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Drug metabolism and transport gene polymorphisms and efavirenz adverse effects in Brazilian HIV-positive individuals

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Objectives: There are limited data regarding efavirenz pharmacogenetics in admixed populations. The Brazilian population is highly admixed. In a Brazilian cohort, we sought to characterize associations between efavirenz adverse effects (all-cause and CNS) and polymorphisms in seven genes known or suspected to affect efavirenz metabolism and transport.

Methods: We studied 225 HIV-positive individuals who had been prescribed efavirenz-containing regimens at a hospital in Rio de Janeiro, Brazil. Eighty-nine cases had efavirenz adverse effects, including 43 with CNS adverse effects, while 136 controls had no adverse effect of any antiretroviral after treatment for at least 6 months. A total of 67 candidate polymorphisms in *ABCB1*, *CYP2A6*, *CYP2B6*, *CYP3A4*, *CYP3A5*, *NR1I2* and *NR1I3* genes were selected for association analysis. Admixture was assessed using 28 ancestry-informative polymorphisms previously validated for the Brazilian population. Associations were evaluated with logistic regression models adjusted for sex and genetic ancestry.

Results: There was extensive African, European and Native American admixture in the cohort. Increased all-cause adverse effects were associated with the *CYP2B6* genotype combination 15582CC-516TT-983TT (OR = 7.26, $P = 0.003$) and with the *CYP2B6* slow metabolizer group 516TT or 516GT-983CT (OR = 3.10, $P = 0.04$). CNS adverse effects were nominally associated with *CYP3A4* rs4646437 (OR = 4.63, $P = 0.014$), but not after adjusting for multiple comparisons.

Conclusions: In a highly admixed Brazilian cohort, the *CYP2B6* slow metabolizer genotype was associated with an increased risk of efavirenz adverse effects.

Introduction

Worldwide, ∼37 million people are living with HIV-1, of whom nearly 50% are receiving ART.[1] The most recent WHO guidelines recommend efavirenz in combination with tenofovir and lamivudine as first-line ART.[2] Intolerance and adverse effects are primary reasons for first regimen discontinuation. Nearly one-half of patients prescribed efavirenz experience adverse effects, particularly CNS disturbances (e.g. insomnia, dizziness, depression, psychosis and suicidal ideation), which has been related to higher plasma efavirenz concentrations.[3–6] In a Brazilian cohort, efavirenz CNS adverse effects were the third most frequent reason for treatment discontinuation.[4]

Cytochrome P450 (CYP2) B6 is primarily responsible for efavirenz metabolism, with accessory pathways involving CYP2A6 and possibly CYP3A4/5.[7,8] The CYP genes are transcriptionally regulated by nuclear receptor genes *PXR*, *CAR* and others.[9] Efavirenz is also directly glucuronidated by uridine 5′-diphospho-glucuronosyltransferase (UGT) 2B7.[10,11] SNPs in genes that encode these enzymes, especially *CYP2B6*, predict higher plasma efavirenz concentrations.[12–15]

In *CYP2B6*, 516G→T (rs3745274) has been most extensively studied for associations with increased plasma efavirenz exposure.[12,16–19] In addition, *CYP2B6* 983T→C (rs28399499) and 15582C→T (rs4803419) have been associated with increased plasma efavirenz exposure.[20–22] Associations with plasma efavirenz concentrations have also been reported in different populations with SNPs in *ABCB1*, which encodes the efflux transporter P-glycoprotein.[23–27] The *CYP2B6* SNPs that predict increased plasma efavirenz concentrations have also been associated with efavirenz CNS adverse effects.[12,17,28]

Polymorphism frequencies and effects on gene expression vary among populations depending on genomic structure. Consequently, associations in one population may not translate to others. In Brazil there is marked ancestry admixture, defined as what occurs when previously isolated populations interbreed. This raises the possibility that genetic associations reported in other less admixed populations may not translate to Brazil.[29] In addition, there are limited studies of efavirenz pharmacogenetics in Brazilian populations. A previous study from our group did not find associations between SNPs in absorption, distribution, metabolism and excretion (ADME) genes and adverse effects of ART regimens containing either efavirenz or nevirapine, but that study only considered all-cause rather than specific adverse effects.[30]

The aim of the present study was to characterize, among Brazilians, associations between SNPs in genes that are known or suspected to affect efavirenz disposition and risk of efavirenz all-cause and CNS adverse effects.

Methods

Ethics statement

This study was approved by the Research Ethics Committee of Hospital Universitário Gaffreé e Guinle (HUGG), number 94/2011. All procedures were performed in accordance with the guidelines of the Helsinki Declaration. Written informed consent was obtained from all subjects.

Study subjects

The present study was based on retrospective review of medical records and included a total of 225 HIV-1-positive individuals who received routine clinic follow-up at the Clinical Immunology Service of HUGG. Individuals at least 18 years of age were eligible regardless of sex. Additional eligibility criteria included complete documentation of ART regimens in the medical record, previous or current use of an efavirenz-containing regimen, documentation of reason for ART change and no treatment for TB or viral hepatitis. Individuals were excluded for adverse drug effects that occurred during pregnancy and for adverse effects that were attributed to antiretrovirals other than efavirenz. Analyses were performed with all eligible participants ($n = 225$). Those who developed any adverse effects due to efavirenz were defined as cases ($n = 89$) while those with no documented adverse effect of any antiretroviral for at least 6 months were defined as controls ($n = 136$). Additional analyses considered only the subset of cases with CNS adverse effects ($n = 43$). Adverse effects were based on self-report by individuals during clinical follow-up. Severity of adverse effects was not reliably documented in medical records. Among controls, the median time on an efavirenz-containing regimen was 63 months. Among cases, the median time on an efavirenz-containing regimen was 5.5 months. Data were obtained by retrospective review of medical records.

Considering a minimum allele frequency of 1% and a 50% incidence of adverse effects due to efavirenz, the minimum OR value to achieve 80% power in our sample size ($n = 225$) would be OR = 3. For analysis including only CNS adverse effects ($n = 179$), with a medium incidence of 30%, the minimum OR value to reach 80% power would be OR = 4.5.

Selection of polymorphisms for analysis

Candidate genes were *ABCB1*, *CYP2A6*, *CYP2B6*, *CYP3A4*, *CYP3A5*, *NR1I2* and *NR1I3*, in which 67 SNPs were selected for genotyping largely based on literature review (Table S1, available as Supplementary data at *JAC* Online).[7–9,11,14] The SNPper tool was used to search for potentially informative SNPs in coding and non-coding regulatory regions. We also used the HapMap data bank to identify tagging SNPs and increase gene coverage. For HapMap, we used a minor allele frequency (MAF) of 0.05 in CEU (Utah residents with Northern and Western European ancestry) or YRI (Yoruba in Ibadan, Nigeria) populations and an r^2 cut-off of 0.8 as parameters. To adjust for genetic ancestry, we used a panel of 28 ancestry-informative SNPs previously validated for the Brazilian population.[31]

DNA extraction and genotyping

Buffy coat samples were obtained from whole blood by centrifugation at 3000 **g** for 10 min and genomic DNA was extracted with a salting-out method. The *CYP2A6*, *CYP3A4*, *CYP3A5*, *NR1I2* and *NR1I3* SNPs and ancestry-informative markers were genotyped with TaqMan® Open Array® technology, using the QuantStudio™ 12K Flex Real-Time PCR System (Thermo Fisher Scientific, MA, USA), according to the manufacturer's instructions. The *CYP2B6* SNPs (except rs3745274, rs4803419, rs4803420 and rs7260525) were genotyped with SNaPshot® (Thermo Fisher Scientific), according to the manufacturer's instructions. Purified products underwent capillary electrophoresis on an ABI3130 Genetic Analyzer (Thermo Fisher Scientific) using the standard fragment analysis protocol. GeneMapper software (version 4.0 Thermo Fisher Scientific) was used for genotyping. *CYP2B6* rs3745274 and rs4803419 were genotyped with the TaqMan® Drug Metabolism SNP Genotyping Assay (Thermo Fisher Scientific), following the manufacturer's instructions. *CYP2B6* rs4803420 and rs7260525, and *ABCB1* markers were genotyped with a Sequenom MassARRAY® iPLEX platform (Agena Bioscience™, CA, USA) at Vanderbilt Technologies for Advanced Genomics (VANTAGE) in Nashville, TN, USA. Data were analysed using Typer software (Agena Bioscience™, CA, USA). Other genotyping was done at Laboratório de Virologia Molecular, Departamento de Genética, Instituto de Biologia, Universidade Federal do Rio de Janeiro.

Estimates of genetic ancestry

Proportions of African, European and Native American genetic ancestries were estimated using Structure software, version 2.3.1.[32–35] Proportions of each ancestry were estimated under an admixture model using data from European and African populations of the 1000 Genomes Project as a reference. Native American ancestry was estimated using an admixed Amazonian population from Santa Isabel do Rio Negro as a reference, all of whom reported recent indigenous ancestry.[36]

Statistical analyses

Statistical analyses were performed using R software (version 2.13.0) and the genetics, gap, SNPassoc, haplo.stats, LDheatmap, grid and coin packages.[37] Deviations from Hardy–Weinberg equilibrium (HWE) were assessed by the χ^2 test. Pairwise linkage disequilibrium (LD) patterns were determined using r^2 statistics (cut-off of $r^2 \geq 0.8$). Stepwise logistic regression analysis was performed to select covariates to include in the model as possible confounders, including age at the start of efavirenz-containing regimen, sex and genetic ancestry. The variables sex, African ancestry and Native American ancestry were considered to be informative and were included in the model. Sex proportions between cases and controls were compared using the χ^2 test. Age and genetic ancestry were compared using the Wilcoxon rank sum test.

Logistic regression models were performed to identify associations between SNPs and adverse effect outcomes. We primarily considered additive genetic models and secondarily considered dominant and recessive models. Bonferroni adjustment was applied to minimize type I error (P value cut-off for 46 SNPs = 1.1×10^{-3}). Haplotype analyses were performed for SNPs with nominal P value <0.05. Haplotype frequencies were estimated through maximum likelihood and compared between cases and controls using logistic regression models, as described for SNP analyses.

CYP2B6 genotype levels were defined as described elsewhere, with each increasing plasma efavirenz concentration stratum predicted by specific combinations of rs4803419 (15582C→T), rs3745274 (516G→T) and

Table 1. Characteristics of study participants

	All-cause cases ($N = 89$)	CNS cases ($N = 43$)	Controls ($N = 136$)	P^c	P^d
Sex[a], *n* (frequency)					
male	43 (0.48)	26 (0.60)	84 (0.62)	0.064	0.978
female	46 (0.52)	17 (0.40)	52 (0.38)		
Age (years), mean ± SD[b]	41.1 ± 11.4	40.9 ± 11.9	40.5 ± 10.3	0.840	0.940
Genetic ancestry (%), mean ± SD[b]					
European	48 ± 28	41 ± 27	51 ± 29	0.540	0.071
African	40 ± 26	45 ± 28	36 ± 28	0.400	0.110
Native American	12 ± 14	14 ± 16	13 ± 14	0.640	0.950

[a]Comparisons were performed using the χ^2 test.
[b]Comparisons were performed using the Wilcoxon rank sum test.
[c]*P* value based on all-cause cases versus controls.
[d]*P* value based on CNS cases versus controls.

rs28399499 (983T→C) alleles.[20] We designated the 10 strata defined by these genotypes as level 1 to level 10, with level 1 being the composite genotype associated with the lowest concentrations and level 10 being associated with the highest concentrations. Levels 1, 2 and 3 are defined by 15582CC, CT and TT, respectively, together with 516GG and 983TT homozygosity. Levels 4 and 5 are defined by 516GT and 983CT heterozygosity, respectively, together with 15582CC homozygosity. Levels 6 and 7 are defined by 516GT and 983CT heterozygosity, respectively, together with 15582CT heterozygosity. Level 8 is defined by 516TT with 983TT, level 9 is defined by 516GT with 983CT and level 10 is defined by 516GG with 983CC, all together with 15582CC homozygosity.

We also assigned *CYP2B6* genotypes into three metabolizer groups: extensive, intermediate and slow. *CYP2B6* levels 1 and 2 were defined as extensive metabolizers, *CYP2B6* levels 3, 4, 5, 6 and 7 were defined as intermediate metabolizers and *CYP2B6* levels 8, 9 and 10 were defined as slow metabolizers. CIs were determined using the Wald modified method. Associations between *CYP2B6* genotype levels and CNS adverse effects were determined by logistic regression models considering *CYP2B6* genotype level both as an ordinal variable and comparing each level separately to the normal metabolizer genotype. *CYP2B6* genotype level was also included as a covariate in analyses for associations between other candidate SNPs and efavirenz adverse effects.

Results

Patient characteristics

The distribution of sex, age at the start of efavirenz-containing regimen and genetic ancestry in all-cause cases, CNS cases and controls is presented in Table 1. The cohort was highly admixed, with the average individual having ∼50% European, ∼45% African and ∼13% Native American genetic ancestry. Covariates did not differ significantly between cases and controls, although there were somewhat fewer males among all-cause cases versus controls.

CNS adverse effects represented 48% of all adverse effect cases, with the most common symptoms being hallucinations (30%), dizziness (26%) and nightmares (16%) (Figure 1). Among the non-CNS cases ($n = 46$), 19% developed a rash, 11% were simply described as intolerant, 6% had malaise and 10% were unique cases of diabetes mellitus, dyslipidaemia, epigastralgia or hypertriglyceridaemia. Specific symptoms were not described for 24 individuals.

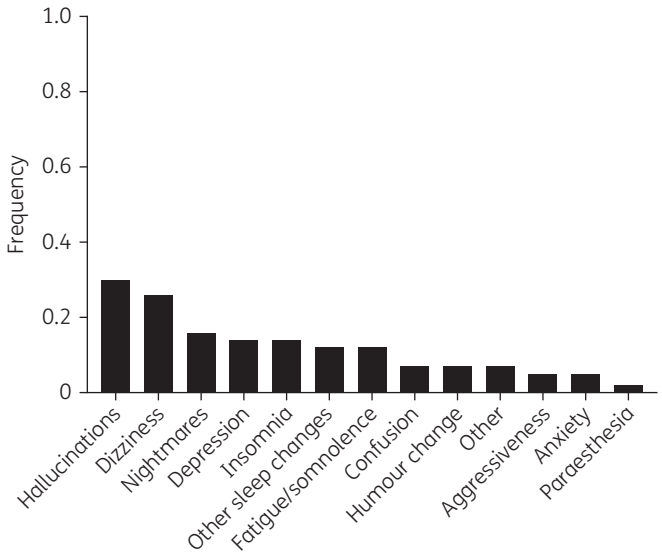

Figure 1. Distribution of reported efavirenz CNS adverse effects among 43 cases. Some cases had more than one type of efavirenz CNS adverse effect documented. Each type of CNS adverse effect was counted separately for such individuals.

Genotypes including HWE and LD

We attempted to genotype 67 SNPs in seven drug metabolism and transport genes. Of these, 5 that deviated from HWE were excluded from analysis, as were an additional 13 monomorphic loci. Of the remaining SNPs, three pairs were in LD at $r^2 \geq 0.8$ in *NR1I2* (rs1523127 and rs1523130) and *ABCB1* (rs4148740 and rs10225473; rs3789244 and rs1128503). Only the most frequent SNP of each pair was maintained. The remaining 46 SNPs were included in association analyses (Table S1).

Association between CYP2B6 genotype levels and efavirenz adverse effects

We assessed whether *CYP2B6* genotype levels previously reported to predict plasma efavirenz concentrations were associated with efavirenz adverse effects.[20] We found no significant association

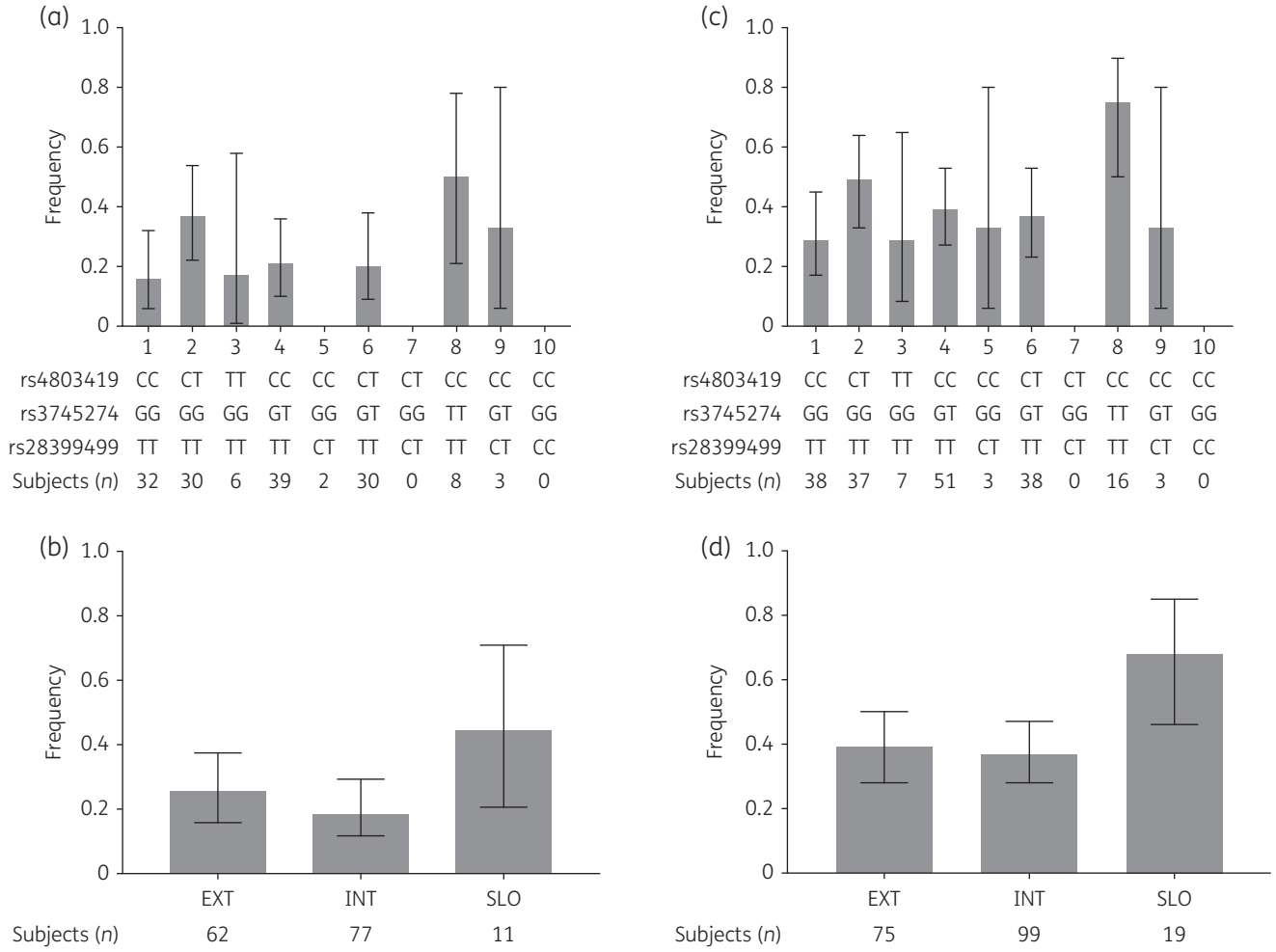

Figure 2. Distribution of *CYP2B6* level frequencies in cases. CIs were calculated using the modified Wald method. (a) Frequencies of *CYP2B6* metabolizer genotype levels in CNS cases based on 10 possible levels ($n = 43$). (b) Frequencies of *CYP2B6* metabolizer genotype levels in CNS cases collapsed into extensive, intermediate and slow metabolizer groups. (c) Frequencies of *CYP2B6* metabolizer genotype levels in all-cause cases based on 10 possible levels ($n = 89$). (d) Frequencies of *CYP2B6* metabolizer genotype levels in all-cause cases collapsed into extensive, intermediate and slow metabolizer groups. EXT, extensive metabolizer genotypes; INT, intermediate metabolizer genotypes; SLO, slow metabolizer genotypes. *CYP2B6* genotype levels were collapsed as follows: extensive (levels 1 and 2), intermediate (levels 3, 4, 5, 6 and 7) and slow (levels 8, 9 and 10).[20]

between *CYP2B6* genotype level, considered as an ordinal variable, and CNS adverse effects by an unadjusted logistic regression model (OR = 1.06, $P = 0.50$) or with adjustment for sex, African ancestry and Native American ancestry (OR = 1.04, $P = 0.67$). Likewise, we found no significant association between *CYP2B6* genotype level and all-cause efavirenz adverse effects in an unadjusted model (OR = 1.11, $P = 0.14$) or after adjustment (OR = 1.10, $P = 0.13$). Proportions of adverse effect cases within each genotype level for CNS adverse effects and for all-cause adverse effects are presented in Figure 2(a and c).

In association analyses that compared each *CYP2B6* genotype level pairwise to genotype level 1, which predicts the lowest plasma efavirenz concentrations, there was an association between the diplotype rs4803419CC-rs3745274TT-rs28399499TT (level 8) and increased risk of CNS adverse effects in an unadjusted model (OR = 8.67, $P = 0.021$). This association was marginally significant after adjustment for sex, African ancestry and Native American ancestry (OR = 5.46, $P = 0.05$). We also found an association between level 8 and all-cause efavirenz adverse effects in both an unadjusted model (OR = 7.26, $P = 0.003$) and after adjustment (OR = 6.83, $P = 0.005$).

We have also performed data analysis after collapsing *CYP2B6* genotype levels into extensive, intermediate and slow metabolizer groups. Individuals with slow metabolizer genotypes were more likely to be among CNS adverse effect cases, although this difference was not statistically significant (Figure 2b). Using extensive metabolizers as reference, we found no significant association between the metabolizer group and CNS adverse effects in an unadjusted model and after adjusting for sex, African ancestry and Native American ancestry. The intermediate group had an OR of 0.68 ($P = 0.34$) and the slow group an OR of 2.29 ($P = 0.23$) in the adjusted model. However, when all-cause adverse effect cases were considered, we found a significant association between slow metabolizer status and increased risk for adverse effects both in unadjusted (OR = 3.44, $P = 0.024$) and adjusted (OR = 3.10, $P = 0.04$) analyses (Figure 2d).

Table 2. Significant associations between SNPs and efavirenz CNS adverse effects

Genotype	Cases[a]	Controls[a]	Unadjusted model OR (95% CI)	Adjusted model OR (95% CI)[c]
SNP rs1882478 (*ABCB1*)				
CC	7 (0.17)	41 (0.31)	reference	reference
CT	22 (0.52)	72 (0.54)	1.79 (0.70–4.55)	1.50 (0.54–4.17)
TT	13 (0.31)	20 (0.15)	3.81 (1.32–11.02)	2.62 (0.80–8.65)
	42	133	$P = 0.040$[b]	$P = 0.267$[b]
CC-CT	29 (0.69)	113 (0.85)	reference	reference
TT	13 (0.31)	20 (0.15)	2.53 (1.13–5.69)	1.94 (0.79–4.80)
			$P = 0.027$[d]	$P = 0.156$[d]
SNP rs4646437 (*CYP3A4*)				
GG	6 (0.17)	47 (0.41)	reference	reference
GA	16 (0.46)	46 (0.40)	2.72 (0.98–7.58)	3.09 (0.96–9.95)
AA	13 (0.37)	22 (0.19)	4.63 (1.55–13.79)	4.95 (1.22–20.05)
	35	115	$P = 0.014$[b]	$P = 0.059$[b]
GG	6 (0.17)	47 (0.41)	reference	reference
GA-AA	29 (0.83)	68 (0.59)	3.34 (1.29–8.68)	3.36 (1.07–10.59)
			$P = 0.007$[d]	$P = 0.029$[d]
SNP rs2740574 (*CYP3A4*)				
TT	14 (0.39)	60 (0.51)	reference	reference
TC	12 (0.33)	42 (0.36)	1.22 (0.52–2.91)	1.03 (0.39–2.70)
CC	10 (0.28)	15 (0.13)	2.86 (1.06–7.68)	2.02 (0.60–6.79)
	36	117	$P = 0.116$[b]	$P = 0.435$[b]
TT-TC	26 (0.72)	102 (0.87)	reference	reference
CC	10 (0.28)	15 (0.13)	2.62 (1.05–6.49)	1.98 (0.71–5.52)
			$P = 0.043$[d]	$P = 0.198$[d]

[a]Results are shown as *n* (frequency) for SNP genotypes.
[b]Overall *P* value for additive model (2 degrees of freedom).
[c]Results adjusted for sex, African ancestry and Native American ancestry.
[d]Overall *P* value for dominant or recessive model (1 degree of freedom).

Adverse effects and polymorphisms beyond CYP2B6

In logistic regression models that considered only CNS adverse effect cases, two SNPs in *CYP3A4*, rs4646437 (OR = 4.63, $P = 0.014$, additive model) and rs2740574 (OR = 2.86, $P = 0.116$, additive model; OR = 2.62, $P = 0.043$, recessive model), were nominally associated with increased risk. There was also a nominal association with *ABCB1* rs1882478 (OR = 3.81, $P = 0.040$, TT genotype, additive model). After adjusting for sex, African ancestry and Native American ancestry, only *CYP3A4* rs4646437 remained associated with efavirenz CNS adverse effects (OR = 4.95, $P = 0.059$, additive model; OR = 3.36, $P = 0.029$, dominant model) (Table 2). This association was not statistically significant after adjusting for multiple comparisons.

Logistic regression analyses were also adjusted for *CYP2B6* genotype level to see whether this affected the apparent associations with *CYP3A4* rs4646437, *CYP3A4* rs2740574 and/or *ABCB1* rs1882478. Such adjustment did not substantially change ORs or *P* values of these associations (data not shown).

No associations were observed when all-cause efavirenz adverse effect cases were considered either before or after adjustment. The lowest *P* value was for *CYP2A6* rs28399433 after adjustment for sex, African ancestry and Native American ancestry ($P = 0.301$).

Haplotype analysis

We tested for associations between *CYP3A4* rs4646437-rs2740574 haplotypes noted above and efavirenz CNS adverse effects, as these two SNPs are in linkage equilibrium and were separately associated with the same risk effect. The haplotype containing both minor alleles, rs4646437A and rs2740574C, was associated with CNS adverse effects in both unadjusted (OR = 2.04, $P = 0.012$) and adjusted (OR = 2.08, $P = 0.045$) models. This haplotype did not have a stronger association with CNS adverse effects than each SNP considered separately (Table 3).

Discussion

Research into the pharmacogenetics of efavirenz among Brazilians is limited. The present study characterized, in a highly admixed Brazilian population, associations between efavirenz adverse effects (both all-cause and CNS adverse effects) and SNPs in genes that are known or suspected to affect efavirenz disposition. In unadjusted analyses, *CYP2B6* genotype rs4803419CC-rs3745274TT-rs28399499TT (genotype level 8) was significantly associated with efavirenz all-cause adverse effects. The *CYP2B6* slow metabolizer group (rs3745274TT or rs3745274GT-rs28399499CT) was also significantly associated with all-cause efavirenz adverse effects. In

Table 3. Association between *CYP3A4* rs4646437 and rs2740574 and CNS adverse effects due to efavirenz

Haplotype rs4646437/rs2740574	Controls[a]	Cases[a]	Unadjusted model OR (95% CI)	Adjusted model OR (95% CI)[b]
G/T	0.6	0.39	reference	reference
A/C	0.29	0.43	2.04 (1.17–3.56; $P = 0.012$)	2.08 (1.02–4.26; $P = 0.045$)
A/T	0.1	0.16	2.70 (1.09–6.70; $P = 0.033$)	2.94 (1.06–8.15; $P = 0.04$)
G/C	0.01	0.02	1.99 (0.18–21.42; $P = 0.571$)	1.83 (0.15–22.02; $P = 0.634$)

[a]Haplotype frequencies were estimated by maximum likelihood.
[b]Results adjusted for sex, African ancestry and Native American ancestry.

addition, *CYP3A4* rs4646437 was nominally associated with efavirenz CNS adverse effects, but not after adjusting for multiple comparisons.

The *CYP2B6* 516G→T (rs3745274) SNP is widely reported to be associated with efavirenz phenotypes, including higher efavirenz plasma concentrations and adverse effects.[12,16–19,28] However, previous reports suggested that this effect may not occur in Brazilian populations.[30,38,39] In a study of 50 individuals from the south of Brazil, Müller *et al.*[38] found no association between rs3745274 and CNS adverse effects due to efavirenz. A limitation of that study was the small sample size. In a previous study by our group, rs3745274 was not associated with intolerance to regimens containing either efavirenz or nevirapine in 395 individuals from Rio de Janeiro, Brazil.[30] Lack of focus on a specific intolerance could have limited our power to find an association. Similarly, Coelho *et al.*[39] found no association between rs3745274 and treatment regimen failure.

In contrast to previous reports from Brazil, our analyses considered *CYP2B6* genotype levels based on combinations of rs3745274, rs28399499 and rs4803419. We found that *CYP2B6* level 8 (rs4803419CC-rs3745274TT-rs28399499TT) was associated with increased risk of efavirenz all-cause adverse effects ($P = 0.003$). In addition, when considering classical metabolizer groups, we also found an association between the slow metabolizer group and efavirenz all-cause adverse effects ($P = 0.04$).

These associations were not significant when considering only CNS adverse effect cases, possibly due to a smaller sample size in that analysis. An additional limitation of our study is the use of a broad case definition, since different types of efavirenz adverse effects can be distinctively associated with the SNPs analysed. Therefore, the analysis of all cases as a single group may have reduced our power to detect associations that might be specific for each adverse effect. Associations between long-term efavirenz use and performance on formal neurocognitive testing have been reported.[40,41] In the present retrospective study, we could not test for associations with neurocognitive performance because such data were not available.

Our findings agree with previous studies that showed associations between *CYP2B6* variants and increased risk for specific efavirenz adverse effects or discontinuation of ART due to CNS symptoms.[42,43] To our knowledge, the present study is the first to show associations between *CYP2B6* genotype and efavirenz adverse effects in a Brazilian population. We cannot explain the stronger association between *CYP2B6* level 8 (homozygosity for 516TT) as compared with level 9 (heterozygosity for 516GT and 983CT), although it might be due to the very few individuals in level 9.

The enzyme CYP3A4 is responsible for metabolizing the largest number of medications and plays a minor role in efavirenz metabolism.[7,44] In our study, *CYP3A4* rs4646437 was nominally associated with increased risk of CNS adverse effects, but not after adjusting for multiple comparisons. We also observed an association with *CYP3A4* rs2740574 in an unadjusted model. The SNP *CYP3A4* rs4646437 has been reported to be associated with decreased efavirenz clearance independent of *CYP2B6* genotype, which could increase risk for efavirenz adverse effects.[45] In the published literature, we found no clear associations reported for rs2740574. An early study suggested that this SNP could be weakly associated with plasma efavirenz concentrations, but a subsequent study did not replicate this finding.[12,18] Furthermore, Haas *et al.*[12] did not find an association between rs2704574 and efavirenz CNS adverse effects.

Although P-glycoprotein is not described as an efavirenz transporter, associations have been reported between *ABCB1* SNPs and efavirenz-related outcomes.[18,23,26,46] In the present study, among 21 candidate SNPs in *ABCB1*, we found an association with rs1882478 in an unadjusted model. To our knowledge, no prior studies have shown associations of *ABCB1* rs1882478 with efavirenz phenotypes, although this SNP was reported to be associated with decreased hepatic *ABCB1* expression among liver transplant donors in China.[47]

The marked ancestry admixture in the Brazilian population may affect SNP frequencies and LD patterns. Consequently, findings from other well-defined ethnic groups may not reliably translate to Brazilians. It is thus important that pharmacogenetic associations reported in other populations be replicated in Brazil, as was done in the present study. Better understanding of pharmacogenetic mechanisms that underlie adverse effects of antiretroviral drugs may help to identify genetic predictors for these outcomes and ultimately lead to better-tolerated and more effective HIV therapies.

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Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at *JAC* Online.

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