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ORIGINAL RESEARCH

Influence of *SULT1A1**2* Polymorphism on Plasma Efavirenz Concentration in Thai HIV-1 Patients

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Purpose: Plasma efavirenz (EFV) concentrations within therapeutic levels are essential to successfully treat patients suffering from human immunodeficiency virus (HIV) type 1. In addition to the drug-metabolizing enzyme CYP2B6, other phase II drug-metabolizing enzymes and transporters may have an important role in the pharmacokinetics of EFV. Thus, the influence of phase II drug-metabolizing enzymes and drug transporters on plasma EFV levels was investigated in Thai HIV patients receiving EFV.

Patients and Methods: Genotyping was performed by TaqMan® real-time PCR in 149 HIV-infected Thai adults, and plasma efavirenz concentration was measured by a validated high-performance liquid chromatography in 12 hours after dosing steady-state plasma samples at week 12 and 24.

Results: Patients with three or more copies of *SULT1A1* had significantly lower median plasma EFV concentrations than those carrying two copies at week 12 (p=0.046) and *SULT1A1*2* (c.638G>A) carriers had significantly lower median plasma EFV concentrations compared to those not carrying the variant at week 24 (p=0.048). However, no significant association was found after adjusting for *CYP2B6* genotype.

Conclusion: Genetic variation in a combination of *SULT1A1*2* and *SULT1A1* copy number may contribute to variability in EFV metabolism and thereby may impact drug response. The influence of a combination between the *SULT1A1* and *CYP2B6* genotype on EFV pharmacokinetics should be further investigated in a larger study population.

Keywords: phase II drug-metabolizing enzymes, transporter genes, efavirenz, HIV-1, Thai

Introduction

Human immunodeficiency virus type 1 infection (from here on forward referred to "HIV") is a major global health problem including Thailand. Co-infections with other viruses including hepatitis B are also common.[1] Efavirenz (EFV), a non-nucleoside reverse transcriptase inhibitor (NNRTI) is a mainstay component in highly active antiretroviral therapy (HAART). EFV is combined with Truvada, which consists of tenofovir and emtricitabine; this triple combination provides the principal HAART in a single, once a day tablet to effectively suppress HIV replication in the majority of patients.[2] This drug combination has been approved by the US Food and Drug Administration (FDA) in July 2006 under the brand name Atripla and is listed as one of the most important medications needed in basic health systems in the Essential Medicines List issued by the World Health Organization. The preferred therapeutic range of EFV plasma concentrations is 1–4 mg/L. Plasma concentrations below 1 mg/L have been associated with virological failure,[3–8] effectively causing a patient to have "treatment failure on an EFV-based

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regimen".[6] In contrast, plasma concentrations >4 mg/L have been associated with a higher risk of central nervous system (CNS) side effects,[6,8] as well as CNS toxicity that often causes patients to discontinue therapy.[3,4,6,7,9–13]

CYP2B6 plays a major role in EFV metabolism.[14] Several previous studies, including our cohort, have reported the influence of CYP2B6 enzyme on EFV pharmacokinetics. Increased plasma EFV concentrations were associated with *CYP2B6* genotype in HIV patients.[12,15,16] A few studies to date have investigated only CYP2B6 and plasma EFV concentrations, however, other enzymes such as CYP3A4/5, CYP1A2 and CYP2A6 in minor pathways[17,18] are still overlooked. Furthermore, EFV is directly conjugated with glucuronic acid via UDP-glucuronosyltransferase (UGT) 2B7 into EFV-*N*-glucuronide, and hydroxyefavirenz metabolites can be further glucuronidated by UGTs shunting them towards urinary excretion.[19–21] In addition, an association between single nucleotide polymorphisms (SNPs) in the *ABCC4* c.3348A>G (rs1751034), c.912G>T (rs2274407) and EFV kinetics was reported.[22] This observation was consistent with an in vitro study describing an association of *ABCC4* c.559G>T (rs11568658) and c.1460A>G (rs11568668) and intracellular accumulation of azidothymidine (AZT).[23] Finally, Belanger et al showed that UGT2B7 can directly conjugate EFV to EFV-N-glucuronide (EFV-G),[21] and that a synonymous *UGT2B7* c.735A>G (rs28365062) SNP that is part of some *UGT2B7*1* sub-alleles as well as the *UGT2B7*4* allele was associated with increased EFV concentrations[24] contrasting other investigations.[25–27] *SULT1A1* has also been reported to influence EFV metabolism.[24] It has never been investigated, however, whether *SULT1A1* and/or *UGT2B17* gene copy number variation (CNV) impact EFV concentrations.

Because there is sparse or no data regarding the impact of phase II drug-metabolizing enzymes and drug transporters on EFV metabolism. We aimed to investigate the influence of sequence variations and copy number in these genes on plasma efavirenz concentrations in Thai HIV-infected adult patients.

Materials and Methods

Patient Samples

One hundred and forty-nine HIV-infected adult Thai patients receiving EFV were recruited from the outpatient unit of the Bamrasnaradura Infectious Diseases Institute, Ministry of Public Health and Nonthaburi, Thailand. All subjects provided written informed consent to participate in the study in accordance with the guidelines of the Declaration of Helsinki. This study was approved by the Ethics Committee of the Faculty of Medicine Ramathibodi Hospital, Mahidol University, Thailand. Enrolled subjects for the study were over 18 years, had no opportunistic infection and receiving efavirenz 600 mg, tenofovir 300 mg, lamivudine 300 mg at bedtime. Mid-dose efavirenz plasma concentration was measured at 12 and 24 weeks following initiation of antiretroviral therapy. Patients receiving concomitant treatments that could potentially affect efavirenz pharmacokinetics were excluded. And the *CYP2B6* genotype results from our previous studies,[15,28,29] which consisted of **1/*1* (52, 34.9%), **1/*2* (11, 7.4%), **1/*4* (3, 2.0%), **1/*6* (61, 40.9%), **2/*4* (2, 1.3%), **2/*6* (2, 1.3%), **4/*4* (5, 3.4%), **5/*6* (2, 1.3%), **6/*6* (11, 7.4%), were used for calculation in multiple regression analysis to control the impact of major CYP2B6 enzyme that may interfere the results in this study.

Genotype of Phase II Drug Metabolizing Enzymes and Transporter Genes

DNA was isolated from the stored EDTA cell pellets (−20°C) using the QIAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany). Genomic DNA was quantified by a UV spectrophotometer ND-1000 at 260 nm (NanoDrop Technologies, Wilmington, DE).

Genotyping with TaqMan® assays was performed on a ViiA7 real-time PCR instrument (Applied Biosystems, Foster City, CA, USA) as previously described.[28] Genotyping included eight SNPs (rs and assay IDs are shown in brackets): *ABCA1* c.4760A>G (rs2230808, C___2741104_1_), *ABCC2* g.68231A>G (rs3740065, C__22271640_10), *ABCC2* c.-24C>T (rs717620, C___2814642_10), *ABCC4* c.3348A>G (rs1751034, C___1901918_30), *SULT1A1* c.638G>A (rs1042028 or rs9282861, AHOJH16), *UGT2B7* c.-161C>T (rs7668258, C__27827970_40, part of some *UGT2B7*1* haplotypes and **2*), *UGT2B7* c.211G>T (rs12233719, C__45181106_10, *UGT2B7*3*) and *UGT2B7* c.372A>G (rs28365063, C__30689135_20, found in some *UGT2B7*1* and **3*).

SULT1A1 and UGT2B17 Copy Number Variation Analysis

CNV analysis was performed by quantitative multiplex PCR amplification (MPA) as previously described by Gaedigk et al.[30] Briefly, PCR fragments were separated

on an ABI 3730 instrument (Applied Biosystems, Foster City, CA, USA) and analyzed with GeneMapper®. *UGT2B17* and *SULT1A1* copy number was determined by normalizing against *UGT2B15* and *SULT1A2*, respectively.

Measurement of Efavirenz Plasma Concentrations

Fasting plasma EFV concentrations 12 hours after dosing were measured at 12 and 24 weeks following antiretroviral therapy initiation. A validated isocratic reversed-phase high-performance liquid chromatography (HPLC) method with ultraviolet detection at 245 nm was used to measure plasma EFV concentrations as previously described.[29] Plasma samples (300 µL pretreated with acetonitrile) were injected into an Agilent 1100 HPLC instrument equipped with an Omnispher C18 (150 x 4.6 mm ID/particle size 5 µm) analytical column (Varian, CA, and USA), and a ChromGuard RP guard column. The mobile phase consisted of 10 mM KH_2PO_4 pH 3.1: acetonitrile (50: 50, v/v). ChromQuest Software version 4.1 was used for processing the sample peak heights. The average accuracy was 102–105% and the coefficient of variation was <5%.

Statistical Analysis

Genotype distributions were tested for Hardy–Weinberg equilibrium using exact tests under a call rate of 95% exemption. Data were summarized using medians and interquartile ranges (IQR) for continuous variables and frequencies and proportions for categorical variables. A Kruskal–Wallis test was performed for the comparison of plasma EFV concentrations among genotype groups. Mann–Whitney *U*-tests were used to compare plasma EFV concentrations between two genotypes. Wilcoxon matched pairs test was used for comparing two groups. A multivariable linear regression was performed for multiple factors including clinical characteristics, genotyping data with *p* values less than 0.20. Statistical significance was defined as $p<0.05$ (STATA 14, StataCorp LP, TX) after non-adjusting and adjusting for *CYP2B6* genotyping that is the main enzyme in efavirenz metabolism.

Results

Demographic Characteristics, Allele and Genotype Frequencies

A total of 149 participants were enrolled in the study. The average age of the patients was 37.4 (min-max; 19–59) years; 116 (77.9%) patients were male and 33 (22.1%) female. Patient demographics are presented in Table 1.

SULT1A1 copy number (CN) ranged from 1 to 6 copies and the frequency distribution of *SULT1A1* CN 1, 2, 3, 4, 5, and 6 was 3.4%, 48.3%, 29.5%, 11.4%, 4.7%, and 2.7%, respectively. The observed *SULT1A1* gene deletion (1 copy) and multiallelic duplications were similar to previous reports in Thai,[31] Caucasian-American,[32] European-Caucasian,[33,34] Indians,[35] Japanese,[36] and Chinese.[37]

For *UGT2B17*, three distinct clusters representing zero (56.4%), one (34.2%) and two gene copies (9.4%) were observed. Over 90% of patients were predicated to have decreased or no activity due to the loss of one or both gene copies. Furthermore, CNV frequencies differed from those reported by others.[24,30,38–43] The distribution of *SULT1A1* and *UGT2B17* gene copy number among different ethnic populations is shown in Table 2.

Genotyping data for the investigated phase II drug-metabolizing enzyme and transporter genes were obtained for all 149 patients. Allele frequencies and copy number variations are summarized in Table 3.

Table 1 Patient Demographics (n=149)

Data Characteristics, (Number of Patients) at Baseline	Value
HIV-1 positive	100%
Age, mean±SD (range; min-max), years	37.4±8.54 (19–59)
Gender, number (%) -Males -Females	 116 (77.9%) 33 (22.1%)
Body weight, mean±SD (min-max), Kg	54.6±9.5 (31–86)
CD4 cell count, median (IQR), cells/mm3	42 (17–109)
Plasma HIV-1 RNA, median (IQR), Log copies/mL	5.8 (5.4–6.2)
Hemoglobin, mean±SD, g/dL	10.8±1.8 (6.5–15.5)
Blood Urea Nitrogen, median (IQR), mg/dL	10 (7–11)
Serum creatinine, mean±SD (min-max), mg/dL	0.7±0.2 (0.3–1.3)
Direct bilirubin, median (IQR), mg/dL	0.2 (0.1–0.4)
Total bilirubin, median (IQR), mg/dL	0.5 (0.3–0.7)
Alkaline phosphatase, median (IQR), mg/dL	105 (74–167)
Aspartate aminotransferase, median (IQR), U/L	37 (27–52)
Alanine aminotransferase, median (IQR), U/L	31 (19–46)

Table 2 *SULT1A1* and *UGT2B17* Gene Copy Number (CN) Distribution Among Different Ethnic Populations

***SULT1A1* gene copy number**	**Present study (n=149)**	**Charoenchokthavee et al 2016[31]**	**Hebbring et al 2007[32]**	**Gjerde et al 2008[33]**	**Gaedigk et al 2012[30]**	**Tremmel et al 2017[34]**	**Almal et al 2017[35]**	**Yu et al 2013[36]**	**Li et al 2010[37]**
Ethnicity	Thai	Thai	Caucasian-American	European-Caucasian	Caucasian	European-Caucasian	Indian	Japanese	Chinese
1	3.4%	0%	4.7%	5.3%	4.3%	3.3%	3.8%	0%	1.7%
2	48.3%	97.1%	69.6%	64.9%	47.2%	60.3%	64.9%	65%	75.7%
3	29.5%	2.9%	21%	21.8%	35.4%	29.8%	21.7%	25.8%	NA
4	11.4%	0%	3.6%	6%	9.3%	NA	5.1%	NA	NA
5	4.7%	0%	NA	2%	3.5%	NA	NA	NA	NA
6	2.7%	0%	NA	0%	0.3%	NA	NA	NA	NA
>2	48.3%	2.9%	25.7%	29.8%	48.5%	36.4%	31.3%	35%	22.6%
≥4	18.8%	NA	4.7%	8%	13.1%	6.6%	CN>4 (4.5%)	9.2%	22.6%
***UGT2B17* gene copy number**		**Angstadt et al 2013[41]**	**Chew et al 2011[42]**	**Haas et al 2014[24]**	**Gaedigk et al 2012[30]**	**Uddin et al 2013[43]**	**Song et al 2017[66]**	**Mafune et al 2015[40]**	**Yang et al 2008[39]**
Ethnicity		American	Caucasian	Hispanic&Non-Hispanic-American	Caucasian	European-Caucasian	Chinese	Japanese	Chinese
0	56.4%	14%	12%	6%	11.6%	13%	NA	80%	76.8%
1	34.2%	47.8%	45.5%	43%	46.7%	51%	CN<2 (73%)	19%	21.6%
2	9.4%	38.2%	42.5%	49%	40.6%	35%	27%	1%	1.6%
>2	0%	0%	0%	1%	1.2%	0%	0%	0%	0%

Abbreviation: NA, not applicable.

All SNPs were in Hardy–Weinberg equilibrium ($p>0.05$).

Efavirenz Plasma Concentration in HIV-Infected Thai Adults

The overall median steady state EFV plasma concentration (12 hours after dosing) was 2.41 mg/L (IQR; 1.46–4.12 mg/L) at week 12 and 2.32 mg/L (IQR; 1.54–3.70 mg/L) at week 24. Of the 149 patients, 100 (67.1%) were within the therapeutic range (1 to 4 mg/L). Large inter-individual variation in EFV plasma concentrations was observed for 49 patients (32.9%), ranging from <1 mg/L (efficacy cut-off value) in 11 cases (7.4%) to >4 mg/L (toxicity cut-off value) in 38 cases (25.5%) at week 12, and 15 cases with <1 mg/L and 34 cases with >4 mg/L at week 24.

Relationship Between SNPs Tested, Gene Copy Number and Efavirenz Plasma Concentration

The SNP defining *SULT1A1*2* (c.638G>A, Arg213His) was associated with plasma EFV concentrations at week 24 ($p=0.048$) but was not significant for week 12 (Table 3, Figure 1A and B). Patients heterozygous for *SULT1A1*2* had significantly lower median plasma EFV concentrations at week 24 (2.18 mg/L, IQR: 1.44–2.60) compared to patients who did not carry this variant (2.33 mg/L, IQR: 1.57–4.21). However, there was no significant effect on plasma EFV concentrations at week 12. An association was also observed for *SULT1A1* gene copy number. Patients with CN>2 predicted to have higher SULT1A1 activity were significantly associated with decreased plasma EFV concentrations at week 12 ($p=0.046$).

Table 3 Relationship Between Genetic Variation in Drug-Metabolizing Enzyme and Transporter Genes and Plasma Efavirenz Concentrations (N=149)

Gene	N=149 (%)	EFV Plasma Concentration (mg/L), Median (IQR), Week 12	EFV Plasma Concentration (mg/L), Median (IQR), Week 24	*P* value[+]
ABCA1 c.4760A>G (rs2230808)				
A/A	32 (21.5)	2.07 (1.29–3.46)	2.62 (1.31–3.81)	0.601
A/G	69 (46.3)	2.23 (1.34–3.53)	2.33 (1.60–4.21)	0.644
G/G	48 (32.2)	2.94 (1.78–4.59)	2.22 (1.54–3.10)	0.056
P-value		0.103	0.775	
ABCC2 g.68231A>G (rs3740065)				
A/A	63 (42.3)	2.43 (1.57–4.10)	2.22 (1.54–3.41)	0.059
A/G	86 (57.7)	2.40 (1.36–4.21)	2.38 (1.55–4.01)	0.728
P-value		0.476	0.596	
ABCC2 c.-24C>T (rs717620)				
C/C	88 (59.1)	2.43 (1.52–4.26)	2.50 (1.56–3.84)	0.943
C/T	56 (37.6)	2.23 (1.41–4.14)	2.22 (1.44–4.04)	0.118
T/T	5 (3.3)	2.29 (1.57–2.38)	2.13 (1.58–3.10)	0.225
P-value		0.688	0.721	
ABCC4 c.3348A>G (rs1751034)				
A/A	97 (65.1)	2.41 (1.57–4.99)	2.36 (1.55–3.67)	0.526
A/G	45 (30.2)	2.29 (1.43–5.02)	2.33 (1.56–4.24)	0.343
G/G	7 (4.7)	2.43 (1.03–2.73)	1.81 (1.07–2.46)	0.735
P-value		0.516	0.322	
SULT1A1 c.638G>A (rs1042028)				
G/G	123 (82.6)	2.39 (1.41–4.54)	2.33 (1.57–4.21)	0.629
G/A	26 (17.4)	2.41 (1.48–3.22)	2.18 (1.44–2.60)	0.109
P-value		0.638	0.048[‡]	
SULT1A1 copy number (CN)				
1	5 (3.4)	2.43 (1.34–4.97)	2.46 (2.22–2.55)	0.500
2	72 (48.3)	2.78 (1.64–4.24)	2.32 (1.61–4.01)	0.143
3	44 (29.5)	2.06 (1.26–2.82)	2.22 (1.47–4.02)	0.524
4	17 (11.4)	2.02 (1.43–3.89)	2.30 (1.47–3.00)	0.756
5	7 (4.7)	2.77 (1.33–5.84)	2.72 (1.09–5.58)	0.398
6	4 (2.7)	3.95 (2.45–5.32)	2.10 (1.60–2.98)	0.068
>2	72 (48.3)	2.07 (1.39–3.22)	2.30 (1.52–3.51)	0.860
P-value		0.194	0.958	
UGT2B7 c.-161C>T (rs7668258)				
C/C	88 (59.1)	2.20 (1.45–3.94)	2.22 (1.57–3.50)	0.126
C/T	52 (34.9)	2.78 (1.56–4.78)	2.33 (1.52–4.24)	0.536
T/T	9 (6)	2.15 (1.37–2.55)	2.85 (1.47–3.87)	0.012[‡]
P-value		0.269	0.776	
UGT2B7 c.211G>T (rs12233719)				
G/G	124 (83.2)	2.43 (1.52–4.12)	2.30 (1.54–3.59)	0.137
G/T	24 (16.1)	1.74 (1.36–3.41)	2.46 (1.95–4.19)	0.317
T/T	1 (0.7)	4.63 (4.63–4.63)	4.17 (4.17–4.17)	0.317
P-value		0.353	0.462	

(*Continued*)

Table 3 (Continued).

Gene	N=149 (%)	EFV Plasma Concentration (mg/L), Median (IQR), Week 12	EFV Plasma Concentration (mg/L), Median (IQR), Week 24	*P* value[+]
UGT2B7 c.372A>G (rs28365063)				
A/A	80 (53.7)	2.57 (1.39–4.16)	2.22 (1.42–3.50)	0.094
A/G	59 (39.6)	2.41 (1.69–3.51)	2.55 (1.84–4.42)	0.786
G/G	10 (6.7)	1.58 (1.30–4.63)	2.22 (1.36–4.17)	0.876
P-value		0.720	0.271	
UGT2B17 copy number				
0	84 (56.4)	2.42 (1.46–3.92)	2.37 (1.54–3.64)	0.450
1	51 (34.2)	2.38 (1.48–4.10)	2.22 (1.52–3.59)	0.297
2	14 (9.4)	2.84 (1.24–5.42)	2.63 (2.05–5.52)	0.875
0–1	135 (90.6)	2.41 (1.48–3.99)	2.30 (1.53–3.62)	0.216
P-value		0.969	0.300	

Notes: [+]Wilcoxon matched pairs test (comparing two groups); [‡]Significance ($P<0.05$); A post-hoc analysis of plasma EFV concentrations and *SULT1A1* copy number at week 12 was as follows: CN=2 vs CN ≥3 ($P = 0.046$), CN=2 vs CN=3 ($p=0.019$), CN=2 vs CN=3+4 ($P = 0.015$).

Table 4 Univariate and Multivariate Analyses of Genetic and Non-Genetic Factors Associated with Plasma Efavirenz Concentrations at Week 12 and 24 in HIV-1 Infected Thai Adults

Characteristics/Duration	Week 12				Week 24			
	Univariate Analysis		Multivariate Analysis		Univariate Analysis		Multivariate Analysis	
	Beta	*P* value	Beta	*P* value	Beta	*P* value	Beta	*P* value
Age, years	0.13	0.115			0.04	0.628		
Gender	0.04	0.653			0.11	0.181		
Body weight, kg	−0.04	0.634			0.01	0.883		
Height, cm	−0.16	0.048			−0.04	0.681		
Hemoglobin, g/dL	−0.04	0.622			−0.23	0.009	−0.27	0.002
Blood Urea Nitrogen, mg/dL	−0.01	0.948			0.02	0.865		
Serum creatinine, mg/dL	−0.06	0.504			−0.21	0.016	−0.25	0.004
Albumin, g/dL	−0.01	0.965			−0.01	0.887		
Globulin, g/dL	0.19	0.022	0.20	0.018	0.19	0.030		
Direct bilirubin, mg/dL	−0.05	0.555			0.03	0.721		
Total bilirubin, mg/dL	−0.06	0.495			0.02	0.806		
Alkaline phosphatase, U/L	0.03	0.747			0.01	0.963		
Aspartate aminotransferase, U/L	−0.06	0.488			−0.09	0.339		
Alanine aminotransferase, U/L	−0.08	0.360			−0.09	0.313		
ABCA1 (c.4760A>G, rs2230808)	0.02	0.791			−0.06	0.458		
ABCC2 (g.68231A>G, rs3740065)	−0.01	0.905			0.07	0.423		
ABCC2 (c.-24C>T, rs717620)	−0.03	0.687			−0.07	0.437		
ABCC4 (c.3348A>G, rs1751034)	−0.01	0.988			−0.07	0.398		
*SULT1A1**2 (c.638G>A, rs1042028)	−0.10	0.220			−0.19	0.027	−0.17	0.049
SULT1A1 copy number	−0.11	0.204			−0.05	0.595		
UGT2B7 (c.-161C>T, rs7668258)	−0.01	0.994			0.11	0.207		
UGT2B7, (c.211G>T, rs12233719)	−0.01	0.898			0.08	0.359		
UGT2B7, (c.372A>G, rs28365063)	−0.04	0.601			0.07	0.421		
UGT2B17 copy number	0.07	0.395			0.02	0.774		

Abbreviations: CD4, cluster of differentiation 4; HIV, human immunodeficiency virus; RNA, ribonucleic acid; ARV, antiretroviral; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ABCB1, ATP Binding Cassette Subfamily B Member 1; ABCA1, ATP binding cassette subfamily A member 1; ABCC2, ATP Binding Cassette Subfamily C Member 2; SULT1A1, sulphotransferase 1A1; UGT2B7, UDP Glucuronosyltransferase Family 2 Member B7.

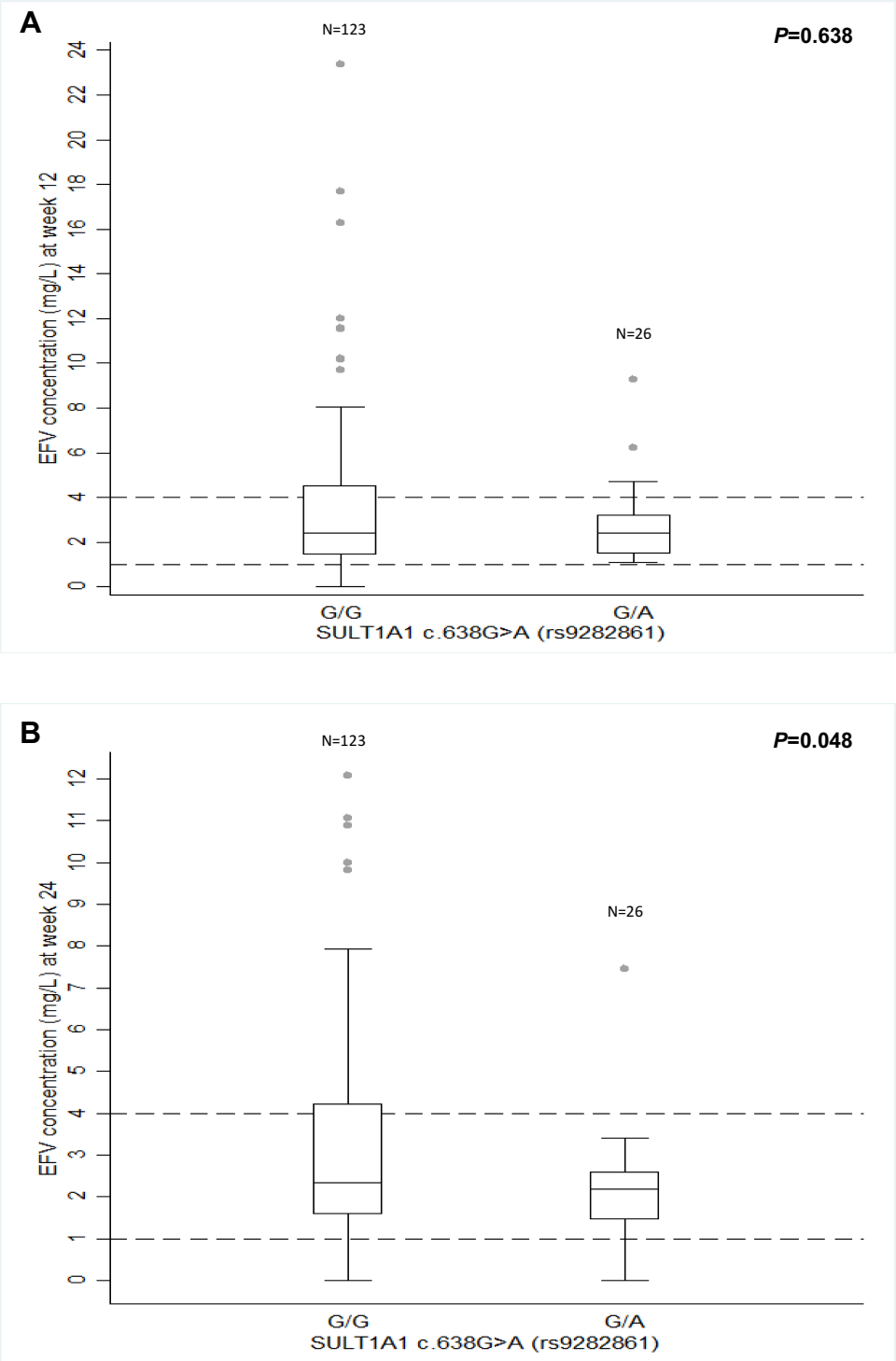

Figure 1 Influence of *SULT1A1**2 (c.638G>A) on median plasma efavirenz (EFV) concentrations at week 12 and 24. Dash lines represent the therapeutic window for EFV (1–4 mg/L). (**A**) Median plasma EFV concentrations compared between groups (p=0.638) at week 12. (**B**) Median plasma EFV concentrations were significantly lower in heterozygous patients (638G/A; 2.18 mg/L, IQR 1.44–2.60, p=0.048) compared to those not carrying the variant (638G/G; 2.33 mg/L, IQR 1.57–4.21).

A comparison of *SULT1A1* CN and plasma EFV concentrations at week 12 was as follows: CN = 3 versus CN = 2 (p=0.019), CN = 4 versus CN = 2 (p=0.192), and CN = 2 versus CN = 3 and 4 (p=0.015), respectively (Figure 2). Copy number of *UGT2B17* was not associated with plasma EFV concentrations.

We also observed a trend for *ABCA1* 4760G/G and higher median EFV concentrations (2.94 mg/L, IQR:

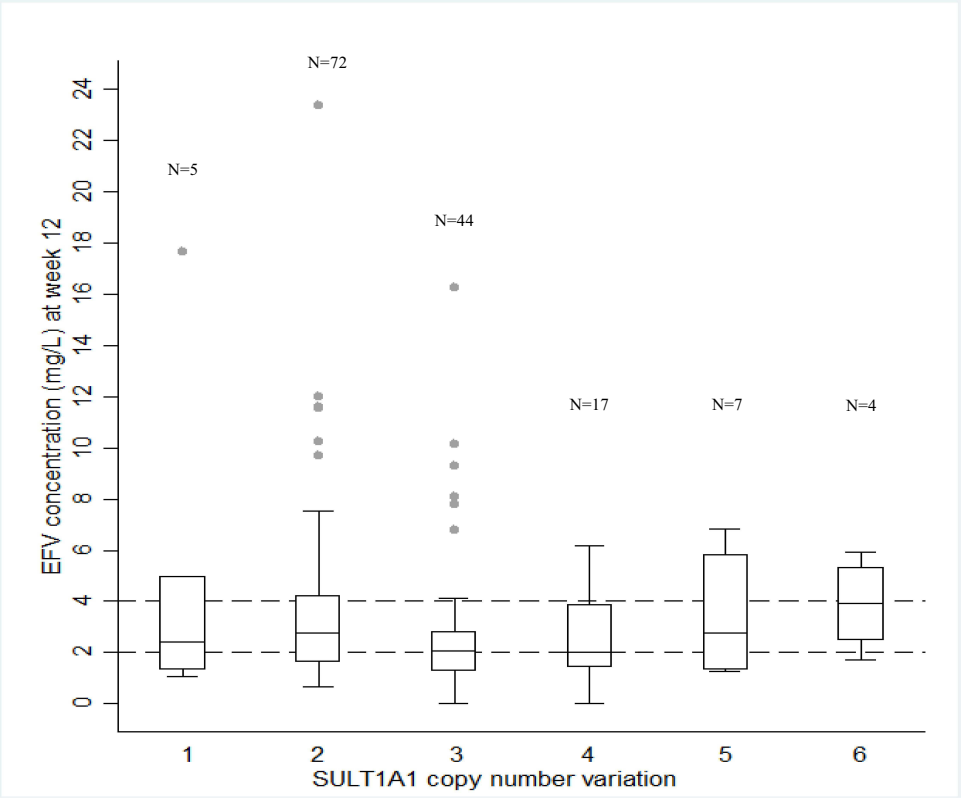

Figure 2 Influence of *SULT1A1* copy number variation on median plasma efavirenz (EFV) concentrations at week 12. Dash lines represent the therapeutic window for EFV (1–4 mg/L). A comparison of *SULT1A1* copy number (CN) and median plasma EFV concentration at week 12. Median plasma EFV concentrations were significantly lower in patients with CN≥3 (p=0.046), CN=3 (p=0.019), and CN=3+4 (p=0.015) compared to those carrying CN=2.

1.78–4.59) compared to subjects homozygous for the reference "A" allele (2.07 mg/L, 1.29–3.46), p=0.059. Conversely, patients genotyped as *ABCC4* 3348G/G tended to have lower EFV concentrations (1.81 mg/L, IQR: 1.07–2.46) compared to those homozygous for the reference "A" (2.36 mg/L, IQR: 1.55–3.67), p=0.140.

In a univariate linear regression model, height, and globulin were significantly associated with plasma EFV concentrations at week 12 and hemoglobin, serum creatinine, globulin, and *SULT1A1*2* were significantly associated with plasma EFV concentrations at week 24 (p<0.05). Our previous studies reported that *CYP2B6* genotypes were significantly associated with higher or lower plasma EFV concentrations,[15,28,29] thus, this variable was used in the composition of the multiple regression analysis. In a multivariable analysis of non-adjusting for *CYP2B6* genotype, globulin was significantly associated with plasma EFV concentrations at week 12 (p=0.018) and hemoglobin, serum creatinine, and *SULT1A1*2* allele were significantly associated with plasma EFV concentrations at week 24 (p=0.049), as presented in Table 4. After adjusting for *CYP2B6* genotype that is a main factor for EFV metabolism in the multivariable linear regression analysis, a trend toward statistical significance of *SULT1A1*2* was observed for patients carrying the *SULT1A1*2* allele (c.638G>A), p=0.089. However, hemoglobin and serum creatinine were still significantly associated with plasma EFV concentrations at week 24 (p=0.006, p=0.010, respectively). Potential differences in demographic and clinical characteristics were also compared between *SULT1A1*2* subgroups (**1/*1, *1/*2*); however, statistically significant differences in these data between the subgroups were not found.

Discussion

Previous studies have established that CYP2B6 is a major route to EFV metabolism and thus plays an important role in EFV pharmacokinetics and pharmacodynamics. The Clinical Pharmacogenetics Implementation Consortium (CPIC) has recently published a CYP2B6-efavirenz guideline.[44] However, despite the prominent role of CYP2B6, notable portions of variability in plasma EFV concentrations cannot be explained by genetic variation in *CYP2B6*. In this study, we set out to explore whether

minor polymorphic phase II pathways (*SULT1A1, UGT2B7* and/or drug transporters *ABCA1, ABCC2* and *ABCC4*) may also contribute to the observed variability.[17]

To that end, eight SNPs were selected in genes that have been shown to influence EFV pharmacokinetics[22,23,45,46] and, therefore, may also be involved in EFV transport and/or metabolism. Notably, *SULT1A1* was previously implicated to be significantly associated with plasma EFV concentrations,[24] although its effects on EFV PK remain unknown. The most striking result was the observation that the *SULT1A1*2* (c.638G>A) allele and *SULT1A1* copy number variation were associated with reduced plasma EFV concentrations. The nonsynonymous SNP causes an Arg213His amino acid substitution and is found at 8.7% in this study, similar to that of other East Asian populations (8%, 12%, 17% in Han Chinese, Korean, Japanese, respectively), while high allelic frequency is shown at 33%, 29% in Caucasian-American and African-Americans.[47–49] The Arg213His substitution negatively affects enzyme activity by decreasing protein thermostability.[50,51] The *SULT1A1*2* allele has been associated with decreased estrogen metabolism and hormone-dependent cancers and has also been described to contribute to the risk of cancer or response to therapy.[52–54] There is no direct evidence supporting that genetic variation of *SULT1A1* contributes to EFV PK, but Haas et al[24] have reported a significant relationship between *SULT1A1* c.667A>G (Met223Val, rs1801030) and plasma EFV concentrations. This study was, however, limited due to the lack of correction for multiple comparisons and another limitation of the Haas et al[24] study is that *SULT1A1* copy number variation was not accounted for pharmacokinetics of EFV. However, *SULT1A1* CNVs have been shown to be the most important underlying genetic variation determining SULT1A1 activity[55] and have been shown to impact the metabolism of several drugs.[36,56,57] Our data suggest that *SULT1A1* copy number and plasma EFV concentrations are indeed correlated contrasting the conclusion drawn by Hass et al.[24] Patients carrying more than 2 *SULT1A1* gene copies had decreased plasma EFV concentrations which were especially evident for subjects with 3 gene copies, the second most frequent CNV allele in our cohort. Based on our findings, the addition of *SULT1A1* CNV testing is strongly encouraged to independently confirm the contribution of this pharmacogene to EFV metabolism and determine whether *SULT1A1* genotype is a clinically useful marker to predict outcome and/or reduce adverse effects. No correlations were detected for *UGT2B17* copy number.

The *SULT1A1* CNV frequencies were markedly different from those previously reported in Thai,[31] a small study that only included 34 subjects. The frequencies we describe here are overall consistent with those found across other populations (Table 2). Regarding the frequency of *UGT2B17* copy number, there appears to be considerable variation among the major population groups.[24,30,39–43] The frequencies we report for our cohort are distinct from those described for other Asian populations, but share the overall lower frequencies of 2-copy alleles compared to populations of European descent.

We also observed trends of association between variants in *ABCA1, ABCC4* transporter genes and EFV concentrations which were consistent with previous reports.[11,22,58] *ABCC4* c.3348A>G carriers (MRP4, multidrug resistance protein 4) had a tendency towards lower plasma EFV concentrations. This transporter, also known as MRP4 is located in the blood–brain barrier and in the kidney.[59] MRP4 is mainly expressed in the blood-facing membrane of the brain capillaries and choroid plexus facilitating the export of substrate drugs via luminal cells.[60] The c.3348A>G SNP may reduce expression concentrations and thereby increase EFV concentrations in cerebrospinal fluid which may in turn cause central nervous system effects.[61] Many SNPs of *ABCC4* have previously been reported to correlate with EFV PK parameters.[22] However, it remains unclear whether genetic variation of *ABCC4* has clinical relevance.

Given the complexity of HIV-1 infection, it is not surprising that multiple factors including patient demographics such as gender, age, body weight, liver, and renal impairment influence plasma EFV concentration.[62] The contribution of these factors is in line with our previous findings[63] as well as the current study (ie, associations between plasma EFV concentrations and body weight, height, viral load, blood urea nitrogen (BUN) and aspartate aminotransferase (AST) were found). In addition, globulin influenced EFV metabolism at week 12. At week 24, however, there were only three factors, hemoglobin, serum creatinine, and *SULT1A1*2* that remained statistically significant with low plasma EFV concentration after multivariate analysis. Lastly, EFV therapy may also influence hemoglobin and albumin,[64] and Yimer et al[65] reported that lower baseline albumin levels are an important predictor for EFV-based HAART-induced liver injury. Although power analysis of the effect size of *SULT1A1*2* allele accounted for 17% of the observed variability in plasma EFV concentrations for multiple linear regression analysis by using non-adjusting for *CYP2B6* genotyping model, this effect was disappeared after adjusting for *CYP2B6* genotype in this

study. The influence of cofactors of EFV biotransformation pathway may be reduced because the number of sample sizes in each group tends to be small. Therefore, the findings of this study need to be viewed as preliminary.

Conclusion

Our findings suggest that *SULT1A1*2* and copy number variation contribute to the metabolism of drug regimens containing EFV. Thus, in addition to patient demographics and *CYP2B6* genotype status, future investigations should include *SULT1A1* to further our understanding of variability of EFV metabolism to ultimately empower us to more accurately predict EFV drug response and avoid adverse events.

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Disclosure

The authors reported no conflicts of interest in this work.

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