Multiphenotype association study of patients randomized to initiate antiretroviral regimens in AIDS Clinical Trials Group protocol A5202

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Background High-throughput approaches are increasingly being used to identify genetic associations across multiple phenotypes simultaneously. Here, we describe a pilot analysis that considered multiple on-treatment laboratory phenotypes from antiretroviral therapy-naive patients who were randomized to initiate antiretroviral regimens in a prospective clinical trial, AIDS Clinical Trials Group protocol A5202.

Participants and methods From among 5 9545 294 polymorphisms imputed genome-wide, we analyzed 2544, including 2124 annotated in the PharmGKB, and 420 previously associated with traits in the GWAS Catalog. We derived 774 phenotypes on the basis of context from six variables: plasma atazanavir (ATV) pharmacokinetics, plasma efavirenz (EFV) pharmacokinetics, change in the CD4 + T-cell count, HIV-1 RNA suppression, fasting low-density lipoprotein-cholesterol, and fasting triglycerides. Permutation testing assessed the likelihood of associations being by chance alone. Pleiotropy was assessed for polymorphisms with the lowest *P*-values.

Results This analysis included 1181 patients. At *P* less than 1.5×10^{-4}, most associations were not by chance alone. Polymorphisms with the lowest *P*-values for EFV pharmacokinetics (*CYPB26* rs3745274), low-density lipoprotein -cholesterol (*APOE* rs7412), and triglyceride (*APOA5* rs651821) phenotypes had been associated previously with those traits in previous studies. The association between triglycerides and rs651821 was present with ATV-containing regimens, but not with EFV-containing regimens. Polymorphisms with the lowest *P*-values for ATV pharmacokinetics, CD4 T-cell count, and HIV-1 RNA phenotypes had not been reported previously to be associated with that trait.

Conclusion Using data from a prospective HIV clinical trial, we identified expected genetic associations, potentially novel associations, and at least one context-dependent association. This study supports high-throughput strategies that simultaneously explore multiple phenotypes from clinical trials' datasets for genetic associations. *Pharmacogenetics and Genomics* 27:101–111 Copyright © 2017 The Author(s). Published by Wolters Kluwer Health, Inc.

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Introduction

Access to safe and effective antiretroviral therapy (ART) is critical in the global response to the AIDS pandemic. Genetic polymorphisms in drug absorption, distribution, metabolism, and elimination (ADME) genes and off-target genes have convincingly been shown to be associated with adverse effects and/or pharmacokinetics of antiretroviral drugs including abacavir (ABC) [1], atazanavir (ATV) [2], dolutegravir [3], efavirenz (EFV) [4], etravirine [5], lopinavir [6], and nevirapine [7], and genetic screening to avoid ABC hypersensitivity reaction is now the standard of care in many resource-abundant countries.

Genome-wide association studies (GWAS) explore whether an individual trait (i.e. phenotype) associates with single-nucleotide polymorphisms (SNPs) across the genome. Only one phenotype is typically considered in a

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GWAS. The term ‘phenome’ describes the aggregate of many phenotypes in a given dataset. Phenome-wide association studies (PheWAS) complement GWAS by testing for genotype–phenotype associations across numerous phenotypes [8–12]. A PheWAS may interrogate a single SNP against the phenome or may interrogate numerous SNPs simultaneously. Also unique to PheWAS is the ability to identify pleiotropy, whereby one SNP is found to be associated with multiple seemingly unrelated phenotypes [13,14].

Context-dependent genetic associations with antiretroviral drugs are well described. Failure to consider context may miss or underestimate important genetic associations. For EFV, some individuals with *CYP2B6* slow metabolizer genotypes experience extremely high plasma EFV exposure only in the context of concomitant isoniazid [15–17]. Among individuals with *CYP2B6* slow metabolizer genotypes, the likelihood of EFV discontinuation for central nervous system side effects appears to be greater in the context of European versus African ancestry [18,19]. For ATV, among individuals with *UGT1A1* low expressor genotypes, the likelihood of bilirubin-related drug discontinuation is considerably greater in the context of European versus African ancestry [20]. With nevirapine, among individuals with *HLA* risk alleles, severe cutaneous reactions occur largely when nevirapine is initiated in the context of higher CD4 T-cell counts [21].

Prospective clinical trials that randomized HIV-infected patients to initiate different antiretroviral regimens, and that involve extensive data collection, offer a special opportunity to apply a multiphenotype analytical approach focused on pharmacogenomics. We previously applied PheWAS to pretreatment (i.e. baseline) laboratory data National Institute of Health-funded AIDS Clinical Trials Group (ACTG) protocols [22]. That analysis established that our analysis pipeline for studying multiple phenotypes is robust, with 20 polymorphisms replicating associations with identical or related phenotypes reported in the National Human Genome Research Institute – European Bioinformatics Institute GWAS Catalog [23], including several not reported previously in HIV-positive cohorts.

The present analyses explored associations with multiple on-treatment phenotypes from ACTG protocol A5202 [24,25]. We considered a total of 774 phenotypes representing ATV pharmacokinetics, CD4 T-cell count, EFV pharmacokinetics, fasting low-density lipoprotein (LDL) cholesterol, HIV-1 RNA, and fasting triglycerides, and that were derived by considering various contexts including sex, race/ethnicity, baseline age, baseline body mass index, baseline CD4+ T-cell count, baseline plasma HIV-1 RNA, randomized antiretroviral regimen, and component antiretroviral drug. These context-dependent phenotypes are useful in interpreting genome–phenome association results and highlight relationships of potential interest between these polymorphisms and phenotypes.

Participants and methods

Study participants

AIDS Clinical Trials Group protocol A5202 (ClinTrials.gov NCT00118898) was a phase IIIb equivalence study of four once-daily regimens for the initial treatment of HIV-1 infection. The primary results of A5202 have been reported previously [24,25]. Patients enrolled from 2005 to 2007 were randomized to open-label ATV (300 mg) plus ritonavir (RTV, 100 mg) or EFV (600 mg) with either placebo-controlled ABC/lamivudine (3TC) (600 mg/300 mg) or tenofovir disoproxil fumarate/emtricitabine (TDF/FTC, 300 mg/200 mg). Study evaluations included laboratory testing at entry, at weeks 4, 8, 16, and 24, and every 12 weeks thereafter until the last enrolled patient was followed for 96 weeks. Analyses included A5202 participants who consented to provide DNA for genetic research under ACTG protocol A5128.

Phenotypes

For this analysis, we considered laboratory data from A5202 at entry and subsequent on-study weeks, and representing immunologic, virologic, metabolic, and pharmacologic domains. Immunologic phenotypes were derived from CD4+ T-cell count data, which are known to correlate with mortality on ART [26–30]. Virologic phenotypes were derived from data on plasma HIV-1 RNA suppression to less than 200 copies/ml, which decreases transmission [31]. Metabolic phenotypes were derived from data on fasting LDL cholesterol and fasting triglyceride levels, which are in the causal pathway to myocardial infarction [32,33]. Pharmacologic phenotypes were derived from data on EFV and ATV pharmacokinetics, which relate to drug efficacy and toxicity [34–41].

We define the terms variable, primary phenotype, and subphenotype as follows: variables represent data without regard to study week or context (e.g. among all study patients, all fasting triglyceride data). Primary phenotypes are derived from variables while also considering study week but without regard to context (e.g. among all study patients, fasting triglycerides at baseline, at study weeks 24, 48, and 96, and change in fasting triglycerides from baseline to week 24, to 48, and to 96). Subphenotypes are derived from primary phenotypes while considering context (e.g. the fasting triglyceride primary phenotype noted above, but only among patients randomized to receive ATV/RTV).

Contexts for subphenotypes were defined as follows: categorical context included sex (male or female), self-identified race/ethnicity (White, Black, or Hispanic), randomized antiretroviral regimen (ATV + RTV + ABC/3TC, EFV + ABC/3TC, ATV + RTV + TDF/FTC, or EFV + TDF/FTC), and component antiretroviral drug (ATV + RTV, ABC/3TC, EFV, or TDF/FTC). Because

ATV/RTV, ABC/3TC, and TDF/FTC were always prescribed as two-drug combinations, the component drugs could not be analyzed individually. For continuous baseline parameters, continuous context was derived on the basis of percentile cut-offs for age, BMI, CD4+ T-cell count, and plasma HIV-1 RNA (10, 25, 33, 50, 67, 75, and 90 percentile for each). With this approach, we generated a total of 774 primary phenotypes and subphenotypes for analysis, as listed in Supplemental Table 1 (Supplemental digital content 1, *http://links.lww.com/FPC/B148*).

For each primary phenotype, we examined frequency distribution plots and reviewed summary information, identified phenotypes requiring transformation to approximate normality to fulfill assumptions for linear regression, assured consistent units of measurement, and censored outliers judged to be biologically implausible.

Imputation and QC of genetic data

Patients from A5202 were genotyped with the Illumina 1M duo array as part of a previous immunogenomics project [42]. The PLINK program and R statistical programming language were used for QC procedures [43,44]. Polymorphisms were censored for call rates below 98%. After excluding 10 samples where genetically inferred sex differed from clinical data, or missing sex status that could not be inferred, 26 samples with overall genotyping call rates below 98%, and one sample with cryptic relatedness on the basis of identity-by-descent estimates of more than 0.3 from ~100 000 pruned SNPs, there were 1221 samples for imputation.

Post-QC data were imputed to 1000 genomes [45] after converting into genome build 37 using liftOver [46] and stratifying by chromosome to parallelize imputation processing. ShapeIt2 [47] was used to check strand alignment and to phase data. The IMPUTE2 algorithm [48] was used to impute additional genotypes that were available in the 1000 genomes reference panel, but not directly genotyped. Each chromosome was segmented into 6 Mb regions with at least 3500 reference variants in each region. Imputed genotypes were included if posterior probabilities exceeded 0.9.

The quality of imputed data was assessed following the Electronic Medical Records and Genomics protocol [49]. Each chromosome from each phase was checked for 100% concordance with genotyped data. We excluded imputed SNPs with imputation scores less than 0.3, genotyping call rates below 98%, and minor allele frequencies (MAF) less than 0.01.

Candidate polymorphisms for analysis

From the set of imputed SNPs, we included in this analysis only SNPs for which there was some a priori evidence of a pharmacogenetic association with any drug and phenotype on the basis of data from PharmGKB (Pharmacogenomics Knowledgebase [50]). There were 2622 such SNPs in 761 genes that were annotated for a possible drug–phenotype association. Of these 2622 SNPs, we included in this analysis only a subset of 2124 SNPs that were also represented in the imputed, post-QC genome-wide data.

In addition to PharmGKB SNPs, from the set of imputed SNPs, we also included SNPs for which previous GWAS had shown evidence of association with any lipid-related trait with a P-value of less than 10^{-8}, as represented in the GWAS Catalog SNPs [23], which includes results from published GWAS fulfilling catalog criteria [51]. There were 447 such SNPs, of which we included in this analysis only a subset of 420 SNPs that were also represented in the imputed, post-QC genome-wide data. A total of 2544 SNPs were included in the analysis (listed in Supplemental Table 2, Supplemental digital content 2, *http://links.lww.com/FPC/B149*).

Statistical analysis

When linked with available laboratory phenotypes, the final analysis dataset included 1181 patients, 2124 PharmGKB SNPs, and 420 GWAS Catalog SNPs. Using the R statistical package, continuous phenotypes were modeled with linear regression and the dichotomous phenotype with logistic regression [44]. The first three principal components, calculated using EIGENSOFT [52], were used to adjust for global ancestry. Each analysis was also adjusted for sex and age. Consideration of context resulted in models of varying sample sizes. For models with at least 100 patients, we excluded SNPs with MAF of less than 0.05. For models with fewer than 100 patients, we excluded SNPs with MAF of less than 0.10. We did not infer or impute missing laboratory data.

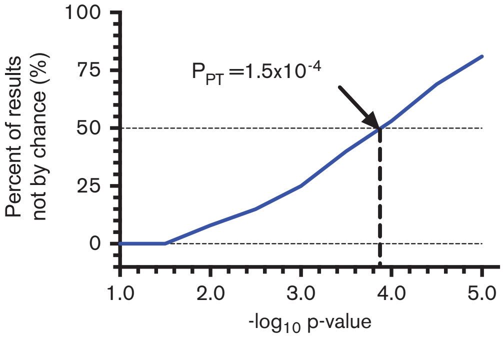
Permutation testing was used to empirically derive P-value cut-offs (P_{PT}) [53]. Briefly, within the analysis dataset, we permuted the connection between genotype and phenotype data. This randomly matches each patient's genotypes to another patient's phenotypes, while preserving relationships between genotypes (e.g. linkage disequilibrium) and between phenotypes (e.g. correlations). Permutation was repeated 1000 times, each generating a new dataset. We then carried out the association analysis on each of the 1000 datasets, from which we determined, at various P-value cut-offs, the average number of SNPs per analysis that pass that cut-off in the permuted data (i.e. by chance alone). We compared this average number with the actual number of SNPs that passed that same cut-off in the unpermuted data. This yields probabilities that SNP–phenotype associations at any given P-value threshold in the unpermuted data were by chance alone. Our approach differs from a more traditional permutation approach that would calculate permuted P-values for each association test, the latter permutation approach being computationally prohibitive.

Table 1 **Baseline characteristics of study patients included in phenome-wide association studies**

Characteristics	PheWAS patients ($N=1181$)	All A5202 patients ($n=1957$)
Race/ethnicity [*n* (%)]		
White	515 (44)	746 (40)
Black	378 (32)	615 (33)
Hispanic	244 (20)	429 (23)
Female [*n* (%)]	183 (15)	322 (17)
Median BMI in kg/m^2 (IQR)	24.8 (22.1–27.9)	
Median age in years (IQR)	38 (31–46)	38 (31–45)
Median baseline plasma HIV-1 RNA copies/ml (IQR)	4.7 (4.3–5.0)	4.7 (4.3–5.0)
Median baseline CD4+ T-cell count in cells/mm^3 (IQR)	232 (98–338)	230 (90–334)

IQR, interquartile range.

Fig. 1



Empirically derived *P*-values on the basis of permutation testing. Permutation testing was used to empirically derive *P*-value cut-offs (P_{PT}). Briefly, within the dataset used for association analysis, we permuted the connection between genotype and phenotype data. Permutation was repeated 1000 times, each generating a new dataset. We then carried out analyses on each of the 1000 datasets, from which we determined, at various *P*-value cut-offs, the average number of single nucleotide polymorphisms (SNPs) per analysis that pass that cut-off in the permuted data. We compared this average number with the actual number of SNPs that passed that same cut-off in the unpermuted data, providing an empiric determination of the probability that SNP–phenotype associations in the unpermuted data were by chance alone.

Results

This multiphenotype analysis included data from 1181 patients from A5202, who had consented to provide DNA for genetic research under ACTG protocol A5128. The characteristics of the study patients are presented in Table 1. The characteristics of patients included in the analysis generally reflected the characteristics of all A5202 study patients. From the available baseline and subsequent on-study data, a total of 774 phenotypes were derived for analysis as described under 'participants and methods' section. These comprised 19 primary phenotypes as well as 755 subphenotypes that were derived on the basis of baseline age, sex, race/ethnicity, BMI, CD4+ T-cell count, plasma HIV-1 RNA, randomized antiretroviral regimen, and component antiretroviral drug. This generated 68 phenotypes for ATV pharmacokinetics, 84 for CD4 T-cell count, 34 for EFV pharmacokinetics, 252 for fasting LDL cholesterol, 84 for HIV-1 RNA, and 252 for fasting triglycerides. Definitions for each of the 774 phenotypes are provided in Supplemental Table 2 (Supplemental digital content 2, *http://links.lww.com/FPC/B149*).

From the imputed genome-wide genotype data on these study patients, a total of 2501 SNPs (which were represented in either PharmGKB or the GWAS Catalog) provided at least one association result with at least one phenotype in this analysis. As noted in 'participants and methods' section, we excluded SNPs with MAF of less than 0.05 from models with at least 100 patients and SNPs with MAF of less than 0.10 from models with fewer than 100 patients. A total of 1 773 707 SNP–phenotype pairs provided *P*-values for association.

To assess the likelihood that associations were by chance alone, permutation testing was used to empirically derive P_{PT} to determine the probability that SNP–phenotype associations in the unpermuted data were by chance alone, as described in 'participants and methods' section. For example, at P_{PT} less than 1.5×10^{-4}, 50% of SNP–phenotype pairs in this analysis are likely not by chance alone (Fig. 1). Of the 1 773 707 SNP–phenotype pairs noted above, *P*-values for 737 (0.04%) were less than this P_{PT} threshold. The number of patients included in each model ranged from 18 (e.g. for EFV concentrations in patients younger than 26 years of age) to 1080 (e.g. for HIV-1 RNA response at 48 weeks), with a median of 242 patients per model.

Within each phenotype domain, association results for the five SNPs with the lowest *P*-value with at least one phenotype are presented in Table 2. For EFV pharmacokinetics, fasting LDL-cholesterol, and fasting triglyceride phenotypes, the SNP with the lowest *P*-value had previously been associated with that trait at *P* less than 5.0×10^{-8} in at least one GWAS [4,54]. For the five SNPs with the lowest *P*-values in EFV pharmacokinetics, fasting LDL-cholesterol, and fasting triglyceride domains (15 SNPs total), Manhattan plots for associations between each SNP and as many as 774 phenotypes across all six domains are shown in Fig. 2.

For EFV concentrations, the lowest *P*-value was with rs3745274 ($P=1.1\times10^{-28}$) among all 351 patients with evaluable data, but rs3745274 was also associated with numerous other context-derived EFV subphenotypes (Fig. 2). Log$_{10}$ *P*-values for association between rs3745274 and EFV concentrations correlated very strongly with sample size in the model (Spearman's $\rho=0.95$, $P<0.0001$), suggesting that this genetic association was present irrespective of context (i.e. sex, race/ethnicity, randomized antiretroviral regimen, component antiretroviral drug, baseline age, BMI, CD4+ T-cell count, and plasma HIV-1 RNA). In contrast, an association between rs10871777 and EFV concentration ($P=2.0\times10^{-5}$) was only observed

Table 2 Association results for the five lowest *P*-value single nucleotide polymorphisms within each phenotype domain

Domains	SNP	Chromosome	Gene[a]	Phenotype	Baseline context[b]	Cases	Controls	MAF	*P*-value
Atazanavir PK	rs12683493	9	*ABO*, *SURF6*	Atazanavir clearance	CD4 < 23	45	NA	0.20	7.54E−06
Atazanavir PK	rs7671266	4	*SLC2A9*, *WDR1*	Atazanavir clearance	Hispanic	115	NA	0.31	1.18E−05
Atazanavir PK	rs1137101	1	*LEPR*	Atazanavir exposure	VL < 3.93	38	NA	0.53	1.74E−05
Atazanavir PK	rs57270423	13	*ABCC4*	Atazanavir clearance	Age < 26	59	NA	0.22	2.85E−05
Atazanavir PK	rs2071427	17	*NR1D1*	Atazanavir exposure	Age > 52	41	NA	0.47	3.58E−05
CD4 T-cells	rs2368393	10	*MIR604*	CD4 change to week 96	All patients	970	NA	0.29	1.67E−06
CD4 T-cells	rs1799964	6	*LTA*, *TNF*	CD4 change to week 48	ATV/r arm	529	NA	0.19	1.99E−06
CD4 T-cells	rs112227868	6	*HLA-DRA*	CD4 change to week 48	Black	337	NA	0.09	7.39E−06
CD4 T-cells	rs1555543	1	*PTBP2*	CD4 change to week 96	VL > 5.71	101	NA	0.43	8.47E−06
CD4 T-cells	rs7941030	11	*UBASH3B*	CD4 change to week 48	CD4 < 23	102	NA	0.38	1.42E−05
Efavirenz PK	rs3745274	19	*CYP2B6*	Efavirenz concentration	All patients	351	NA	0.28	1.08E−28
Efavirenz PK	rs8192719	19	*CYP2B6*	Efavirenz concentration	All patients	359	NA	0.29	9.22E−28
Efavirenz PK	rs2279345	19	*CYP2B6*	Efavirenz concentration	All patients	359	NA	0.33	8.40E−22
Efavirenz PK	rs7746993	6	*GSTA5*, *GSTA10P*	Efavirenz concentration	BMI < 23	108	NA	0.06	7.35E−06
Efavirenz PK	rs10871777	18	None	Efavirenz concentration	BMI < 20.1	34	NA	0.20	2.00E−05
LDL-cholesterol	rs7412	19	*APOE*	LDL at week 96	All patients	853	NA	0.09	2.85E−10
LDL-cholesterol	rs9644568	8	*SLC18A1*, *LPL*	LDL change to week 96	BMI < 20.1	63	NA	0.10	5.38E−08
LDL-cholesterol	rs6731242	2	*UGT1A10*	LDL change to week 96	VL > 5.71	69	NA	0.13	1.02E−07
LDL-cholesterol	rs2725252	4	*ABCG2*	LDL change to week 96	BMI < 20.1	63	NA	0.40	2.14E−07
LDL-cholesterol	rs16998073	4	*ABCG2*	LDL at week 48	Age < 38	416	NA	0.21	2.87E−07
HIV-1 RNA	rs7865618	9	*CDKN2B-AS1*	HIV RNA < 200 at week 96	VL > 5.0	247	12	0.29	6.20E−07
HIV-1 RNA	rs2270777	12	*CDK4*	HIV RNA < 200 at week 96	BMI > 31.9	93	6	0.34	9.86E−07
HIV-1 RNA	rs1491850	11	*BDNF*, *KIF18A*	HIV RNA < 200 at week 48	CD4 > 302	326	20	0.39	2.16E−06
HIV-1 RNA	rs324026	3	*DRD3*	HIV RNA < 200 at week 96	VL > 5.0	247	12	0.46	8.68E−06
HIV-1 RNA	rs6280	3	*DRD3*	HIV RNA < 200 at week 96	VL > 5.0	247	12	0.46	8.68E−06
Triglycerides	rs651821	11	*APOA5*	TG at week 96	ATV/r arm	439	NA	0.12	4.33E−07
Triglycerides	rs6589566	11	*ZPR1*	TG at week 96	ATV/r arm	437	NA	0.07	6.18E−07
Triglycerides	rs2302821	9	*PTGES*	TG change to week 96	CD4 > 302	275	NA	0.17	7.82E−07
Triglycerides	rs10790162	11	*BUD13*	TG at week 24	BMI > 26.7	317	NA	0.07	8.21E−07
Triglycerides	rs1558861	11	*BUD13*	TG at week 24	BMI > 26.7	317	NA	0.06	8.21E−07

CD4, absolute CD4+ T-cell count; LDL, low-density lipoprotein; MAF, minor allele frequencies; NA, not available; PK, pharmacokinetics; SNP, single nucleotide polymorphism; VL, plasma HIV-1 RNA (i.e. viral load).

[a] When there are two genes named, the SNP is in the intergenic region, or within overlapping genes.

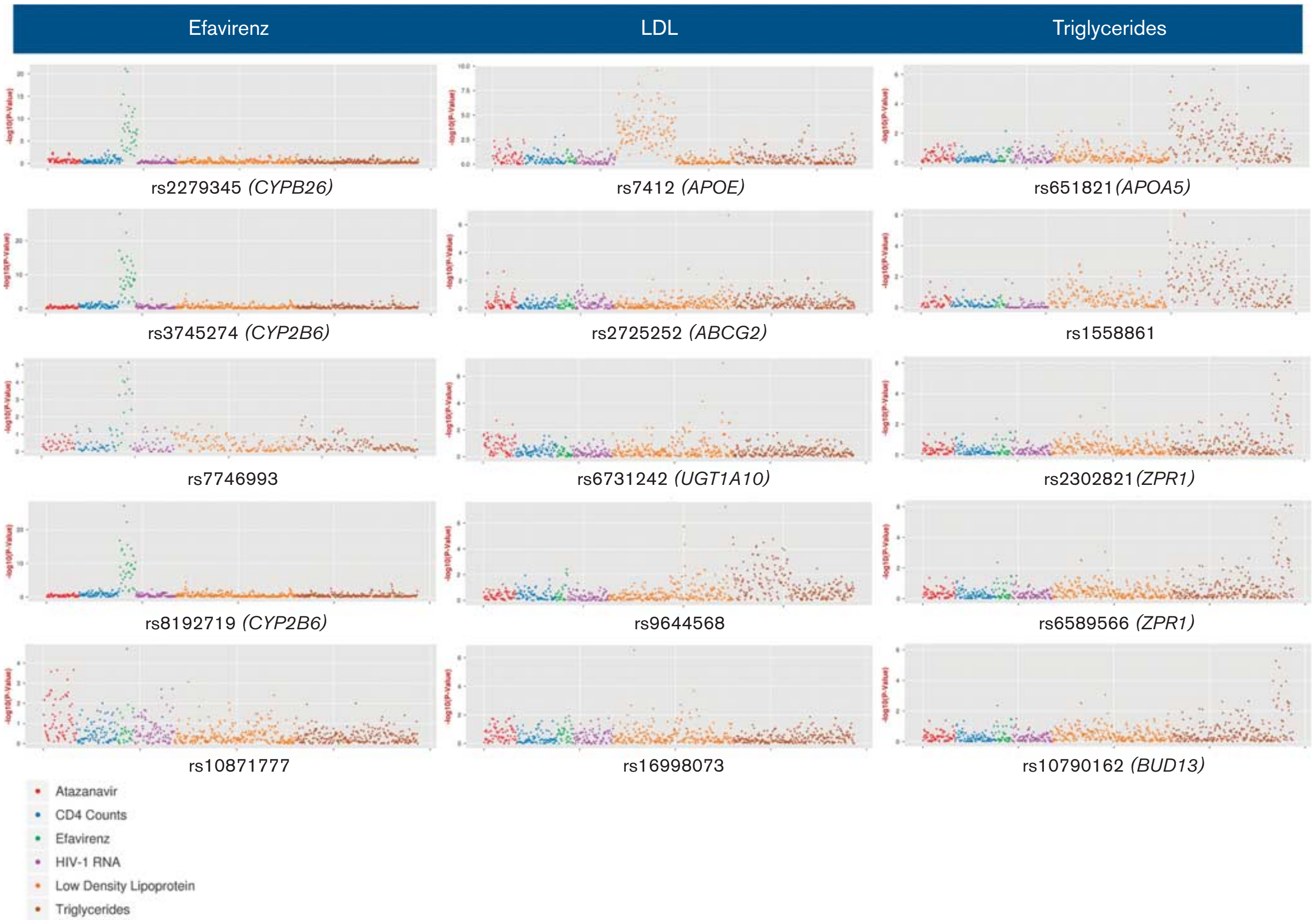
[b] Units are as follows: CD4, T-cells/mm^3; BMI, kg/m^2; VL, $\log_{10}$ HIV-1 RNA copies/ml; age, years.

among 34 individuals with baseline BMI in the lowest 10th percentile, but not among 80 individuals with BMI in the lowest 25th percentile ($P=0.01$), nor among 108 individuals with BMI in the lowest 33rd percentile ($P=0.25$) (Fig. 2). Furthermore, there was no hint of association between rs10871777 and EFV concentration within any other decile of BMI (i.e. 10th to 20th decile, 20th to 30th decile, etc.), considering both P-values and β coefficients (data not shown).

For fasting LDL-cholesterol, the lowest P-value was between rs7412 in *APOE* and week 96 LDL-cholesterol among all 853 evaluable patients. As shown in Fig. 2, rs7412 was associated with numerous context-derived LDL-cholesterol phenotypes. Associations were only with absolute values of LDL-cholesterol at individual study weeks, not with LDL-cholesterol change from baseline. Log_{10} P-values for association between rs7412 and LDL-cholesterol correlated directly with sample size in the model (Spearman's $\rho=0.64$, $P<0.0001$), without strong evidence for context dependence. For example, rs7412 was associated with week 96 LDL-cholesterol among patients randomized to either ATV/RTV-containing ART ($n=419$, $P=2.0\times10^{-7}$) or to EFV-containing ART ($n=435$, $P=2.7\times10^{-4}$). In addition, rs9644568 (near *LPL*) was associated with LDL-cholesterol change to week 96 among 63 individuals with baseline BMI in the lowest 10th percentile, but few other LDL-cholesterol phenotypes, but was more broadly associated with triglyceride phenotypes. In contrast, an association between rs16998073 and LDL-cholesterol at week 48 ($P=2.9\times10^{-7}$) was only at week 48 among 416 individuals in the lower 50th percentile for age (less than 38 years), but less so among individuals in the lowest 33rd percentile for age ($n=270$; $P=3.6\times10^{-3}$) or in the top 33rd percentile for age ($n=277$; $P=0.042$) (Fig. 2).

For fasting triglycerides, the lowest P-value was between rs651821 in *APOA5* and week 96 triglycerides among 439 individuals randomized to the ATV/RTV-containing ART. As shown in Fig. 2, rs651821 was associated with numerous context-derived triglyceride subphenotypes, including both absolute values at individual study weeks and change from baseline. Although $\log_{10}$ P-values for associations between rs651821 and triglycerides tended to correlate with phenotype sample size (Spearman's $\rho=0.42$, $P<0.0001$), there was some evidence for context dependence. For example, rs651821 was associated with week 96 triglycerides among patients randomized to ATV/RTV-containing ART ($n=439$, $P=4.3\times10^{-7}$), but not EFV-containing ART ($n=543$, $P=0.24$). Furthermore, among patients randomized to ATV/RTV-containing ART, this association between rs651821 and week 96 triglycerides was also observed with concomitant TDF/FTC ($n=219$, $P=2.3\times10^{-4}$), with concomitant ABC/3TC ($n=221$, $P=2.5\times10^{-4}$), and was also observed at week 48 ($n=481$, $P=3.2\times10^{-4}$). Among patients randomized to

Fig. 2



Manhattan plots representing all phenotype associations for the five single nucleotide polymorphisms (SNPs) with the lowest *P*-values for efavirenz pharmacokinetic, fasting low-density lipoprotein (LDL) cholesterol, and fasting triglyceride phenotypes. We analyzed SNPs that were annotated previously for any drug in the PharmGKB or associated previously with any trait in the GWAS Catalog, and that were also represented in the imputed, post-QC genome-wide data. Each marker represents, for each phenotype, the $-\log_{10}$ *P*-value for association with the indicated SNP. Color-coded phenotype categories are indicated at bottom left of figure. Note that the scale of the *Y*-axis differs between plots.

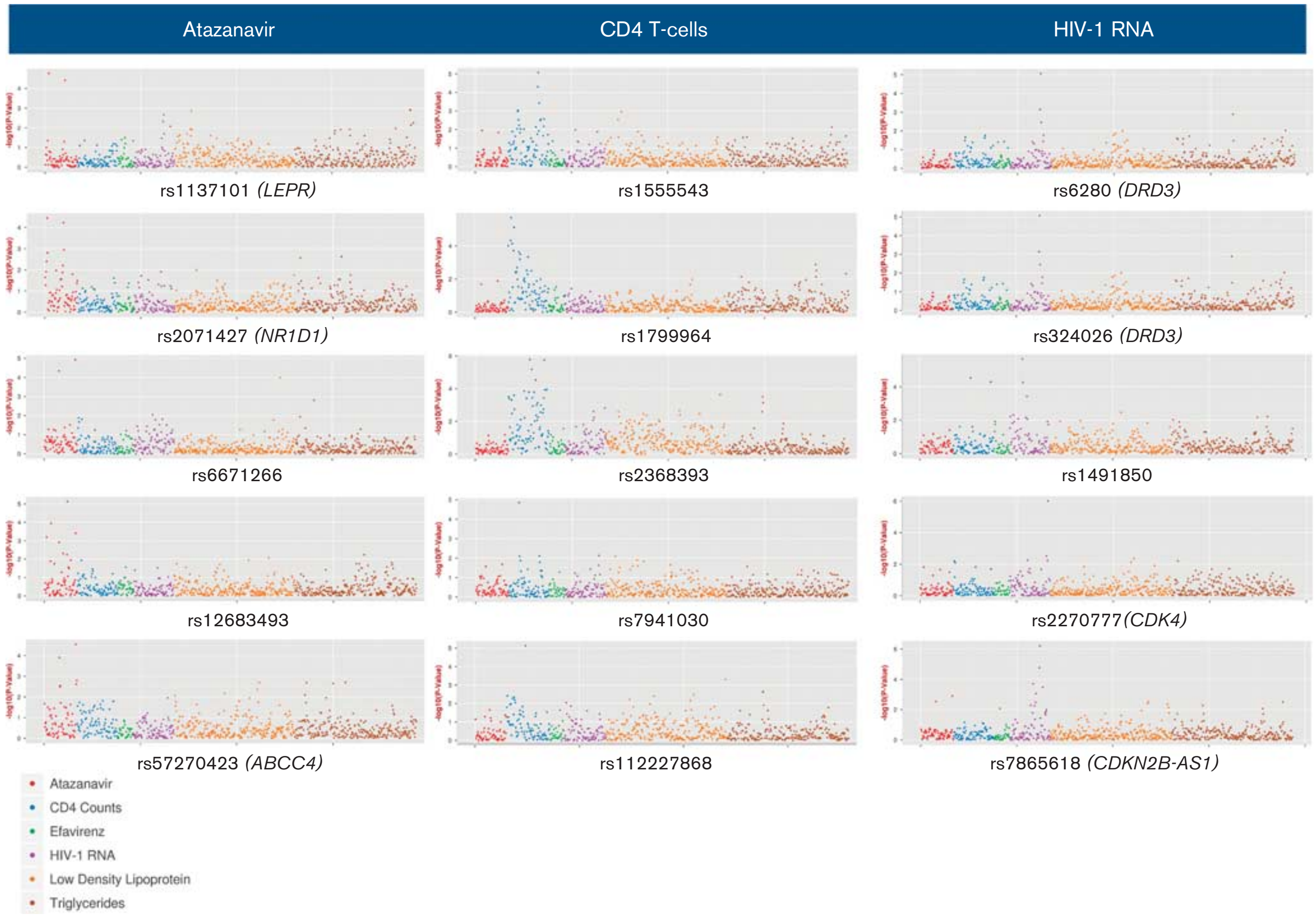
EFV-containing ART, an association between rs651821 and week 96 triglycerides was also absent with concomitant TDF/FTC ($n=224$, $P=0.04$), with concomitant ABC/3TC ($n=230$, $P=0.86$), and at week 48 ($n=488$, $P=0.02$). In contrast, among individuals with baseline CD4 count of more than 302, there was an association between rs2302821 and change in triglycerides at week 96 ($n=275$, $P=7.8\times10^{-7}$), but not at week 48 ($n=296$, $P=0.45$).

For ATV pharmacokinetics, CD4 T-cell count, and HIV-1 RNA phenotypes, the SNP with the lowest *P*-value had not been reported previously to be associated with that trait (Table 2). For the five SNPs with the lowest *P*-values in ATV pharmacokinetics, CD4 T-cell count, and HIV-1 RNA domains (15 SNPs total), Manhattan plots for associations between each SNP and as many as 774 phenotypes across all six domains are shown in Fig. 3. For ATV pharmacokinetics, the lowest *P*-value was with ATV clearance among patients with baseline CD4 T-cell count of less than 23 cells/mm^3 ($n=45$), and was with rs12683493, which is intergenic between *ABO* and *SURF6* ($P=7.5\times10^{-6}$). For CD4 T-cells, the lowest *P*-value was with change in patients CD4 T-cell count from baseline to week 96 among all patients ($n=970$) and was with rs2368393 in both *MIR604* and *SVIL* ($P=1.7\times10^{-6}$). For HIV-1 RNA, the lowest *P*-value was with HIV-1 RNA control at week 96 among patients with baseline HIV-1 RNA of more than 5.0 $\log_{10}$ copies/ml ($n=247$) and was with rs7865618 in *CDKN2B-AS1* ($P=6.2\times10^{-7}$).

Discussion

Phenome-wide association studies typically rely on observational data collected from electronic medical records, which may be subject to variability in the timing and completeness of data collection. The present multiphenotype analysis is unique in that it is the first to explore on-treatment data from a prospective clinical trial. The

Fig. 3



Manhattan plots representing all phenotype associations for the five single nucleotide polymorphisms (SNPs) with the lowest *P*-values for atazanavir pharmacokinetic, HIV-1 RNA, and CD4 T-cell phenotypes. We analyzed SNPs that were annotated previously for any drug in the PharmGKB or previously associated with any trait in the GWAS Catalog, and that were also represented in the imputed, post-QC genome-wide data. Each marker represents, for each phenotype, the $-\log_{10}$ *P*-value for association with the indicated SNP. Color-coded phenotype categories are indicated at the bottom left of the figure. Note that the scale of the *Y*-axis differs between plots.

collection of specific data elements at predetermined intervals before and after initiation of therapy makes clinical trials an attractive resource of structured longitudinal data to evaluate pharmacogenomic associations.

The present study characterized associations between 2544 SNPs from the PharmGKB and the GWAS Catalog and 774 context-derived phenotypes among 1181 HIV-infected participants from ACTG protocol A5202. Several associations replicated previous reports. We readily replicated the known association between *CYP2B6* variants and plasma EFV concentrations [4,55,56]. The lowest *P*-value was with rs3745274, which was associated with numerous context-derived EFV phenotypes. This genetic association appeared to persist irrespective of context (i.e. sex, race/ethnicity, randomized antiretroviral regimen, component antiretroviral drug, baseline age, BMI, CD4+ T-cell count, and plasma HIV-1 RNA). In contrast, the association between EFV concentration and rs10871777 (an SNP previously associated with obesity [57]) is very likely spurious, as this was only observed among individuals with baseline BMI in the lowest 10th percentile.

For LDL-cholesterol, rs7412 in *APOE* has been associated with LDL-cholesterol levels in previous GWAS [54,58], and was associated with numerous context-derived LDL-cholesterol phenotypes in this analysis. However, it was only associated with absolute values of LDL-cholesterol at individual study weeks, not with LDL-cholesterol change from baseline. In addition, there was no strong evidence for context dependence. An advantage of PheWAS is the ability to detect pleiotropy. In this respect, although rs9644568 (near *LPL*) was associated with LDL-cholesterol change at week 96 among 63 individuals with baseline BMI in the lowest

10th percentile, but very few other LDL-cholesterol phenotypes, it was associated more with numerous triglyceride phenotypes, consistent with its previously reported association with triglycerides in GWAS [59]. In contrast, an association between LDL-cholesterol and rs16998073 (an SNP associated previously with diastolic blood pressure [60]) is very likely spurious as an association was observed only at week 48 among individuals in the lower 50th percentile for age.

For triglycerides, rs651821 in *APOA5* has been associated with triglycerides in previous GWAS [61], as have three of our other top five SNPs (rs6589566 [62]; rs10790162 [63]; and rs1558861 [64]). The SNP rs651821 was associated with numerous context-derived triglyceride phenotypes, representing both absolute values at individual study weeks and change from baseline. In addition, there was some evidence that this association was context dependent, with rs651821 associated with week 96 LDL-cholesterol among patients randomized to ATV/RTV-containing ART, but not EFV-containing ART. An association between and change in triglycerides and rs2302821 (an SNP associated weakly with cardiovascular toxicity in patients treated with celecoxib [60]) is very likely spurious, as this was observed in a phenotype at week 96, but not at week 48.

In contrast to the above findings, SNPs with the lowest *P*-values for association with ATV pharmacokinetics, CD4 T-cell count, and HIV-1 RNA phenotypes had not been reported previously to be associated with that trait. The validity of multiple other associations, such as those between ATV pharmacokinetics and rs12683493 (intergenic between *ABO* and *SURF6*, in a haplotype implicated in cough with enalapril [65]), change in CD4 T-cell count from baseline and rs2368393 (in both *MIR604* and *SVIL*, not associated with risk of drug toxicity in children with lymphoblastic leukemia–lymphoma [66]), and HIV-1 RNA control and rs7865618 (in *CDKN2B-AS1*, associated with cardiovascular disease [67] and glaucoma in GWAS [68]) may be spurious. Replication for these and other SNP associations (many of which may be spurious) in independent cohorts is warranted.

In this analysis, we used context as a strategy to derive multiple subphenotypes from each primary phenotype. Our rationale is that genetic associations for a given SNP–trait association may differ depending on context; thus, we expect that context-dependent associations may be more readily identified and understood using this approach. One advantage of this approach is that it allows for a very granular exploration of SNP–genotype associations that may be influenced by context. We found such context dependence in the association between rs651821 and triglyceride phenotypes among patients randomized to ATV/RTV-containing ART regimens, but not among patients randomized to EFV-containing ART regimens. The random assignment of A5202 participants to receive either ATV/RTV-containing or EFV-containing ART decreases the likelihood that our finding was because of confounding as unrecognized confounders should be equally distributed across arms. This is an advantage of using clinical trials datasets for genetic association analyses. Another advantage of granular exploration of SNP–genotype associations is the ability to discern associations that are almost certainly spurious by comparing strengths of association between closely related phenotypes. For example, among individuals with baseline CD4 count of more than 302, the association between rs2302821 and change in triglycerides at week 96 ($P=7.8\times10^{-7}$) is almost certainly spurious as there was no such association at week 48 ($P=0.45$).

Multiple hypothesis testing is inherent in approaches that examine multiple phenotypes such as PheWAS, but Bonferoni correction is not appropriate because the assumption that tests are independent is violated. To address this issue, we performed 1000 permutations of the analysis to create an empirical null distribution. This showed that in the present analysis, the majority of models with *P*-value less than 1.5×10^{-4} were unlikely to be by chance alone.

This analysis leveraged extensive a priori knowledge of genes and phenotypes from PharmGKB and the GWAS Catalog. This increases the likelihood of validity, biological plausibility, and supportive publications. As was apparent in both our previous PheWAS [22] and the present study, disease-specific knowledge is useful in interpreting genetic associations and to prioritize associations for further replication and study. Because every phenome is unique, analyses that consider large numbers of phenotypes may benefit more so than GWAS from disease-specific knowledge and understanding, including relationships among phenotypes.

This study had limitations. A larger sample size may have shown additional associations and we have not yet sought to replicate associations in other datasets. We considered a limited number of phenotypes and contexts. We considered ART as intent to treat. We only used available SNPs that were available or imputed from genome-wide genotyping, without additional genotyping. We also focused analyses on individual SNPs, whereas multiple SNPs considered in combination may more strongly associate with some phenotypes. Data from prospective, randomized clinical trials offer distinct advantages (e.g. randomization tends to evenly distribute covariates across study arms), but there are limitations. Although data of ACTG clinical trials are rigorously collected and validated, electronic medical records datasets will likely contain a wider range of variables. In addition, eligibility criteria for clinical trials may exclude some individuals who would otherwise be included in electronic medical records datasets.

In summary, this pilot study supports a multiphenotype analysis strategy to explore clinical trials datasets for genetic associations and to ultimately identify genetic associations with the potential to optimize ART safety and efficacy. This approach complements more established GWAS by performing simultaneous calculations for identifying genotype–phenotype associations across numerous phenotypes. Work is ongoing to further evaluate and optimize multiphenotype analyses for clinical trials datasets. On the basis of results from this pilot study, we plan to extend the PheWAS approach both to a much more extensive set of traits and to multiple other clinical trials datasets. This will include replication of associations identified here.

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Conflicts of interest

Eric S. Daar has been principal investigator on research grants to Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center from Gilead, Merck, and ViiV, and a consultant/advisor to Bristol Myers Squibb, Gilead, Janssen, Merck, Teva, and ViiV. For the remaining authors there are no conflicts of interest.

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