Medicine, Komfo Anokye Teaching Hospital, Kumasi, Ghana;* [2]*Department of Molecular and Clinical Pharmacology, University of Liverpool, Liverpool, UK;* [3]*Kwame Nkrumah University of Science and Technology, School of Medical Sciences, Kumasi, Ghana;* [4]*Centre for Clinical Infection, The James Cook University Hospital, Middlesbrough, UK*

*Corresponding author. Tel: +44-1642-854429; Fax: +44-1642-854017; E-mail: davidr.chadwick@stees.nhs.uk

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Objectives: Efavirenz is widely used in first-line antiretroviral therapy in sub-Saharan Africa. However, exposure to efavirenz shows marked interindividual variability that is genetically mediated with potential for important pharmacodynamic consequences. The aims of this study were to assess the frequencies of *CYP2B6*, *CYP2A6*, *UGT2B7* and *CAR* single nucleotide polymorphisms (SNPs) and their impact on plasma efavirenz concentration and clinical/immunological responses in Ghanaian patients.

Methods: Genomic DNA from 800 HIV-infected patients was genotyped for selected SNPs by real-time PCR-based allelic discrimination. Mid-dose plasma efavirenz concentrations were measured for 521 patients using HPLC with UV detection. Clinical outcomes in 299 patients on efavirenz were retrospectively assessed. Univariate and multivariate linear regression were performed using best subset selection. Time-to-event outcomes were analysed using a Cox proportional hazards regression model.

Results: The variant allele frequencies for CYP2B6 516G>T (rs3745274), CYP2B6 983T>C (rs28399499), CYP2A6 −48T>G (CYP2B6*9B; rs28399433), UGT2B7 802C>T (UGT2B7*2; rs7439366), UGT2B7 735A>G (UGT2B7*1c; rs28365062) and CAR 540C>T (rs2307424) were 48%, 4%, 3%, 23%, 15% and 7%, respectively. CYP2B6 516G>T, CYP2B6 983T>C and CYP2A6 −48T>G were associated with significantly elevated efavirenz concentrations. A trend towards association between plasma efavirenz concentration and CAR 540C>T was observed. CYP2B6 516G homozygosity was associated with immunological failure [adjusted hazards ratio compared with T homozygosity, 1.70 (1.04–2.76); $P=0.03$].

Conclusions: *CYP2B6* and *CYP2A6* SNPs were associated with higher plasma efavirenz concentrations due to reduction in major and minor phase I routes of elimination, respectively. Further prospective studies are needed to validate the pharmacodynamic correlates of these polymorphisms in this population.

Keywords: non-nucleoside reverse transcriptase inhibitors, Africa, pharmacokinetics, pharmacodynamics

Introduction

Efavirenz is an essential component of the preferred non-nucleoside reverse transcriptase inhibitor regimens for initial treatment of HIV-1 infection in both industrialized[1] and developing[2] countries. Despite the proven potency and favourable tolerability of efavirenz-based regimens, interindividual variability in plasma efavirenz concentrations predisposes to the development of treatment-limiting toxicity or failure to achieve durable viral load suppression.[3,4] Efavirenz is administered orally as a single fixed dose of 600 mg in adults and undergoes phase I oxidative metabolism primarily by the hepatic CYP2B6 enzyme,[5] with minor contributions from CYP3A4 and CYP2A6. Subsequent phase II metabolism involves glucuronidation of oxidized efavirenz metabolites by the UGT2B7 enzyme.[6] The constitutive androstane receptor (CAR) is known to regulate the expression of many of the enzymes that contribute to efavirenz metabolism.[7–9] Therefore, genetic variation in the enzymes responsible for the efavirenz metabolism and transcription factors that regulate them, such as CAR, may partially explain the interindividual variability in plasma efavirenz concentrations.

It has become apparent that the profound interindividual differences in hepatic CYP2B6 expression and enzymatic activity may

result in variable systemic exposure and therapeutic response to the drugs metabolized by CYP2B6. Indeed, the *CYP2B6* gene is considerably polymorphic[10] and encodes the major enzyme for hepatic elimination of efavirenz and nevirapine. The allelic variant 516G>T (rs3745274) is more common in African Americans (TT 20%) than in Hispanics (6.7%) or Caucasians (3.4%) and has been reproducibly associated with slower clearance and ethnic difference in efavirenz plasma concentrations.[11] Another polymorphism (983T>C; rs28399499) within the *CYP2B6* gene has also been associated with efavirenz and nevirapine concentrations and the minor allele of this variant is more common among Ghanaians than among Caucasians or other African ethnicities.[12,13] The *CYP2B6* gene is regulated at the transcriptional level through complex interactions of nuclear factors such as CAR and pregnane X receptor. Therefore, differences in the expression or function of CAR are predicted to influence the expression/function of drug metabolism proteins and, therefore, the pharmacokinetics of their substrates. A variant polymorphism within the CAR gene has also been associated with higher efavirenz plasma concentrations[14,15] as well as early treatment discontinuation of efavirenz-based regimens.[14] Other studies have also indicated that polymorphisms within the *CYP2A6* and *UGT2B7* genes may influence efavirenz plasma concentrations.[16]

The aims of this study were to investigate the frequencies of *CYP2B6* 516G>T (rs3745274), *CYP2B6* 983T>C (rs28399499), *CYP2A6* −48T>G (CYP2B6*9B; rs28399433), *UGT2B7* 802C>T (UGT2B7*2; rs7439366), *UGT2B7* 735A>G (UGT2B7*1c; rs28365062) and *CAR* 540C>T (rs2307424) in a cohort of 800 Ghanaian HIV-infected patients. The impact of these genetic variants on plasma efavirenz concentrations was also assessed in a subset of 521 (65%, $n=800$) patients. Finally, we conducted a *post hoc* retrospective analysis to examine the clinical correlates of deaths/loss to follow-up, AIDS progression, immunological failure and documented CNS toxicity among a subset of 299 (57%, $n=521$) with complete pharmacogenetic, plasma efavirenz concentrations and clinical data.

Methods

Study patients

Eight hundred patients with confirmed HIV infection who had plasma and sera in a sample repository at the Komfo Anokye Teaching Hospital, Kumasi, Ghana were included to evaluate the frequencies of candidate single nucleotide polymorphisms (SNPs) within the Ghanaian population. In a subset of patients receiving efavirenz-based antiretroviral therapy ($n=521$) in the post-induction phase of therapy, with a median (range) duration of 12 (2–24) months on efavirenz, the impact of selected polymorphisms on steady-state mid-dose concentrations of plasma efavirenz was investigated. Patients included in the 'genetic' study were aged ≥15 years and a subset who were taking efavirenz-based antiretroviral therapy were included in the 'pharmacokinetic' study. To assess the clinical impact of the pharmacogenetic data, a retrospective cohort data analysis was performed for patients by reviewing their medical charts.

Pharmacokinetic sampling

Mid-dose sampling is frequently used in clinical studies of efavirenz disposition for patient convenience, as the drug is invariably taken at bedtime to minimize CNS side effects during the day.[3] Mid-dose blood samples were obtained, typically between 08:00 and 13:00 h. Plasma and sera were separated by centrifugation at 4°C and have previously been successfully used for determining the impact of genetics on plasma efavirenz concentrations.[17,18] Aliquots were collected and stored at −20°C. Samples were shipped from Ghana to the Department of Molecular and Clinical Pharmacology at the University of Liverpool, UK on dry ice for subsequent analyses.

Quantification of plasma efavirenz concentration

Plasma concentrations of efavirenz were measured using reverse-phase HPLC with UV detection of efavirenz at 250 nm by a validated assay.[13] The laboratory at the University of Liverpool participates in an external international quality assurance scheme (KKGT, The Netherlands).

Genotyping of CYP2B6, CYP2A6, UGT2B7 and CAR SNPs

Genomic DNA was extracted from the serum at the University of Liverpool using standard methodology. Pre-amplification for exon 4 and exons 7 and 8 (combined) was first conducted to discriminate from the *CYP2B6* pseudogene (*CYP2B7*). Genotyping for 516G>T and 983T>C was then performed on the resultant amplicons by real-time PCR allelic discrimination. Genotypes for *CYP2A6* −48T>G (CYP2B6*9B; rs28399433), *UGT2B7* 802C>T (UGT2B7*2; rs7439366), *UGT2B7* 735A>G (UGT2B7*1c; rs28365062) and *CAR* 540C>T (rs2307424) were determined by real-time PCR using standard methodology (95°C for 15 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min) in a DNA Engine Opticon® 2 system (MJ Research Inc., USA). The full PCR conditions as well as primer and probe sequences are available upon request.

Retrospective analysis of clinical outcomes

The medical records of 299 patients with plasma efavirenz concentration and genotype data were retrieved and assessed for the following clinical events: documented neuropsychiatric toxicity, treatment discontinuation due to efavirenz-associated toxicity, immunological failure, WHO-defined clinical failure, death and loss to follow-up. Attrition was defined as either confirmed death of a patient or loss to follow-up for >6 months from the date of the last scheduled visit. Neuropsychiatric toxicity was defined by the treating clinician as any CNS toxicity attributable to efavirenz. All antiretroviral therapy-related toxicity and treatment-limiting toxicity are routinely documented in patients' medical records. Clinical failure was defined as the development of new or recurrent WHO-defined stage 3 or 4 opportunistic disease whilst on antiretroviral therapy.[19] Immunological failure was defined according to WHO criteria as failure to achieve an increase in CD4 counts by >100 CD4 cells/mm^3 after 1 year of therapy or a decline of >50% of the peak CD4 count on antiretroviral therapy.[19] The HIV viral load was not routinely tested in the clinic. The baseline variables collected included CD4 counts, WHO clinical stage, weight and body mass index (BMI).

Statistical analyses

The concentration of efavirenz was log transformed to achieve normality and equal variance. A χ^2 test was employed to assess whether the observed and expected genotype frequencies were in Hardy–Weinberg equilibrium. Bivariate statistical analyses examining the effects of patient demographics and genotypes on efavirenz mid-dose concentrations were assessed by an unpaired Student's *t*-test (for gender and for two genotype groups), analysis of variance (ANOVA) (for three genotype groups) or simple linear regression (continuous data such as age, body weight, height and BMI) using Pearson's r correlation coefficient. A multivariate model was then constructed using patient demographic, anthropometric and genotype data as independent variables and efavirenz concentrations as the dependent variable. Dichotomous variables, including gender, *CYP2B6* 983T>C, *CYP2A6* −48T>G and *CAR* 540C>T genotypes were coded as 0 or 1 while *CYP2B6* 516G>T, *UGT2B7* 735A>G and *UGT2B7* 802C>T were coded as 0, 1 and 2 for patients homozygous for the common allele, heterozygous or

homozygous for the variant allele, respectively. Age, weight and height were included as continuous variables. Thus, rare genotypes (defined by <3 patients in the total sample population) were treated as one group along with the heterozygotes in these analyses (carrier/non-carrier analysis). A multiple linear regression using best subset selection was then performed with stepwise removal of non-significant variables until all remaining independent variables had coefficients with P values <0.05.

A Cox proportional hazards multivariate regression analysis was conducted to assess the impact of plasma efavirenz concentration and selected SNPs on time-to-event clinical outcomes, namely neuropsychiatric toxicity, immunological failure and clinical failure defined as a composite of death, loss to follow-up and AIDS progression on antiretroviral therapy. The data were censored at the date of event of interest or at 31 December 2011 for the remainder. For this analysis, any factor reaching a 10% level of significance on univariate analysis was included in the final multivariable model. No adjustments were made for multiple comparisons. All statistical analyses were performed using SPSS version 17 and GraphPad Prism 4.

Ethics approval

Ethics permission for this study was given by the Committee on Human Research Publications and Ethics of the Kwame Nkrumah University of Science and Technology and the Komfo Anokye Teaching Hospital, Kumasi, Ghana (CHRPE44/09).

Results

Demographic and anthropometric data

Eight hundred patients were sampled for the pharmacogenetic study, whilst 521 and 299 were included in the efavirenz pharmacokinetic and pharmacodynamic studies, respectively, as shown in Figure 1. Of the 800 patients, the median (range) age of the patients sampled was 40 (17–68) years, with a female-to-male ratio of 2:1. The mean (±SEM) BMI of the males [28.1 (±0.46) kg/m^2] was significantly different from that of the females [25.9 (±0.36) kg/m^2; P<0.0002]. Some 578 (72.3%) patients were on antiretroviral therapy, while 222 (27.8%) were antiretroviral therapy naive at the time of sampling. Of those on antiretroviral therapy, 521 were on efavirenz-based therapy, 56 were on nevirapine-based therapy and 1 was on nelfinavir-based therapy; 277 (47.9%) were on zidovudine plus lamivudine, 300 (51.9%) on stavudine plus lamivudine and 1 on a didanosine plus lamivudine nucleoside backbone.

Frequencies of genetic polymorphisms in efavirenz metabolizing enzymes

Of the 800 samples from which genomic DNA was extracted, genotyping was successful in 705 (88%) for *CYP2B6* 516 G>T, 701 (88%) for *CYP2B6* 983 T>C, 678 (85%) for *CYP2A6* −48T>G, 704 (88%) for *UGT2B7* 735A>G, 697 (87%) for *UGT2B7* 802C>T and 695 (87%) for *CAR* 540C>T, respectively (Figure 1). When a χ^2 test of observed versus predicted genotype frequencies was conducted, all polymorphisms were found to be in Hardy–Weinberg equilibrium. The allele and genotype frequencies of the SNPs analysed in this study are shown in Table 1.

Mid-dose plasma efavirenz concentrations

Five hundred and twenty-one patients on efavirenz-containing antiretroviral therapy had mid-dose efavirenz concentrations determined. The median (IQR) concentration of plasma efavirenz was not significantly different in males [1090 (533.3–2173) ng/mL] compared with females [1083 (559.9–2102) ng/mL]. Some (46%) had mid-dose efavirenz concentrations below the proposed minimum effective concentration (<1000 ng/mL), 44% were within the proposed therapeutic range (1000–4000 ng/mL) and 10% had concentrations of efavirenz above the proposed maximum tolerated concentration (>4000 ng/mL). The median (IQR) concentration of plasma efavirenz in patients on zidovudine plus lamivudine [1094 (546–2188) ng/mL] was not significantly different from that in patients on stavudine plus lamivudine [1193 (641–2350) ng/mL; P>0.05].

Selected SNPs and impact on plasma efavirenz concentrations

The impact of each selected SNP on the mid-dose plasma concentration of efavirenz is depicted in Figure 2(a–f). The median concentration of efavirenz was significantly higher in individuals homozygous for the variant allele (TT) of *CYP2B6* at position 516 [TT (n=133): 1800 versus 1073 and 929 ng/mL for GT (n=235) and GG (n=128) individuals, respectively; P<0.0001]. Similarly, the concentration of efavirenz was significantly higher in individuals homozygous or heterozygous for the variant allele (CC) of *CYP2B6* at position 983 [CC (n=1) and TC (n=42): 3235 versus 1053 ng/mL for TT (n=451) individuals; P<0.0001]. Also, the concentrations of efavirenz were significantly higher in individuals homozygous or heterozygous for the variant allele (GG) of *CYP2A6* at position −48 [GG (n=1) and GT (n=37): 2192 versus 1093 ng/mL for TT (n=435) individuals; P<0.0001]. However, the concentrations of efavirenz were not significantly different in individuals homozygous or heterozygous for the variant allele (GG) of *UGT2B7* at position 735 [GG (n=14) and AG (n=117): 802.9 versus 1160 ng/mL] and for normal AA individuals (n=367: 1107 ng/mL; P=0.84). Concentrations of efavirenz were not significantly different in individuals homozygous or heterozygous for the variant allele (TT) of *UGT2B7* at position 802 [TT (n=37) and CT (n=186): 823.3 versus 1034 ng/mL] and for normal CC individuals (n=273: 1172 ng/mL; P=0.67). Finally, the concentration of efavirenz of 1001 ng/mL was not significantly different in individuals either homozygous or heterozygous for the variant allele of *CAR* at position 540 [TT (n=2) and CT (n=82)] and for normal CC individuals (n=416: 1130 ng/mL; P=0.3).

Multivariate analysis

The median (range) plasma concentrations of efavirenz were 1087 (110.0–12146.0 ng/mL) and the mean±SD was 1792±2024 ng/mL with a coefficient of variation of 113%. In univariate analyses, polymorphisms in *CYP2B6* 516G>T (P<0.0001), *CYP2B6* 983T>C (P<0.0001), *CYP2A6* −48T>G (P=0.002) and body weight in kilograms (P=0.008) were all significantly correlated with log-transformed plasma efavirenz concentrations. In multivariate analysis, *CYP2B6* 516G>T (P<0.0001), *CYP2B6* 983T>C (P<0.0001) and body weight (P=0.016) were identified as independently associated with efavirenz exposure, as shown in Table 2. The *CAR* 540C>T polymorphism was marginally significant (P=0.07), while increasing weight was inversely correlated with efavirenz exposure.

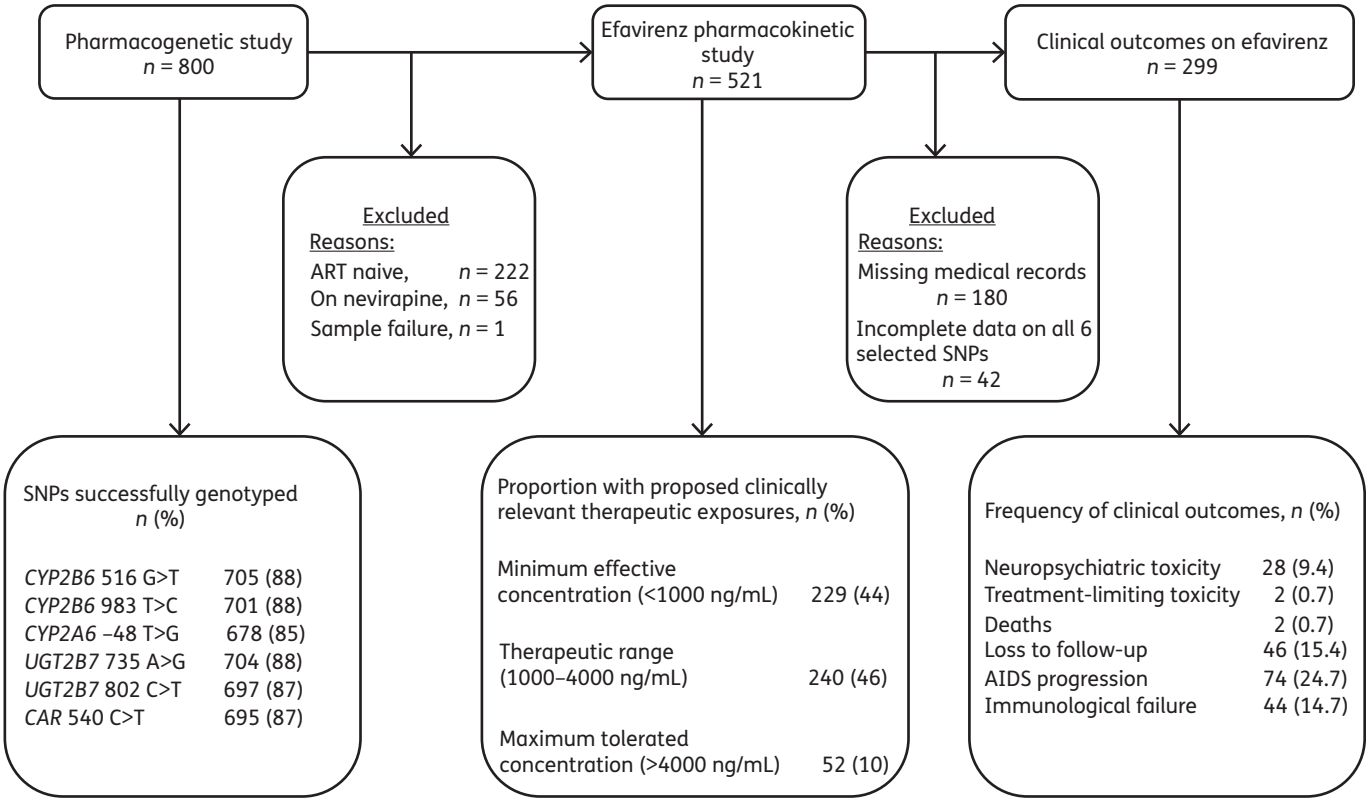

Figure 1. Description and main outcome variables of the study population. ART, antiretroviral therapy.

Table 1. Genotype and allele frequencies of selected SNPs of enzymes involved in metabolism of efavirenz

SNP	Genotype			Allele, *n* (%)	
CYP2B6 516G>T	GG	GT	TT	G	T
	208	320	177	730 (52)	674 (48)
CYP2B6 983T>C	TT	TC	CC	T	C
	639	61	1	1339 (96)	63 (4)
CYP2A6 −48T>G	TT	TG	GG	T	G
	635	42	1	1312 (97)	44 (3)
UGT2B7 735A>G	AA	AG	GG	A	G
	507	172	25	1186 (85)	222 (15)
UGT2B7 802C>T	CC	CT	TT	C	T
	391	287	19	1069 (77)	325 (23)
CAR 540C>T	CC	CT	TT	C	T
	602	89	4	1293 (93)	97 (7)

Associations between plasma efavirenz concentrations, pharmacogenetics and clinical outcomes

The medical records of 341 patients receiving efavirenz were available, but 299 patients were evaluable because they had data on all selected SNPs as well as plasma efavirenz concentrations and were analysed for clinical events. The medical records of 180 patients from the efavirenz pharmacokinetic study were missing. The median (IQR) age of this subset of patients of 40 (33–47) years and the male-to-female ratio of 1 : 2 were not significantly different from either the cohort of 521 who were on efavirenz or the 800 patients involved in the 'genetic' study. The median (IQR) CD4 count at the initiation of antiretroviral therapy was 123 (55–188) cells/mm^3 and 84% had WHO AIDS-defining conditions.

Risk of neurotoxicity

Twenty-eight out of 299 (9.4%) had documented neuropsychiatric toxicities. The median (range) time to the first episode of neuropsychiatric toxicity was 2 months (2–84 months). The following were reported: 10 had insomnia, 5 had severe headaches, 5 had vivid nightmares, 2 had drowsiness, 2 had ataxia and there was 1 case each of dystonia and dizziness. On univariate and multivariate analyses, low baseline CD4 count was significantly associated with CNS toxicity, as shown in Table 3. Neither efavirenz concentration nor any of the selected SNPs were significantly associated with documented CNS toxicity, although the *CYP2B6* 516G>T variants showed a trend towards higher risk. Only two patients with documented neuropsychiatric toxicity had efavirenz substituted for nevirapine.

Risk of immunological failure

Forty-four out of 299 (14.7%) had immunological failure over a median follow-up of 66 months (range 2–90 months). On adjusted analysis, immunological failure was significantly associated with the GG genotype of *CYP2B6* 516G>T compared with the TT genotype hazards ratio of 1.70 (1.04–2.76; $P=0.03$), as

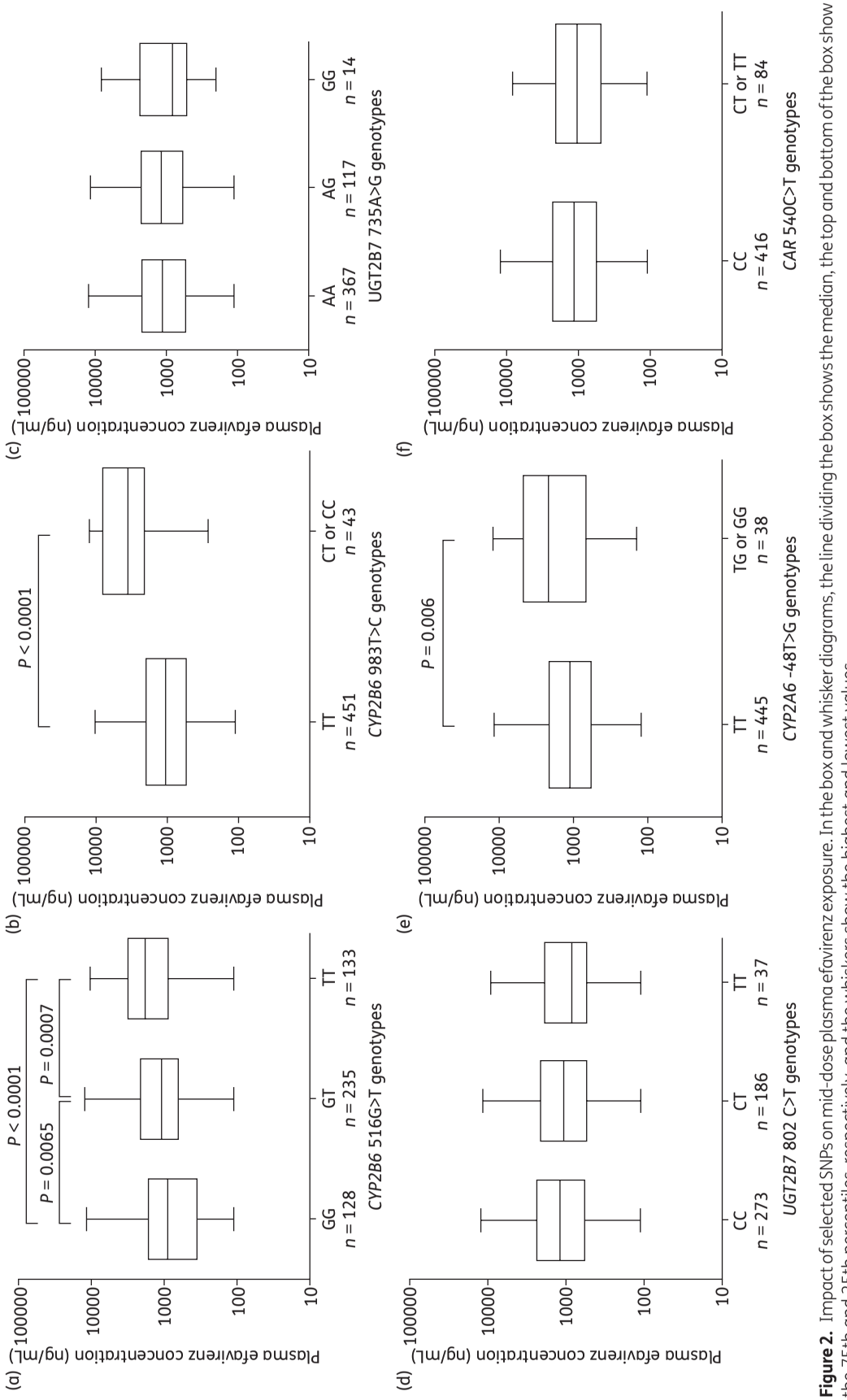

Figure 2. Impact of selected SNPs on mid-dose plasma efavirenz exposure. In the box and whisker diagrams, the line dividing the box shows the median, the top and bottom of the box show the 75th and 25th percentiles, respectively, and the whiskers show the highest and lowest values.

Table 2. Univariate and multivariate analyses of the effects of different variables on the log concentration of efavirenz

Covariate	Univariate probability	Multivariate probability	Unstandardized coefficient (95% CI)	Standardized coefficient (β)
CYP2B6 516G>T	2.5×10^{-7}	1.4×10^{-11}	0.43 (0.31–0.55)	0.32
CYP2B6 983T>C	4.5×10^{-14}	1.3×10^{-15}	1.31 (1.00–1.62)	0.38
CYP2A6 −48T>G	0.002	0.60		—
UGT2B7 735A>G	0.84	—		—
UGT2B7 802C>T	0.24	—		—
CAR 540C>T	0.12	0.07	0.22 (0.02–0.45)	0.08
Age	0.23	—		—
Gender	0.84	—		—
Weight	0.008	0.016	−0.01 (−0.02–0)	−0.11
Height	0.45	—		—

Table 3. Cox proportional hazards regression analysis of selected SNPs and baseline patient characteristics on risk of CNS toxicity

Covariate	Unadjusted hazards ratio (95% CI)	*P* value	Adjusted hazards ratio	*P* value
CYP2B6 516G>T				
GT or TT	2.12 (0.73–6.10)	0.16	—	—
GG	1.00			
CYP2B6 983T>C				
CT or CC	0.44 (0.06–3.25)	0.42	—	—
TT	1.00			
CYP2A6 −48T>G				
GT or GG	0.57 (0.08–4.21)	0.58	—	—
TT	1.00			
CAR 540C>T				
TC/TT	1.0 (0.38–2.63)	1.00	—	—
CC	1.00			
UGT2B7 735A>G				
GA/GG	1.08 (0.48–2.46)	0.85	—	—
AA	1.00			
UGT2B7 802C>T				
TC/TT	1.33 (0.63–2.80)	0.45	—	—
CC	1.00			
Gender				
male	1.25 (0.59–2.68)	0.56	—	—
female	1.00			
Age (per 10 year increase)	1.16 (0.80–1.69)	0.43	—	—
WHO clinical stage				
stage 3 or 4	0.54 (0.23–1.27)	0.16	—	—
stage 1 or 2	1.00			
Baseline CD4 count (per 50 cells/mm^3 increase)	0.74 (0.57–0.96)	0.02	0.74 (0.57–0.96)	0.02

were adherence <95% and low baseline CD4 counts, as shown in Table 4. Furthermore, as depicted in the Kaplan–Meier survival analysis in Figure 3, the risk of immunological failure was highest for patients with plasma efavirenz exposure below the proposed minimum effective concentration of 1000 ng/mL; the converse was observed for those above the maximum tolerated concentration of >4000 ng/mL.

Risk of clinical failure

Attrition and disease progression is a composite endpoint of patients who died ($n=2$), who were lost to follow-up ($n=46$) and who developed AIDS-defining events during follow-up ($n=74$). There were a total of 102 events (in 34.1% of patients studied). Neither the selected SNPs nor the plasma efavirenz concentration predicted clinical failure in univariate analyses.

Discussion

This study constitutes the largest study to date to assess the frequencies of selected polymorphisms in three enzymes involved in the phase I and II metabolism of efavirenz—*CYP2B6*, *CYP2A6* and *UGT2B7*—and one nuclear receptor (*CAR*, which modulates *CYP2B6* expression) amongst HIV-infected Ghanaian patients. In this study population of 800 patients, between 85% and 88% of selected SNPs were successfully genotyped. Five hundred and twenty-one patients were receiving efavirenz with an interindividual coefficient of variance of 113% corroborating the well-established wide variance in steady-state plasma efavirenz concentrations.[3,4]

CYP2B6 is highly polymorphic with >100 SNPs described, numerous complex haplotypes and different frequencies in different populations.[20] However, evidence from several studies shows that the 516G>T and 983T>C polymorphisms are most strongly associated with efavirenz pharmacokinetics.[21,22] In the present study involving only patients of black ethnicity, the frequency of *CYP2B6* 516G>T homozygosity was 25% and comparable to the 23% found among a South African cohort,[23] 20% amongst an African American cohort[5] and 19% in another Ghanaian cohort.[17]

Table 4. Cox proportional hazards regression analysis of selected SNPs, plasma efavirenz exposure and baseline patient characteristics on risk of immunological failure

Covariate	Unadjusted hazards ratio (95% CI)	*P* value	Adjusted hazards ratio	*P* value
CYP2B6 516G>T				
GG	1.53 (0.97–2.42)	0.07	1.70 (1.04–2.76)	0.03
GT	2.08 (0.88–4.93)	0.10	5.11 (0.66–39.50)	0.12
TT	1.00		1	
CYP2B6 983T>C				
TT	0.88 (0.31–2.48)	0.81	—	—
TC/CC	1.00			
CYP2A6 −48T>G				
GT or GG	1.23 (0.30–5.09)	0.70	—	—
TT	1.00			
CAR 540C>T				
CC	0.64 (0.31–1.36)	0.25	—	—
CT/TT	1.00			
UGT2B7 735A>G				
GA/GG	1.06 (0.53–2.13)	0.87	—	—
AA	1.00			
UGT2B7 802C>T				
TC/TT	0.84 (0.45–1.58)	0.59	—	—
CC	1.00			
Plasma efavirenz concentration				
<1000 ng/mL	1.34 (0.99–1.82)	0.06	1.57 (0.83–2.97)	0.17
≥1000 ng/mL	1.00		1	
Adherence to cART				
≤95%	2.30 (1.25–4.24)	0.008	2.19 (1.16–4.12)	0.02
>95%	1.00		1	
Gender				
female	0.74 (0.40–1.36)	0.34	—	—
male	1.00			
WHO clinical stage				
stage 3 or 4	1.13 (0.47–2.68)	0.79	—	—
stage 1 or 2	1.00			
Baseline CD4 count (per 50 cells/mm^3 increase)	0.66 (0.53–0.82)	0.0002	0.65 (0.52–0.82)	0.0002

cART, combination antiretroviral therapy.

Similarly, the allelic frequency of *CYP2B6* 983T>C homozygosity was comparable to that observed in another Ghanaian cohort.[18] Only one patient was homozygous for the C allele for 983T>C. Thus, the minor allele frequency of 0.04 in the 983T>C SNP in our study closely agrees with that found by Kwara *et al.*[18] among Ghanaians and the 0.045 among West Africans reported by Mehlotra *et al.*[12]

In vitro data have shown that *CAR* is able to transcriptionally regulate the *CYP2B6* gene[7] and, in liver tissue from patients, *CAR* and *CYP2B6* are strongly correlated.[8,9] Therefore, interpatient variability in *CAR* expression or activity is likely to impact *CYP2B6* expression, activity and variability in plasma exposure to *CYP2B6* substrates. Until recently, no studies had assessed the contribution of *CAR* 540C>T in efavirenz steady-state exposure, particularly among West Africans. In a German cohort where 25.5% (n=373) were of black ethnicity, the variant allele of *CAR* 540C>T was present at a frequency of 0.15 among blacks compared with 0.33 among whites.[14] Thus, the minor allele frequency in the population of 0.07 in the present study shows that this SNP is less common in Ghana. The minor allele frequency of the *CYP2A6* −48T>G polymorphism of 0.03 is comparable to the 0.05 found in another Ghanaian study.[18] Hydroxylated metabolites of efavirenz are subsequently glucuronidated to form an *N*-glucuronide of hydroxyefavirenz via UGT2B7.[6] Kwara *et al.*[18] reported *UGT2B7* 802C>T and *UGT2B7* 735A>G as important determinants of efavirenz mid-dose plasma concentrations. The minor allele frequencies of these variants were 0.26 and 0.46 among Ghanaians,[18] while 0.15 and 0.23, respectively, were found in the present study.

As expected, *CYP2B6* 516G>T and 983T>C variant allele carriers (Figure 2a and b) were associated with higher efavirenz mid-dose concentrations, as has been noted in several other studies across several ethnicities.[11,21,22,24–27] It was also found that variant allele carriers (GT/GG) of *CYP2A6* −48T>G had a 2-fold higher median concentration of efavirenz compared with homozygotes for the T allele (P=0.006). This study is the first to examine the impact of variants of the nuclear receptor *CAR* on efavirenz exposure among Ghanaian patients and shows overall (Figure 2f) that the 540C>T variant did not significantly influence efavirenz exposure in this population. However, it should be noted that a trend was observed and that this variant was rare in this population. The precise mechanistic role of this variant remains to be elucidated. Kwara *et al.*[18] were the first to report on the effect of *UGT2B7* variants on plasma efavirenz exposure and noted that while carriers of the 735A>G variant had higher efavirenz concentrations, 802C>T variant carriers were exposed to lower concentrations of efavirenz compared with individuals homozygous for the common allele. We could not confirm this association in our study population. It should be noted that the sample size in the present study is larger than that in the previous study. Nonetheless, further work is required to resolve the impact of *UGT2B7* variants on efavirenz exposure in other populations.

We also evaluated three clinically relevant outcome measures of efavirenz-based antiretroviral therapy within a resource-constrained setting in relation to the selected SNPs and plasma efavirenz exposure. We found that only 9.4% had documented neuropsychiatric toxicity on efavirenz, of which only 2 led to discontinuations. The low rates of discontinuation in this cohort could mean that patients tolerated neuropsychiatric toxicity associated with efavirenz with long-term usage. A lower rate of early treatment discontinuation has previously been reported in black patients compared with white patients, despite the higher plasma efavirenz concentrations.[14] Patients with advanced baseline immunosuppression were significantly more likely to experience CNS toxicity whilst those with the *CYP2B6* 516G>T SNP demonstrated a trend towards this toxicity. This would support the notion that *CYP2B6* SNPs predisposing to higher plasma efavirenz exposure as well as advanced HIV disease are important determinants of the risk of CNS toxicity in this population. An interesting trend was observed between *CYP2B6* 516G>T and the risk of immunological failure, a commonly used outcome measure of treatment effectiveness in settings where virological monitoring is not routine. It is notable that compared with the TT homozygotes, individuals homozygous for the G allele of *CYP2B6* 516G>T were at 70% higher risk of immunological failure (P=0.03) on adjusted

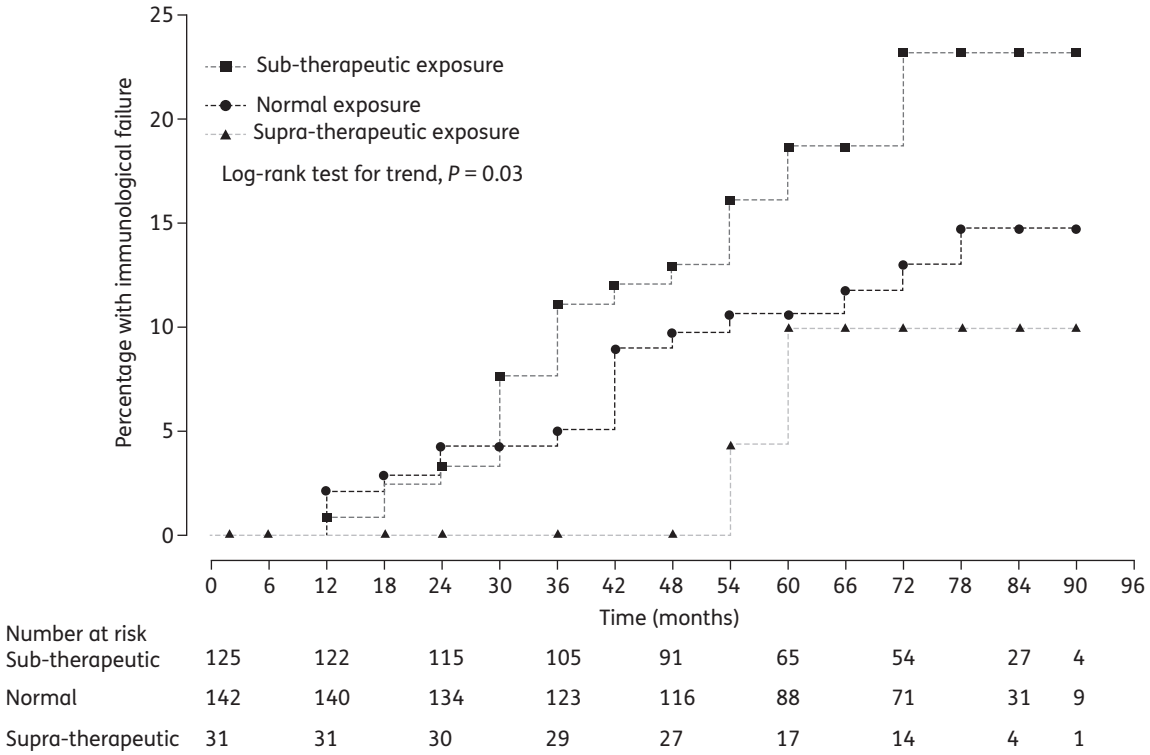

Figure 3. Kaplan–Meier survival analysis of risk of immunological failure on efavirenz-based antiretroviral therapy according to plasma exposure of efavirenz. Sub-therapeutic, therapeutic and supra-therapeutic plasma exposures refer to plasma efavirenz concentrations of <1000 ng/mL, between 1000 and 4000 ng/mL, and >4000 ng/mL, respectively.

analysis (Table 4), suggesting a potential protective effect of the TT genotype over the long term. Indeed, Ribaudo *et al*.[28] have reported that one of the pharmacodynamic effects of the composite 516/983 genotypes of *CYP2B6* among black patients is a decreased virological failure rate. Furthermore, the subtherapeutic concentrations of efavirenz observed in up to 46% of the study participants were associated with an increased risk of immunological failure over the long term (Figure 3). Our findings suggest pharmacogenetics as an important predictor of efavirenz pharmacodynamics via pharmacokinetic exposure among this cohort of Ghanaian HIV-infected patients.

Despite these observations, evidence that genotyping and measurement of efavirenz plasma concentrations actually improve patient outcome is lacking. For example, it has been recently shown among HIV-positive Haitians that the likelihood of virological response at 48 weeks was not predicted by 49 *CYP2B6* haplotypes known to predict plasma efavirenz concentrations,[29] whilst in another cohort from British Columbia, early discontinuation of efavirenz was not associated with variants in *CYP2A6*, *CYP2B6* and *CYP3A4*.[30]

The following limitations should be noted. Samples for this study were collected from a repository without assessing patients' adherence or other clinical events at the time of sample collection and the time from last dose of efavirenz was not known. Therefore, adherence was assessed retrospectively by patient self-reports and pill counts, which is not ideal but has been shown to be reasonably reliable for predicting treatment response.[31,32] Between 12% and 15% of the participants could not be successfully genotyped because insufficient genomic DNA was isolated from serum samples. Whole blood samples provide better yields of genomic DNA and this should be considered in future studies. Missing data were therefore missing completely at random. The clinical correlates were analysed *post hoc* in a retrospective manner and subject to confounding, which we attempted to minimize by performing adjusted analysis; however, data on other potential confounders, such as additional medications patients were taking, were not available. Thus, the clinical correlations presented are best viewed as exploratory and associational but not causational. Finally, due to collinearity between weight and gender, it is not possible to be absolutely certain which of these factors predominantly drive the association with efavirenz pharmacokinetics.

In conclusion, this study shows a high frequency of loss-of-function polymorphisms among Ghanaian HIV-infected patients and association with high exposure to efavirenz. SNPs in *UGT2B7* and *CAR* appear to be less important determinants of efavirenz exposure among Ghanaians.

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Transparency declarations

None to declare.

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