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Genome-wide association study of plasma efavirenz pharmacokinetics in AIDS Clinical Trials Group protocols implicates several *CYP2B6* variants

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Abstract

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Conflicts of interest

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Objectives—Prior candidate gene studies have associated *CYP2B6* 516G→T [rs3745274] and 983T→C [rs28399499] with increased plasma efavirenz exposure. We sought to identify novel variants associated with efavirenz pharmacokinetics.

Materials and methods—Antiretroviral therapy-naive AIDS Clinical Trials Group studies A5202, A5095, and ACTG 384 included plasma sampling for efavirenz pharmacokinetics. Log-transformed trough efavirenz concentrations (C_{min}) were previously estimated by population pharmacokinetic modeling. Stored DNA was genotyped with Illumina HumanHap 650Y or 1MDuo platforms, complemented by additional targeted genotyping of *CYP2B6* and *CYP2A6* with MassARRAY iPLEX Gold. Associations were identified by linear regression, which included principal component vectors to adjust for genetic ancestry.

Results—Among 856 individuals, *CYP2B6* 516G→T was associated with efavirenz estimated C_{min} ($P = 8.5 \times 10^{-41}$). After adjusting for *CYP2B6* 516G→T, *CYP2B6* 983T→C was associated ($P = 9.9 \times 10^{-11}$). After adjusting for both *CYP2B6* 516G→T and 983T→C, a *CYP2B6* variant (rs4803419) in intron 3 was associated ($P = 4.4 \times 10^{-15}$). After adjusting for all the three variants, non-*CYP2B6* polymorphisms were associated at *P*-value less than 5×10^{-8}. In a separate cohort of 240 individuals, only the three *CYP2B6* polymorphisms replicated. These three polymorphisms explained 34% of interindividual variability in efavirenz estimated C_{min}. The extensive metabolizer phenotype was best defined by the absence of all three polymorphisms.

Conclusion—Three *CYP2B6* polymorphisms were independently associated with efavirenz estimated C_{min} at genome-wide significance, and explained one-third of interindividual variability. These data will inform continued efforts to translate pharmacogenomic knowledge into optimal efavirenz utilization.

Keywords

CYP2B6; efavirenz; HIV; pharmacogenomics; pharmacokinetics

Introduction

Efavirenz is one of the most extensively prescribed medications for HIV-1 infection worldwide. Although generally safe and effective, some efavirenz recipients experience virologic failure [1] and/or central nervous system symptoms [2]. Efavirenz is metabolized primarily by cytochrome P450 (CYP) 2B6 [3]. Analyses involving AIDS Clinical Trials Group (ACTG) protocol A5097s first associated the nonsynonymous variant *CYP2B6* 516G→T (rs3745274) with increased plasma efavirenz exposure [4]. Subsequent studies have consistently replicated this pharmacokinetic association [5–8]. The *CYP2B6* 516T allele is considerably more frequent in individuals of African ancestry than those of European ancestry [9], which largely explains the somewhat greater mean plasma efavirenz concentrations among populations of African descent [10,11]. A less frequent *CYP2B6* polymorphism, 983T→C (rs28399499), also predicts increased plasma efavirenz exposure [12–14]. The *CYP2B6* 983C allele is also more frequent with African ancestry, although far less frequent overall than 516G→T, and is virtually absent from populations of European ancestry [9]. Functional studies have associated the *CYP2B6* 516G→T variant with reduced hepatic CYP2B6 mRNA levels *in vivo*, and with aberrant splicing *in vitro* [15]. The *CYP2B6* 983TC variant has also been associated with reduced CYP2B6 mRNA levels *in vitro* [12,16].

Data are scant regarding efavirenz pharmacokinetic associations beyond *CYP2B6* 516G→T and 983T→C. Additional *CYP2B6* polymorphisms suggested to affect *CYP2B6* activity have been extremely infrequent [12,17,18] or have not predicted plasma efavirenz exposure [18,19]. Polymorphisms in genes beyond *CYP2B6* reported to affect interindividual

variability in efavirenz pharmacokinetics include *CYP2A6* [20,21], *UGT2B7* [21], *CYP3A4* [22], *CYP3A5* [4], and *CAR* [23], although such associations have yet to be replicated.

All previous analyses to identify genetic associations with efavirenz pharmacokinetics have relied upon candidate gene strategies. To determine whether genetic variants beyond *CYP2B6* 516G→T and 983T→C are associated with interindividual variability in plasma efavirenz exposure, we apply a retrospective genome-wide analysis approach to data from multiple prospective clinical trials of the ACTG.

Materials and methods

Study participants

Treatment-naive individuals were randomized to efavirenz-containing regimens in ACTG studies 384 [24,25], A5095 [1] (including its neurologic substudy A5097s [2]), and A5202 [26,27], with DNA obtained under protocol A5128 [28]. The present analyses involved individuals from these clinical trials who also had available plasma efavirenz assay data. Some participants from 384 and A5097s in these analyses were also included in previous candidate gene studies describing associations between *CYP2B6* 516G→T, 983T→C, and plasma efavirenz exposure [4,6,14]. Self-identified race/ethnicity categories 'white, non-Hispanic', 'black, non-Hispanic', and 'Hispanic' are hereafter referred to as white, black, and Hispanic, respectively. This study complied with the Helsinki Declaration, was approved by institutional review boards for each site, and participants gave written informed consent.

Identifying genetic polymorphisms

This clinical trials population has undergone whole genome analysis for a project related to immune control of HIV-1 replication [29]. A total of 1739 individuals received genome-wide genotyping. Genotyping was conducted in three phases, where the first phase utilized the Illumina 650Y Beadchip and the latter two phases, the Illumina Human-1M Duo Beadchip (Illumina Inc., San Diego, California, USA). For statistical analyses we used only those polymorphisms that were common to both platforms and were successfully genotyped. The NIH grant that supported A5202 was submitted to NIH before 2008, and support for genome-wide genotyping was not from NIH, so this study is not subject to NIH policy that requires submission of genome-wide association study data to the database of Genotypes and Phenotypes (dbGaP). The ACTG has well-established procedures to facilitate data and specimen sharing with non-ACTG investigators.

Genome-wide data were complemented by additional targeted genotyping in a subset of 891 participants. A total of 73 polymorphisms in *CYP2B6* (n=47), *CYP2A6* (n=21), *CYP3A4* (n=1), *CYP3A5* (n=1), and *ABCB1* (n=3) were genotyped by MassARRAY iPLEX Gold (Sequenom Inc., San Diego, California, USA). The Sequenom assay design was as follows: We tagged the entire *CYP2B6* gene using SeattleSNPs [30], including 5 kB in each 5′ and 3′ untranslated regions (UTR), using a cosmopolitan strategy across populations (Yoruba, Asian, African-American, European-American, and Hispanic) with a 5% allelic frequency cutoff, a 0.80 threshold for r^2, 85% data convergence for tagging polymorphisms, and 70% data convergence for clustering. Additional polymorphisms of interest (but that were not extremely infrequent) were added based on previous reports [12,13]. We also added polymorphisms with at least 5% allelic frequency in 20 kB of the 5′ UTR identified using Ensembl Genome Browser [31], as well as upstream polymorphisms possibly associated with *CYP2B6* expression based on a previous report [28]. (Final Sequenom assay design available upon request.) Genotypes were confirmed by visual inspection of plots. The MassARRAY iPLEX Gold were merged with the genome-wide data for statistical analyses.

In addition, a custom MassARRAY iPLEX Gold assay was designed for test whether selected polymorphisms from whole genome analyses would replicate in a separate group of individuals.

Laboratory personnel with no knowledge of clinical data performed genotyping. Quality control of genotype data was performed using PLINK [32] and EIGENSTRAT [33]. Samples which were potential sex misclassifications, population outliers according to principal components analysis projected onto HapMap Phase III data [29,34], individuals with estimated IBD greater than or equal to 0.25, and those with exceptionally high or low heterozygosity values ($|F|>0.1$) were excluded. Subsequently, genetic markers were excluded if the genotyping completion rate was lower than 98% or if minor allele frequency was below 1%.

Pharmacokinetic endpoint

Plasma efavirenz concentrations were assayed by high-performance liquid chromatography at treatment weeks 1, 4, 12, and 24. Sampling times were not prespecified, and the time of prior dose was by patient report. For association analyses, efavirenz C_{min} values were estimated based on measured efavirenz concentrations at times in the dosing interval before C_{min}, adjusted for dosing time using simulated efavirenz concentration–time curve percentiles, and generated by pharmacokinetic model simulations [35]. On the basis of dosing time, each observed concentration was assigned to a percentile. For each individual the summary statistic for analysis was median of observed time-adjusted percentiles over up to four time points for each individual. To minimize confounding by nongenetic factors and to assure steady state, we excluded individual efavirenz concentrations if beyond 24 h postdose or before at least 2 weeks of efavirenz, individuals with only one evaluable efavirenz value, and those with greater than 30-percentile difference between any two concentrations. For each participant, the median efavirenz percentile was used to estimate a 24-h postdose plasma efavirenz concentration (C_{min}), which was then used in all statistical analyses.

Statistical analysis

Linear regression was used to test for association between each genetic variant and efavirenz estimated C_{min}. Before regression analysis, estimated C_{min} values were log-transformed because of non-normality. Next, the residual from regression with the top 10 principal component vectors was taken to adjust for potential confounding by ancestry. The number of statistically significant principal components was computed using twstats in the EIGENSOFT software package [33,36]. Linear regression was conducted first using this residual phenotype and then adjusting for rs3745274 (*CYP2B6* 516G→T), then for rs3745274 and rs28399499 (*CYP2B6* 983T→C), and then for rs3745274, rs28399499, and rs4803419 (*CYP2B6* 15582C→T). Linear regression was performed using PLINK, whereas residuals were taken using the lm command in the R stats package [37]. In the replication analysis, the linear regression process was repeated adjusting for ethnicity and the three single-nucleotide polymorphisms as categorical variables instead of computing residuals from the phenotype. Manhattan plots were generated using the R stats package [37].

Results

Genome-wide association analyses of efavirenz estimated C_{min}

Initial analyses comprised 856 individuals (16% women, 50% self-reported white, 33% self-reported black, 18% self-reported Hispanic). Associations between genetic polymorphisms and efavirenz estimated C_{min} values by linear regression, correcting for the top 10 principal components, are shown in Fig. 1a. There is a strong genome-wide significant ($P<5\times10^{-8}$)

peak of polymorphisms involving the *CYP2B6* locus on chromosome 19. The lowest *P*-value represented *CYP2B6* 516G→T (rs3745274, $P=8.5\times10^{-41}$). To investigate associations beyond this peak, we repeated this linear regression while additionally adjusting for 516G→T. This analysis showed significant associations that included *CYP2B6* 983T→C (rs28399499, $P=9.9\times10^{-11}$) and *CYP2B6* 15582C→T (rs4803419, $P=3.4\times10^{-11}$). In addition, there were apparent associations with polymorphisms on chromosome 8 (rs7818576, $P=7.2\times10^{-9}$) and on chromosome 18 (rs288982, $P=1.5\times10^{-8}$; rs2959521, $P=2.2\times10^{-8}$), as shown in Fig. 1b. We continued to investigate associations by again repeating linear regression, this time adjusting for both *CYP2B6* 516G→Tand 983T→C. (This polymorphism took priority over rs4803419 because of the former's strong a-priori evidence of association with efavirenz pharmacokinetics.) This analysis continued to show a significant association with *CYP2B6* 15582 C→T ($P=4.4\times10^{-15}$), along with variants on chromosomes 8 and 18 (Fig. 1c). In a final analysis adjusted for 516G→T, 983T→C, and rs4803419, there appeared to be a continued significant association with rs7818576 on chromosome 8 ($P=1.1\times10^{-9}$), as well as an association with rs2288657 on chromosome 2 ($P=3.6\times10^{-8}$). The polymorphisms on chromosome 18 no longer exceeded the threshold for genome-wide significance (Fig. 1d). Of note, after adjusting for 516G→T, 983T→C, and rs4803419 there was no association detected between efavirenz estimated C_{min} values and *CYP2B6* rs2279343 ($P=0.49$). The latter polymorphism defines *CYP2B6*4*, and was recently reported to be associated with increased plasma clearance of the CYP2B6 substrate nevirapine among HIV-infected Cambodians [38].

Replication or genotype–phenotype associations

In the clinical trials studied herein there were 240 individuals with efavirenz pharmacokinetic data, but who were not included in genome-wide association analyses. We used this group to attempt replication of the above associations with *CYP2B6* polymorphisms as well as with 18 other polymorphisms with *P*-value less than 10^{-5} in the final genome-wide analysis above (Table 1). Of note, none of the 18 polymorphisms were close to any gene with clear plausibility for affecting efavirenz disposition. Each polymorphism was examined for association with efavirenz estimated C_{min} individually, and with correction for *CYP2B6* 516G→T. Self-identified race/ethnicity (rather than principal components because genome-wide genotype data were not available) was used to adjust for systematic ancestry differences that might constitute population stratification. In the analysis unadjusted for *CYP2B6* genotype, 516G→Tremained strongly associated with efavirenz estimated C_{min} ($P=6.1\times10^{-14}$). A chromosome 5 polymorphism (rs1897833) was also nominally associated ($P=0.004$), however, its effect was in the opposite direction as in the genome-wide analysis. After adjusting for 516G→T, *CYP2B6* rs4803419 C→T was significantly associated with efavirenz estimated C_{min} ($P=2.3\times10^{-5}$), with a trend toward significance for *CYP2B6* 983T→C ($P=0.070$). In a final analysis that adjusted for both *CYP2B6* 516G→T and rs4803419 C→T, *CYP2B6* 983T→C was nominally associated with efavirenz estimated C_{min} ($P=0.017$). Thus, all three *CYP2B6* polymorphisms replicated, whereas no polymorphism beyond *CYP2B6* replicated.

Linkage disequilibrium with other *CYP2B6* variants

To better understand relationships between efavirenz estimated C_{min} and the three *CYP2B6* polymorphisms that were independently associated in genome-wide analyses, we considered their linkage disequilibrium (LD) with other polymorphisms. In the first genomewide analyses, without adjusting for any *CYP2B6* polymorphism, two groups of polymorphisms were associated with efavirenz estimated C_{min} (Fig. 2, blue markers), the first comprising 13 polymorphisms in strong LD with *CYP2B6* 516G→T and with minor alleles associated with higher efavirenz estimated C_{min} values (i.e. positive β values), and the second comprising 13 polymorphisms in weaker LD with *CYP2B6* 516G→Tand with minor alleles

associated with lower efavirenz estimated C_{min} values (i.e. negative β values). In the second analyses, which adjusted for *CYP2B6* 516G→T, only four of the above 26 polymorphisms remained associated with efavirenz estimated C_{min} values, whereas eight additional polymorphisms became significant (Fig. 2, red markers). All but *CYP2B6* 983T→C were all in strong LD with rs4803419 C→T. In the third analyses, which adjusted for *CYP2B6* 516G→Tand 983T→C, only rs4803419 C→Tand seven polymorphisms in strong LD with rs4803419 were associated with efavirenz estimated C_{min} values (Fig. 2, orange markers).

The rs4803419 polymorphism had not been previously associated with efavirenz pharmacokinetics. We therefore used 1000 Genomes data to further characterize LD with rs4803419 C→T in different populations. For polymorphisms within 200 kB of rs4803419 and that are in LD at r^2 values of at least 0.5 among individuals of African, Asian, European, and Hispanic ancestry, r^2 values are shown in Online Supplemental Materials (Supplementary digital content 1, http://links.lww.com/FPC/A523). Of note, the many polymorphisms in strong LD with rs4803419 included rs7251950 (r^2=0.88 in Asians, r^2=0.68 in Europeans, r^2=1.00 in Hispanics). As reported elsewhere in this journal issue, rs7251950 was associated with decreased plasma nevirapine clearance among HIV-1-infected patients in Cambodia [38].

Associations with efavirenz estimated C_{min} values

Relationships between each of the three significant *CYP2B6* polymorphisms and efavirenz estimated C_{min} values among all evaluable study participants are shown in Fig. 3. Homozygosity for any one of these polymorphisms appears to preclude the presence of the other two, suggesting that *CYP2B6* 516T, 983C, and rs4803419T may be on mutually exclusive haplotypes. Both *CYP2B6* 516G→T and 983T→C were associated with markedly increased median efavirenz estimated C_{min} values. The median estimated C_{min} among individuals homozygous for *CYP2B6* 516 TTwas 3.98 μg/ml, 5.4 times higher than for individuals lacking variant alleles at all three loci (0.74 μg/ml). This ratio was 7.1 among individuals who were concomitantly heterozygous for both 516Tand 983C (5.38 μg/ml). In contrast, compared with individuals lacking variant alleles at all three loci, homozygosity for rs4803419 TT was associated with only 1.7 times higher median estimated C_{min} values (1.24 μg/ml), comparable with *CYP2B6* 516 GT heterozygosity (1.27 μg/ml). Among individuals heterozygous for *CYP2B6* 516T or 983C, the concomitant presence of a rs4803419 T allele conferred somewhat higher median estimated C_{min} values. In the genome-wide association analyses described above, the somewhat smaller *P*-value for rs4803419 C→T than for 983T→C is explained by the former's greater allelic frequency, despite its lesser magnitude of effect.

In the genome-wide analysis cohort, a linear regression model that included the three significant *CYP2B6* polymorphisms and the top 10 principal components vectors explained 34% of the variance in log-transformed C_{min}. A model that considered the *CYP2B6* polymorphisms without principal component vectors explained 33% of the variance, whereas in a model that considered the top 10 principal component vectors without *CYP2B6* polymorphisms explained only 3% of the variance. In the replication cohort, the three significant *CYP2B6* polymorphisms and self-identified race/ethnicity indicators (rather than principal component vectors) explained 31% of the variance in log-transformed C_{min}.

Previously reported associations beyond *CYP2B6*

Previous reports have suggested associations between polymorphisms in *CYP2A6* [20,21], *UGT2B7* [21], *CYP3A4* [22], *CYP3A5* [4], and *CAR* [23] and increased plasma efavirenz exposure. Genotype results for six previously implicated polymorphisms were available in the present genome-wide dataset. After adjusting for *CYP2B6* 516G→T, 983T→C, and

rs4803419, no association was detected between efavirenz estimated C_{min} values and 28399433 (*CYP2A6*9*, *CYP2A6*13*, and *CYP2A6*15*, P=0.82), rs1801272 (*CYP2A6*2*, P=0.07), rs28399454 (*CYP2A6*17*, P=0.39), rs4646437 (*CYP3A4*1B*, P=0.91), rs776746 (*CYP3A5*3*, P=0.59), or rs2307424 (*CAR*, P=0.99).

Discussion

Here we present results of the first genome-wide association analyses of efavirenz pharmacokinetics. We readily show at genome-wide significance that both *CYP2B6* 516G→T and 983T→C are independently associated with efavirenz estimated C_{min} values. After controlling for these two polymorphisms we identify an independent association with a third *CYP2B6* variant (rs4803419), which replicated in a separate group of study participants. A multivariable model that included all three *CYP2B6* polymorphisms and the top 10 principal components vectors explained approximately one-third of variance in efavirenz log-transformed estimated C_{min} value. It is noteworthy that two of these three genomewide significant polymorphisms were already known to predict efavirenz pharmacokinetics based on prior candidate gene studies [5–8,12–14].

Several lines of evidence support the validity of association between rs4803419 and efavirenz C_{min}. First, this association achieved genome-wide significance in our discovery cohort, and replicated in a separate group of clinical trials participants. Second, this polymorphism has recently been associated with increased plasma clearance of the CYP2B6 substrate nevirapine among patients in Cambodia, as reported elsewhere in this journal issue [38]. Third, in a previous ex-vivo analysis of human liver tissue samples, this polymorphism was associated with decreased CYP2B6 activity among women [39]. As this polymorphism is adjacent to an intron 3 splicing branch site, it has been speculated that this may cause aberrant splicing with resultant decreased expression of the functional enzyme [39]. However, Hofmann *et al.* [15] found no association between rs4803419 and splice variants in human liver. We cannot exclude the possibility of an indirect effect through LD with other polymorphisms. In this regard, Lang *et al.* [40] reported that rs4803419 resides on several haplotypes (*CYP2B6*13B*, **15A*, and **15B*) that include nonsynonymous polymorphisms, several of which (K139E, Q172H, I391N, and K262R) were associated with decreased CYP2B6 protein expression and/or decreased enzymatic activity in COS-1 cells and in ex-vivo liver samples. Our query of 1000 Genomes data showed rs4803419 to be in strong LD with many polymorphisms, although none in exons. The effect size for this polymorphism was modest in comparison with those for *CYP2B6* 516G→Tand 983T→C, and rs4803419 appears to reside on a haplotype(s) separate from the former two polymorphisms. The implication is that rs4803419 does not improve our ability to predict very high plasma efavirenz concentrations (e.g. >4.0 μg/ml) beyond 516G→T and 983T→C, because rs4803419 T cannot be present among individuals who already have two variant alleles at *CYP2B6* 516G→Tand/or 983T→C. The rs4803419 polymorphism does, however, improve considerably the overall description of efavirenz estimated C_{min} values, especially at lower concentrations. Individuals homozygous for rs4803419 CC who also lacked 516T and 983C had the lowest median efavirenz estimated C_{min} values (Fig. 3).

In multivariable analyses that controlled the three significant *CYP2B6* polymorphisms, no additional *CYP2B6* polymorphisms reached genome-wide significance. Although several polymorphisms beyond chromosome 19 achieved genome-wide significance in the genome-wide dataset, none of these replicated in the targeted analyses of a separate group of study participants. These polymorphisms were therefore almost certainly spurious. There may very well be other polymorphisms in the present cohort that are independently associated with substantially increased plasma efavirenz C_{min} values. These must, however, be very infrequent, because among 383 individuals lacking variant alleles at both *CYP2B6*

516G→T and 983T→C only one (0.26%) had an estimated C_{min} greater than 4.0 μg/ml (Fig. 3). Alternatively, other yet-to-be-identified variants (not assayed herein) may have modest effects alone, however, with much greater impact when combined with *CYP2B6* 516G→T or 983T→C.

There were limitations to this study. We largely studied individuals of European descent, African descent, and Hispanics. Different associations may be identified in other populations. We limited our statistical analyses to polymorphisms that were shared across the two genomewide assay platforms (plus additional targeted genotyping of *CYP2B6* and selected other genes). More in-depth genotyping might identify additional polymorphisms associated with efavirenz pharmacokinetics, particularly if such polymorphisms are infrequent or were poorly tagged by our assays. Efavirenz was not directly quantified at the time of C_{min}, but rather C_{min} values were estimated by applying pharmacokinetic model simulations to assays obtained earlier in the dosing interval, and the time since previous dose was by self-report. Therefore, actual efavirenz C_{min} values may differ from these estimated values. Several previous associations between plasma efavirenz exposure and polymorphisms in *CYP2A6* [20,21], *CYP3A4* [22], *CYP3A5* [4], and *CAR* [23] did not replicate herein. However, genotype data were unavailable for the previously implicated *CYP2A6*1H, *1J* [20], and *UGT2B7* [21] variants.

Conclusion

To date it has been difficult to replicate putative associations between human genetic variants and efavirenz efficacy and/or toxicity. Previous studies based on candidate gene approaches have consistently associated *CYP2B6* 516G→T [4,6–8] and *CYP2B6* 983T→C [12–14] with higher plasma efavirenz concentrations. Additional *CYP2B6* polymorphisms suggested to affect *CYP2B6* activity have been extremely infrequent [12,17,18] or have not predicted plasma efavirenz exposure [18,19], and data for associations beyond *CYP2B6* are limited [4,20,21,23]. The present study provides a more comprehensive understanding of genetic determinants of efavirenz plasma exposure. These results should support continued work to define the potential utility of human genetic testing to inform the prescribing of efavirenz.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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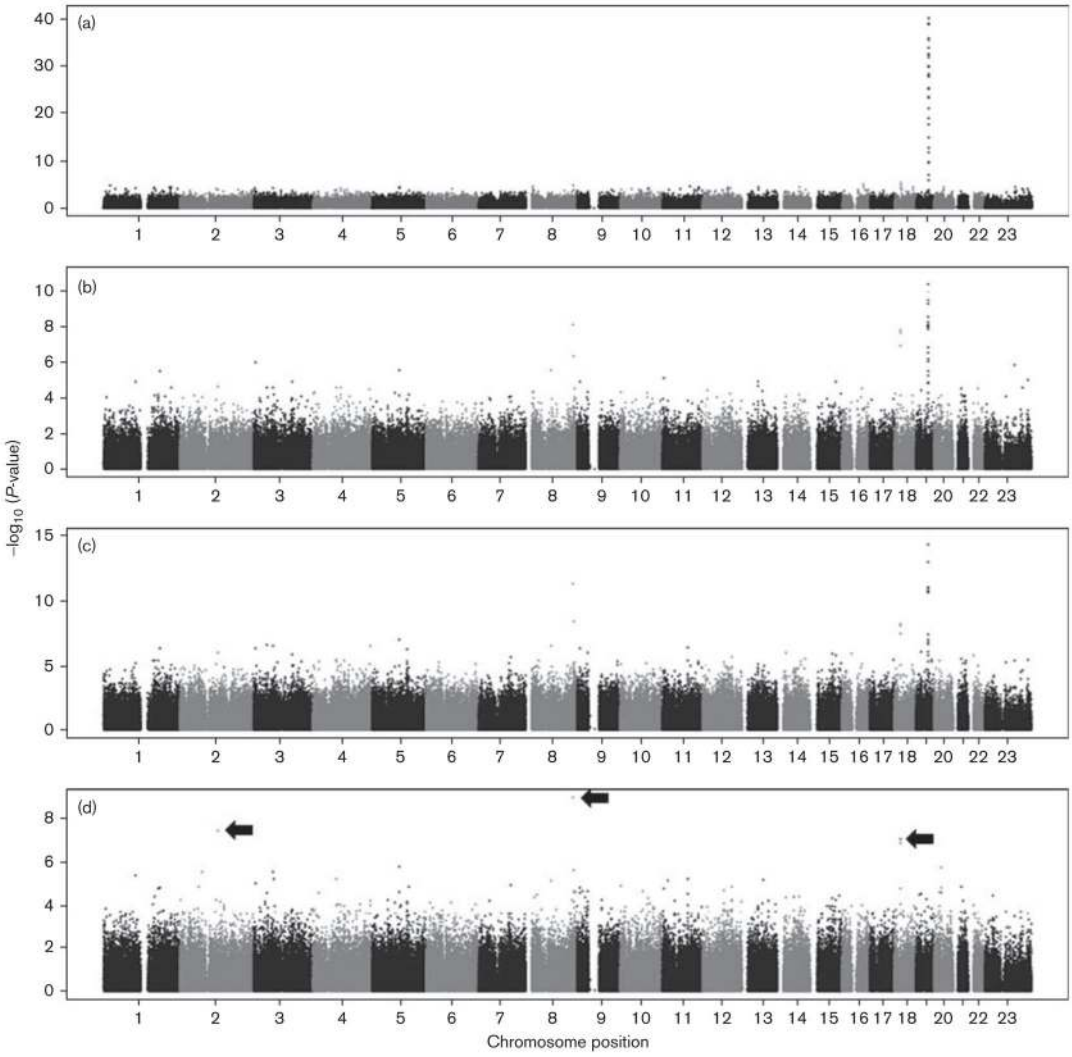

Fig. 1.

Genome-wide associations with efavirenz estimated C_{min} values by multiple linear regression analysis. Manhattan plots of associations between genetic polymorphisms and efavirenz log-transformed estimated C_{min} values. Linear regression was used. Each analysis includes the top 10 principal component vectors to adjust for potential confounding by ancestry. The $-\log_{10}$ of *P*-values are shown. Note the different *y*-axis scale on each panel. (a) Adjusting only for principal component vectors. (b) Also adjusting for *CYP2B6* 516G→T. (c) Also adjusting for *CYP2B6* 516G→T and 983TC. (d) Also adjusting for *CYP2B6* 516G→T, 983T→C, and rs4803419 C→T. Arrows indicate non-*CYP2B6* polymorphisms that achieved (or nearly achieved) $P=5\times10^{-8}$ genome-wide significance in the final model.

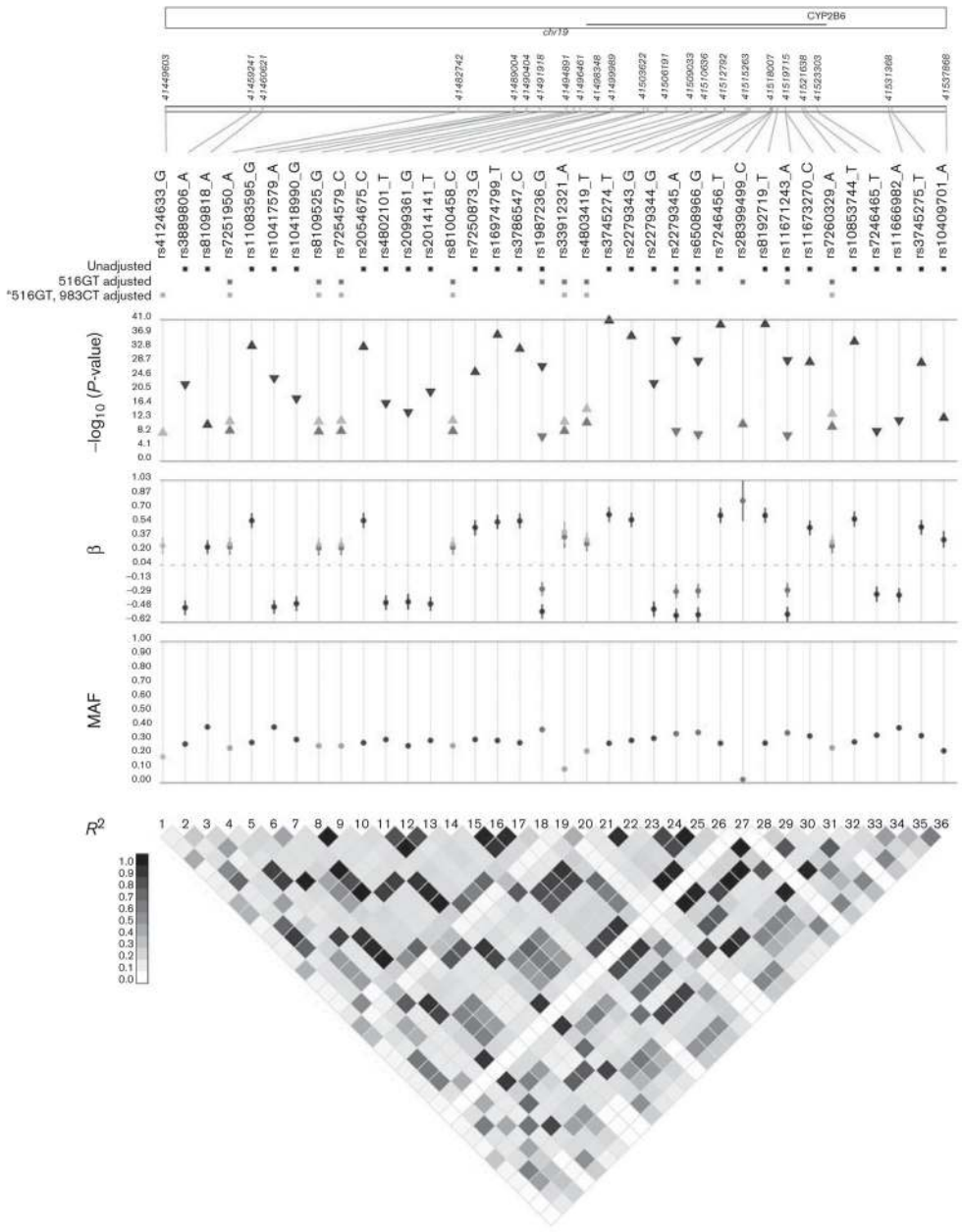

Fig. 2.

Association between polymorphisms in the *CYP2B6* locus and efavirenz estimated C_{min} values, considering linkage disequilibrium (LD). Synthesis-View plots [40] of associations for the chromosome 19 region of interest are shown. At the top are chromosome positions, below which are panels showing – $\log_{10}$ *P*-values, β values, and minor allele frequencies (MAF). At the bottom shown LD between polymorphisms. Blue markers: Adjusting only for principal component vectors; Red markers: Also adjusting for *CYP2B6* 516G→T; Orange markers: Also adjusting for *CYP2B6* 516G→T and 983T→C. The blue, red, and orange markers correspond to Fig. 1a–c, respectively. Only polymorphisms with $P \leq 5 \times 10^{-8}$ are shown.

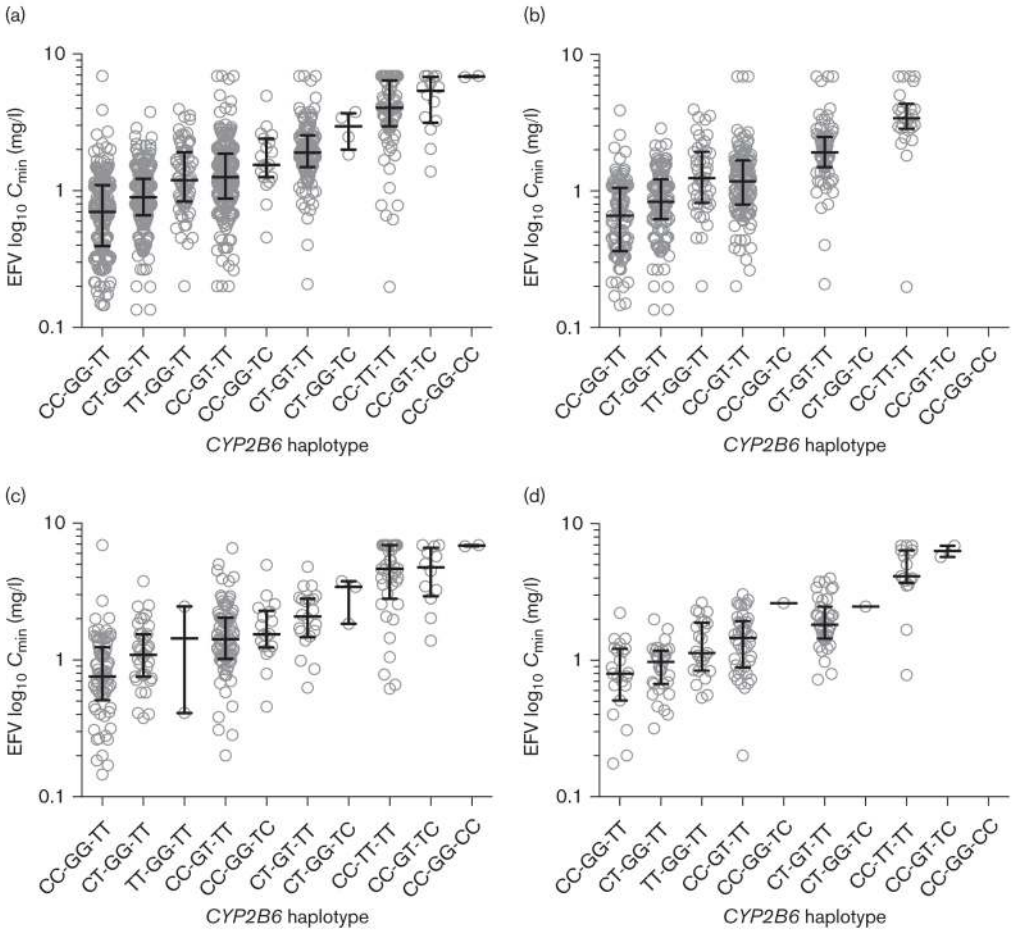

Fig. 3.

Relationships with *CYP2B6* variants and efavirenz estimated C_{min} values. Efavirenz C_{min} values were estimated as described in the Materials and Methods section. (a) All participants (self-identified white, black, and Hispanic); (b) Self-identified white participants; (c) Self-identified black participants; (d) Self-identified Hispanic participants. On *x*-axis, *CYP2B6* haplotypes represent (in order) *CYP2B6* rs4803419 C→T (CC, CT, TT), 516G→T (GG, GT, TT), and 983T→C (TT, TC, CC). The need to convert measured efavirenz concentrations to percentiles to derive estimated C_{min} values creates a spurious plateau of concentrations at approximately 7 mg/l. Actual C_{min} values for some individuals undoubtedly exceed this value.

Table 1

Associations between genetic polymorphisms with lowest *P*-values and efavirenz estimated C_{min} values in genome-wide analysis cohort and in replication cohort

		Primary analysis[a]		Replication unadjusted		Replication 516G-T adjusted	
Chromosome	**Polymorphism**	**β**	***P*-value**	**β**	***P*-value**	**β**	***P*-value**
19	*CYP2B6* 516G→T	0.63	8.5×10^{-41}	0.68	6.1×10^{-14}	NA	NA
19	*CYP2B6* 983T→C	0.79	9.9×10^{-11}	0.52	0.444	1.09	0.070
19	rs4803419	0.34	4.4×10^{-15}	0.03	0.769	0.41	2.3×10^{-5}
1	rs2034525	− 0.33	3.8×10^{-6}	0.10	0.509	0.05	0.695
2	rs13386913	− 0.68	2.7×10^{-6}	0.12	0.798	0.15	0.727
2	rs2288657	− 0.44	3.6×10^{-8}	0.24	0.224	0.14	0.423
3	rs7625304	− 0.42	9.0×10^{-6}	− 0.40	0.129	− 0.38	0.101
3	rs17070662	− 0.45	2.70×10^{-6}	0.61	0.055	0.43	0.130
3	rs17295791	− 0.28	6.0×10^{-6}	0.03	0.846	0.03	0.823
4	rs16999951	− 0.37	6.0×10^{-6}	− 0.17	0.413	− 0.30	0.118
5	rs1897833	− 0.23	1.5×10^{-6}	0.33	0.003	0.29	0.004
8	rs11988660	− 0.53	6.9×10^{-6}	0.69	0.149	0.54	0.207
8	rs7818576	− 0.47	1.1×10^{-9}	− 0.01	0.954	− 0.06	0.780
8	rs10086443	− 0.23	2.3×10^{-6}	− 0.02	0.895	0.02	0.879
11	rs10834309	− 0.23	7.1×10^{-6}	− 0.05	0.660	− 0.05	0.636
11	rs4556569	− 0.23	6.0×10^{-6}	0.18	0.180	0.08	0.523
13	rs2439613	− 0.42	6.3×10^{-6}	0.50	0.103	0.15	0.588
18	rs288982	− 0.63	8.2×10^{-8}	0.16	0.633	− 0.01	0.971
18	rs4800112	− 0.63	8.9×10^{-8}	0.51	0.080	0.26	0.314
18	rs2959521	− 0.87	1.3×10^{-7}	0.45	0.412	0.30	0.543
20	rs6050259	0.18	1.8×10^{-6}	0.02	0.799	0.02	0.853

NA, not available.

[a]Results for the three *CYP2B6* polymorphisms reflect the β coefficient and *P*-value from the analysis in which they were significant.