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Cytochrome P450 2B6 (*CYP2B6*) and constitutive androstane receptor (*CAR*) polymorphisms are associated with early discontinuation of efavirenz-containing regimens

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Objectives: Cytochrome P450 2B6 (CYP2B6) is responsible for the metabolic clearance of efavirenz and single nucleotide polymorphisms (SNPs) in the *CYP2B6* gene are associated with efavirenz pharmacokinetics. Since the constitutive androstane receptor (CAR) and the pregnane X receptor (PXR) correlate with CYP2B6 in liver, and a CAR polymorphism (rs2307424) and smoking correlate with efavirenz plasma concentrations, we investigated their association with early (<3 months) discontinuation of efavirenz therapy.

Methods: Three hundred and seventy-three patients initiating therapy with an efavirenz-based regimen were included (278 white patients and 95 black patients; 293 male). DNA was extracted from whole blood and genotyping for *CYP2B6* (516G→T, rs3745274), *CAR* (540C→T, rs2307424) and *PXR* (44477T→C, rs1523130; 63396C→T, rs2472677; and 69789A→G, rs763645) was conducted. Binary logistic regression using the backwards method was employed to assess the influence of SNPs and demographics on early discontinuation.

Results: Of the 373 patients, 131 withdrew from therapy within the first 3 months. Black ethnicity [odds ratio (OR)=0.27; *P*=0.0001], *CYP2B6* 516TT (OR=2.81; *P*=0.006), *CAR* rs2307424 CC (OR=1.92; *P*=0.007) and smoking status (OR=0.45; *P*=0.002) were associated with discontinuation within 3 months.

Conclusions: These data indicate that genetic variability in *CYP2B6* and *CAR* contributes to early treatment discontinuation for efavirenz-based antiretroviral regimens. Further studies are now required to define the clinical utility of these associations.

Keywords: pharmacogenetics, pharmacokinetics, metabolism, drug disposition

Introduction

Efavirenz treatment is associated with CNS or neuropsychiatric side effects in a large proportion of patients (~25%–70%), and can give symptoms such as dizziness, headache, abnormal dreams and sleep disturbance, mood changes, anxiety, dizziness, and impaired concentration.[1–3] CNS side effects normally arise in the first days/weeks of treatment and for a small minority of patients can be prolonged for several months or emerge after numerous weeks of treatment,[4] but do not always result in discontinuation of therapy. Discontinuation of efavirenz-containing regimens has been reported with various frequencies in different studies, ranging from 5% to 20%, and neuropsychiatric side effects account for the majority of discontinuation cases.[3,5–7] The causes of CNS and other side effects related to efavirenz-containing regimens have not been fully characterized, but some studies indicate that neuropsychiatric side effects are associated with high plasma concentrations, particularly in the first weeks of treatment.[8–10]

Phase I metabolism of efavirenz is predominantly catalysed by cytochrome P450 2B6 (CYP2B6), and single nucleotide polymorphisms (SNPs) within the *CYP2B6* gene are associated with altered hepatic CYP2B6 expression and activity.[11] The role of the 516G→T (rs3745274) SNP in the pharmacokinetics of efavirenz has been studied extensively.[12–19] In addition to CYP2B6, a secondary route of phase I metabolism of efavirenz is catalysed by CYP2A6 and *CYP2A6* polymorphisms have also recently been identified as determinants of efavirenz plasma concentrations.[20–23]

The primary metabolite of phase I metabolism, 8-hydroxy efavirenz, then undergoes glucuronidation prior to excretion in the urine and bile. This phase II metabolism has recently been attributed to UDP-glucuronosyltransferase isoform 2B7 (UGT2B7)[24] and evidence is emerging that *UGT2B7* polymorphisms are also associated with efavirenz plasma concentrations.[20] Hence, a thorough knowledge of the routes of metabolism and elimination of efavirenz have successfully identified candidate genes for pharmacogenetic studies.

The constitutive androstane receptor (CAR) and the pregnane X receptor (PXR) are known to regulate a complex network of phase I and phase II metabolic enzymes in response to xenobiotics. Importantly, CAR and PXR expression also correlate with CYP2B6[25] and CYP2A6[26] expression in liver, even in the absence of enzyme inducers and xenobiotics. Activators of CAR have also been shown to induce UGT2B genes *in vivo*,[27] and so CAR appears to play a role in the basal and inducible regulation of the enzymes involved in efavirenz metabolism.

Therefore, we hypothesized that interindividual variability in CAR and PXR caused, for example, by polymorphisms, would result in additional interindividual variability in CYP2B6, CYP2A6 and UGT2B7. Single nucleotide polymorphisms have been reported in PXR[28] and CAR[29] genes, and we have previously shown an association of a *PXR* SNP (63396C→T, rs2472677) with unboosted atazanavir plasma concentrations[30] and an association of a *CAR* SNP (540C→T, rs2307424) with plasma concentrations of efavirenz.[31]

The aim of this study was to investigate the association of *CYP2B6* (516G→T, rs3745274), *CYP2A6* (*CYP2A6**9B, rs8192726), *UGT2B7* (735A→G, rs28365062; and 802T→C, rs7439366), *CAR* (540C→T, rs2307424) and *PXR* (44477T→C, rs1523130; 63396C→T, rs2472677; and 69789A→G, rs763645) polymorphisms with discontinuation within 3 months of initiating efavirenz-containing regimens. In addition, the associations with demographic factors, including ethnicity, smoking habits and gender, were also investigated.

Patients and methods

Patients

A total of 373 patients who were on stable efavirenz-containing highly active antiretroviral therapy (HAART) for ≥3 months (n=242; control group) or who discontinued efavirenz-containing HAART within 3 months (n=131; early discontinuation group) were included in this cohort study.

We included patients of the cohort of the German Competence Network for HIV/AIDS (KompNet Cohort) with documented stable efavirenz treatment for ≥3 months and compared with those who discontinued efavirenz-containing HAART within 3 months. A further inclusion criterion was the availability of already stored EDTA samples. All concomitant medications were documented and patients were excluded from the analysis if they received drugs with known drug interactions. No patients were receiving anti-TB treatments.

Out of 480 patients with stable efavirenz-containing HAART identified as a potential control group, stored EDTA samples were available from 255 patients. In 13 of these patients, genotyping for one or more SNP was not successful. Therefore, 242 patients on stable therapy were included in the final analysis. The median duration of efavirenz therapy in this group was 48 months (range 5–99 months). Two hundred and thirteen patients were identified with early discontinuation of efavirenz-containing HAART, therefore fulfilling the inclusion criteria. In 131 patients, EDTA samples were available and were therefore included in the analysis. The median duration of efavirenz exposure in this group was 1.1 months (range 0.2–3 months).

Whole blood was provided by the KompNet Cohort. In this prospective multicentre, Germany-wide cohort, semi-annual follow-up visits are documented, and clinical and demographic data are collected. EDTA samples are taken at enrolment and 3 years afterwards; serum samples are collected at every follow-up visit. Demographic and clinical data were collected on age, gender, weight, height, adherence (evaluated through pharmacy reports), ethnicity and qualitative smoking status.[32] Ethical approval was granted by the ethics committee of the Ruhr-Universität Bochum, Germany, and local ethics committee approval and informed consent were obtained at each site.

Genotyping

Total genomic DNA was isolated using the QIAamp DNA mini kit according to the manufacturer's instructions. Following extraction, purity was assessed by comparing the A_{260} and A_{280} ratio. DNA was quantified using the PicoGreen® dsDNA Quantitation Reagent (Molecular Probes, CA, USA) and normalized to 20 ng/μL. For *CYP2B6*, pre-amplification was first conducted to discriminate from the *CYP2B6* pseudogene (*CYP2B7*) by modification of previously reported methods.[11] Genotyping for *CYP2B6* (516G→T), *CYP2A6* (*9B), *UGT2B7* (735A→G and 802T→C), *CAR* (540C→T) and *PXR* (44477T→C, 63396C→T and 69789A→G) was then performed by real-time PCR allelic discrimination 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). For *CYP2B6* 516G→T (rs3745274), *CYP2A6**9B (rs8192726), *CAR* 540C→T (rs2307424), *PXR* 44477T→C (rs1523130) and *PXR* 7635A→G (rs6785049), Applied Biosystems pre-validated assays were utilized. The assay IDs were C__7817765_60, C__29560333_20, C__25746794_20, C__9152783_20 and C__29280426_10, respectively. For *UGT2B7* 735A→G (rs28365062) the

forward primer, reverse primer, A probe and G probe were 5′-CCTAAAGTAATTATCTTGTGTCATCCACCTT-3′, 5′-CGTCAGCTTTCCCCATTGTCT-3′, 5′-ACCCACTACATTATCTGT-VIC and 5′-CCCACTACGTTATCTGT-FAM, respectively. For *UGT2B7* 802T→C (rs7439366) the forward primer, reverse primer, C probe and T probe were 5′-CTGACGTATGGCTTATTCGAAACTC-3′, 5′-TGGAGTCCTCCAACAAAATCAACAT-3′, 5′-AGTGGATGAGGAAACTT-VIC and 5′-AGAGTGGATAAGGAAACTT-FAM, respectively. For *PXR* 63396C→T (rs2472677) the forward primer, reverse primer, C probe and T probe were 5′-GCACAAACATTTTCAATTTCAATGAAGTTCA-3′, 5′-CATTCGGAAGACTTATTCTATTCCTGTCT-3′, 5′-CCATATTTTTTCTGATTAAA-VIC and 5′-CCATA TTTTTTTTGATTAAA-FAM, respectively.

Statistical analysis

All data are given as frequency number (percentage), unless otherwise stated. Genotypes were tested for Hardy–Weinberg equilibrium by χ^2 test of observed versus predicted (from allele frequency) genotype frequencies. Statistical significance was assessed using the χ^2 test, Cramer's *V*-test or the linear by linear test for differences in qualitative variables. Univariate and multivariate associations were assessed with SPSS v16 (SPSS Inc., Chicago, IL, USA) using backward logistic regression. Continuous variables are expressed as median [interquartile range (IQR)]. A statistical trend was defined as a P value <0.1. Statistical significance was set at $P<0.05$.

Results

This was a cohort study and all patients were recruited between 1 January 2006 and 1 October 2009. Two hundred and forty-two patients, who were on stable efavirenz-containing HAART for ≥3 months, were included in the control group and 131 patients were included in the early discontinuation group (discontinuation of efavirenz-containing HAART within 3 months). Treatment was discontinued for unknown reasons in 55 patients (42%), for self-reported CNS-related side effects in 72 cases (55%), virological failure in 1 case (1%) and elevated liver enzymes or other toxicities in 4 cases (3%). No documented cases of depression were encountered in these patients. The baseline characteristics of the patients entered in the study are presented in Table 1.

The backbone therapy was comparable between the discontinuation group and the control group. Preferential nucleoside reverse transcriptase inhibitor (NRTI)-based backbone therapies consisting either of tenofovir/emtricitabine or lamivudine/zidovudine were used. The remaining patients were receiving alternative backbone or NRTI-free therapies. Within the early discontinuation group, 42 of 131 patients were taking lamivudine/zidovudine (or zidovudine) (32%) and 59 of 131 patients were taking tenofovir/emtricitabine (45%). Within the control group, 63 of 242 patients were taking lamivudine/zidovudine (26%) and 114 of 242 patients were receiving tenofovir/emtricitabine (47%).

Association of demographics and backbone with early (<3 months) treatment discontinuation

A higher proportion of white patients (39.2%; 109 out of 278) discontinued efavirenz-containing HAART within 3 months compared with black patients (23.2%; 22 out of 95; $P=0.005$). Smokers exhibited a lower frequency of discontinuation (30.9%; 73 out of 236) compared with non-smokers (42.3%; 58 out of 137; $P=0.026$). No other associations ($P<0.05$) or trends ($P<0.10$) were evident with demographic factors, such as age, sex, weight, height or body mass index (BMI), even when stratified for ethnicity. Two hundred and seventy-eight patients out of 373 had zidovudine, zidovudine/lamivudine or tenofovir/emtricitabine as backbone therapy. The frequency of zidovudine/lamivudine (or zidovudine) administration did not differ between patients who discontinued (32%; 42 out of 131) and patients who were stable on efavirenz (26%; 63 out of 242; $P=0.21$). Similarly, the frequency of tenofovir/emtricitabine administration did not differ between patients who discontinued (45%; 59 out of 131) and patients who were stable on efavirenz (47%; 114 out of 242; $P=0.71$).

Allele frequencies according to ethnicity

The frequencies of the allelic variants in white and black patients are summarized in Table 2. Differences in the frequencies of the variant alleles between ethnicity groups (black ethnicity as reference) were evident for *CAR* rs2307424C→T [odds ratio (OR)=2.69; $P=0.0001$], *CYP2B6* 516G→T (OR=0.73; $P=0.087$), *CYP2B6* 983T→C (OR=0; NA), *UGT2B7* 802T→C (OR=2.4; $P=0.0001$), *PXR* 7635A→G (OR=0.14; $P=0.0001$), *PXR* 44477T→C (OR=7.38; $P=0.0001$) and *PXR* 63396C→T (OR=1.77; $P=0.009$).

Association of genetic factors with early (<3 months) treatment discontinuation

When considering the entire population, *CYP2B6* 516G→T was statistically associated with early (<3 months) treatment

Table 1. Baseline characteristics of patients with stable efavirenz therapy and with early treatment discontinuation of efavirenz

	On therapy >3 months	On therapy <3 months	Total
Number of patients	242	131	373
Male gender	189	104	293 (78.6%)
White ethnicity	169	109	278 (74.5%)
Median age, years (IQR)	45 (40–51)	47 (41–53)	46 (26–82)
Median BMI, kg/m^2 (IQR)	23.5 (20.5–27.5)	23.5 (20.5–28)	24 (21–28)
Smoking	163 (67.4%)	73 (55.7%)	236 (63.3%)
Median years since diagnosis (range)	6 (1–12)	6 (1–12)	6 (1–12)
Viral load, copies/mL (range)	<50 (<50–8×10^5)	325 (<50–1×10^6)	<50 (<50–1×10^6)
CD4 count, cells/mm^3 (range)	449 (12–3233)	325 (0–1260)	407 (0–3233)

discontinuation. The discontinuation rate was 33.5% (57 out of 170) in the 516GG group compared with 32.5% (55 out of 169) in the 516GT group and 44.1% (15 out of 34) in the 516TT group ($P=0.03$). Patients characterized by T homozygosity had a higher probability of early treatment discontinuation (55.9% versus 33%; $P=0.008$). A trend towards a higher frequency of discontinuation among patients characterized by *CAR* rs2307424CC was also observed, with 38.9% (74 out of 190) of CC patients versus 30% (54 out of 180) of CT/TT patients discontinuing therapy before three months ($P=0.071$; *CAR* genotype data were unavailable for 3 patients). Patients with the TT genotype for *PXR* 63396C→T had a trend towards a higher frequency of discontinuation (41.5%; 51 out 123) compared with patients with the CT/CC genotype (32%; 80 out of 250; $P=0.072$). Similarly, for *UGT2B7* 802T→C, patients with the TC or CC genotype had a trend towards higher frequency of discontinuation (37.5%; 101 out of 269) compared with patients with the TT genotype (28.2%; 29 out of 103; $P=0.089$; the UGT2B7 802 genotype was unavailable for 1 patient). No association ($P<0.05$) or trend ($P<0.10$) was observed for other polymorphisms.

When considering only white patients ($n=278$), patients with *CYP2B6* 516TT had a higher discontinuation rate (58.3%; 14 out of 24) compared with patients with *CYP2B6* 516GG/GT (95 out of 254 patients; $P=0.045$). Moreover, white patients with *CAR* rs2307424CC had a frequency of early discontinuation of 46.3% (56 out of 121) versus 32.5% (50 out of 154) for CT/TT patients ($P=0.019$). In black patients, the *CYP2B6* 516TT genotype was associated with a higher discontinuation rate (50%; 5 out of 10) than *CYP2B6* 516GG/GT (20%; 17 out of 85; $P=0.033$). No other associations ($P<0.05$) or trends ($P<0.10$) were evident in either white or black patients.

Table 2. Allele frequencies in patients, considering ethnicity

Polymorphism	Variant allele in white patients (%)	Variant allele in black patients (%)	OR	*P* value
CYP2B6 516G→T	167/556 (30%)	70/190 (37%)	0.73	0.087
CYP2B6 983T→C	0/556 (0%)	8/190 (4%)	NA	NA
CAR rs2307424C→T	180/550 (32.7%)	29/190 (15%)	2.69	0.0001
PXR 44477T→C	329/556 (59.1%)	30/190 (15.7%)	7.38	0.0001
PXR 63396C→T	339/556 (60.9%)	89/190 (46.8%)	1.77	0.009
PXR 7635A→G	237/556 (42.6%)	158/188 (84%)	0.14	0.0001
*CYP2A6**9B	40/556 (7.8%)	8/190 (4.2%)	1.78	0.17
UGT2B7 735A→G	69/556 (12.4%)	31/190 (16.3%)	0.68	0.101
UGT2B7 802T→C	296/556 (53.2%)	61/188 (32.4%)	2.4	0.0001

NA, not applicable.

Multivariate analysis by binary logistic regression

Multivariate logistic regression in the entire cohort confirmed the independent association of *CYP2B6* 516 and *CAR* rs2307424 genotypes with early (<3 months) treatment discontinuation. In addition to these genetic factors, ethnicity and smoking status were also identified as independent predictors. As shown in Table 3, the following parameters were independently associated with treatment discontinuation: *CYP2B6* 516TT [OR=2.81; 95% confidence interval (CI)=1.34–5.9; $P=0.006$]; *CAR* rs2307424CC (OR=1.92; 95% CI=1.2–3.07; $P=0.007$); black ethnicity (OR=0.27; 95% CI=0.14–0.50; $P=0.0001$); and smoking status (OR=0.45; 95% CI=0.28–0.74; $P=0.002$).

Table 3. Univariate and multivariate backward logistic regression for the identification of factors associated with early treatment discontinuation of efavirenz

	Univariate		Multivariate	
Covariate	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Gender (female)	0.92 (0.55–1.56)	0.77		—
Ethnicity (black)	**0.47 (0.27–0.80)**	**0.05**	**0.27 (0.14–0.50)**	**0.0001**
Age (years)	1 (0.99–1)	0.25		—
Height (cm)	0.99 (0.97–1.01)	0.57		
Weight (kg)	0.99 (0.98–1.01)	0.58		
Smoking (non-smoker)	**0.61 (0.39–0.94)**	**0.03**	**0.45 (0.28–0.74)**	**0.002**
***CYP2B6* 516TT**	**2.56 (1.25–5.2)**	**0.01**	**2.81 (1.34–5.90)**	**0.006**
CYP2B6 983C carrier	0.25 (0.031–2.12)	0.21		—
***CAR* rs2307424CC**	**1.49 (0.96–2.3)**	**0.07**	**1.92 (1.20–3.07)**	**0.007**
PXR 63396C carrier	0.66 (0.43–1.04)	0.07	ND	0.112
*CYP2A6**9B carrier	1.4 (0.75–2.65)	0.29		
UGT2B7 735G carrier	0.75 (0.45–1.25)	0.28		
UGT2B7 802C carrier	1.53 (0.93–2.5)	0.09	ND	0.22

ND, not done.
Rows with a significant *P* value ($P<0.05$) in the multivariate analysis are shown in bold.
Constant of the final multivariate model=0.74; $P=0.21$.
Measure of goodness of fit was conducted using the Hosmer–Lemeshow test: $\chi^2=4.8$; $P=0.56$.

Composite analysis for genetic and demographic risk factors

To further confirm the association of these two polymorphisms with early discontinuation, the *CYP2B6* 516T and *CAR* rs2307424C alleles were classified as discontinuation-associated alleles. As shown in Figure 1(a), the number of high discontinuation-associated alleles correlated with the rate of early discontinuation. The rate of treatment discontinuation was 25% (4 out of 16) for patients with no discontinuation-associated alleles, 29.6% (24 out of 81) for patients with one discontinuation-associated allele, 32.4% (48 out of 148) for patients with two alleles, 39.4% (43 out of 109) for patients with three alleles and 56.2% (9 out of 16) for patients with four alleles ($P=0.023$; *CAR* genotype data were unavailable for 3 patients).

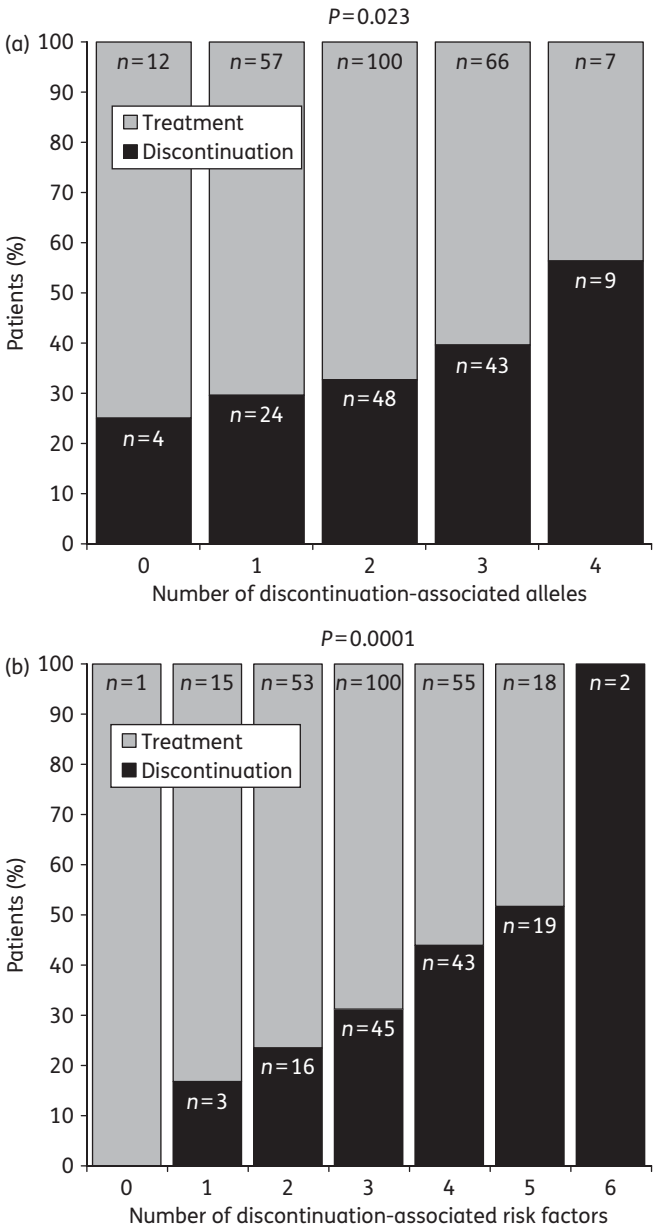

Figure 1. Bar chart showing the percentage of patients discontinuing treatment within 3 months and (a) number of discontinuation-associated alleles (*CYP2B6* 516T and *CAR* rs2307424C) and (b) number of discontinuation-associated risk factors (*CYP2B6* 516T and *CAR* rs2307424C, white ethnicity, and smoking).

As shown in Figure 1(b), the number of early discontinuation risk factors harboured by a patient [including *CYP2B6* and *CAR* genetics (with each allele counting as one risk factor), ethnicity, and smoking status] was associated with early discontinuation. The rate of early treatment discontinuation was 0% ($n=1$) for patients with no risk factors, 16.7% (3 out of 18) for patients with one risk factor, 23% (16 out of 69) for patients with two risk factors, 31% (45 out of 145) for patients with three risk factors, 43.9% (43 out of 98) for patients with four risk factors, 51.4% (19 out of 37) for patients with five risk factors and 100% ($n=2$) for patients with six risk factors ($P=0.0001$; *CAR* genotype data were unavailable for 3 patients).

Discussion

Several studies have described an elevated frequency of CNS side effects in patients treated with efavirenz-based regimens, leading to treatment discontinuation primarily in the first week of treatment.[3,5–7] Interindividual variability in genes involved in the metabolism and disposition of efavirenz may influence the onset of side effects and, therefore, therapy discontinuation. *CYP2B6* 516 has been identified as a major predictor of efavirenz plasma concentrations,[12–19] and a correlation between the frequency of CNS side effects in white patients and slow-metabolizer genotypes (*CYP2B6* 516TT and 983TT) was recently described.[33] In the current cohort, TT homozygosity for the *CYP2B6* 516G→T polymorphism was independently associated with early treatment discontinuation.

A novel association between a *CAR* polymorphism (rs2307424) and plasma efavirenz concentrations was recently reported by some of the investigators involved in the present study.[31] The discontinuation data presented here extend these findings to a clinical phenotype, showing significant association of the CC genotype with early treatment discontinuation. These findings strengthen the role of CAR in efavirenz disposition and pharmacokinetics, presumably through its role in the regulation of CYP2B6.

A genetic composite of these two polymorphisms (*CYP2B6* 516C→T and *CAR* rs2307424) was assessed and patients with more discontinuation-associated alleles (also the same as the high concentration-associated alleles[31]) had a higher rate of discontinuation. This finding reinforces the hypothesis that early treatment discontinuation is associated with higher plasma concentrations. However, it must be noted that plasma efavirenz concentrations are higher in black patients than in white patients,[34] yet treatment discontinuation rates appear to be lower in black patients.[35] In the present study, white patients had a higher frequency of early discontinuation than black patients. Furthermore, the frequency of *CYP2B6* and *CAR* alleles associated with higher plasma efavirenz concentrations are more frequent in black patients. We hypothesize that this apparent paradox may be explained by interethnic variability in other unknown genes involved in the aetiology of CNS toxicity.

Smoking status was also identified as being independently associated with early treatment discontinuation. We previously reported a trend towards an association between smoking and efavirenz plasma concentrations in the German Competence Network for HIV/AIDS[19] and in a Chilean cohort.[31] Interestingly, nicotine has previously been shown to activate nuclear receptors such as PXR and CAR,[36] and induction of CYP2B6 has been described.[37] Furthermore, nicotine and efavirenz have an overlapping elimination pathway, both being metabolized by CYP2B6 and CYP2A6, providing another putative mechanism for the interaction.[38] Nicotine exposure has also been shown to influence blood–brain barrier permeability *in vivo*[39,40] and may therefore have an effect on efavirenz diffusion in the CNS, independently of effects on pharmacokinetics.

It must be noted that a major limitation of the current study is that the reasons for early discontinuation were not known for 42% of the cohort. However, of those discontinuing therapy for a known reason, 94.7% discontinued due to CNS toxicity. Other reasons for early treatment discontinuation (e.g. virological failure) usually lead to later (>3 months) discontinuation or modification of therapy. For this reason, it seems likely that in the majority of patients the reason for the treatment discontinuation was CNS toxicity. Moreover, the reported associations were also present when univariate logistic analysis was conducted for patients who discontinued due to known CNS toxicity ($n=72$): *CAR* rs2307424CC (OR=1.86; $P=0.037$); *CYP2B6* 516TT (OR=2.1; $P=0.122$); and black ethnicity (OR=0.42; $P=0.013$). A potential additional limitation of this study is that no information was collected with respect to their adherence to medication.

In conclusion, our findings indicate that genetic variability in genes involved in the metabolism and disposition of efavirenz are determinants of early (<3 months) treatment discontinuation. These data provide evidence that smoking status may be associated with discontinuation. *In vitro* studies to identify the mechanisms underpinning these associations and to identify toxicological targets in the brain are now warranted.

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