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Meta-analysis of the effect of *CYP2B6, CYP2A6, UGT2B7 and CAR* polymorphisms on efavirenz plasma concentrations

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Background: Efavirenz primary metabolism is catalysed by CYP2B6 with minor involvement of CYP2A6. Subsequently, phase I metabolites are conjugated by UGT2B7, and constitutive androstane receptor (CAR) has been shown to transcriptionally regulate many relevant enzymes and transporters. Several polymorphisms occurring in the genes coding for these proteins have been shown to impact efavirenz pharmacokinetics in some but not all studies.

Objectives: A meta-analysis was performed to assess the overall effect of *CYP2B6* rs3745274, *CYP2A6* (rs28399454, rs8192726 and rs28399433), *UGT2B7* (rs28365062 and rs7439366) and *NR1I3* (rs2307424 and rs3003596) polymorphisms on mid-dose efavirenz plasma concentrations.

Methods: Following a literature review, pharmacokinetic parameters were compiled and a meta-analysis for these variants was performed using Review Manager and OpenMetaAnalyst. A total of 28 studies were included.

Results: Unsurprisingly, the analysis confirmed that individuals homozygous for the T allele for *CYP2B6* rs3745274 had significantly higher efavirenz concentrations than those homozygous for the G allele [weighted standard mean difference (WSMD)=2.98; 95% CI 2.19–3.76; $P<0.00001$]. A subgroup analysis confirmed ethnic differences in frequency but with a similar effect size in each ethnic group ($P=0.96$). Associations with *CYP2A6* and *UGT2B7* variants were not statistically significant, but T homozygosity for CAR rs2307424 was associated with significantly lower efavirenz concentrations than in C homozygotes (WSMD=−0.32; 95% CI −0.59 to −0.06; $P=0.02$).

Conclusions: This meta-analysis provides the overall effect size for the impact of *CYP2B6* rs3745274 and *NR1I3* rs2307424 on efavirenz pharmacokinetics. The analysis also indicates that some previous associations were not significant when interrogated across studies.

Introduction

HIV is one of the world's most serious health and development challenges. In 2017, there were 36.9 million people living with HIV and around 2.5% of these people died from AIDS-related illness. Despite increased access to antiretroviral therapy, it is still only available to around 53% of infected people.[1] Efavirenz (600 mg once daily) has been a cornerstone of first-line antiretroviral therapy for the last two decades and is still often used in certain contexts,[2,3] with a number of studies demonstrating the applicability of its use at a lower dose (400 mg) during pregnancy and TB coinfection.[4,5] Efavirenz is metabolized mainly in the liver (90%) through the cytochrome P450 (CYP) system. CYP2B6 is responsible for efavirenz hydroxylation, which produces 8-hydroxyefavirenz, whereas CYP2A6 catalyses formation of 7-hydroxyefavirenz.[6] Efavirenz phase II metabolism includes the production of oxidized efavirenz metabolites produced through conjugation by UDP-glucuronosyltransferase (UGT). Furthermore, efavirenz can be conjugated directly to efavirenz-*N*-glucuronide by UGT2B7.[7]

Expression of CYP2B6 shows inter- and intra-individual pharmacokinetic variability. The most studied polymorphism to date is *CYP2B6*6*, defined by the rs3745274 (516G>T) and rs2279343 (785A>G) variants. These polymorphisms are in strong linkage disequilibrium, and the rs3745274 variant is often studied in isolation.[8] Accordingly, the impact of rs3745274 on efavirenz pharmacokinetics has been widely explored in many studies and has been associated with alterations in efavirenz metabolism, pharmacokinetics and toxicity in a variety of populations. Thus, individuals that are homozygous for the T allele have consistently demonstrated higher efavirenz plasma concentrations and a

decreased rate of 8-hydroxyefavirenz formation than individuals homozygous for the G allele.[9–15]

The secondary route of efavirenz metabolism via CYP2A6 has been proposed to be more important in the case of patients homozygous for the T variant for *CYP2B6* rs3745274,[16] and accordingly certain CYP2A6 polymorphisms have been demonstrated to affect efavirenz plasma concentrations in some studies. The *CYP2A6* rs8192726 and *CYP2A6* rs28399454 variants (493+23G>T; *CYP2A6*9B*, and 40850474A>C; *CYP2A6*17*; respectively) have been significantly associated with higher efavirenz plasma concentrations in a Ghanaian population.[17] Moreover, the *CYP2A6* rs28399433 variant (1093C>T *CYP2A6*48*) has been reported to be associated with altered efavirenz plasma concentrations.[18] However, these associations have not been reported in studies of other African,[19,20] American[21] or European populations.[22]

Recently it was reported that the products of efavirenz phase II metabolism are present in plasma at higher concentrations than the oxidized species produced by phase I metabolism.[23] *UGT2B7* rs28365062 and rs7439366 genetic variants (735A>G; *UGT2B7*1c* and 802T>C; *UGT2B7*2*, respectively) have been highlighted as contributing to inter-patient variation in efavirenz plasma concentration.[17,24] However, conflicting results have been reported.[7,19]

Nuclear receptors such as the constitutive androstane receptor (CAR) are transcription factors that regulate drug metabolism enzymes and drug transporters.[25] The *NR1I3* rs2307424 variant (540C>T) has been associated with altered efavirenz plasma concentrations in Chilean patients[21] and early discontinuation of efavirenz therapy in Caucasian patients.[26] Furthermore, an association between the *NR1I3* rs3003596 (1089T>C) variant and reduced plasma efavirenz concentration has been suggested.[27] However, a more recent extended study in the same cohort did not find a statistically significant association,[28] and other studies in African and Caucasian populations have not corroborated these associations.[28,29]

The effect of *CYP2A6*, *UGT2B7* and *NR1I3* genetic variants on efavirenz pharmacokinetic remains controversial. Hence, to evaluate the correlation between these genetic variants and efavirenz plasma concentration, a meta-analysis was conducted to systematically review the published evidence and assess an overall effect of their impact.

Methods

Search strategy and study selection

This meta-analysis was conducted in line with the statement outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[30] (Table S1, available as Supplementary data at *JAC* Online). PubMed was searched until October 2017, without language, publication date or publication status restrictions, to identify prospective studies, observational cohort studies, cross-sectional studies and randomized controlled trials addressing the association between *CYP2B6*, *CYP2A6*, *UGT2B7* or *CAR* polymorphisms and efavirenz pharmacokinetics in HIV-positive patients. The following search terms were used: efavirenz, HIV, CYP2B6, CYP2A6, UGT2B7, CAR, SNP, polymorphism, genotype, plasma, concentration and pharmacokinetics (see Supplementary Methods). In addition, relevant papers were manually screened to identify potentially relevant studies. Studies were considered for inclusion if (i) they explored the association between *CYP2B6*, *CYP2A6*, *UGT2B7* or *NR1I3* polymorphisms and pharmacokinetics of efavirenz in HIV patients of any age; (ii) the pharmacokinetics of efavirenz were expressed as efavirenz concentration in plasma at 12 h after dosing (C_{12}) (12±2 h); and (iii) efavirenz pharmacokinetic parameters were described separately according to different genotypes. The original authors were contacted for more detailed information when the articles that met the inclusion criteria provided insufficient pharmacokinetic data. If there were studies with overlapping subject groups, the study with the highest number of patients was included in the meta-analysis. According to the above criteria, two reviewers evaluated the studies and extracted the data independently. Disagreements between reviewers were resolved by consensus.

Data extraction

The following information was collected and included in a data extraction form by one review author and a second author checked the extracted data: (i) the basic characteristics of eligible studies, including first author's name, publication year, patient ethnicity and efavirenz dose; (ii) the number of subjects for each genotype for all the polymorphisms; and (iii) the mean values and standard deviation of efavirenz concentration in plasma at 12 h after dosing. All concentrations were expressed in ng/mL. If the studies provided the median (range), minimum and maximum values or 25th and 75th percentile range, the method reported by Wan *et al.*[31] was employed to estimate the mean and standard deviation. Disagreements were discussed and resolved by consensus.

To assess the risk of bias, pairs of reviewers worked independently and determined the adequacy of each study.[32] Study design, sample size, reliability of genotypes, population stratification, Hardy–Weinberg equilibrium and definition of outcome were evaluated. Disagreements were resolved through discussion.

The primary analysis compared the mean efavirenz concentration between genotype groups for *CYP2B6*, *CYP2A6*, *UGT2B7* and *NR1I3* SNPs. For each SNP, three pairwise comparisons were undertaken: heterozygous genotype versus homozygous common allele; homozygous rare allele versus homozygous common allele; and heterozygous genotype versus homozygous rare allele. A secondary analysis was performed to evaluate the effect of the association between *CYP2B6* rs3745274 and efavirenz plasma concentration within different ethnicities. This was conducted by exploring: (i) the overall efavirenz plasma concentration according to genotype and ethnicity; and (ii) the *CYP2B6* rs3745274 frequency within each ethnic group.

Statistical analysis

Standardized mean difference and 95% CIs were calculated to assess the difference between genotypes in the pharmacokinetics of efavirenz, based on efavirenz concentration in plasma at 12 h after dosing. *Z*-tests were performed to determine the statistical significance of the results. Statistical significance was defined as $P<0.05$.

To measure heterogeneity across the included studies the I^2 statistic was used. An I^2 value of $\geq$50% was considered a substantial level of heterogeneity, and the random-effects model was used. When the I^2 value was <50%, a fixed-effects model was applied. We produced a funnel plot and conducted an Egger's test to assess the risk of bias across studies. All statistical analysis were performed using Review Manager,[33] Comprehensive Meta-Analysis[34] and OpenMetaAnalyst.[35]

Results

Literature search

All studies included patients aged $\geq$18 years, with the exception of two paediatric studies. The dose range in both studies was 200–600 mg of efavirenz per day. In the case of studies including HIV-positive pregnant women, the efavirenz concentration data used were obtained *post partum*. Efavirenz concentration data obtained

from patients on tuberculosis treatment were not included in the study.

A total of 106 publications were identified that included the *CYP2B6* rs3745274. After screening the titles and abstracts, 67 studies were excluded (reviews, letters, articles not directly assessing the *CYP2B6* rs3745274 variant). The full texts of the remaining 39 studies were further evaluated. Twenty studies were excluded for the following reasons: a focus on associations between *CYP2B6* rs3745274 and efavirenz minimum concentration or AUC; use of a dominant or recessive model to study genotypes; and absence of standard deviation associated with mean values. Nineteen studies were included in the final meta-analysis (Table 1).

For *CYP2A6* the same criteria were followed, with 42 publications identified and 8 studies included in the final meta-analysis (Table 1). For *UGT2B7* genetic variants and their effect on efavirenz pharmacokinetics, the initial search yielded 21 publications. After reviewing the study selection process, four studies were included in the meta-analysis (Table 1). Sixteen publications were identified for *NR1I3* genetic variants and their effect on efavirenz pharmacokinetics. Using the criteria described above, the final meta-analysis included six studies investigating *NR1I3* genetic variants (Table 1). Several original authors were contacted for missing or specific data[17–22,24,27–29,36–39] and 11 replied.[17,18,21,22,24,27–29,36,37,39] As a result, 24 different studies of *CYP2B6*, *CYP2A6*, *UGT2B7* and *NR1I3* polymorphisms were included in the meta-analysis (Table 1). A PRISMA flow chart showing the literature search for each gene is provided in the Supplementary materials (Figures S1–S4).[30]

Effect of the CYP2B6 rs3745274 variant on efavirenz concentration in HIV-positive patients

Nineteen studies involving a total of 2391 individuals were analysed. Of these, 42% were African, 21% Caucasian, 26% Asian and 11% were of other ethnic populations. Through heterogeneity testing ($I^2 > 50\%$) a random effects model was applied to analyse differences in efavirenz plasma concentration between the following genotypes: GG and GT; GG and TT; and GT and TT. Following these principles, weighted standard mean differences (WSMDs) and 95% CIs were computed in different groups. Results of this meta-analysis assessing the influence of *CYP2B6* rs3745274 on efavirenz concentration are presented in Figure 1. The heterogeneity observed may have been due to the variable distribution of genotypes in different geographic areas. The analysis confirmed that individuals with a TT genotype had a significantly higher efavirenz concentration than G-homozygous patients (WSMD=2.98; 95% CI 2.19–3.76; $P<0.00001$). Similarly, individuals that were heterozygous had a significantly higher efavirenz concentration than G homozygotes (WSMD=0.74; 95% CI 0.29–1.19; $P<0.001$), and T homozygotes had a significantly higher efavirenz concentration than heterozygotes (WSMD=1.51; 95% CI 0.99–2.02; $P<0.00001$). Thus, we observed a large effect of TT genotype and a moderate effect of GT genotype with respect to higher efavirenz concentrations.

Table 1. Basic information on all studies included in the meta-analysis to investigate the effect of *CYP2B6*, *CYP2A6*, *UGT2B7* and *NR1I3* polymorphisms on efavirenz pharmacokinetics

Study	Year	Cases	Ethnicity	Gene
Tsuchiya *et al.*[40]	2004	35	Asian	*CYP2B6*
Wang *et al.*[41]	2006	46	Caucasian	*CYP2B6*
Gatanaga *et al.*[42]	2007	101	Asian	*CYP2B6*
Wyen *et al.*[13]	2008	170	Caucasian	*CYP2B6*
Uttayamakul *et al.*[43]	2010	65	Asian	*CYP2B6*
Gounden *et al.*[44]	2010	80	African	*CYP2B6*
Elens *et al.*[19]	2010	50	Caucasian	*UGT2B7*
Habtewold *et al.*[45]	2011	160	African	*CYP2B6*
Heil *et al.*[22]	2012	51	Caucasian	*CYP2A6*
Sukasem *et al.*[46]	2012	52	Asian	*CYP2B6*
Mutwa *et al.*[47]	2012	80	African	*CYP2B6*
Cortes *et al.*[21]	2013	202	South American	*CYP2A6*, *UGT2B7*, *NR1I3*
Sukasem *et al.*[48]	2013	100	Asian	*CYP2B6*
Sarfo *et al.*[18]	2014	474	African	*CYP2B6*, *CYP2A6*, *UGT2B7*, *NR1I3*
Bienvenu *et al.*[49]	2014	28	African	*CYP2B6*, *CYP2A6*
Sukasem *et al.*[50]	2014	97	Asian	*CYP2A6*
Olangunju *et al.*[39]	2014	92	Caucasian	*CYP2B6*, *NR1I3*
Olangunju *et al.*[29]	2015	117	African	*CYP2B6*, *CYP2A6*, *NR1I3*
Dickinson *et al.*[51]	2016	273	Mixed	*CYP2B6*
Cusato *et al.*[52]	2016	201	Caucasian	*CYP2B6*, *CYP2A6*, *UGT2B7*, *NR1I3*
Swart *et al.*[28]	2015	293	African	*CYP2A6*, *NR1I3*
Bienczak *et al.*[36]	2016	162	African	*CYP2B6*
Sankovsky *et al.*[37]	2017	30	American	*CYP2B6*
Neary *et al.*[53]	2017	20	African	*CYP2B6*

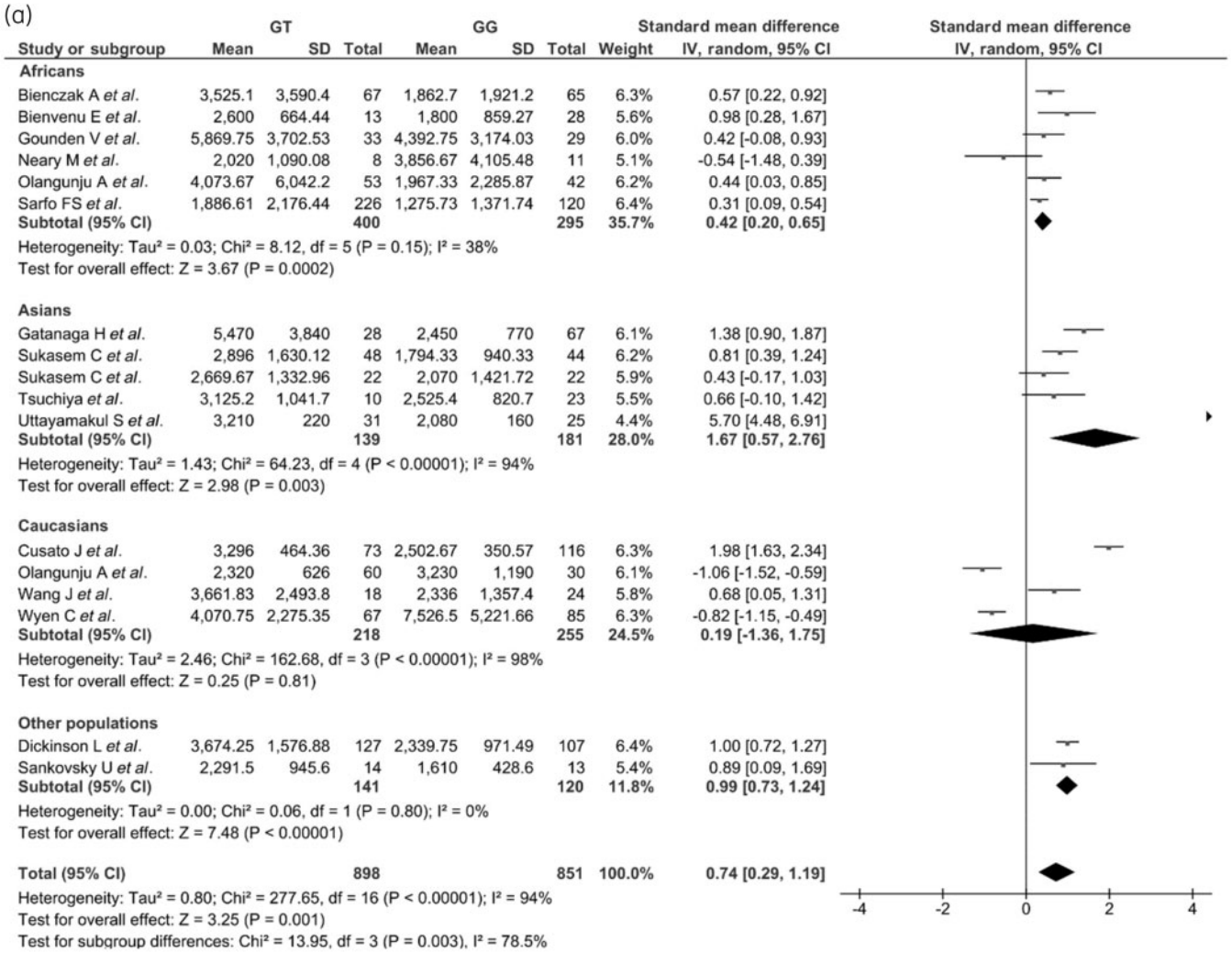

Figure 1. Forest plots of the association between *CYP2B6* 516GT and efavirenz plasma concentration: (a) GT versus GG; (b); TT versus GG; (c) TT versus GT. The centre of each square represents the WSMD. The area of the square is the number of samples and thus the weight used in the meta-analysis, and the horizontal line indicates the 95% CI.

Therefore, there may be a role for tailored efavirenz dosing in HIV patients according to *CYP2B6* rs3745274 genotype.

The funnel plot and an Egger's test for this analysis provided evidence of considerable asymmetry due to differences in ethnicity of participants (Figure S5). Stratification by ethnicity was deemed the suitable approach to correct for risk of bias. The funnel plot for each ethnicity and the Egger's test provided no evidence of bias (Figures S6–S8). To correct for risk of bias for African ethnicity, the Habtewold *et al.*[45] and Mutwa *et al.*[47] studies were excluded.

The subgroup analysis that was performed based on ethnicity detected a significant association amongst Asian patients who showed higher WSMD values (Figure 1).

The secondary measure showed that the overall frequency of the variant allele for *CYP2B6* rs3745274 was 0.322 (95% CI 0.281–0.362; Table 2). The subgroup analysis demonstrated differences in the polymorphism frequency within different ethnicities. African *CYP2B6* rs3745274 T homozygotes showed a significantly higher frequency than Asian $P<0.0001$ or Caucasian individuals ($P<0.0001$; Table 2). Moreover, this subgroup meta-analysis demonstrated a statistically significant higher efavirenz concentration in T-homozygous patients (Table 2), and this was observed within all ethnicity groups included in the analysis (overall efavirenz concentration in T homozygotes was comparable between different ethnic groups; $P=0.992$; Table 2).

Effect of CYP2A6 genetic variants on efavirenz concentration in HIV-positive patients

We analysed three different *CYP2A6* polymorphisms: rs28399454, rs8192726 and rs28399433. For *CYP2A6* rs28399454, three studies comprising a total of 468 individuals were analysed (Table 1). A fixed-effects model was applied to efavirenz plasma concentration compared by genotypes GG and GA. Two studies reported a zero frequency for the AA genotype.[21,22] Thus, these studies were removed for the GG–AA and GA–AA comparisons. The meta-analysis revealed no statistically significant association between

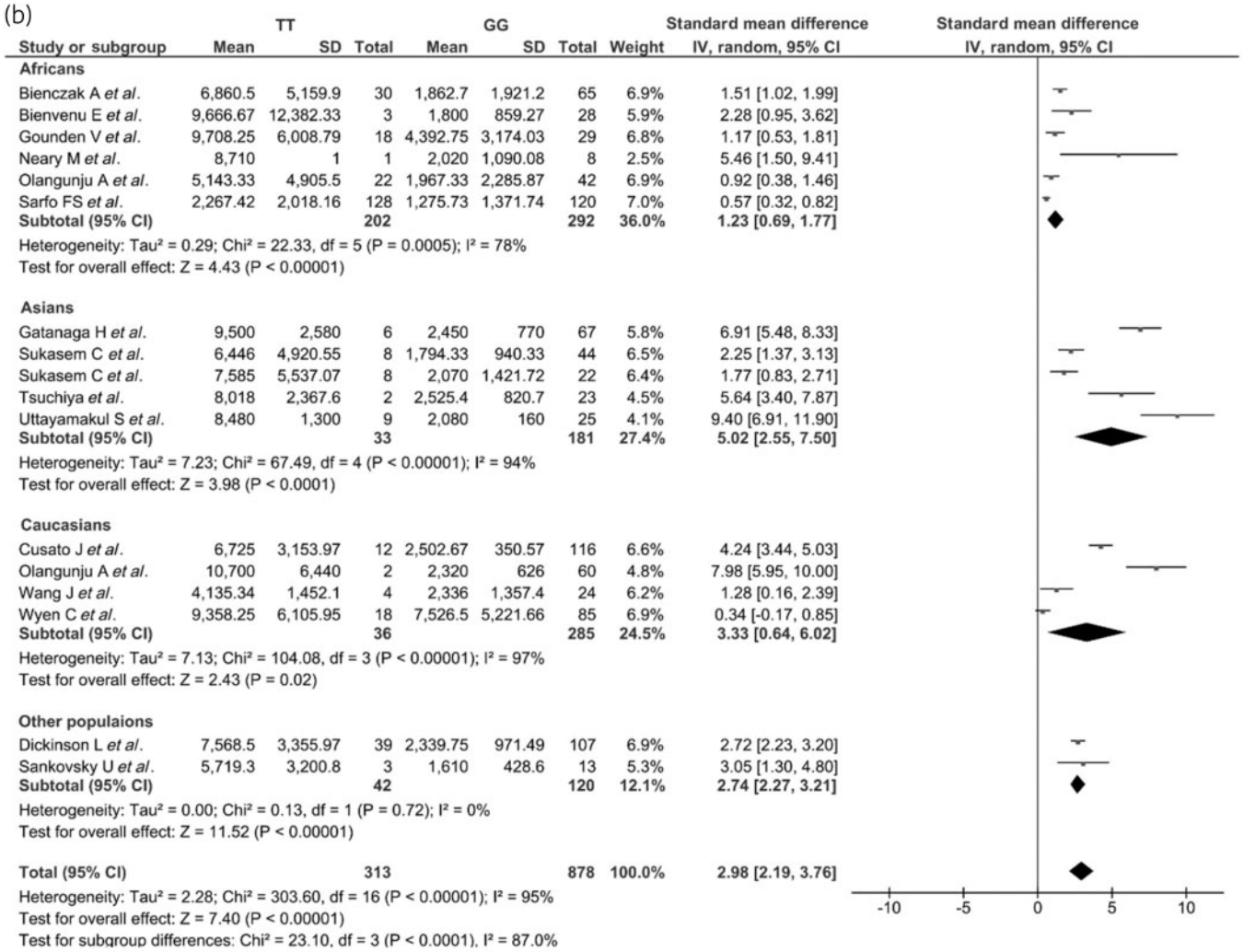

Figure 1. Continued

CYP2A6 rs28399454 and efavirenz concentration for this comparison (Figure S9). No risk of bias was observed (Figure S10). In addition, following the same procedure, *CYP2A6* rs8192726 was analysed. Two studies involving 403 individuals were included and a random-effects model was used (Table 1). WSMDs and 95% CIs were computed. One study reported the frequency of the AA genotype to be zero.[21] The meta-analysis showed no statistically significant effect on efavirenz concentration (Figure S11). Finally, the effect of *CYP2A6* rs28399433 was analysed through the inclusion of four studies comprising 693 individuals (Table 1). The funnel plot and the Egger's test indicated a small risk of bias for *CYP2A6* rs28399433, which was corrected by excluding the Sarfo *et al.*[18] study (Figure S12). A fixed-effects model was performed for all the comparisons. The meta-analysis revealed no statistically significant differences for these comparisons (Figure S13).

Effect of UGT2B7 genetic variants on efavirenz concentration in HIV-positive patients

We analysed two *UGT2B7* polymorphisms, rs28365062 and rs7439366. For *UGT2B7* rs28365062, four studies involving 949 individuals were analysed (Table 1). A fixed-effects model was used for AA–GG, AA–AG and AG–GG comparisons. The meta-analysis demonstrated no statistically significant association between *UGT2B7* rs28365062 and efavirenz concentration for any comparison (Figure S14). Similarly, for *UGT2B7* rs7439366 three studies involving 1099 individuals were included in the meta-analysis. A fixed-effects model was applied for CC–TT and CT–TT comparisons and a random effects model for CC–CT. Although individuals with a TT genotype had a notably lower efavirenz concentration than homozygotes for the C allele, this difference was not statistically significant (WSMD=−0.23; 95% CI −0.49–0.04; *P*=0.09). For the CT–TT and CC–CT comparisons, the meta-analysis did not show a statistically significant association between *UGT2B7* rs7439366 and efavirenz concentrations (Figure S15). No risk of bias was observed (Figures S16 and S17).

Effect of NR1I3 genetic variants on efavirenz concentration in HIV-positive patients

Two *NR1I3* polymorphisms were analysed, rs2307424 and rs3003596. In the case of *NR1I3* rs2307424, six studies

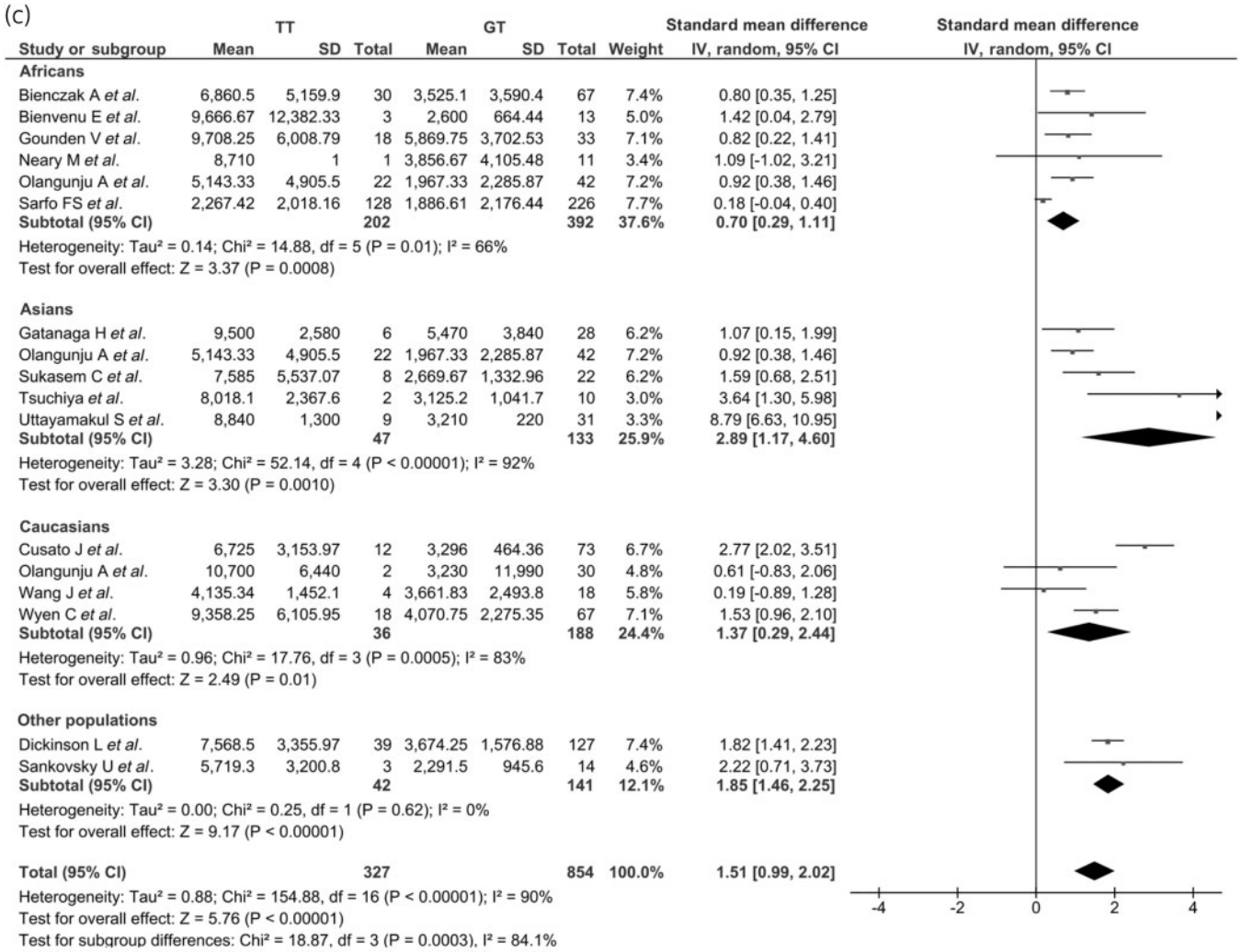

Figure 1. Continued

Table 2. Subgroup analysis of *CYP2B6* rs3745274 frequency and its effects on efavirenz concentration throughout African, Asian and Caucasian populations

Subjects	Study citations	Overall T allele frequency estimated (95% CI)	Test heterogeneity (I^2, *P* value)	Overall TT genotype frequency estimated (95% CI)	Test heterogeneity (I^2, *P* value)	Overall efavirenz concentration estimated in TT homozygous individuals (95% CI)	Test heterogeneity (I^2, *P* value)
African	18, 29, 36, 44, 49, 53	0.393 (0.371–0.416)	0.0, 0.697	0.168 (0.097–0.240)	0.86, 0.001	6661.7 (2836.88–10486.50)	0.99, 0.0001
Asian	40, 42, 43, 46, 48	0.290 (0.213–0.368)	0.81, 0.0001	0.085 (0.052–0.118)	0.22, 0.274	8458.96 (7727.25–9190.66)	0.0, 0.629
Caucasian	13, 39, 41, 52	0.251 (0.199–0.303)	0.71, 0.016	0.063 (0.024–0.102)	0.70, 0.018	6824.01 (4171.68–9476.35)	77.87, 0.004
Mixed population	37, 51	0.371 (0.333–0.410)	0.0, 0.512	0.137 (0.099–0.176)	0.0, 0.466	7424.32 (6412.96–8435.68)	0.0, 0.337
Overall		0.322 (0.281–0.362)	0.86, 0.001	0.117 (0.077–0.156)	0.88, 0.001	7193.252 (5252.24–9134.26)	0.99, 0.0001

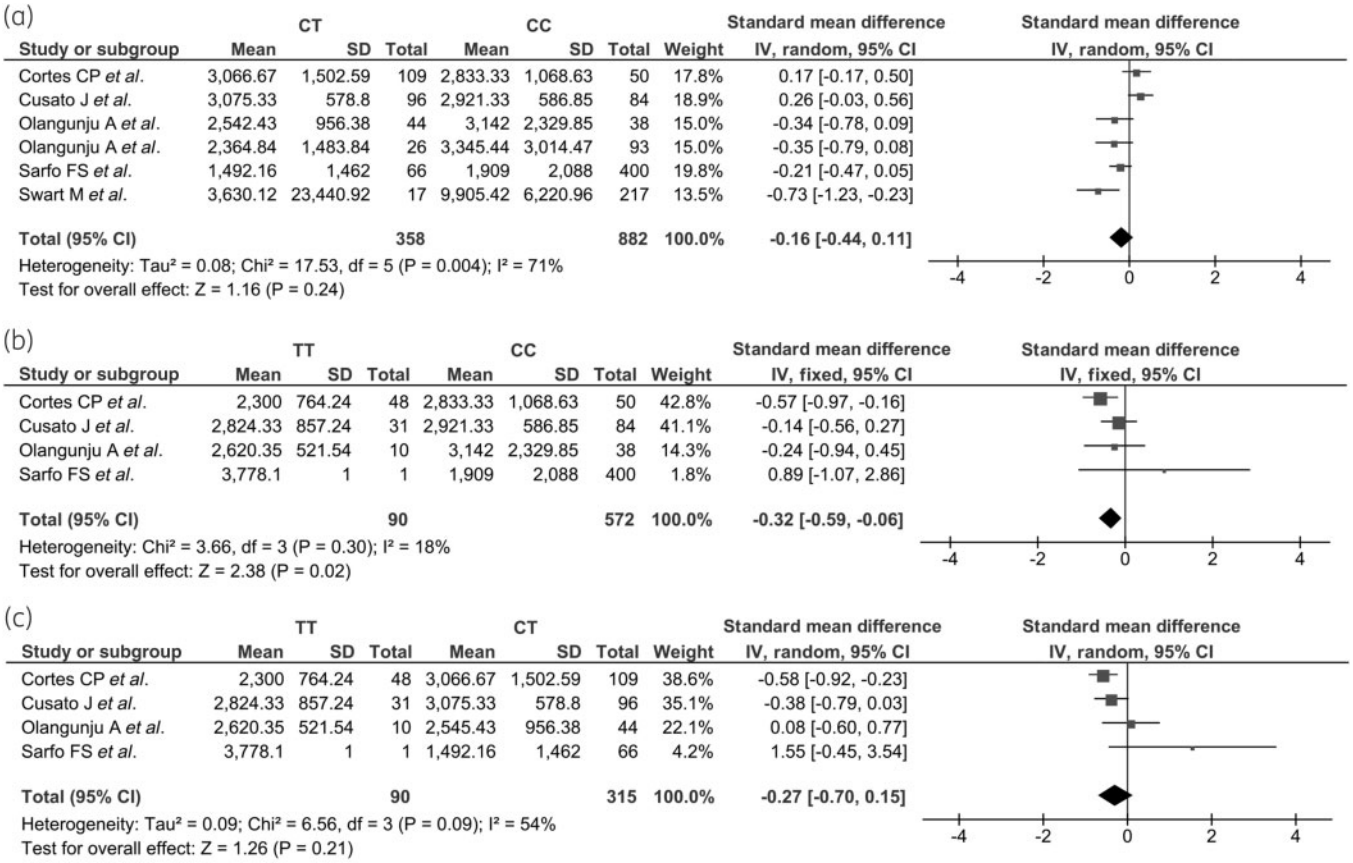

Figure 2. Forest plots of the association between *NR1I3* rs2307424 and efavirenz plasma concentration: (a) CC versus CT; (b) CC versus TT; (c) CT versus TT. The centre of each square represents the WSMD. The area of the square is the number of sample and thus the weight used in the meta-analysis, and the horizontal line indicates the 95% CI.

involving 1330 individuals were included (Table 1). A random-effects model was used for CC–CT and CT–TT comparisons and a fixed-effects model for CC–TT. Two of these studies reported zero individuals with the TT genotype.[28,29] Therefore, four studies were included in the CC–TT and CT–TT comparisons. The meta-analysis revealed no statistically significant differences for CC–CT and CT–TT comparisons. However, when comparison was performed between TT and CC subjects, the meta-analysis indicated that individuals with a TT genotype had a significantly lower efavirenz concentration than individuals homozygous for the C allele (WSMD=−0.32; 95% CI −0.59 to −0.06; *P*=0.02; Figure 2). Hence, a moderate effect of TT genotype on lower efavirenz concentrations was observed. The rs2307424 is associated with a CAR gain of function and would therefore be expected to result in higher CYP2B6 activity and lower efavirenz concentrations.

For the *NR1I3* rs3003596 polymorphism, three studies comprising 440 individuals were analysed (Table 1). Following the same procedure, a random-effects model was performed for TT–CC and CT–CC comparisons and a fixed-effects model for TT–CT. The meta-analysis showed no statistically significant differences for any comparisons (Figure S18). No risk of bias was observed (Figures S19 and S20).

Discussion

Variability in efavirenz plasma concentrations influences its safety and efficacy.[14] Genetic polymorphisms in drug-metabolizing enzymes, including CYP2B6, CYP2A6 and UGT2B7 and transcriptional regulators such as CAR, have been shown to affect efavirenz pharmacokinetics. However, some associations have yielded conflicting results between studies. Therefore, we performed a meta-analysis to systematically assess previously reported associations and provide further clarity on the relevance of *CYP2B6*, *CYP2A6*, *UGT2B7* and *NR1I3* polymorphisms.

A large number of *CYP2B6* SNPs have been identified,[54] but rs3745274 and rs2839949 (983T>C) have been most extensively studied for their impact upon efavirenz pharmacokinetics. *CYP2B6* rs2839949 is exclusively found in patients of African ancestry. This polymorphism produces an amino acid change in the CYP2B6 protein, resulting in a reduction in enzyme function and subsequently an alteration in efavirenz pharmacokinetics.[28,36,55,56] *CYP2B6* rs2839949 and rs3745274 have been reported to explain 8% and 19% of efavirenz variance, respectively, in a South African population.[57] However, *CYP2B6* rs2839949 was not included in the current analysis because the aim was to characterize the effect of *CYP2B6* polymorphisms within multiple ethnic groups. For this reason, *CYP2B6* rs3745274 was the focus of the analysis.[9–15] This

meta-analysis demonstrates that individuals with a TT or GT genotype had a significantly higher efavirenz concentration than patients homozygous for the G allele in a gene-dose-dependent manner, and is in concordance with a previous meta-analysis conducted with a smaller number of studies.[58] African *CYP2B6* T homozygotes had a significantly higher frequency than Asian or Caucasian individuals, but the effect of this genotype on efavirenz plasma concentration was similar between different ethnicities. These results reinforce the important role of *CYP2B6* rs3745274 on efavirenz pharmacokinetics, variability in which has been associated with CNS side effects and virological failure.[59]

A reduced dose of 400 mg of efavirenz was demonstrated to be non-inferior to the standard 600 mg dose in the ENCORE1 trial. Moreover, adverse events related to efavirenz were more frequent in recipients of 600 mg of efavirenz than in recipients of 400 mg.[60] Recent data have suggested that 400 mg doses may be appropriate during therapy with rifampicin during TB coinfection and during pregnancy.[4,5] A *CYP2B6* genotype-guided efavirenz dose reduction in pregnant women also suggested keeping the standard 600 mg dose for fast and intermediate metabolizers while reducing to 400 mg in slow metabolisers.[61] Given that the magnitude of effect of *CYP2B6* rs3745274 is similar between different ethnicities, the appropriateness of rolling out validated clinical strategies across populations is de-risked.

The effect of *CYP2A6* rs28399433 on efavirenz pharmacokinetics was associated with individuals with concomitant *CYP2B6* slow-metabolizer genotypes.[24,62] However, this effect has also been described in individuals independently of *CYP2B6* genotype and extended to *CYP2A6* rs8192726 and rs28399454 with some conflicting results.[17–21] The findings of the current meta-analysis indicate no significant effect of *CYP2A6* rs8192726, rs28399433 and rs2839945. However, it should be noted that only a comparatively small number of studies have investigated *CYP2A6*, and the frequency of these SNPs is somewhat lower than those of *CYP2B6* alleles.

This meta-analysis also failed to find an association between *UGT2B7* rs28365062 and efavirenz concentrations. The association with this genetic variant was also initially described in individuals with *CYP2B6* slow-metabolizer genotypes.[24] However, all the studies included in our analysis researched the effect of this polymorphism independently of *CYP2B6* status. In addition to rs28365062, we also analysed the effect of *UGT2B7* rs7439366, which was previously associated independently with alterations in efavirenz pharmacokinetics.[17] The meta-analysis also did not find a statistically significant effect, although individuals with a TT genotype had lower efavirenz concentrations than patients that were homozygous for the C allele. Again, these observations should be interpreted in the context that very few studies have been conducted, and this association was only identified in a Ghanaian cohort.[17]

CAR is a ligand-activated transcription factor but also regulates expression of *CYP2B6* and other genes in a constitutive manner.[63] *NR1I3* rs2307424 has been associated with low efavirenz concentrations in Chilean HIV patients, [21] but other studies in African or Caucasian populations failed to demonstrate the association.[29,52] However, the presented meta-analysis suggests that individuals with a TT genotype have significantly lower efavirenz concentrations than individuals that are homozygous for the C allele. This is in line with the association reported between the *NR1I3* rs2307424 CC genotype and early discontinuation of efavirenz treatments.[26] Neuropsychiatric side effects in patients on efavirenz treatment that lead to early discontinuation have been associated with high plasma concentrations,[64–66] and the *NR1I3* rs2307424 CC genotype is linked to higher efavirenz plasma concentrations.

The polymorphism *NR1I3* rs2307424 is synonymous (proline/proline)[67] and there is a lack of functional studies that have demonstrated how this SNP might affect CAR functionality. *NR1I3* rs2307424 TT could be in linkage disequilibrium with an undefined causative polymorphism that evokes an increase in CAR activity; this would result in elevated CYP2B6 activity and consequently lower efavirenz concentrations.

Conversely, *NR1I3* rs3003596 has been suggested to increase CAR expression and therefore elicit an effect through promoting expression of enzymes that participate in efavirenz metabolism.[27] However, our meta-analysis failed to demonstrate a definitive correlation between *NR1I3* rs3003596 and efavirenz concentrations. The initial report of this association was conducted in a small cohort[28] and other studies including this SNP are scarce. Therefore, further research to clarify the role of this polymorphism is required.

There are limitations to this meta-analysis. Firstly, the mean and standard deviation values were unavailable in several studies and some of these were excluded from analysis because attempts to contact the authors failed. Secondly, the sample size for some polymorphisms was small. Thirdly, most eligible studies were conducted in African and Asian population while few were conducted in Caucasians.

In conclusion, this meta-analysis reinforces the well-reported effect of *CYP2B6* rs3745274 on efavirenz plasma concentrations across different ethnicities. Despite the different allele frequencies observed within different populations, the effect size for *CYP2B6* rs3745274 is similar. A significant association between *NR1I3* rs2307424 and lower efavirenz concentrations was also reinforced by the meta-analysis, but no association between *CYP2A6* or *UGT2B7* genetic variants was evident.

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Supplementary data

Supplementary Methods, Table S1 and Figures S1–S20 are available as Supplementary data at *JAC* Online.

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