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Genome-wide Association Study of Virologic Response with Efavirenz- or Abacavir-containing Regimens in AIDS Clinical Trials Group Protocols

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Abstract

Background—Efavirenz and abacavir are components of recommended first-line regimens for human immunodeficiency virus (HIV)-1 infection. We used genome-wide genotyping and clinical data to explore genetic associations with virologic failure among subjects randomized to efavirenz- or abacavir-containing regimens in AIDS Clinical Trials Group (ACTG) protocols.

Methods—Virologic response and genome-wide genotype data were available from treatment-naive subjects randomized to efavirenz-containing (n=1,596) or abacavir-containing (n=786) regimens in ACTG protocols 384, A5142, A5095, and A5202.

Results—Meta-analysis of association results across race/ethnic groups showed no genome-wide significant associations (p<5×10\textsuperscript{−8}) with virologic response for either efavirenz or abacavir. Our sample size provided 80% power to detect a genotype relative risk of 1.8 for efavirenz, and 2.4 for abacavir. Analyses focused on \textit{CYP2B} genotypes that define the lowest plasma efavirenz exposure stratum did not reveal associations, nor did analysis limited to gene sets predicted to be relevant to efavirenz and abacavir disposition.

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Conclusions—No single polymorphism is strongly associated with virologic failure with efavirenz- or abacavir-containing regimens. Analyses to better consider context, and that minimize confounding by non-genetic factors, may reveal associations not apparent herein.

Keywords
HIV-1; efavirenz; abacavir; pharmacogenomics; virologic failure

Introduction
The non-nucleoside reverse transcriptase inhibitor (NNRTI) efavirenz, and the nucleoside reverse transcriptase inhibitor (NRTI) abacavir are components of recommended first-line regimens for human immunodeficiency virus (HIV-1) infection [1]. Approximately 15–20% of individuals randomized to efavirenz-containing regimens in prospective clinical trials met virologic failure endpoints at week 48 [2–9]. Limited data from candidate gene studies suggest that genetic polymorphisms may affect virologic response. An initial report identified an association between an \textit{ABCB1} polymorphism (which encodes P-glycoprotein) and virologic response to efavirenz-containing regimens in the Swiss HIV Cohort Study [10]. A subsequent analysis involving two AIDS Clinical Trials Group (ACTG) protocols suggested increased virologic failure of efavirenz-containing regimens associated with \textit{CYP2B6} polymorphisms in African Americans, but not in whites or Hispanics [11]. This gene encodes cytochrome P450 (CYP) 2B6, the primary metabolic pathway for efavirenz [12]. In contrast, a recent analysis of data from prospective randomized clinical trial HT 001 in Port au Prince, Haiti found no association between \textit{CYP2B6} polymorphisms and likelihood of virologic failure [13].

Increased plasma efavirenz exposure is associated with several \textit{CYP2B6} loss-of-function polymorphisms, 516G→T (rs3745274) [14–19], 983T→C (rs28399499) [11, 19–21], and 15582C→T (rs4803419) [19]. The greater frequency of \textit{CYP2B6} 516G→T in individuals of African ancestry compared to European ancestry [22] largely explains the greater mean plasma efavirenz concentrations in the former group [23, 24]. \textit{CYP2B6} 983T→C is also more frequent in Africans, although far less frequent overall than 516G→T, and is virtually absent from populations of European ancestry [22]. \textit{CYP2B6} 15582C→T is more frequent with European or Asian ancestry than with African ancestry [22], and its effect on efavirenz concentrations is modest compared to those of \textit{CYP2B6} 516G→T and 983T→C [19]. The three \textit{CYP2B6} polymorphisms reside on mutually exclusive haplotypes, and various two-way combinations of these polymorphisms define plasma efavirenz exposure strata that span an approximately 10-fold range [19].

All recommended initial regimens for HIV-1 infection include either abacavir/lamivudine or tenofovir disoproxil fumarate (TDF)/emtricitabine (FTC), each in combination with a third drug from a different class (an NNRTI, a protease inhibitor with low-dose ritonavir as a pharmacokinetic enhancer, or an integrase inhibitor) [1]. Abacavir is metabolized primarily by alcohol dehydrogenase and glucuronyl transferase, with minimal metabolism by CYP enzymes. Its active 5-triphosphate metabolite, carbovir triphosphate, is generated intracellularly [25]. While carriage of \textit{HLA-B}*57:01 is a strong predictor of hypersensitivity
reactions to abacavir [26–28], genetic predictors of other responses or pharmacokinetic parameters with abacavir have not been reported.

Randomized clinical trials have assessed the safety and efficacy of abacavir in combination with other drugs. Study A5202 compared abacavir/lamivudine with TDF/FTC, each in combination with either efavirenz or atazanavir/ritonavir. In A5202, among subjects with pre-treatment plasma HIV-1 RNA ≥100,000 copies/mL, virologic response was inferior with abacavir/lamivudine versus TDF/emtricitabine, but responses were equivalent with lower pre-treatment plasma HIV-1 RNA concentrations [29]. A study of NRTIs in combination with lopinavir/ritonavir found no difference in virologic response between abacavir/lamivudine and TDF/FTC [30]. In the SINGLE trial, which compared abacavir/lamivudine + dolutegavir with TDF/FTC/efavirenz, virologic responses between abacavir/lamivudine and TDF/FTC did not differ in patients with high pre-treatment plasma HIV-1 RNA concentrations [31]. Thus, differences in responses with ABC-containing regimens could be due to the third drug, but current data does not exclude potential pharmacogenomics effects on outcome.

In the present study we used a genome-wide approach to evaluate whether common human genetic variants were associated with virologic failure among treatment-naive subjects who initiated efavirenz- or abacavir-containing regimens in prospective, randomized ACTG clinical trials.

**Methods**

**Study Participants**

Treatment-naive subjects were randomized to efavirenz-containing regimens in ACTG studies 384 [32, 33], A5095 [2], A5142 [4], and A5202 [7, 29], and to abacavir-containing regimens in A5095 [2] and A5202 [7, 29], with DNA obtained under protocol A5128 [34]. Some subjects from ACTG 384 and A5095 were also included in previous candidate gene analyses of virologic response to efavirenz-containing regimens [11]. For efavirenz, concomitant antiretrovirals included once-daily TDF/FTC [7, 29]; once-daily abacavir/lamivudine [7, 29]; twice-daily zidovudine/lamivudine [2, 4, 32, 33], twice-daily zidovudine/lamivudine/abacavir [2], once daily stavudine (d4T)/lamivudine [4, 32, 33]; once-daily TDF/lamivudine [4]; and twice-daily lopinavir/ritonavir [4]. For abacavir, concomitant antiretrovirals included once-daily efavirenz/lamivudine [7, 29]; once-daily atazanavir/ritonavir/lamivudine [7, 29]; once daily efavirenz with twice-daily zidovudine/ lamivudine [2]; and twice-daily zidovudine/lamivudine [2]. Abacavir was prescribed once daily in A5202, and twice daily in A5095. 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 subjects gave written informed consent.
Definitions of virologic response

Three complementary definitions for virologic response were used for case/control analyses. These included 1) HIV-1 RNA <50 copies/mL at ≤16 weeks, 2) HIV-1 RNA >200 copies/mL after HIV-1 RNA <200 copies/mL and 3) virologic response at week 48 (HIV-1 RNA <50 copies/mL at 48 weeks). The week 48 virology response definition was based on a single, cross-sectional plasma HIV-1 RNA determination. The other two virology response definitions required confirmation by a second HIV-1 RNA determination at a separate study visit. The first two of these endpoints were defined to reflect 1) rapidity and 2) maintenance of virologic response while accommodating the differing visit schedules across the studies. The third endpoint was defined for its clinical relevance in the setting of treatment for HIV-1. To limit confounding of non-virologic factors, analyses of the first endpoint were limited to individuals who remained on the drug of interest for at least 8 weeks, analyses of the second endpoint were limited to individuals who achieved HIV-1 RNA <200 copies/ml while on initial treatment, and analyses of the third endpoint were limited to individuals who remained on randomized treatment for 48 weeks. To address possible confounding by differential adherence, sub-analyses including only patients with no reported missing doses were also carried out.

Genotyping

The Vanderbilt Institutional Review Boards and the ACTG approved this use of DNA. Genome-wide polymorphism data was obtained as part of the International HIV Controllers study as described [35]. Briefly, samples were genotyped on either the Illumina HumanHap 650Y or 1M-Duo platform. Data quality control included removal of population outliers, highly related individuals and individuals with highly missing genotype data. Polymorphisms were excluded for low frequency (<1%), high missingness (>5%) or deviation from Hardy-Weinberg expectation (Fisher’s exact test p<5e-6) using PLINK version 1.07 [36]. Genetic ancestry was determined using principal components analysis in EIGENSTRAT [37]. Based on comparison with HapMap phase 3 populations, data were clustered into Black, White and Hispanic groups for analysis. After quality control, unobserved genotypes were imputed using the 1,000 Genomes Project Phase I release integrated polymorphisms and indels (March 2012) and BEAGLE software version 4 [38]. After imputation, >8 million high-quality polymorphisms were tested for association.

Association testing

Per ethnic group and per response phenotype, association was tested at each polymorphism using logistic regression including top principal components as covariates to correct for residual population structure. To control for the impact of concomitant medication on association signals we included categorical covariates depending on the full ART regimen per patient. For EFV-containing regimens we defined four groups: 1) TDF/FTC/EFV containing regimens 2) once-daily ABC/3TC/EFV regimens 3) PI-containing regimens 4) twice-daily ZDV or d4T containing regimens without PIs. For ABC-containing regimens, we defined two groups: 1) ABC/3TC/EFV-containing regimens 2) ABC/3TC/ZDV regimens. Additionally, we included pre-treatment CD4+ T cell count and viral load as covariates to account for their potential influence on virologic response. This was sufficient
to correct for inflation in all cases (genomic inflation factor ~1, Figure 1.2). Data were combined across ethnic groups using inverse-variance weighted meta-analysis. Association results for all variants tested in each analysis are available upon request.

Power for variant detection was calculated post-hoc using the online genetic power calculator [39] and assumed a 10% causal allele frequency, a trait prevalence of 0.1, the observed sample size and genome-wide significance level for detection of association (p<5×10^{-8}).

**Targeted analysis of CYP2B6 haplotypes**

Since combinations of alleles at rs3745274, rs28399499, and rs4803419 are known to influence plasma EFV exposure we directly tested these SNPs in a targeted analysis. We built haplotypes spanning these SNPs, testing individuals homozygous for the CC/GG/CC haplotype (known to result in the lowest stratum of plasma EFV levels) against all others using the same testing framework and covariates as the genome-wide analysis.

**Gene set enrichment testing**

To assess for enrichment of association signal across pathways we used MAGENTA [40]. A custom gene set of 240 ADME genes was compiled (list of genes in Supplemental Material). Based on a survey of the literature, we broadly included ADME genes of potential relevance to efavirenz or abacavir, and excluded ADME genes almost certain to not be relevant to each drug. Ninety-eight of these genes were implicated in abacavir metabolism, and 145 in efavirenz metabolism (of which 7 were considered strongly influential, Table S1). In addition we tested sets of pre-defined pathways from sources including Gene Ontology (GO) terms, PANTHER (biological processes, molecular functions, metabolic and signaling pathways), KEGG, and Ingenuity. We used an FDR cutoff of 95% to consider pathways or custom gene sets as significantly associated.

**Results**

**Genetic associations with virologic failure**

A total of 1,596 subjects randomized to efavirenz-containing regimens, and 786 subjects randomized to abacavir-containing regimens had sufficient clinical and genome-wide polymorphism data for analysis in the three phenotypic strata (Table 1). After genotype imputation, we tested >8 million polymorphisms for association with the three virologic response categories in all ethnic groups by logistic regression, including covariates to account for genetic population structure, concomitant medications and pre-treatment clinical markers (see methods). Evidence was combined across ethnic groups by meta-analysis.

For efavirenz, we observed good behavior of the test statistic as visualized by QQ plots for each of the three virologic response definitions, indicating no residual inflation due to population structure (Figure 1). There were no genome-wide significant associations in any virologic response phenotype for efavirenz in any of the ethnic groups individually. Meta-analysis of association results across ethnic groups did not uncover any associations for efavirenz (Figure 1).
For abacavir, we also observed good behavior of the test statistic as visualized by QQ plots for each virologic response definition (Figure 2). There were no genome-wide significant associations for any virologic response phenotype for abacavir. Meta-analysis of association results across ethnic groups did not uncover any associations for abacavir (Figure 2). For both efavirenz and abacavir analyses, a list of SNPs with p-values below 1×10\(^{-5}\) (top SNP per locus, pruned by linkage disequilibrium) is provided in Table S2.

To assess whether accounting for differential adherence may improve our ability to detect associations we restricted our analysis to subjects who were prescribed efavirenz or abacavir for at least 16 weeks (endpoint #1), or only subjects who reported no missed doses (endpoint #3). Neither sub-analysis revealed significant associations (data not shown).

Given the available sample sizes, we calculated the genotypic relative risk required to provide ~80% power for detection. We were limited to detecting polymorphisms with very strong individual effects (Table 1). For efavirenz, the lowest genotype relative risk (GRR) that could be detected with ~80% power was HIV-1 RNA suppression at 48 weeks by meta-analysis across groups (detected GRR = 1.8). For abacavir, the lowest genotype relative risk (GRR) that could be detected with ~80% power was also HIV-1 RNA suppression at 48 weeks by meta-analysis across groups (detected GRR = 2.4).

**Genotypes that predict lower plasma efavirenz exposure do not impact virologic response**

Pairwise combinations of three CYP2B polymorphisms (rs3745274, rs28399499, and rs4803419) are known to predict plasma efavirenz exposure [19], with the lowest exposure stratum defined by homozygosity for the haplotype CC, GG, and CC, respectively, at these loci [19]. We therefore tested whether carrying this haplotype was associated with increased risk for virologic failure. In analyses that compared this stratum to all other strata combined, there was no significant association with virologic failure (Table 2).

**Gene set association analysis**

We next tested for enrichment of genetic signal across sets of genes important for efavirenz and/or abacavir metabolism and disposition. Per drug, we defined custom sets of 147 (efavirenz) and 99 (abacavir) absorption, distribution, metabolism, and excretion (ADME) genes from the literature based on their importance to drug metabolism (Table S1). We used MAGENTA to assess the statistical enrichment of signal in these genes sets against a permuted null distribution. There was no evidence for association in cumulative variation across these gene sets with any of the phenotypes under study (Table 3). Similarly, there was no evidence for enrichment of association signal in a discovery screen using canonical pathways defined by several databases (Ingenuity, KEGG, GO).

**Discussion**

Efavirenz and abacavir are antiretroviral agents that are often included as components of recommended first-line regimens for human immunodeficiency virus (HIV)-1 infection [1], based on data from multiple prospective, randomized clinical trials [2–9, 29–31, 41, 42]. Interindividual variability in virologic efficacy of HIV-1 treatment responses may be influenced to polymorphisms in drug absorption, distribution, metabolism, and elimination.
(ADME) genes as well as off-target genes. We used a genome-wide approach to investigate associations between common human genetic variants and virologic responses to initial regimens that included efavirenz or abacavir. No polymorphism achieved genome-wide significance, either considering the entire genome, or in analyses focused on numerous ADME genes relevant to efavirenz or abacavir. In addition we did not find associations with the CYP2B6 genotype that is known to predict the lowest stratum of plasma efavirenz exposure [19].

In an effort to capture different underlying aspects of virologic response we separately analyzed based on three different virologic response phenotypes. The first definition, virologic response to HIV-1 RNA <50 copies/mL at ≤16 weeks, was chosen to capture genetic effects on antiretroviral potency while shortening the window of opportunity for confounding by non-adherence. The second definition, virologic relapse to HIV-1 RNA >200 copies/mL after being <200 copies/mL, was chosen to capture possible genetic effects on reducing durability of virologic control. The third definition, virologic response to HIV-1 RNA <50 copies/mL at 48 weeks, was chosen to reflect a more standard definition of virologic response. Lack of association with any of these phenotypes reinforces that there is not a single strong genetic predictor of virologic response for efavirenz or abacavir in the total population. Importantly, these results didn’t change when further restricting to the analyses to further limit the impact of missed doses of treatment.

Prospective, randomized clinical trials data offer a unique window to genetic associations, but it is possible that true genetic influences on virologic response to efavirenz and/or abacavir were missed by our GWAS. Our sample size was modest compared to many GWAS of complex traits. We were limited to detecting common variants (>1%) with relatively large effect sizes (in most cases OR > 2). However, associations with drug ADME and off-target genes may have large effect sizes, so may be apparent with small sample sizes. For example, genetic prediction of abacavir hypersensitivity is genome-wide significant (P<5.0×10^{-8}) with 15 cases and 200 controls [43], hepatitis C treatment response with 303 cases [44], statin response with 85 cases and 90 controls [45], nevirapine rash with 71 cases and 77 controls [46], and tamoxifen response with 240 cases [47].

A challenge in quantifying the impact of human genetics on HIV treatment response is that associations are often context-dependent. For example, an association between HLA type and nevirapine hypersensitivity reaction is only seen with high pre-treatment CD4 T-cells counts [48]. In CYP2B6 slow metabolizers, a paradoxical association between concomitant rifampicin and increased plasma efavirenz concentrations is only seen with concomitant isoniazid [49]. An association between UGT1A1 genotype and elevated indirect hyperbilirubinemia with atazanavir/ritonavir is exacerbated by higher pre-treatment bilirubin and hemoglobin concentrations [50]. Additionally, the variable efficacy of different regimens may also impact overall virologic outcome. In this study, we addressed this by adjusting for concomitant medication and pre-treatment clinical markers in our association tests. However, directed analysis per-regimen may be necessary to uncover subtle genetic signals masked in such a pooled analysis. Thus, associations with virologic response to efavirenz or abacavir may only be detected when considering appropriate covariables.
The impact of human genetic variants of virologic failure with efavirenz and/or abacavir containing regimens may be masked by non-adherence and other non-genetic factors. For example, in the seminal study that identified an association between $IL28$ genotype and virologic response to therapy for hepatitis C virus, removing non-adherent subjects yielded a substantial improvement in power for detection of associated variants [J Fellay, personal communication]. Analyses that can control for important confounders will increase power to detect associations with antiretroviral drugs.

Access to safe and effective antiretroviral therapy is a cornerstone in the global struggle against HIV/AIDS, which now affects ~35 million worldwide. Because suboptimal treatment response can have devastating consequences, it is critical to continue efforts to define the predictive value of human genetics for HIV treatment efficacy and toxicity.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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39. Purcell S, Sham PC. Available at: http://pngu.mgh.harvard.edu/~purcell/gpc/.


Figure 1. Associations between genetic polymorphisms and virologic response phenotypes in subjects randomized to efavirenz-containing regimens

Results visualized by QQ plots (left panels) and Manhattan plots (right panels) represent meta-analysis involving all three ethnic groups. Association results for each group, calculated by logistic regression, were meta-analyzed using inverse-variance weighting for the phenotypes: (A) plasma HIV-1 RNA <50 copies/mL at ≤16 weeks; (B) HIV-1 RNA >200 copies/mL after being <200 copies/mL; (C) HIV-1 RNA <50 copies/mL at 48 weeks. On each QQ plot the dashed line indicates the null expectation. On each Manhattan plot the dashed line indicates the threshold of genome-wide significance (p<5×10^{-8}).
Figure 2. Associations between genetic polymorphisms and virologic response phenotypes in subjects randomized to abacavir-containing regimens

Results visualized by QQ plots (left panels) and Manhattan plots (right panels) represent meta-analysis involving all three ethnic groups. Association results for each ethnic group calculated by logistic regression were meta-analyzed using inverse-variance weighting for the phenotypes: (A) plasma HIV-1 RNA <50 copies/mL at ≤6 weeks; (B) HIV-1 RNA >200 copies/mL after being <200 copies/mL; (C) HIV-1 RNA <50 copies/mL at 48 weeks.
On each QQ plot the dashed line indicates the null expectation. On each Manhattan plot the dashed line indicates the threshold of genome-wide significance (p<5×10^{-8}).
Table 1

Sample numbers and power estimates in each analysis stratified by antiretroviral, phenotype, and race/ethnicity group.

<table>
<thead>
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<th>Group</th>
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<th>Controls</th>
<th>Total</th>
<th>GRR(^a) for 80% Power</th>
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<td>374</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>Rebound</td>
<td>92</td>
<td>206</td>
<td>298</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>Suppression at 48 weeks</td>
<td>220</td>
<td>566</td>
<td>786</td>
<td>2.4</td>
</tr>
</tbody>
</table>

\(^a\) GRR, genotype relative risk.
na indicates the required GRR is out of range for the calculator.
Table 2

Efavirenz virologic response comparison of lowest drug exposure genotype versus other genotypes.

<table>
<thead>
<tr>
<th></th>
<th>Suppression at 16 weeks</th>
<th>Rebound</th>
<th>Suppression at 48 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR¹</td>
<td>p-value</td>
<td>OR¹</td>
</tr>
<tr>
<td>White</td>
<td>0.77</td>
<td>0.24</td>
<td>1.04</td>
</tr>
<tr>
<td>Black</td>
<td>1.60</td>
<td>0.12</td>
<td>1.88</td>
</tr>
<tr>
<td>Hispanic</td>
<td>0.42</td>
<td>0.08</td>
<td>1.02</td>
</tr>
<tr>
<td>Meta-analysis</td>
<td>0.90</td>
<td>0.53</td>
<td>1.31</td>
</tr>
</tbody>
</table>

¹Odds ratio (OR) is set such that an OR > 1 indicates a higher genotype frequency in cases compared to controls.
Table 3

Association results (p-values) for gene set enrichment analysis of an impact of genetic variation in selected ADME genes on virologic response categories

<table>
<thead>
<tr>
<th></th>
<th>Efavirenz</th>
<th>Abacavir</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Suppression at 16 weeks</td>
<td>Rebound</td>
</tr>
<tr>
<td>White</td>
<td>0.23</td>
<td>0.77</td>
</tr>
<tr>
<td>Black</td>
<td>0.82</td>
<td>0.41</td>
</tr>
<tr>
<td>Hispanic</td>
<td>0.81</td>
<td>0.51</td>
</tr>
<tr>
<td>Meta-analysis</td>
<td>0.61</td>
<td>0.73</td>
</tr>
</tbody>
</table>