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Genome-wide Association Study of Atazanavir Pharmacokinetics and Hyperbilirubinemia in AIDS Clinical Trials Group Protocol A5202

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Abstract

Background—Atazanavir-associated hyperbilirubinemia can cause premature discontinuation of atazanavir and avoidance of its initial prescribing. We used genome-wide genotyping and clinical data to characterize determinants of atazanavir pharmacokinetics and hyperbilirubinemia in AIDS Clinical Trials Group protocol A5202.

Methods—Plasma atazanavir pharmacokinetics and indirect bilirubin concentrations were characterized in HIV-1-infected subjects randomized to atazanavir/ritonavir-containing regimens. A subset had genome-wide genotype data available.

Results—Genome-wide assay data were available from 542 subjects, of who 475 also had estimated atazanavir clearance and relevant covariate data available. Peak bilirubin concentration and relevant covariates were available for 443 participants. By multivariate analysis, higher peak on-treatment bilirubin was associated with *UGT1A1* rs887829 T allele ($P=6.4\times 10^{-12}$), higher baseline hemoglobin ($P=4.9\times 10^{-13}$), higher baseline bilirubin ($P=6.7\times 10^{-12}$), and slower plasma atazanavir clearance ($P=8.6\times 10^{-11}$). For peak bilirubin >3.0 mg/dL, the positive predictive value

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of baseline bilirubin 0.5 mg/dL with hemoglobin 14g/dL was 0.51, which increased to 0.85 with rs887829 TT homozygosity. For peak bilirubin 3.0 mg/dL, the positive predictive value of baseline bilirubin <0.5 mg/dL with hemoglobin <14 g/dL was 0.91, which increased to 0.96 with rs887829 CC homozygosity. No polymorphism predicted atazanavir pharmacokinetics at genome-wide significance.

Conclusions—Atazanavir-associated hyperbilirubinemia is best predicted by considering *UGT1A1* genotype, baseline bilirubin, and baseline hemoglobin values in combination. Use of ritonavir as a pharmacokinetic enhancer may have abrogated genetic associations with atazanavir pharmacokinetics.

Keywords

HIV; atazanavir; pharmacogenomics; pharmacokinetics; *UGT1A1*

Introduction

The once-daily HIV-1 protease inhibitor atazanavir with low-dose ritonavir as a pharmacokinetic enhancer (atazanavir/r) is generally safe, effective, and well tolerated [1-3] in first-line regimen for HIV-1 infection [4]. Atazanavir increases plasma indirect bilirubin concentrations by inhibiting uridine diphosphate glucuronosyltransferase (UGT) 1A1-mediated bilirubin glucuronidation [5]. Approximately 40% of patients prescribed atazanavir/r experience at least one indirect bilirubin elevation greater than 2.5 times the upper limit of normal (i.e. grade 3) [1, 6-8], and approximately 5% at least 5 times the upper limit of normal (i.e. grade 4) [1, 8]. While atazanavir-associated hyperbilirubinemia does not indicate hepatic injury, some patients discontinue atazanavir due to icterus [3, 9, 10], or are not prescribed atazanavir to avoid this possibility. Reliable baseline predictors of subsequent hyperbilirubinemia could inform atazanavir/r prescribing.

Greater plasma atazanavir concentrations are associated with greater plasma bilirubin elevations [7, 11]. Atazanavir undergoes phase I metabolism by hepatic cytochrome P450 (CYP) 3A isoforms [12] and is a substrate for membrane transporters including P-glycoprotein (encoded by *ABCB1*), multidrug resistance-associated proteins (MRPs, encoded by *ABCC* genes), and organic anion transporting polypeptides (OATPs, encoded by *SLCO* genes) [13]. Candidate gene studies, most involving unboosted atazanavir, have suggested associations between atazanavir pharmacokinetics and genetic polymorphisms in *ABCB1* [14-16], *CYP3A5* [16-18], and *NR1I2* (encoding pregnane X receptor) [16, 19]. Plasma atazanavir exposure is also influenced by non-genetic factors including concomitant antiretrovirals [20-22], other medications such as rifampin [Acosta, 2007 #2192], food [23], and gastric acid blocking medications [12, 24].

Interindividual differences in plasma indirect bilirubin concentrations have been associated with a *UGT1A1* promoter tandem TA repeat. The *UGT1A1**28 (TA)₇ allele is associated with reduced *UGT1A1* transcription as compared to *UGT1A1**1 (TA)₆ [25, 26]. Among atazanavir recipients, *UGT1A1**28 has been strongly associated with unconjugated hyperbilirubinemia [27, 28], but inconsistently with atazanavir discontinuation [28, 29].

The present study used a genome-wide approach to investigate genetic and non-genetic associations with hyperbilirubinemia, and genetic predictors of plasma atazanavir pharmacokinetics among subjects randomized to atazanavir/r-containing regimens in a prospective clinical trial.

Methods

Study Participants

AIDS Clinical Trials Group (Protocol) A5202 (ClinTrials.gov NCT00118898) was a phase IIIb equivalence study of four once-daily regimens for initial treatment of HIV-1 infection. Primary results of A5202 have been previously reported [3, 30]. Briefly, A5202 subjects enrolled from 2005 to 2007 were randomized to open-label atazanavir (300 mg) plus ritonavir (100 mg), or efavirenz (600 mg), with either placebo-controlled abacavir/lamivudine (600 mg/300 mg) or tenofovir DF/emtricitabine (300 mg/200 mg). Study evaluations including indirect bilirubin and hemoglobin determinations were performed before entry, at entry, at weeks 4, 8, 16 and 24, and every 12 weeks thereafter until the last enrolled subject was followed 96 weeks. Bilirubin and hemoglobin were assayed at research site clinical laboratories. Subjects in the present study were also the basis of a previous analysis focused on *UGT1A1**28 and premature discontinuation of atazanavir[28].

Atazanavir assays and plasma sampling

In A5202, plasma samples for atazanavir assays were obtained during any two scheduled visits during the first 24 weeks of study. At one visit a sample was to be drawn immediately before an observed dose and again 3-4 hours later. During the second visit a sample was drawn between 5 and 12-15 hours post-dose (12 versus 15 hours depending on whether the dose was in the morning or evening). Additional samples for atazanavir assay were collected at week 48 and every 48 weeks thereafter, at final study visit, with first documented virologic failure, and with medication change due to virologic failure.

Atazanavir was quantified using a previously reported reverse phase high performance liquid chromatography (HPLC) method utilized at the University at Buffalo. Atazanavir was separated on a Waters 5 μ m Symmetry™ shield RP C8, 3.0 \times 150 mm column, with a chromatographic system consisting of Waters 2695 Alliance Separations Module, and a 996 Photodiode Array Detector. The system was controlled by Waters Empower 2 software Version 6.20.00.00 that collected all chromatographic data for analysis, generating a calibration standard curve that was linear from 100-16,000 ng/mL.

Derivation of pharmacokinetic parameters

A model-based population pharmacokinetic analysis was performed using pharmacokinetic samples collected at steady-state during the first 24 weeks of therapy. Concentration-time data were analyzed using the first-order conditional estimation with interaction (FOCE-I) method of the non-linear mixed effects modeling program NONMEM (version VII; ICON, Ellicott City, MD). One- and two-compartment models were tested to identify an appropriate structural model. A single compartment model with first-order absorption and elimination was ultimately selected based on successful convergence (which two-

compartment models did not) and goodness-of-fit plots (Supplementary Figure, Supplemental Digital Content 1, <http://links.lww.com/FPC/A688>). Estimation of population pharmacokinetic parameters included clearance, volume of distribution, and absorption rate constant. Interindividual random effects were described on an exponential scale, while a proportional error model was used to describe residual variability. Individual Bayesian estimates of oral clearance values of atazanavir were estimated from the structural model.

Identifying genetic polymorphisms

Consent for genetic analyses was obtained under ACTG protocol A5128 [31]. The Vanderbilt Institutional Review Boards and the ACTG approved this use of DNA. Genome-wide data from the Illumina Human-1M-Duo platform were available from a separate immunogenomics project [32]. Genetic data management and association analyses were performed with PLINK version 1.07 [33]. Genetic ancestry was assigned using principal components as described elsewhere [32]. Samples that did not group with African American, European, or Hispanic populations were excluded. Analyses were performed separately for African American, European, and Hispanic ancestry populations, and for all three populations combined. Within each population, only polymorphisms with >98% genotyping efficiency and >2% minor allele frequency were included in analyses. Samples were censored for >2% missingness, excessive heterozygosity ($|F| > 0.1$), or possible sex misclassification. For sample pairs with high estimated relatedness as determined by identity by descent ($\pi\text{-hat} > 0.125$), only one sample was included in analyses. Final analyses included as many as 925,738 assayed polymorphisms.

Targeted genotyping of *UGT1A1* rs8175347 (i.e. (TA)₅ *36, (TA)₆ *1, (TA)₇ *28, and (TA)₈ *37) was by fragment analysis as described elsewhere [34]. Laboratory personnel with no knowledge of clinical data performed genotyping. Genotype assignment was confirmed by visual inspection for electrophoresis peaks representing promoter TA tandem repeats *36 (TA)₅, *1 (TA)₆, *28 (TA)₇, and *37 (TA)₈.

Association testing

Genetic associations were assessed using an uncorrected Wald test, as well as a linear regression model that adjusted for covariates that by univariate analysis had $P < 0.05$. For atazanavir clearance, covariates included were body mass index, abacavir/lamivudine versus tenofovir DF/emtricitabine assignment at randomization, sex, and occurrence of an atazanavir concentration below limit of quantification. Peak bilirubin was defined as the highest concentration during the first 24 weeks of atazanavir/r prescribing in A5202. For peak bilirubin, univariate analyses showed associations with age, baseline bilirubin concentration, baseline hemoglobin concentration, atazanavir clearance, and sex; after correcting for hemoglobin, sex was no longer associated so was not included as a covariate.

To correct for population stratification, principal components were determined separately for each population by EIGENSTRAT [35]. The first two principal components were included as covariates for African American, European, and Hispanic groups. The first four principal components generated with the combined population were included as covariates in the

combined group analyses. P values were adjusted for genomic inflation factor based on median chi-squared value.

From our genome-wide dataset, 13 functional polymorphisms in genes suggested to affect atazanavir disposition were chosen a priori from PharmGKB[36] (*CYP3A4* [12], *CYP3A5* [12, 17, 18], *ABCB1* [13], *SLCO1B1*[13], *ABCC1* [13], *ABCC2* [13], and *NR1I2* [16, 37]) and previous atazanavir pharmacogenetic studies [14-19]. Similarly, a targeted set of 27 polymorphisms previously associated with bilirubin metabolism was assembled a priori.

Non-genetic statistical analyses were performed using R version 2.15.0 [38], using two-sided tests for P values and confidence intervals, without adjusting for multiple comparisons. Bonferroni-corrected genome-wide significance was defined as 5.0×10^{-8} giving a genome-wide family error rate of 0.05 for 925,738 comparisons. For the 13 polymorphisms in atazanavir disposition genes and 27 in bilirubin associated genes, Bonferroni corrected P value thresholds for the single combined group were 0.0038 and 0.0019, respectively. For analyses of these a priori designated polymorphisms in all three ethnic groups, Bonferroni corrected P values were 0.0013 and 0.00062 for pharmacokinetic and bilirubin associated genes respectively.

Results

Study Participants

Among 928 subjects randomized to receive atazanavir/r-containing regimens in A5202, atazanavir plasma concentration data were available from 815 subjects for population pharmacokinetic modeling. Forty-eight out of 2195 atazanavir concentrations were below the lower limit of quantitation (< 3%), and were substituted with concentration values of 50 ng/mL. Genome-wide assay data were available from 542 subjects [32], of who 475 (88%) also had estimated atazanavir clearance and relevant covariate data available. Peak bilirubin concentration and relevant covariates were available for 443 participants. Derivation of the study population is shown in **Figure 1**. Baseline characteristics of study participants are shown in **Table 1**.

Genetic associations with atazanavir pharmacokinetics

The parameter estimates (and relative standard error as percentage) for the one-compartment pharmacokinetic model were 0.47 h^{-1} (11%) for the first-order absorption rate constant, 86.7 L (5%) for the volume of distribution, and 7.86 L/h (2%) for apparent oral clearance with an interindividual variability of 36% (8%). The median estimated atazanavir clearance for subjects with genome-wide assay data was 7.75 L/hr (95% C.I. 7.54 to 7.96 L/hr). No polymorphism was associated with clearance at genome-wide significance, whether considering each population separately, or in a combined analysis including all three populations. Of the 10 polymorphisms with the lowest P values in the combined population, none were in genes with apparent relevance to drug disposition (**Table 2, top**). Data for all polymorphisms associated with estimated atazanavir clearance at $P < 5.0 \times 10^{-5}$ are listed in Supplemental Table 1, Supplemental Digital Content 2, <http://links.lww.com/FPC/A689>. Of the 13 polymorphisms designated a priori for possible associations with atazanavir

clearance, none were significant after correcting for multiple comparisons in the combined population. There were trends toward nominal associations with rs4149056 in *SLCO1B1* ($\beta = -0.089$, $P = 0.049$) and rs776746 in *CYP3A5* ($\beta = 0.076$, $P = 0.097$), as shown in **Table 3**.

When each population was analyzed separately, of the 13 targeted polymorphisms, rs2235015 in *ABCB1* was nominally associated with clearance among Hispanics ($P_{\text{Clearance}} = 0.013$). Additionally, clearance was nominally associated in African Americans with rs1722273 in *ABCC2* ($P = 0.033$) and in Hispanics with rs776746 in *CYP3A5* ($P = 0.046$). None were significant after Bonferroni correction.

Associations with indirect bilirubin concentrations

Among the 443 participants included in analyses for genomic associations with peak bilirubin concentrations, median baseline indirect bilirubin was 0.5 mg/dL (IQR 0.3 - 0.8 mg/dL), and median peak indirect bilirubin during the first 24 weeks of therapy was 2.2 mg/dL (IQR 1.6 - 3.4 mg/dL). By univariate analysis, greater baseline (i.e. pre-atazanavir) bilirubin concentrations were significantly associated with male sex ($P = 1.4 \times 10^{-5}$) and greater baseline hemoglobin concentration ($P = 7.1 \times 10^{-6}$, $\beta = 0.094$ bilirubin mg/dL per hemoglobin g/dL). In genome-wide analysis of baseline indirect bilirubin in the combined population, 10 polymorphisms were genome-wide significant, each in or near *UGT1A1*. The smallest P value was for rs6742078 ($P = 1.8 \times 10^{-9}$, $\beta = 0.30$ mg/dL per T allele).

Clinical variables associated with greater on-treatment peak indirect bilirubin concentration by univariate analysis included greater baseline indirect bilirubin concentration ($P = 2.61 \times 10^{-25}$, $\beta = 0.36$), greater baseline hemoglobin concentration ($P = 1.37 \times 10^{-13}$, $\beta = 0.12$ (mg/dL)/(g/dL)), slower estimated atazanavir clearance ($P = 8.98 \times 10^{-9}$, $\beta = -0.55$ (mg/dL) per (L/h)), and younger age ($P = 4.93 \times 10^{-4}$, $\beta = -0.0097$ mg/dL per year). There was no association between on-treatment peak indirect bilirubin concentration and either baseline alanine aminotransferase (ALT, $P = 0.72$) or baseline hepatitis C seropositivity ($P = 0.16$). In multivariate genome-wide analysis of peak on-treatment indirect bilirubin concentration in the combined population (adjusting for baseline bilirubin concentration, baseline hemoglobin concentration, age, atazanavir clearance, and the top 4 principal components), 10 polymorphisms in or near *UGT1A1* were genome-wide significant, the smallest P value being for rs887829. In this analysis, greater peak bilirubin was associated with rs887829 T allele ($P = 6.4 \times 10^{-12}$, $\beta = 0.30$ mg/dL per T allele), greater baseline bilirubin concentration ($P = 6.7 \times 10^{-12}$, $\beta = 0.22$), slower estimated atazanavir clearance ($P = 8.6 \times 10^{-11}$, $\beta = -0.51$ (mg/dL)/(L/hr)), and greater baseline hemoglobin concentration ($P = 4.9 \times 10^{-13}$, $\beta = 0.098$ (mg/dL)/(g/dL)). After controlling for rs887829, no additional polymorphisms were genome-wide significant. Of 27 targeted polymorphisms selected a priori for possible association with bilirubin, only those in or near *UGT1A1* were significant after Bonferroni correction (Supplemental Table 2, Supplemental Digital Content 3, <http://links.lww.com/FPC/A690>).

In addition to genome-wide assay data, the *UGT1A1* tandem TA repeat (rs8175347 which defines *UGT1A1* *28, *36 and *37 alleles) was directly assayed. Among A5202 subjects, rs8175347 was in almost complete linkage disequilibrium with rs887829 ($R^2 = 0.99$), so was not informative beyond rs887829.

Prediction of peak on-treatment bilirubin

Associations between peak plasma bilirubin >3.0 mg/dL (an approximate concentration above which jaundice icterus tends to be noticeable) and rs887829 genotype were considered in relation to a baseline bilirubin threshold of 0.5 mg/dL and a baseline hemoglobin threshold of 14 g/dL. These baseline thresholds approximate median values. As shown in **Figure 2**, the positive predictive value of baseline bilirubin 0.5 mg/dL and baseline hemoglobin 14 g/dL for peak bilirubin >3.0 mg/dL was 0.51 (95% C.I. 0.40 to 0.57). This increased to 0.85 (95% C.I., 0.65 to 0.96) by also including rs887829 TT homozygosity. Conversely, the positive predictive value of baseline bilirubin <0.5 mg/dL and baseline hemoglobin <14 g/dL for peak bilirubin 3.0 mg/dL was 0.91 (95% C.I., 0.85 to 0.95). This increased to 0.96 (95% C.I., 0.88 to 0.99) by also including rs887829 CC homozygosity. Among individual with rs887829 CT heterozygosity and/or representing discordant baseline bilirubin and hemoglobin strata, the likelihood of peak bilirubin greater or less than 3.0 mg/dL reflected ordinal effects of each of the three variables (**Figure 2**). Additional analyses that considered several alternative peak plasma bilirubin thresholds greater than 3.0 mg/dL did not substantially improve prediction (data not shown).

Multivariable models assessed the extent to which interindividual variability in peak on-treatment bilirubin concentrations could be explained by genetic and non-genetic factors, based on adjusted-R² values (**Table 4**). A model that included all four variables (baseline bilirubin concentration, baseline hemoglobin concentration, estimated plasma atazanavir clearance, and rs887829 genotype) explained 41.3% of variability in peak on-treatment bilirubin concentrations (Model 6). In models that included baseline hemoglobin concentration and estimated plasma atazanavir clearance, adding either baseline bilirubin concentration or rs887829 genotype contributed comparably to peak on-treatment bilirubin concentrations (33.4% in Model 1 vs. 33.6% in Model 2). In models that included baseline hemoglobin concentration, adding either baseline bilirubin concentration or rs887829 genotype contributed comparably to peak on-treatment bilirubin concentrations (30.3% in Model 3 vs. 26.4% in Model 4).

Discussion

We used a genome-wide approach to investigate associations between human genetic variants, pharmacokinetics, and indirect bilirubin concentrations among subjects randomized to receive atazanavir/r in ACTG protocol A5202. In a multivariate model, variables independently associated with peak on-treatment indirect bilirubin concentrations were *UGT1A1* rs887829 genotype, baseline bilirubin concentration, baseline hemoglobin concentration, and atazanavir clearance, which together explained 41.3% of interindividual variability. The best pre-treatment predictor of peak on-treatment indirect bilirubin 3.0 mg/dL (i.e. excluding atazanavir pharmacokinetic data) was the combination of rs887829 CC homozygosity, lower bilirubin concentration, and lower hemoglobin concentration. Conversely, the best pre-treatment predictor of peak on-treatment indirect bilirubin >3.0 mg/dL was the combination of rs887829 TT homozygosity, greater bilirubin concentration, and greater hemoglobin concentration.

Previous candidate gene studies have suggested associations between atazanavir pharmacokinetics and polymorphisms in drug metabolism and/or transporter genes. The present study did not replicate such associations, nor did we identify new genetic associations with atazanavir pharmacokinetics among targeted polymorphisms in genes relevant to atazanavir disposition or in genome-wide analyses. We hypothesize that ritonavir, by inhibiting the activity of CYP isoforms including CYP3A5, may have masked potential genetic associations with atazanavir pharmacokinetics. Drug metabolism and transporter gene polymorphisms were therefore not included in multivariate models for on-treatment bilirubin concentrations.

In the combined population analysis of atazanavir clearance there were trends toward nominally significant associations with *SLCO1B1* 521T→C (rs4149056) and the *CYP3A5**3 loss-of-function polymorphism (rs776746). Among Hispanics, rs776746 was also nominally associated with clearance. However, neither polymorphism was significant after Bonferroni correction among Blacks, Whites, or Hispanics analyzed separately. Multiple prior studies have associated *SLCO1B1* rs4149056 with increased plasma lopinavir concentrations among patients prescribed lopinavir/ritonavir [39-42][43] and one study associated this polymorphism with decreased plasma amprenavir concentrations among patients prescribed fos-amprenavir/ritonavir [41]. A prior study of 31 HIV-negative individuals suggested an association between *CYP3A5* genotype and plasma clearance of atazanavir (without ritonavir), which lessened when combined with ritonavir [17]. The present study did not replicate *ABCB1* 3435C→T (rs1045642), which has been reported to be associated with pharmacokinetics for atazanavir without ritonavir [14] and with ritonavir [15]. While we cannot explain the failure of *ABCB1* 3435C→T to replicate in our study, reported associations of this polymorphism with pharmacokinetics and other phenotypes for many drugs have failed to replicate. The *NR1I2* polymorphism 63396C→T (rs2472677) reported to be associated with atazanavir pharmacokinetics [19, 37] was not assayed in the present study.

The *UGT1A1* rs887829 polymorphism is a well-established predictor of increased plasma indirect bilirubin concentrations in the general population (i.e. Gilbert trait) [44-46], and accounts for approximately 15% of variation in African Americans [44] and Europeans [46]. In the present study rs887829 was in almost complete linkage disequilibrium with *UGT1A1**28 (rs8175347) genotype. Previous studies have associated *UGT1A1**28 with greater indirect bilirubin elevations among HIV-infected individuals prescribed indinavir [27] or atazanavir [27, 28]. In a study of patients HIV-infected patients prescribed indinavir and HIV-negative individuals, increased likelihood of hyperbilirubinemia was associated with multiple polymorphisms in *UGT1A* genes including *UGT1A1**28, *UGT1A7* (rs7586110), *UGT1A7* (rs17868323), and *UGT1A3* (rs3806596). These associations were also seen in the present study, but disappeared after correcting for rs887829.

Polymorphisms in genes beyond *UGT1A1* have been reported to be associated with serum bilirubin concentrations in the general population, including *ABCC2*, *ABCB4*, *ABCB11*, *ATP8B1*, *SLCO1B1* [46], *SLCO1B3* and *G6PD* [45]. In addition, bilirubin concentrations have been associated with *ABCB1* 3435C→T among patients prescribed atazanavir without ritonavir but not with ritonavir [17], although results have been inconsistent [14]. The

present study did not replicate genetic predictors of hyperbilirubinemia beyond rs887829 (Supplemental Table 2, Supplemental Digital Content 3, <http://links.lww.com/FPC/A690>).

It is not unexpected that baseline bilirubin is associated with peak on-treatment indirect bilirubin concentrations, independent of *UGT1A1* rs887829 genotype. In population-based studies, genetic polymorphisms have explained less than 50% of interindividual variability in bilirubin concentrations. Non-genetic factors such as increased rates of red blood cell turnover (i.e. increased bilirubin production) are certain to affect baseline bilirubin concentrations. The association between peak on-treatment indirect bilirubin concentrations and higher hemoglobin concentrations may be the consequence of a greater mass of red blood cells from which to produce bilirubin.

In genetic association studies, unrecognized population stratification can lead to false discovery. This was addressed in the present study by correcting for genetic ancestry using principal components in the total population analysis as well as in each race/ethnicity group analyzed separately.

There were limitations to the present study. Some previously reported pharmacogenetic associations have involved atazanavir without ritonavir [14, 16, 19, 47], and a decreased effect of genetic variants has been suggested for atazanavir with ritonavir [15, 17]. In the present study, genetic associations with atazanavir pharmacokinetics may have been abrogated by the use of atazanavir with ritonavir. Clearance was estimated from sparse pharmacokinetic data applied to a population pharmacokinetic model, and errors in the model would have misestimated pharmacokinetic parameters. However, Colombo et al compared population pharmacokinetic modeling for a rich versus a sparse data set and found similar individual variances between the two groups [24]. Although we used a genome-wide approach, it is possible that polymorphisms not assayed herein, such as rare variants and copy number polymorphisms, or with weaker effect sizes than can be detected with this sample size. Our sample size of 443 participants for bilirubin analyses and 475 participants for pharmacokinetic analyses may have limited our ability to identify polymorphisms with small effects. However, unlike GWAS of complex traits such as diabetes, large effect sizes with drug metabolism/transport genes and off-target genes often reveal genome-wide significant associations with modest sample sizes. For example, genetic prediction of abacavir hypersensitivity is genome-wide significant ($P < 5.0 \times 10^{-8}$) with only 15 cases and 200 controls [48], statin response with only 85 cases and 90 controls [49], and nevirapine rash with only 71 cases and 77 controls [50].

In summary, peak plasma indirect bilirubin concentrations during the first 24 weeks of therapy are associated with *UGT1A1* rs887829, baseline bilirubin concentrations, baseline hemoglobin concentrations, and atazanavir clearance. It is possible that therapeutic drug monitoring with atazanavir dose reduction in selected individuals could reduce hyperbilirubinemia, but this question was not addressed by the present study. Consideration of genetic and clinical laboratory data at baseline best stratifies risk for subsequent development of on-treatment hyperbilirubinemia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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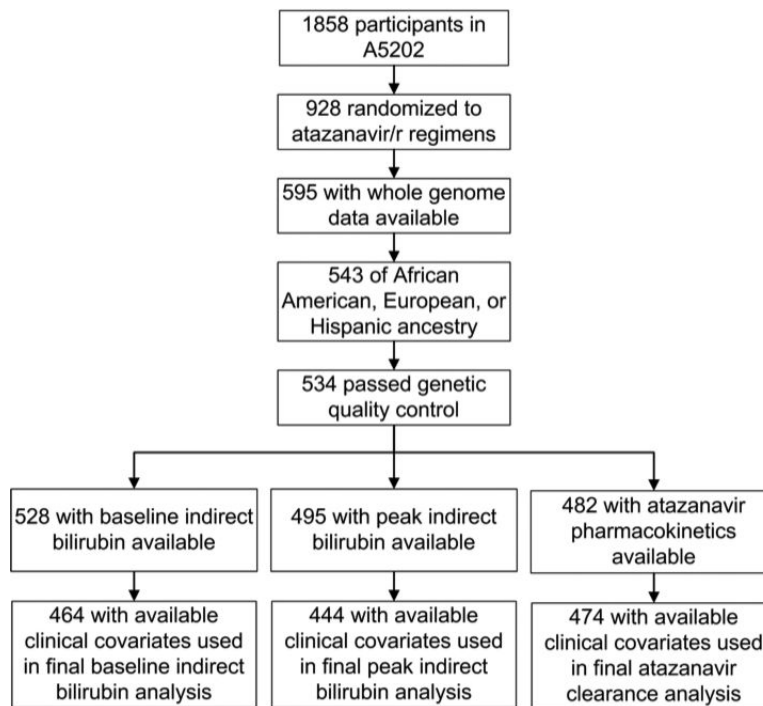


Figure 1. Derivation of the study population

Of 928 individuals randomized to atazanavir-containing regimens in A5202, a total of 464 were ultimately included in analyses for genetic associations with baseline indirect bilirubin, 444 in analyses for genetic associations with peak indirect bilirubin, and 474 in analyses for genetic associations with atazanavir clearance.

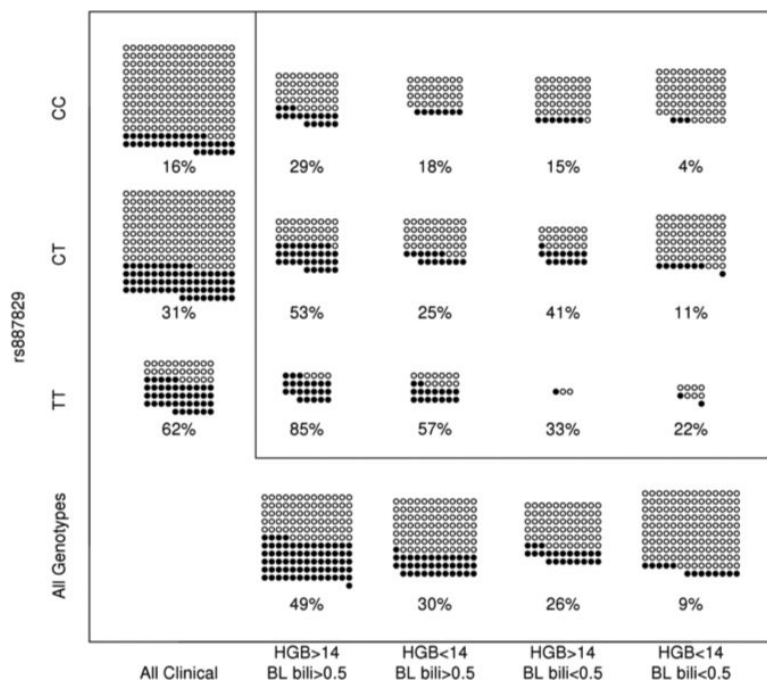


Figure 2. Predictive values of baseline bilirubin, baseline hemoglobin, and rs887829 genotype for peak on-treatment bilirubin

For these analyses a cut-off of 3.0 mg/dL was used. Each dot represents a different individual. Positive predictive values and 95% confidence intervals are provided. Positive predictive value = (number of participants with peak bilirubin > 3.0 mg/dL) / (all individuals with the predictor variables indicated). BBR, baseline bilirubin; HGB, baseline hemoglobin.

Table 1

Baseline characteristics of study subjects

	Pharmacokinetic association analyses (N = 475)	Bilirubin association analyses (N = 443)
Race/ethnicity		
Black, n (%)	145 (31%)	135 (30%)
White, n (%)	212 (45%)	204 (46%)
Hispanic, n (%)	118 (25%)	104 (23%)
Female	13.5%	14.2%
Randomized to TDF/FTC ^a	51%	51%
Median BMI ^b in kg/m ² (SD ^c)	25.7 (5.15)	25.8 (5.19)
Median age in years (SD)	38.5 (10.3)	38.8 (10.3)
Median baseline bilirubin in mg/dL (IQR ^d)	-	0.5 (0.3 - 0.8)
Median baseline hemoglobin in g/dL (IQR)	-	13.8 (12.4 - 14.9)

^aTDF/FTC, tenofovir DF/emtricitabine;^bBMI, body mass index^cSD, standard deviation^dIQR, interquartile range

Table 2

Polymorphisms with the lowest P-values for association with estimated plasma atazanavir clearance in the combined population analysis.

SNP	Overall	Overall β	Gene	Distance	AfAm MAF	AfAm β	Hisp MAF	Hisp β	Euro MAF	Euro β
<i>Associations with clearance</i>										
rs1899979	2.38×10^{-6}	0.21	<i>BICC1</i>	0	0.34	0.18	0.26	0.23	0.24	0.22
rs2335462	3.81×10^{-6}	0.21	<i>FLYWCH2</i>	0	0.25	0.2	0.3	0.16	0.27	0.2
rs17156916	5.05×10^{-6}	-0.2	<i>NUDT12</i>	1158305	0.1	-0.25	NA	NA	0.15	-0.26
rs4719772	6.07×10^{-6}	0.2	<i>MPP6</i>	-184933	0.44	0.11	0.48	0.3	0.41	0.19
rs8041911	1.26×10^{-5}	0.19	<i>DUOX1</i>	0	0.17	0.26	0.22	0.3	0.22	0.1
rs540171	1.28×10^{-5}	0.19	<i>LPAR1</i>	-53662	0.2	0.3	0.1	0.25	0.1	0.1
rs575107	1.28×10^{-5}	0.19	<i>LPAR1</i>	-52145	0.2	0.3	0.1	0.25	0.1	0.1
rs211069	1.33×10^{-5}	0.19	<i>PRKG1</i>	0	0.46	0.14	0.5	0.21	0.44	0.2
rs6929400	1.37×10^{-5}	-0.19	<i>C6orf105</i>	0	0.18	-0.17	0.28	-0.15	0.42	-0.22
rs10902646	1.42×10^{-5}	-0.19	<i>SFN</i>	-6648	0.19	-0.25	0.24	-0.1	0.42	-0.21

Footnote: Overall P and β values are based on combined population analyses with African Americans, Europeans and Hispanics. Distance reflects base pairs from the nearest gene, with zero indicating location within the gene. AfAm, Hisp and Euro MAF indicate minor allele frequencies in the African-American, Hispanic and European populations. AfAm, Hisp and Euro β are calculated β for each race/ethnicity group analyzed separately.

Table 3

Associations between targeted drug metabolism and transporter gene polymorphisms and plasma atazanavir clearance.

SNP	P _{clearance}	β _{clearance}	CHR	Position (bp)	Gene	AfAm MAF	Hisp MAF	Euro MAF	AfAm β _{clearance}	Hisp β _{clearance}	Euro β _{clearance}
rs10264272	0.73	-0.016	7	99100771	CYP3A5	0.13	-	-	-0.026	-	-
rs1045642	0.23	-0.054	7	86976581	ABCB1	0.23	0.41	0.43	-0.015	-0.082	0.038
rs1128503	1	-0.00018	7	87017537	ABCB1	0.23	0.41	0.45	-0.037	-0.046	0.047
rs17222723	0.36	-0.041	10	101585986	ABCC2	0.061	0.044	0.05	-0.18	0.078	0.0023
rs2229109	0.76	-0.013	7	87017745	ABCB1	-	0.042	0.039	-	-0.13	0.11
rs2235015	0.49	0.031	7	87037500	ABCB1	0.33	0.19	0.18	-0.083	0.22	0.03
rs2238476	0.39	-0.038	16	16121373	ABCC1	0.045	0.11	0.048	-0.059	-0.11	-0.00078
rs2273697	0.61	0.023	10	101553805	ABCC2	0.15	0.16	0.2	0.044	0.056	0.0024
rs246240	0.48	0.032	16	16026525	ABCC1	0.1	0.2	0.17	0.013	0.04	0.05
rs35592	0.62	-0.022	16	16049324	ABCC1	0.5	0.3	0.25	-0.059	0.0068	-0.045
rs4149056	0.049	-0.089	12	21222816	SLCO1B1	0.031	0.15	0.17	-0.093	-0.082	-0.11
rs776746	0.097	0.076	7	99108475	CYP3A5	0.31	0.23	0.071	-0.11	0.18	-0.014
rs8187710	0.98	0.00085	10	101601284	ABCC2	0.16	0.054	0.051	-0.077	0.07	0.028

Footnote: The P and β values at the left are based on combined population analyses with African Americans, Europeans Americans, and Hispanics. Distance reflects base pairs from the nearest gene, with zero indicating location within the gene. AfAm, Hisp and Euro MAF indicate minor allele frequencies in the African-American, Hispanic and European populations. AfAm, Hisp and Euro β are calculated β for each race/ethnicity group analyzed separately.

Table 4Adjusted R² for prediction of peak bilirubin of various models

	Model #							
	1	2	3	4	5	6	7	8
HGB	+	+	+	+	+	+	+	+
ATV clearance	+	+	0	0	0	+	+	0
Baseline Bilirubin	+	0	+	0	+	+	0	0
rs887829	0	+	0	+	+	+	0	0
R ² Adjusted*	0.334	0.336	0.303	0.264	0.368	0.413	0.181	0.125

* Adjusted to compensate for the number of variables included in the model.