AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 28, Number 9, 2012

DOI: 10.1089/aid.2011.0261

Short Communication
*UGT1A1*28* Variant Allele Is a Predictor of Severe Hyperbilirubinemia in HIV-Infected Patients on HAART in Southern Brazil

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Abstract

Highly active antiretroviral therapy (HAART) has increased the survival of HIV-infected patients. However, adverse effects play a major role in adherence to HAART. Some protease inhibitors (mainly atazanavir and indinavir) act as inhibitors of uridine diphosphate-glucuronosyltransferase (UGT1A1), the enzyme responsible for hepatic conjugation of bilirubin. Variations in the promoter region of the *UGT1A1* gene (*UGT1A1*28*, rs8175347) can influence bilirubin plasma levels, modulating the susceptibility to hyperbilirubinemia. Aiming to analyze the association between *UGT1A1*28* allele and hyperbilirubinemia in individuals exposed to HAART, we evaluated 375 HIV-positive individuals on antiretroviral therapy. Individuals carrying the *UGT1A1*28* allele had a higher risk of developing severe hyperbilirubinemia [prevalence ratio (PR) = 2.43, 95% confidence interval (CI) 1.08–5.45, $p = 0.032$] as well as atazanavir users (PR = 7.72, 95% CI = 3.14–18.98, $p < 0.001$). This is the first description of such an association in Brazilian HIV patients, which shows that in African-American and Euro-american HAART users, the *UGT1A1*28* allele also predisposes to severe hyperbilirubinemia, especially in those exposed to atazanavir.

Highly active antiretroviral therapy (HAART) has completely changed the prognosis of HIV-infected individuals.[1,2] However, drug toxicity and adverse effects are still major challenges to treatment success. Nevertheless, not all people exposed to the same antiretrovirals may present the same adverse effect, and genetic variations between humans might be responsible for the difference rates and expression of these adverse events.[1]

Hyperbilirubinemia is one of these adverse effects and the development of jaundice could lead to adherence problems and treatment failure.[3,4] This is particularly true in HAART regimens containing indinavir, and, mainly, atazanavir (ATV), which inhibit the enzyme uridine diphosphate-glucuronosyltransferase A family, polypeptide A1 (UGT1A1).[4–6] The development, frequency, and severity of hyperbilirubinemia differ between individuals and one of the possible explanations might be related to genetics.[4–6] The combination of genetic variants added to exposure to xenobiotics and environmental factors can influence the activity of glucuronidation.[4]

UGT1A1 gene promoters containing seven TA repeats $A(TA)_7TAA$, also known as *UGT1A1*28* (rs8175347), cause a reduction of approximately 50% in enzyme activity, in comparison with the wild-type six TA repeats-containing allele (*UGT1A1*1*).[7] Also, the *UGT1A1*28* polymorphism has been related to adverse drug effects such as toxicity and predisposition to cancer.[4] Other rarer variants also reduce (eight repeats, UGT1A1*37) or increase (five repeats, UGT1A1*36) enzyme activity.[7] Based on this rationale, this study aimed to analyze the association between the *UGT1A1*28* allele and the frequency and severity of hyperbilirubinemia in HIV-infected patients on HAART, previously unexplored in Brazilian patients.

We developed a cross-sectional study in which we analyzed consecutively 375 HIV-infected individuals in government-supported reference treatment services in three different cities (Porto Alegre, Pelotas, and Rio Grande) from the Brazilian southernmost state. This study included only people aged over 18 years, with viral load below the detection limit of the test

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Table 1. Median Bilirubin Levels Compared Between the Common *UGT1A1* rs8175347 Genotypes

Genotypes	*Total bilirubin*	p	*Indirect bilirubin*	p
TA_6/TA_6 ($n=169$)	0.67 mg/dl (IQR, 0.40–1.20 mg/dl)	0.848[a]	0.47 mg/dl (IQR, 0.30–0.89 mg/dl)	0.617[a]
TA_6/TA_7 ($n=145$)	0.61 mg/dl (IQR, 0.40–1.23 mg/dl)		0.40 mg/dl (IQR, 0.30–0.87 mg/dl)	
TA_7/TA_7 ($n=39$)	0.80 mg/dl (IQR, 0.40–1.30 mg/dl)		0.50 mg/dl (IQR, 0.30–1.00 mg/dl)	
Whole sample ($n=353$)	0.67 mg/dl (IQR, 0.40–1.20 mg/dl)		0.40 mg/dl (IQR, 0.30–0.85 mg/dl)	

[a]Kruskal–Wallis test.
IQR, interquartile range.

(bDNA, 50 copies/ml) and using antiretroviral therapy regularly for at least 1 year. Indirect and total bilirubin levels were measured through standard methods in each service. For the definition of hyperbilirubinemia, the AIDS Clinical Trials Group guideline criterion was used.[8] All patients signed the Free and Informed Consent Form. This study was approved by the Research Ethics Committees from the institutions involved.

Genomic DNA was extracted from leukocytes by a standard salting out methodology. The fragment containing the chromosomal region of interest of the *UGT1A1* gene was amplified by polymerase chain reaction (PCR), using the primers described by Smiderle *et al.*[9] The size of the amplicons was determined by capillary electrophoresis on the ABI PRISM 310 Genetic Analyzer (Applied Biosystems, USA).

To check whether the genotype frequencies were in agreement with those expected under Hardy–Weinberg equilibrium (HWE), the Roff and Bentzen (1989) chi-square test was used.[10] Due to their asymmetric distribution, mean bilirubin levels were compared among genotypes by Kruskal–Wallis tests. Poisson regression models with robust variance were used to assess the predictor variables for the development of severe hyperbilirubinemia. The variables ethnic group, gender, indinavir use, atazanavir use, age, and presence of the *UGT1A1*28* allele were included in the regression model and removed stepwise. Only those that were significant predictors were kept in the final model. Statistical analyses were performed with Statistical Package for Social Sciences Version 16.0 (SPSS, Chicago, IL). Differences were considered significant when $p<0.05$.

We analyzed a total of 375 HIV-infected individuals: 60.3% were Euro-Brazilians and 39.7% Afro-Brazilians, classified according to the phenotypic definition by the interviewer.[11] Males comprised 54.4% of our sample. The mean age was 43.2 ± 9.6 years. The median time on antiretrovirals was 58.0 months (interquartile range, IQR, 34 to 105 months). Regarding treatment, 51.7% were protease inhibitor (PI) users. ATV users comprised 25.1% (94) of our sample and IDV users comprised 1.6% (6).

UGT1A1 allele frequencies were 0.015, 0.665, 0.306, and 0.014 for alleles TA_5 (*36), TA_6 (*1), TA_7 (*28), and TA_8 (*37), respectively. Genotype frequencies were distributed according to those expected under HWE. The observed allele frequencies were not significantly different between ethnic groups. On the 375 patients evaluated, the majority of genotypes were TA_6/TA_6, TA_6/TA_7, and TA_7/TA_7 ($n=353$). Other genotypic combinations of TA_5 and TA_8 alleles were also present, but the rarer TA_5/TA_5 and TA_8/TA_8 genotypes were not observed. Due to the small number of individuals bearing TA_5 and TA_8 alleles, these rare allele combinations were excluded from the association analysis. A previous study of *UGT1A1* $(TA)_n$ polymorphism performed in the same geographic region but regarding hemolytic anemia patients and healthy controls[12] found allelic and genotypic frequencies similar to those found herein.

The medians of total and indirect bilirubin levels in the whole sample and among the common genotypes are shown in Table 1. Bilirubin levels were not different among the three genotypes.

According to the AIDS Clinical Trials Group, severe hyperbilirubinemia was defined as total bilirubin levels >3.1 mg/dl, and 6.7% ($n=25$) of patients analyzed presented this outcome. From these 25 patients, 72% were atazanavir users and 60% carried at least one *UGT1A1*28* allele.

The predicting variables that contributed to the development of severe hyperbilirubinemia were evaluated through multivariate Poisson regression analyses. The variables ethnic group, gender, and indinavir use were not significant contributors to this outcome, while the most parsimonious model included only atazanavir use, age, and presence of the *UGT1A1*28* allele (Table 2).

Hepatic uridine diphosphate-glucuronosyltransferase catalyzes the conjugation of bilirubin with glucuronic acid to form the more water-soluble bilirubin diglucuronide, which is excreted into the bile.[3] It is well established that the insertion of a TA dinucleotide in the TATA box of the UGT1A1 promoter results in an enzyme with reduced activity, leading to elevation of unconjugated bilirubin levels. However, correlation of bilirubin levels with the presence of the *UGT1A1*28* allele is not necessarily obvious in all the populations studied[3] due to several environmental factors that can also affect this

Table 2. Poisson Regression Models and Predicting Variables for Development of Severe Hyperbilirubinemia in HIV-Infected Individuals on HAART

Outcome	*Predicting variable*	*PR*	*95% CI*	p
Severe hyperbilirubinemia	ATV use	7.72	3.14–18.98	<0.001
	Age	1.04	1.01–1.08	0.029
	*UGT1A1*28* allele	2.43	1.08–5.45	0.032

PR, prevalence ratio; 95% CI, confidence interval; ATV, atazanavir.

phenotype, such as alcohol, drugs, smoking, age, and gender. Our results in a sample of HIV-infected individuals exposed to HAART are in line with these findings in general populations, as total and indirect bilirubin levels were only mildly elevated in TA_7 allele homozygotes, being not significantly different among genotypes.

Moreover, the results of the multivariate Poisson regression analysis showed that the presence of the *UGT1A1*28* allele is a significant risk factor for the development of the more extreme phenotype, severe hyperbilirubinemia, in HAART users. These results are in agreement with other studies[5,6] and demonstrate that when we control for environmental variables that can also affect bilirubin levels, such as age and atazanavir use, the consequences of the presence of this risk allele can be highlighted. Furthermore, it is important to take into consideration that atazanavir is widely used as part of HAART, being associated with unconjugated hyperbilirubinemia due to the competition between this drug and the physiologic binding of bilirubin to the UGT1A1 enzyme.[13]

This is the first description of the influence of this gene variant on the development of severe hyperbilirubinemia in HIV-infected individuals in Brazil. This study is relevant especially because the Brazilian population is a very ethnically admixed population, which is the result of five centuries of interethnic crosses of peoples from three continents: the European colonizers, mainly represented by the Portuguese, the African slaves, and the autochthonous Amerindians.[14] In the South of Brazil, where our study was performed, the Amerindian contribution is very low, and African influence is reduced in comparison to all other geographic regions. Published studies on this same gene evaluated a smaller number of patients and more ethnically restricted populations, such as Rodrigues-Novoa *et al.*, which analyzed a total of 118 HIV-infected patients, all white; Rotger *et al*. evaluated 96 HIV-infected individuals, with 96% being of white ethnicity, and Anderson *et al.* analyzed 33 HIV-infected subjects, 79% of whom were white.[5,6,15] Our data suggest that the effect of the *UGT1A1*28* allele may also be observed in African and Euroamerican populations. This is even more important when we take into consideration the high number of HIV-infected individuals in these populations. In Brazil, it is estimated that about 630,000 people are infected with the virus.[16]

There are other *UGT1A1* gene variants that have also been related to decreased enzymatic activity: *UGT1A1*6* (211 G > A), *UGT1A1*27* (686C > A), and *UGT1A1*37* (TA_8). Although we have not analyzed the *6 and *27 alleles, according to previous studies,[17] they have been found exclusively in Asian populations. Regarding the *37 variant, in our sample we found only 11 carriers of this allele. Therefore, although this is a functional variant, we had no power to detect its effect due to the low number of individuals with this genotype.

The limitations of this study should also be considered, including its cross-sectional design and the inclusion of patients using different HAART regimens. However, due to the large number of drugs available for HIV therapy, it is very difficult to study a considerable number of patients on the same antiretroviral combinations. On the other hand, this heterogeneity allows us to highlight the effect of the *UGT1A1*28* allele in the ATV-containing regimens in contrast to the other drug combinations.

In conclusion, the presence of the *UGT1A1*28* allele with ATV use increases the risk of developing severe hyperbilirubinemia. Although hyperbilirubinemia is considered a mild adverse effect, it has clinical implications. Jaundice causes discomfort due to the yellowish appearance of the skin, which may affect the quality of life of these patients and may lead to treatment discontinuation.[2,5] This finding is a good example of how pharmacogenomic studies can be useful and the consistency among findings in different populations indicates that perhaps the time has come to transfer these results from basic research to clinical practice. It is important to keep in mind that the variant allele frequencies should be considered in each population before initiating a genotyping program. Evidently, cost-effectiveness analyses are needed to determine the utility of genotyping as a screening measure previous to atazanavir use.

Acknowledgments

This study was supported by Departamento de DST, Aids e Hepatites Virais, Ministério da Saúde, Brazil, Conselho Brasileiro de Desenvolvimento Científico e Tecnológico, PRONEX/CNP_q/FAPERGS and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

Author Disclosure Statement

No competing financial interests exist.

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