Impact of *UGT1A1* Gilbert Variant on Discontinuation of Ritonavir-Boosted Atazanavir in AIDS Clinical Trials Group Study A5202

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The *UGT1A1*28* variant has been associated with hyperbilirubinemia and atazanavir discontinuation. Protocol A5202 randomly assigned human immunodeficiency virus type 1 (HIV-1)–infected patients to receive atazanavir/ritonavir (atazanavir/r) or efavirenz, with tenofovir/emtricitabine or abacavir/lamivudine. A total of 646 atazanavir/r recipients were evaluable for *UGT1A1*. Homozygosity for *28/*28* was present in 8% of whites, 24% of blacks, and 18% of Hispanics and was associated with increased bilirubin concentrations. There was an association between *28/*28* and increased atazanavir/r discontinuation among Hispanic participants ($P = .005$) but not among white or black participants ($P = .79$ and $P = .46$, respectively). The positive predictive value of *28*/28** for atazanavir/r discontinuation among Hispanic participants was only 32% (95% confidence interval, 16%–52%).

Keywords. Gilbert syndrome; pharmacogenetics; atazanavir; UGT1A1; HIV therapy.

Bilirubin elimination in bile requires glucuronidation by hepatic UDP-glucuronosyltransferase (UGT). Interindividual differences in plasma unconjugated bilirubin concentrations are due in part to a *UGT1A1* promoter tandem (TA) repeat that varies from 5 to 8 repeats. The *UGT1A1*28* $(TA)_7$ variant reduces *UGT1A1* transcription as compared to the *UGT1A1*1* $(TA)_6$ allele [1, 2].

Hyperbilirubinemia with the human immunodeficiency virus type 1 (HIV-1) protease inhibitor atazanavir results from inhibition of UGT1A1-mediated bilirubin glucuronidation [3]. Although such hyperbilirubinemia does not indicate hepatic injury, some patients discontinue atazanavir because of jaundice. Among patients receiving once-daily atazanavir 300 mg with ritonavir 100 mg, the reported frequency of grade ≥3 elevation in total bilirubin level at least once is approximately 40% [4–6] and the reported frequency of grade 4 elevation is approximately 5% [5, 6]. Atazanavir discontinuation due to hyperbilirubinemia, however, is infrequent [7–9].

Among atazanavir recipients, *UGT1A1*28* has been associated with unconjugated hyperbilirubinemia [10] and increased likelihood of atazanavir discontinuation. In the Swiss HIV Cohort, among 121 patients (80% of whom were white) receiving atazanavir boosted with ritonavir (atazanavir/r), homozygosity for low-expresser *UGT1A1* alleles (*28/*28 or *28/*37) was associated with increased risk of treatment discontinuation over 1 year, with cumulative rates of 62.5% among 18 homozygous, 23.8% among 48 heterozygous, and 14.6% among 55 noncarrier individuals [11].

We sought to replicate the association between low-expresser *UGT1A1* genotypes and atazanavir/r treatment discontinuation.

MATERIALS AND METHODS

Study Participants

Protocol A5202 (ClinTrials.gov identifier NCT00118898) was a phase IIIb randomized study of 4 once-daily regimens for initial treatment of HIV-1 infection [8, 12]. Participants were randomly assigned to receive open-label 300-mg atazanavir plus 100-mg ritonavir or 600-mg efavirenz, with placebo-controlled 600-mg abacavir–300-mg lamivudine or 300-mg tenofovir DF–200-mg emtricitabine. Study drugs were provided at no cost. Study evaluations were done before entry; at entry; at weeks 4, 8, 16, and 24; and every 12 weeks until the last enrolled subject had been followed for 96 weeks. Site personnel were required to report all grade ≥2 central nervous system adverse events, nausea, diarrhea, all other grade ≥3 signs, symptoms and toxicities, and all signs or symptoms that caused a change in study treatment. Approximately 2.5 years

after the study opened, in response to a planned interim data safety monitoring board review, participants with a screening HIV-1 RNA level of ≥100 000 copies/mL were unblinded, and those receiving abacavir/lamivudine could switch to tenofovir/emtricitabine or continue to receive their current regimen [12].

Decisions to discontinue atazanavir/r for bilirubin-related issues were at the discretion of study participants and site investigators, in consultation with the protocol team. Participants who discontinued atazanavir/r could switch to other antiretrovirals (eg, lopinavir/r or fosamprenavir), which were provided at no cost.

Identifying Genetic Polymorphisms

Consent for genetic testing was obtained under AIDS Clinical Trials Group protocol A5128 [13]. Genotyping of *UGT1A1* rs8175347 (ie, *28, *36, and *37) by fragment analysis involved polymerase chain reaction followed by capillary electrophoresis. Laboratory personnel with no knowledge of clinical data performed genotyping. Genotype assignment was based on visual inspection for peaks representing promoter TA tandem repeats *36 $(TA)_5$, *1 $(TA)_6$, *28 $(TA)_7$, and *37 $(TA)_8$.

Statistical Analyses

The primary study end point for this analysis was time from documented first dose of atazanavir/r to permanent discontinuation of atazanavir/r. Additional end points of interest included maximum total bilirubin concentration from baseline to week 24, incident cases of reported jaundice, and time to bilirubin-associated atazanavir/r discontinuation. For the latter, attribution of association to bilirubin was based on site report. Questionable attributions were reviewed for final determination by 2 investigators (E. S. D. and D. W. H.). Since follow-up in A5202 was truncated for some individuals because of closure of clinical research sites, participants discontinuing treatment due to site closure were censored for all end points [8, 12, 14]).

Deviation from Hardy–Weinberg equilibrium expectations was evaluated using exact tests. Proportions of subjects with incident cases of hyperbilirubinemia-attributed atazanavir/r discontinuations across ordered genotypes were compared with a Cochran-Mantel Haenszel test for non-0 correlation stratified by race; comparisons within racial subgroups used the Cochran-Mantel Haenszel trend test. The distribution of time to atazanavir/r discontinuation was estimated using Kaplan-Meier methods and compared across ordered genotypes, using the exact Tarone test for trend. Cox proportional hazards models were used to estimate the relative hazards of atazanavir/r discontinuation by *UGT1A1* genotype (low-risk *1/*1 as reference), with adjustment for randomized nucleoside reverse-transcriptase inhibitor (NRTI; abacavir/lamivudine vs tenofovir/emtricitabine), sex, age, and body mass index (BMI [defined as the weight in kilograms divided by the height in meters squared]; <18, 18–25, >25–30, and >30), and plasma HIV-1 RNA concentration at study entry. The discriminatory properties (sensitivity, specificity, and positive predictive value) of the *UGT1A1* high-risk 28*/*28* genotype for predicting atazanavir/r discontinuation and hyperbilirubinemia-attributed atazanavir/r discontinuation within 48 weeks of antiretroviral initiation (binary outcome) were estimated with exact 95% confidence intervals (CIs).

On the basis of the low expected frequencies of *UGT1A1*36 $(TA)_5$ and *37 $(TA)_8$ and their known effects on UGT1A1 expression, *UGT1A1*36 $(TA)_5$ and *37 $(TA)_8$ were grouped with *1 $(TA)_6$ and *28 $(TA)_7$ alleles, respectively. This provided a 3-level ordered genotype based on (allele 1)/(allele 2): (*1 or *36)/(*1 or *36), (*1 or *36)/(*28* or *37), and (*28* or *37)/(*28* or *37). These are hereafter referred to as *1/*1, *1/*28*, and 28*/*28*, respectively.

Genetic association analyses involved all self-identified non-Hispanic and Hispanic white and black individuals who were randomly assigned to and initiated an atazanavir/r-containing regimen, had consented to genetic testing under A5128, and were successfully genotyped for *UGT1A1* rs8175347. Self-identified race/ethnicity categories "white, non-Hispanic," "black, non-Hispanic," and "Hispanic" are hereafter referred to as white, black, and Hispanic, respectively. All analyses are performed separately by race/ethnicity groups and overall with stratification by race/ethnicity.

This study complied with the Helsinki Declaration and was approved by institutional review boards for each site, and subjects gave written informed consent.

RESULTS

Study Participants and *UGT1A1* Genotypes

A total of 928 participants in A5202 were randomly assigned to receive atazanavir/r, of whom 646 were included in the present analyses. Derivation of these 646 study participants and baseline characteristics are provided in Supplementary Figure 1. Among these 646 participants, 544 (84%) were male, 291 (45%) were white, 202 (31%) were black, and 153 (24%) were Hispanic. The *UGT1A1*1 $(TA)_6$ allele was most frequent overall (69% in whites, 47% in blacks, and 60% in Hispanics), followed by *UGT1A1*28 $(TA)_7$ (31% in whites, 40% in blacks, and 39% in Hispanics). The *UGT1A1*36 $(TA)_5$ and *UGT1A1*37 $(TA)_8$ alleles were extremely infrequent in white and Hispanic participants (only 3 white participants and 1 Hispanic participant had at least 1 such allele). Allelic frequencies of *UGT1A1*36 $(TA)_5$ and *UGT1A1*37 $(TA)_8$ were 7% and 6%, respectively, in black participants. After grouping *36 with *1 and *37 with *28, homozygosity for *UGT1A1*28/*28 was present in 100 individuals, including 24 (8%), 48 (24%), and 28 (18%) of white, black, and Hispanic

participants, respectively. Genotype frequencies did not deviate significantly from Hardy-Weinberg equilibrium.

UGT1A1 Genotype, Serum Bilirubin Concentrations, and Jaundice

During the first 24 weeks of atazanavir/r treatment, across all race/ethnicity groups, higher proportions of individuals homozygous for *UGT1A1*28/*28* experienced grade 4 elevations in bilirubin level (defined as a level ≥5 times the upper limit of normal). Relationships between maximum total bilirubin concentration and *UGT1A1* genotype are shown in Figure 1. Incident reports of jaundice among individuals homozygous for *UGT1A1*28/*28* were significantly higher among white (25%) and Hispanic (29%) participants, but not among black participants (10%; Supplementary Table 1).

UGT1A1 Genotype and Atazanavir/r Discontinuation

Among the 646 qualifying participants, 27% prematurely discontinued atazanavir/r (24%, 32%, and 27% among white, black, and Hispanic participants, respectively). Among 177 participants who prematurely discontinued atazanavir/r, this was attributed to bilirubin-associated issues in 3 (4%) of 70 white participants, 7 (11%) of 65 black participants, and 9 (21%) of 42 Hispanic participants ($P = .046$). Most atazanavir/r discontinuations (79%) were determined to not be due to bilirubin-associated issues (Supplementary Table 2).

The number of *UGT1A1*28* alleles was associated with time to discontinuation across the study population as a whole ($P = .02$), but there was no apparent association among white participants and black participants ($P = .79$ and $P = .46$ respectively). Among Hispanic participants, having a greater number of *UGT1A1*28* alleles was associated with shorter time to atazanavir/r discontinuation, driven by **28/*28* homozygosity ($P = .005$; Figure 2). Findings were similar in Cox proportional hazards models stratified by race/ethnicity and adjusted for age, BMI, concomitant NRTI use, baseline HIV-1 RNA concentration, and sex ($P = .003$). Over >3 years of follow-up, the estimated cumulative incidence of atazanavir/r discontinuation attributed to bilirubin-associated issues among all participants was <3% for the combined *UGT1A1 *1/*1* and **1/*28*
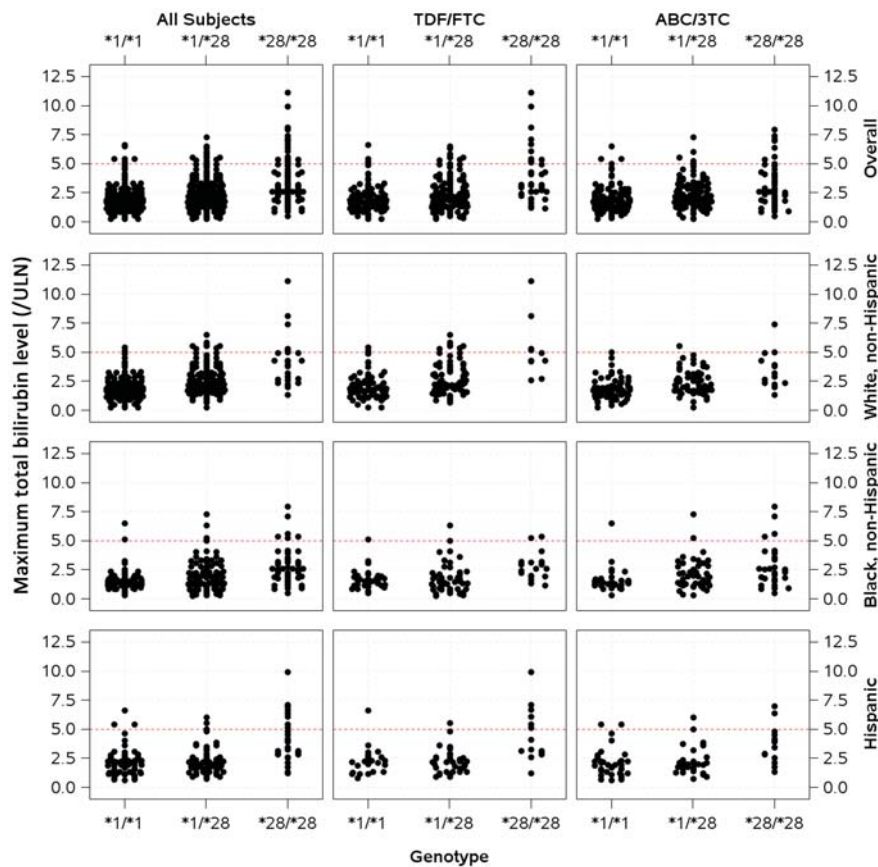

Figure 1. Relationships between *UGT1A1* genotype and maximum total bilirubin level during the first 24 weeks of atazanavir/ritonavir therapy. Data are stratified by race/ethnicity and by nucleoside reverse-transcriptase inhibitor to which participants were randomly assigned. The dashed horizontal line indicates grade 4 total bilirubin level (defined as 5 times the upper limit of normal [ULN]). Abbreviations: TDF/FTC, tenofovir DF/emtricitabine; ABC/3TC, abacavir/lamivudine.
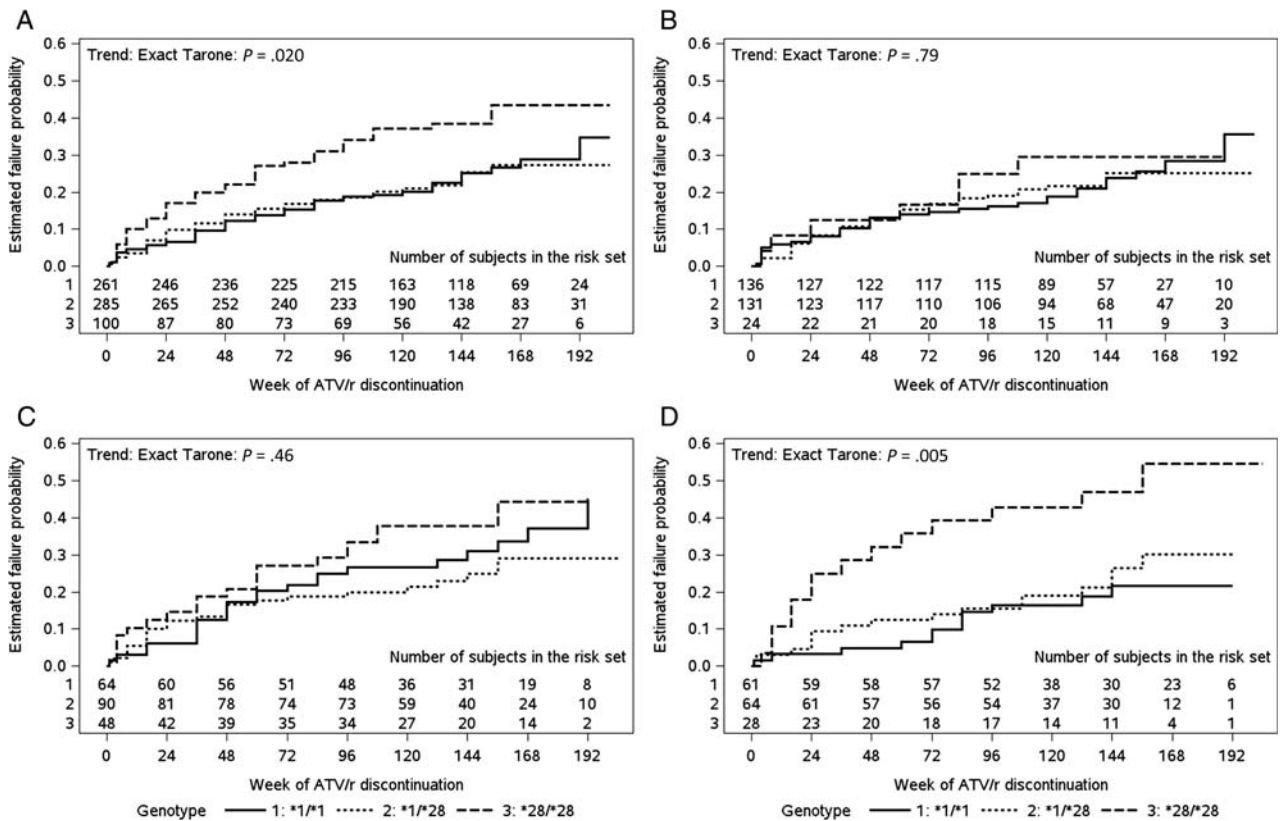
Figure 2. Time to premature atazanavir/ritonavir (ATV/r) therapy discontinuation, stratified by *UGT1A1* genotype. According to *UGT1A1* genotype, within each panel the lines estimate the cumulative probability of discontinuing ATV/r overall (*A*) and among whites (*B*), blacks (*C*), and Hispanics (*D*). *P* values are given by exact Tarone tests. Solid lines represent *UGT1A1*1/*1*, dotted lines represent *1/*28*, and dashed lines represent *28/*28*. On each panel, numbers of participants in the risk set at each time point are provided.

genotypes and 8% for *UGT1A1* *28/*28*. The positive predictive value of the *UGT1A1* *28/*28* genotype for atazanavir/r discontinuation was low among white (13%; 95% CI, 3%–32%), black (21%; 95% CI, 10%–35%), and Hispanic (32%; 95% CI, 16%–52%) participants.

It was unclear why *UGT1A1* *28/*28* would be associated with atazanavir/r discontinuation only in Hispanic participants. We found no suggestive trends regarding patterns of atazanavir/r discontinuations across clinical study sites and potential associations with race/ethnicity and clustering of *UGT1A1* genotypes. Furthermore, results were unchanged in sensitivity analyses that adjusted for population stratification by incorporating EIGENSTRAT values generated from whole genome data [13]. To investigate whether associations in white or black participants may have been masked by discontinuations that were possibly attributable to clinical trial participation, potential clinical-trial-associated reasons (eg, inability to get to study visits, nonadherence with study visits, and withdrawal of consent) were censored in a post hoc competing risks approach. A higher proportion of clinical-trial-associated reasons for discontinuation were observed among black participants (20% vs 11% and 12% for white and Hispanic

participants, respectively). After censoring these events, there was a trend toward a higher hazard of non–clinical-trial-associated atazanavir/r discontinuation with a greater number of *28* alleles among black participants (*P* = .1), while results for Hispanic and white participants were unchanged.

DISCUSSION

In a previous observational study involving Swiss HIV Cohort Study patients who had initiated atazanavir-containing regimens, low-expresser *UGT1A1* genotypes (primarily *UGT1A1*28*) were reported to be associated with increased likelihood of atazanavir/r discontinuation during the first year of therapy [11]. In the present study, the low-expresser *UGT1A1* genotype (primarily *UGT1A1*28*) was not significantly associated with an increased likelihood of atazanavir discontinuation among either white or black participants. Although an association was seen among Hispanics, the *28/*28* genotype did not reliably predict atazanavir/r discontinuation. We presently cannot explain why this association was seen only among Hispanics.

There are several possible reasons for the discrepant findings of the present study with the Swiss HIV Cohort Study [11]. The present analyses involved participants in a prospective clinical trial in the United States, rather than patients in clinical care in Switzerland. It is possible that clinical trial participants were for some reason more inclined to continue assigned study medications despite similar degrees of elevated bilirubin levels, and despite availability of alternative antiretrovirals at no cost. It is well appreciated that unrecognized population stratification can generate spurious associations, which could have contributed to discrepant findings between the 2 studies. A post hoc sensitivity analysis also suggested that a genotype association may have been masked because of discontinuations that were possibly attributable to clinical trial participation rather than to tolerability.

The present study had several limitations. While we focused on the UGT1A1 promoter tandem TA repeat, a more comprehensive, high-throughput genotyping approach might identify novel predictors of atazanavir/r discontinuation. However, as our goal was to replicate the Swiss HIV Cohort Study findings, the present genotyping strategy was appropriate. In addition, while UGT1A1 is the dominant genetic determinant of bilirubin concentrations, we cannot exclude the possibility that additional polymorphisms could improve prediction of bilirubin-driven discontinuations. Nongenetic causes of atazanavir/r discontinuation (eg, nonadherence) could obscure genetic associations.

In summary, the UGT1A1*28 genotype was associated with an increased likelihood of hyperbilirubinemia in a large, prospective clinical trial and was associated with an increased likelihood of atazanavir/r discontinuation among Hispanic participants but not among white or black participants. The association among Hispanic participants did not appear to be driven by decisions related to bilirubin level. The low positive predictive value from these data does not support routine testing for UGT1A1*28 before starting atazanavir/r-based regimens.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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