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Research Article

Association between the UGT1A1*28 allele and hyperbilirubinemia in HIV-positive patients receiving atazanavir: a meta-analysis

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Objectives The uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1)*28 allele in HIV-positive patients receiving atazanavir (ATV) might be associated with the risk of hyperbilirubinemia. Owing to mixed and inconclusive results, a meta-analysis was conducted to systematically summarize and clarify this association.

Methods Based on a comprehensive search of PubMed, Embase and Web of Science databases, studies investigating the association between UGT1A1 alleles and hyperbilirubinemia was retrieved. We evaluated the strength of this relationship using odds ratios (ORs) with 95% confidence intervals (CIs). Sensitivity analysis was performed by removing each study one at a time and calculating the pooled ORs of the remaining studies to test the robustness of the meta-analysis results. The Q statistic and the I^2 index statistic were used to assess heterogeneity. Publication bias was evaluated using Orwin's fail-safe N test.

Results A total of six individual studies were included in this meta-analysis. A significantly increased risk of hyperbilirubinemia was observed in HIV-positive patients receiving ATV with the UGT1A1*1/*28 or UGT1A1*28/*28 genotype, and the risk was higher with the UGT1A1*28/*28 genotype than with the UGT1A1*1/*28 genotype. (UGT1A1*28/*28 versus UGT1A1*1/*28: OR = 3.69, 95%CI = 1.82–7.49; UGT1A1*1/*28 versus UGT1A1*1/*1: OR = 3.50, 95%CI = 1.35–9.08; UGT1A1*28/*28 versus UGT1A1*1/*1: OR = 10.07, 95%CI = 4.39–23.10). All of the pooled ORs were not significantly affected by the remaining studies and different modeling methods, indicating robust results.

Conclusions This meta-analysis suggests that the UGT1A1*28 allele represents a biomarker for an increased risk of hyperbilirubinemia in HIV-positive patients receiving ATV.

Introduction

Atazanavir (ATV) is currently one of the most widely used protease inhibitors in the treatment of HIV infection. It displays several advantages over other protease inhibitors, such as a favorable lipid profile, once daily dosing, low capsule burden, and a relatively distinct resistance profile [1]. The most frequent adverse effect associated with ATV use is an elevation of bilirubin. ATV causes hyperbilirubinemia due to inhibition of the enzyme uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1), which is involved in the bilirubin conjugation [2]. The risk of hyperbilirubinemia is dependent on ATV plasma concentrations as well as genetic factors influencing UGT1A1 function [3,4].

UGT1A1 is involved in the glucuronidation of several commonly used drugs, including gemfibrozil, ezetimibe, simvastatin, atorvastatin, cerivastatin, ethinylestradiol, buprenorphine, ibuprofen and ketoprofen [5–14]. An outstanding example is the glucuronidation and disposition to the side effects of the

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anticancer drug irinotecan [15–17]. Regarding the pharmacogenetics of Gilbert's syndrome and drug side effects, there are two examples with mechanisms that are likely to influence the future administration of drugs, and potentially the drug-licensing process as well: irinotecan, the SN-38 metabolite of which is a UGT substrate, and ATV, which is not a substrate for glucuronidation, but nevertheless conveys a significant risk of unwanted jaundice by means of UGT inhibition [18].

There is some clinical controversy over the relevance of ATV-associated hyperbilirubinemia; it is benign and reversible upon discontinuation of the drug. Thus, some believe a proactive test in an attempt to predict and prevent its occurrence may not be warranted because they consider this adverse effect is not clinically relevant [19], whereas others maintain that this effect can be distressing and stigmatizing to patients and lead to increased discontinuation rates, so a preemptive screening test would be useful [20]. Up to now, there is no meta-analysis investigating this association. Therefore, we performed a meta-analysis to evaluate the association between the UGT1A1*28 allele and the associated hyperbilirubinemia risk.

Methods

Identification of eligible studies

Two independent investigators (P.D. and A.W.) carried out a systematic search in PubMed, Embase and Web of Sciences databases, with the last search update on 20 February 2019. The following search terms were used: ('UGT1A1' OR 'UGT1A1*28') AND 'ATV' AND ('unconjugated hyperbilirubinemia' OR 'hyperbilirubinemia'), without any limitation applied. The reference lists of retrieved studies and recent reviews were also manually searched for further relevant studies.

Inclusion and exclusion criteria

Hyperbilirubinemia refers to the accumulation of bilirubin in the blood causing jaundice. The clinical manifestations of jaundice are the skin, sclera and mucous membranes appear yellow. Studies in this meta-analysis must meet the following inclusion criteria: (i) evaluation of the association between UGT1A1*28 and hyperbilirubinemia; (ii) case–control study or cohort study; (iii) studies focusing on humans; and (iv) detailed genotype data in HIV-positive patients. Exclusion criteria: (i) duplication of previous publications; (ii) comment, review and editorial; and (iii) study with no detailed genotype data. Study selection was achieved by two investigators independently according to the inclusion and exclusion criteria by screening the title, abstract and full text. Any dispute was solved by discussion.

Data extraction and quality assessment

The data of the eligible studies were extracted in duplicate by two investigators independently (P.D. and X.L.). The following contents were collected: PMID, first author name, year of publication, country of origin, the characteristics of cases and controls, ethnicity, total bilirubin levels, sample size, Hardy–Weinberg equilibrium, number of cases and controls, study type and Newcastle–Ottawa Scale (NOS). The two authors checked the extracted data and reached a consensus on all data. If a dissent existed, they would recheck the original data of the included studies and have a discussion to reach a consensus. The authors used a quality score, ranging from 0 to 9, with higher scores associated with better study quality. Two investigators scored the studies independently and solved disagreements through discussion using the NOS Scoring system [21].

Statistics analysis

Our analysis was conducted using the Review Manager Software 5.3 (Cochrane Collaboration, Oxford, U.K.). A chi-square-based Q test was used to measure heterogeneity between studies. In addition, an I^2 statistic was calculated to quantitate the proportion of the total variation across studies due to heterogeneity [22]. Fixed-effects and random-effects models were selected to analyze the data. Random-effects models were used only when there was a considerable heterogeneity ($P<0.1$ or $I^2 > 50\%$) among the studies. The overall effects were calculated by a combined Z value, with $P<0.05$ indicating statistical significance.

Odds ratios (OR) and 95% confidence intervals (95%CI) were calculated to evaluate the strength of the association between the UGT1A1*28 allele and its susceptibility to hyperbilirubinemia. The UGT1A1*28 allele contained UGT1A1 *1/*28 and *28/*28, and the common genotype was UGT1A1*1/*1. For the present study, we used the following group: (i) UGT1A1*28/*28 versus UGT1A1*1/*28; (ii) UGT1A1*1/*28 versus UGT1A1*1/*1; (iii) UGT1A1*28/*28 versus UGT1A1*1/*1; (iv) UGT1A1*28/*28 versus UGT1A1(*1/*28 + *1/*1); and (v) UGT1A1(*1/*28 + *28/*28) versus UGT1A1*1/*1.
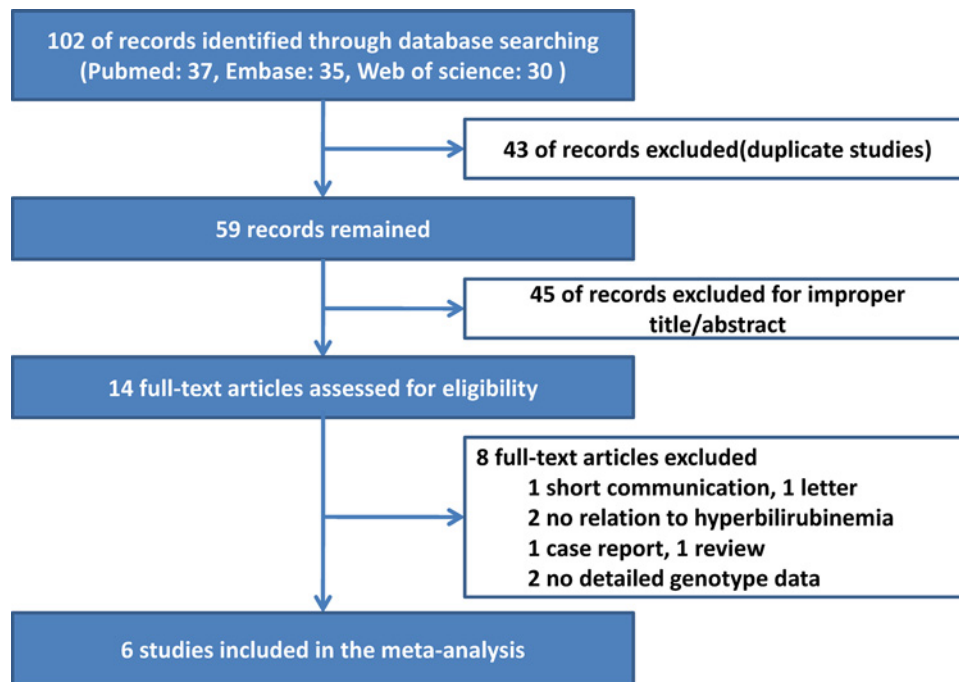
Figure 1. Flow chart for study identification, inclusion, and exclusion criteria

Sensitivity analysis and publication bias

Sensitivity analysis was conducted by the removal of each study, one at a time, understanding the effect of exclusion of respective studies by calculating the pooled ORs of the remaining studies. Heterogeneity was measured using I^2 values. If $I^2 > 50\%$ or $P<0.1$, we considered the study to have a great degree of heterogeneity and used a random-effects model. Otherwise, a fixed-effects model would be more appropriate.

In addition, the fail-safe number, with significance set at 0.05 ($Nfs_{0.05}$) for each meta-analysis, was applied to assess the publication bias. As a rule of thumb, if the $Nfs_{0.05}$ was higher, the authors of the meta-analysis could be more comfortable assuming that the sample of studies was not likely to be overwhelmed by a future influx of studies with no significant relationship [23]. $Nfs_{0.05}$ was calculated according to the formula:

$$Nfs_{0.05} = (SZ/1.64)^2 - k \qquad (1)$$

where k represents the number of included studies, Z represents the Z value of the independent study, and SZ is the sum of Z values [24].

Results
Characteristics of studies

A total of 102 studies were acquired from PubMed and Embase databases (PubMed: 37, Embase: 35, Web of Science: 30). Eighty-eight articles were excluded, of which 43 were duplicate articles and 45 had no relation to this topic. The remaining 14 studies were full-text reviewed, and 8 studies were excluded. Among these excluded studies were one having no relation to hyperbilirubinemia [25,26], one letter [27], one short communication [28], one case report [29], one systematic review [30], and two studies with no detailed genotype data [4,31]. The literature selection process is shown in Figure 1. Finally, six eligible studies [20,32–36] that met the inclusion criteria were included in our meta-analysis. The characteristics of each study are shown in Supplementary Material S1.

UGT1A1*28/*28 versus UGT1A1*1/*28

Relevant data for the comparison of the risk of hyperbilirubinemia between HIV-positive patients with a UGT1A1*28/*28 genotype and those with a UGT1A1*1/*28 genotype was available in four of the included trials [33–36]. Pooled data from all studies showed that the risk of toxicity was higher among patients with a

Table 1 Sensitivity analysis of association between UGT1A1 alleles and hyperbilirubinemia

UGT1A1*28/*28 versus UGT1A1*1/*28		UGT1A1*1/*28 versus UGT1A1*1/*1		UGT1A1*28/*28 versus UGT1A1*1/*1		UGT1A1*28/*28 versus UGT1A1(*1/*28 + *1/*1)		UGT1A1 (*1/*28 + *28/*28) versus UGT1A1*1/*1	
Study exclusion	*P*-value	Study exclusion	*P*-value	Study exclusion	*P*-value	Study exclusion	*P*-value	Study exclusion	*P*-value
22661571	0.0003	22661571	0.01	22661571	<0.00001	22661571	<0.00001	22661571	<0.00001
17058217	0.002	17058217	<0.00001	17058217	0.0002	17058217	<0.0001	17058217	<0.00001
20504240	0.0005	20504240	0.002	20504240	<0.00001	21288825	<0.00001	20504240	<0.00001
23148286	0.0004	23148286	0.0001	23148286	<0.00001	20504240	<0.00001	23148286	<0.00001
						23148286	<0.00001	16170755	<0.00001

UGT1A1*28/*28 genotype than among those with a UGT1A1*1/*28 genotype (OR = 3.69, 95%CI = 1.82–7.49; P=0.0003). Heterogeneity was not statistically significant across all studies (I^2 = 27%, P=0.25) (Figure 2A).

UGT1A1*1/*28 versus UGT1A1*1/*1

A random-effects model was used due to the heterogeneity of $I^2 > 50\%$. Four included trials compared the risk of hyperbilirubinemia between HIV-positive patients with a UGT1A1*1/*28 genotype and those with a wild-type allele [33–36]. A high level of heterogeneity was detected among these trials (I^2 = 61%, P=0.05). Overall analyses suggested an increased risk of hyperbilirubinemia in HIV-positive patients with a UGT1A1*1/*28 genotype as compared with those with a wild-type allele (OR = 3.50, 95%CI = 1.35–9.08; P=0.01) (Figure 2B).

UGT1A1*28/*28 versus UGT1A1*1/*1

Four included trials compared the risk of unconjugated hyperbilirubinemia between HIV-positive patients with a UGT1A1*28/*28 genotype and those with a wild-type genotype [33–36]. Overall analyses suggested an increased risk of hyperbilirubinemia in HIV-positive patients with a UGT1A1*28/*28 genotype as compared with those with a wild-type genotype (OR = 10.07, 95%CI = 4.39–23.10; P<0.00001). No statistical heterogeneity was detected across all studies (I^2 = 0, P=0.55) (Figure 2C).

UGT1A1*28/*28 versus UGT1A1(*1/*28 + *1/*1)

Five included trials compared the risk of hyperbilirubinemia between HIV-positive patients with a UGT1A1*28/*28 genotype and those with a UGT1A1*1/*28 or UGT1A1*1/*1 genotype [20,33–36]. Overall, analyses suggested an increased risk of hyperbilirubinemia in HIV-positive patients with a UGT1A1*28/*28 genotype as compared with those with a UGT1A1*1/*28 or UGT1A1*1/*1 genotype (OR = 5.91, 95%CI = 3.30–10.58; P<0.00001). No statistical heterogeneity was detected across all studies (I^2 = 0, P=0.46) (Figure 2D).
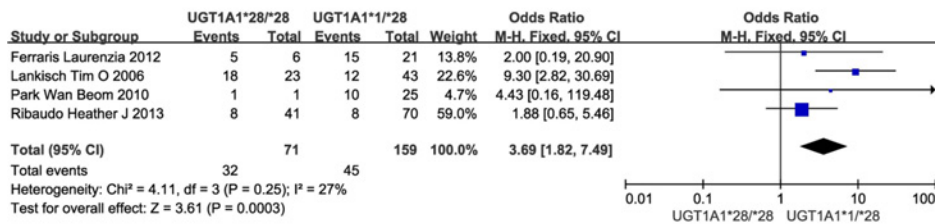
UGT1A1(*1/*28 + *28/*28) versus UGT1A1*1/*1

Five included trials compared the risk of hyperbilirubinemia between HIV-positive patients with a UGT1A1*28 allele and those with a wild-type allele [32–36]. Four trials included patients with the UGT1A1*28 allele and wild-type allele that received ATV, and one trial compared receiving ATV with not receiving ATV among patients with a UGT1A1*28 allele and a wild-type allele. Four analyses suggested an increased risk of hyperbilirubinemia in HIV-positive patients with the UGT1A1*28 allele as compared with those with a wild-type allele (OR = 4.34, 95%CI = 2.55–7.39; P<0.00001). Moderate heterogeneity was detected across all studies (I^2 = 40%, P=0.17); and five analyses combined subgroup also suggested an increased risk of hyperbilirubinemia in HIV-positive patients with UGT1A1*28 allele as compared with those with a wild-type allele (OR = 4.89, 95%CI = 2.94–8.14; P<0.00001). Large heterogeneity was detected across all studies (I^2 = 54%, P=0.07) (Figure 2E).

Sensitivity and publication bias

All of the pooled ORs were not significantly affected by the remaining studies and different modeling methods, indicating robust results (Table 1). Moreover, the statistical outcomes of Orwin's fail-safe N test are as follows: (1) UGT1A1*28/*28 versus UGT1A1*1/*28: $Nfs_{0.05}$ = 2.50; (2) UGT1A1*1/*28 versus UGT1A1*1/*1: $Nfs_{0.05}$ = 11.97; (3) UGT1A1*28/*28 versus UGT1A1*1/*1: $Nfs_{0.05}$ = 23.94; (4) UGT1A1 (*1/*28 + *28/*28) versus UGT1A1*1/*1: $Nfs_{0.05}$ = 48.03; and (v) UGT1A1*28/*28 versus UGT1A1 (*1/*28 + *1/*1): $Nfs_{0.05}$ = 31.09. Based on these comprehensive quantitative evaluations, we concluded that no obvious publication bias existed in the assessed research.
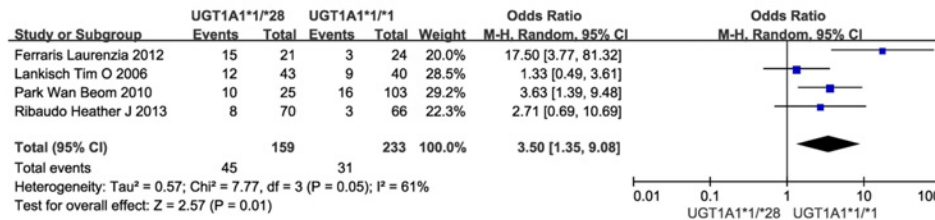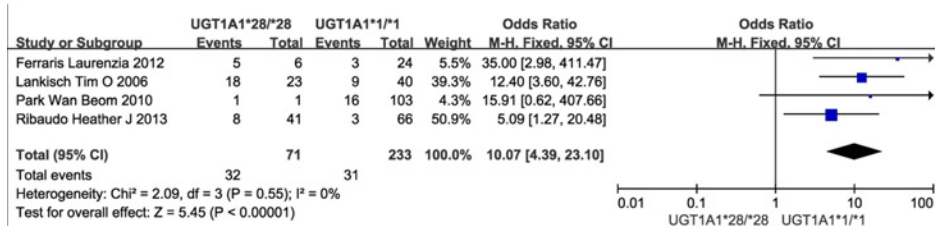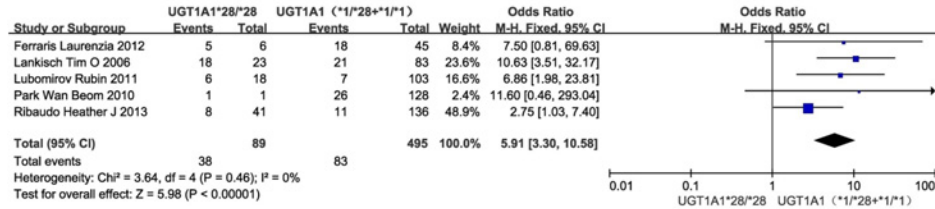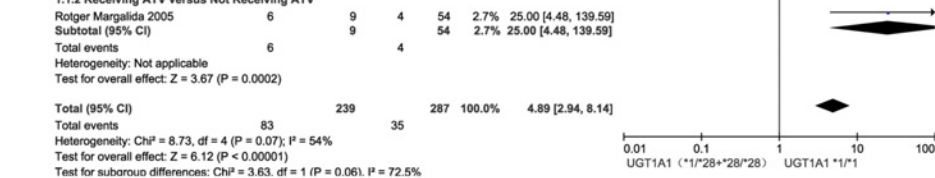
Figure 2. Forest plots demonstrating the association between UGT1A1 allele and hyperbilirubinemia

(**A**) UGT1A1*28/*28 versus UGT1A1*1/*28; (**B**) UGT1A1*1/*28 versus UGT1A1*1/*1; (**C**) UGT1A1*28/*28 versus UGT1A1*1/*1; (**D**) UGT1A1*28/*28 versus UGT1A1(*1/*28 + *1/*1); and (**E**) UGT1A1(*1/*28 + *28/*28) versus UGT1A1*1/*1.

Discussion

To the best of our knowledge, this is the first meta-analysis examining the effect of UGT1A1*28 polymorphism on the risk of hyperbilirubinemia in HIV-positive patients receiving ATV. The unconjugated bilirubin level was calculated by subtracting the conjugated bilirubin level from the total bilirubin level. Hyperbilirubinemia severity was classified on the basis of the AIDS Clinical Trial Group guidelines [37] for total bilirubin levels as follows: grade 1 (mild), 23–32 μM (1.3–1.9 mg/dl); grade 2 (moderate), 33–53 μM (1.9–3.1 mg/dl); grade 3 (severe), 54–105 μM (3.1–6.1 mg/dl); and grade 4 (serious), >105 μM (>6.1 mg/dl). This article refers specifically to severe and serious hyperbilirubinemia which was defined as grades 3 and 4 hyperbilirubinemia, respectively. Rotger et al. [32] provided the analysis that ATV increased bilirubin levels by 0.87 mg/dl, and indinavir increased bilirubin levels by 0.46 mg/dl. Ritonavir, lopinavir, saquinavir and nelfinavir had no or minimal effect on bilirubin levels. Ferraris et al. [34] provided the analysis that ritonavir removal was associated with a significant decrease in total bilirubin levels from 4.09 mg/dl (IQR: 3.14–5.73 mg/dl) to 1.82 mg/dl (IQR: 1.53–2.33 mg/dl) (P=0.001) in the remaining 24 patients in the switch arm, 12 months after therapy simplification, leading to a significant reduction in the number of patients presenting ≥ grade 3 hyperbilirubinemia. That means the combination of ritonavir and ATV increased the plasma concentration of ATV, which resulted in a higher bilirubin level than ATV alone. Bioequivalence between cobicistat and ritonavir as a pharmacoenhancer of ATV was established. Cobicistat shows increased advantages over ritonavir, such as no activity against HIV, fewer drug–drug interactions and better solubility, which promotes coformulation strategies with less pill burden, better tolerability, and, potentially, higher life-long treatment adherence [38]. Lankisch et al. [36] clarified that ATV therapy did not lead to hepatic toxicity indicated by aminotransferase elevations but rather resulted in a significantly higher rate of hyperbilirubinemia. The primary finding of the present study is that HIV-positive patients carrying UGT1A1*28 allele(s) including UGT1A1*1/*28 and UGT1A1*28/*28 are at an increased risk of hyperbilirubinemia than those carrying the UGT1A1*1/*1 allele. A secondary finding is that the UGT1A1*28/*28 allele has an increased risk compared with UGT1A1*1/*28 in HIV-positive patients receiving ATV.

It is important to note that four additional important articles were not included in this meta-analysis (one letter, one short communication and two articles). The letter [25] and the short communication [26] were excluded because both studies were published as abstracts and the data were not presented in detail, but these conclusions confirmed a direct correlation between ATV plasma level and bilirubinemia that was influenced by the presence of a homozygosis or heterozygosis UGT1A1-TA7 (seven thymine–adenine) allele, and also confirmed our results that there was a significant association between UGT1A1*28 allele(s) and hyperbilirubinemia. Two articles [29,30] provided incomplete data concerning the association between UGT1A1*28 mutation and incidence of hyperbilirubinemia. However, taking these results into consideration will not change the result of our meta-analysis. For example, Javelle et al. [29] provided the analysis that having at least one of seven alleles at UGT1A1 was independently associated with severe hyperbilirubinemia (OR, 2.96; 95%CI, 1.29–6.78; P=0.01). Culley et al. [30] confirmed the association of hyperbilirubinemia with the use of an ATV/r-containing regimen in individuals carrying the UGT1A1 polymorphism, highlighting also the fact that carriers of the polymorphism develop total bilirubin levels >5 mg/dl in the majority of cases when on ATV/r. The risk for bilirubin-related ATV discontinuation is substantial, Lubomirov et al. [20] found that first-year cumulative rates of treatment discontinuation about UGT1A1 alleles were 62.5% for homozygous, 23.8% for heterozygous and 14.6% for noncarrier individuals. And for the four centers prescribing ATV to more than ten individuals, there was a statistically significant correlation between discontinuation rates and frequency of UGT1A1*28 homozygocity.

UGT1A1 is a major conjugating enzyme responsible for bilirubin homeostasis [39], and it is expressed in the liver and gastrointestinal tract. One of the main functions of UGT1A1 lies within the liver, where it is the sole enzyme responsible for the metabolism of bilirubin, the hydrophobic breakdown product of hemecatabolism [18,40]. In general, UGT1A enzymes have considerable overlap in substrate specificities [41], however, no other isozyme can substitute for the bilirubin glucuronidation activity of UGT1A1 [18]. Additionally, no effective alternative pathways exist for the detoxification and elimination of bilirubin, excluding that of photoisomeration, a relatively inefficient pathway as compared with UGT1A1 glucuronidation [42]. Its activity is essential in the metabolism of bilirubin [43]. To date, more than 100 variants have been reported in the *UGT1A1* gene [44]. Some have been associated either with a decrease (e.g. UGT1A1*28, UGT1A1*6) or with an increase (e.g. UGTA1*36) in UGT1A1 metabolic function. The most thoroughly studied variant of UGT1A1 is termed as UGT1A1*28 (rs8175347) and is associated with Gilbert's syndrome. This variant corresponds to a TA7 dinucleotide repeat in the TATA box at the promoter region of the *UGT1A1* gene as opposed to six (TA6) that characterizes the wild-type allele (UGT1A1*1) [45]. The distribution of the UGT1A1*28 allele varies across the globe with a minor allelic frequency (MAF) of 26–31% in Caucasians, 42–56% in African-Americans and only 9–16% in Asian populations [45,46]. Gilbert's syndrome is characterized by

mild and intermittent elevations of bilirubin caused by homozygosity of the c.-53-52 (TA)6 > (TA)7 allele in UGT1A1 at rs8175347 (*28). The UGT1A1 *28 allele consists of TA7 tandem repeats in the promoter region of UGT1A1 where normally there are six (UGT1A1*1 allele). The *28 allele causes approximately 50% decrease in UGT1A1 protein expression. Similarly, the *37 ((TA)8) allele also decreases UGT1A1 transcriptional activity relative to *28, whereas the *36 ((TA)5) allele in UGT1A1 leads to increased transcriptional activity relative to *28 [47]. The *36 and *37 alleles are rare in White and Asian populations, but are more common in West and sub-Saharan African populations [48]. The UGT1A1*6 allele (c.211 G>A at rs4148323), which causes a missense mutation (G71R), is more prevalent in individuals of East Asian descent, but has not been found to be associated with ATV-associated hyperbilirubinemia [35].

Polymorphisms in UGT1A1 are associated with indirect bilirubin concentrations in the general population (i.e. Gilbert's syndrome). 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 [49], SLCO1B3 and G6PD [50]. In addition, bilirubin concentrations have been associated with ABCB1 3435C>T among patients prescribed ATV without ritonavir but not with ritonavir alone [26], although results have been inconsistent [4].

Limitations of this meta-analysis must be considered. First, the possibility of information and selection biases cannot be completely excluded because some of the included studies were retrospective. Second, we restricted our search to articles published in English or Chinese. Articles with potentially high-quality data that were published in other languages were not included because of anticipated difficulties in obtaining accurate medical translation. Third, our study did not make the correlation analysis of ethnicity and drug doses. Finally, the association of hyperbilirubinemia and ATV mainly occurs when ATV is boosted. Hypothetical selection bias could have selected patients all with boosted ATV, and that this association might not exist in non-boosted ATV regimens.

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. It is important to keep in mind that the variant allele frequencies should be considered in each population before initiating a genotyping program.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Funding

Author Contribution

P.Q.D. conceived and designed the study. P.Q.D. and A.F.W. carried out a systematic search. X.G.L. and P.Q.D. extracted the data of the eligible studies. Y.C.M contributed to quality control and review of the data. X.G.L. revised the manuscript. All authors read and approved the final manuscript.

Abbreviations

ATV, atazanavir; 95%CI, 95% confidence interval; NOS, Newcastle–Ottawa Scale; OR, odds ratio; TA7, seven thymine–adenine; UGT1A1, uridine diphosphate glucuronosyltransferase 1A1.

References

1 Rivas, P., Morello, J., Garrido, C., Rodriguez-Novoa, S. and Soriano, V. (2009) Role of atazanavir in the treatment of HIV infection. *Ther. Clin. Risk Manag.* **5**, 99–116

2 Zhang, D., Chando, T.J., Everett, D.W., Patten, C.J., Dehal, S.S. and Humphreys, W.G. (2005) In vitro inhibition of UDP glucuronosyltransferases by atazanavir and other HIV protease inhibitors and the relationship of this property to in vivo bilirubin glucuronidation. *Drug Metab. Dispos.* **33**, 1729–1739, https://doi.org/10.1124/dmd.105.005447

3 Rodriguez Novoa, S., Barreiro, P., Rendon, A., Barrios, A., Corral, A., Jimenez-Nacher, I. et al. (2006) Plasma levels of atazanavir and the risk of hyperbilirubinemia are predicted by the 3435C−>T polymorphism at the multidrug resistance gene 1. *Clin. Infect. Dis.* **42**, 291–295

4 Rodriguez-Novoa, S., Martin-Carbonero, L., Barreiro, P., Gonzalez-Pardo, G., Jimenez-Nacher, I., Gonzalez-Lahoz, J. et al. (2007) Genetic factors influencing atazanavir plasma concentrations and the risk of severe hyperbilirubinemia. *AIDS* **21**, 41–46, https://doi.org/10.1097/QAD.0b013e328011d7c1

5   Sparks, R., Ulrich, C.M., Bigler, J., Tworoger, S.S., Yasui, Y., Rajan, K.B. et al. (2004) UDP-glucuronosyltransferase and sulfotransferase polymorphisms, sex hormone concentrations, and tumor receptor status in breast cancer patients. *Breast Cancer Res.* **6**, R488–R498, https://doi.org/10.1186/bcr818

6   Adegoke, O.J., Shu, X.O., Gao, Y.T., Cai, Q., Breyer, J., Smith, J. et al. (2004) Genetic polymorphisms in uridine diphospho-glucuronosyltransferase 1A1 (UGT1A1) and risk of breast cancer. *Breast Cancer Res. Treat.* **85**, 239–245, https://doi.org/10.1023/B:BREA.0000025419.26423.b8

7   Cecchin, E., Russo, A., Corona, G., Campagnutta, E., Martella, L., Boiocchi, M. et al. (2004) UGT1A1*28 polymorphism in ovarian cancer patients. *Oncol. Rep.* **12**, 457–462

8   Chouinard, S., Tessier, M., Vernouillet, G., Gauthier, S., Labrie, F., Barbier, O. et al. (2006) Inactivation of the pure antiestrogen fulvestrant and other synthetic estrogen molecules by UDP-glucuronosyltransferase 1A enzymes expressed in breast tissue. *Mol. Pharmacol.* **69**, 908–920

9   Ebner, T., Remmel, R.P. and Burchell, B. (1993) Human bilirubin UDP-glucuronosyltransferase catalyzes the glucuronidation of ethinylestradiol. *Mol. Pharmacol.* **43**, 649–654

10   Ghosal, A., Hapangama, N., Yuan, Y., Achanfuo-Yeboah, J., Iannucci, R., Chowdhury, S. et al. (2004) Identification of human UDP-glucuronosyltransferase enzyme(s) responsible for the glucuronidation of ezetimibe (Zetia). *Drug Metab. Dispos.* **32**, 314–320, https://doi.org/10.1124/dmd.32.3.314

11   King, C.D., Rios, G.R., Green, M.D., MacKenzie, P.I. and Tephly, T.R. (1997) Comparison of stably expressed rat UGT1.1 and UGT2B1 in the glucuronidation of opioid compounds. *Drug Metab. Dispos.* **25**, 251–255

12   Kuehl, G.E., Lampe, J.W., Potter, J.D. and Bigler, J. (2005) Glucuronidation of nonsteroidal anti-inflammatory drugs: identifying the enzymes responsible in human liver microsomes. *Drug Metab. Dispos.* **33**, 1027–1035, https://doi.org/10.1124/dmd.104.002527

13   Ogilvie, B.W., Zhang, D., Li, W., Rodrigues, A.D., Gipson, A.E., Holsapple, J. et al. (2006) Glucuronidation converts gemfibrozil to a potent, metabolism-dependent inhibitor of CYP2C8: implications for drug-drug interactions. *Drug Metab. Dispos.* **34**, 191–197, https://doi.org/10.1124/dmd.105.007633

14   Prueksaritanont, T., Subramanian, R., Fang, X., Ma, B., Qiu, Y., Lin, J.H. et al. (2002) Glucuronidation of statins in animals and humans: a novel mechanism of statin lactonization. *Drug Metab. Dispos.* **30**, 505–512, https://doi.org/10.1124/dmd.30.5.505

15   Lankisch, T.O., Vogel, A., Eilermann, S., Fiebeler, A., Krone, B., Barut, A. et al. (2005) Identification and characterization of a functional TATA box polymorphism of the UDP glucuronosyltransferase 1A7 gene. *Mol. Pharmacol.* **67**, 1732–1739, https://doi.org/10.1124/mol.104.007146

16   Innocenti, F., Vokes, E.E. and Ratain, M.J. (2006) Irinogenetics: what is the right star? *J. Clin. Oncol.* **24**, 2221–2224, https://doi.org/10.1200/JCO.2005.05.2464

17   Ando, Y., Saka, H., Asai, G., Sugiura, S., Shimokata, K. and Kamataki, T. (1998) UGT1A1 genotypes and glucuronidation of SN-38, the active metabolite of irinotecan. *Ann. Oncol.* **9**, 845–847, https://doi.org/10.1023/A:1008438109725

18   Strassburg, C.P. (2008) Pharmacogenetics of Gilbert's syndrome. *Pharmacogenomics* **9**, 703–715, https://doi.org/10.2217/14622416.9.6.703

19   Nettles, R.E., Child, M.J., Bertz, R.J. and Schnittman, S. (2006) Gilbert syndrome and the development of antiretroviral therapy-associated hyperbilirubinemia: genetic screening is unnecessary. *J. Infect. Dis.* **193**, 1611–1612, https://doi.org/10.1086/503814

20   Lubomirov, R., Colombo, S., di Iulio, J., Ledergerber, B., Martinez, R., Cavassini, M. et al. (2011) Association of pharmacogenetic markers with premature discontinuation of first-line anti-HIV therapy: an observational cohort study. *J. Infect. Dis.* **203**, 246–257, https://doi.org/10.1093/infdis/jiq043

21   Lo, C.K., Mertz, D. and Loeb, M. (2014) Newcastle-Ottawa Scale: comparing reviewers' to authors' assessments. *BMC Med. Res. Methodol.* **14**, 45, https://doi.org/10.1186/1471-2288-14-45

22   Higgins, J.P., Thompson, S.G., Deeks, J.J. and Altman, D.G. (2003) Measuring inconsistency in meta-analyses. *BMJ* **327**, 557–560, https://doi.org/10.1136/bmj.327.7414.557

23   Li, X., Yu, K., Mei, S., Huo, J., Wang, J., Zhu, Y. et al. (2015) HLA-B*1502 increases the risk of phenytoin or lamotrigine induced Stevens-Johnson Syndrome/toxic epidermal necrolysis: evidence from a meta-analysis of nine case-control studies. *Drug Res.* **65**, 107–111

24   Shen, S.D., Zhong, S.Z., Wang, C.Z. and Huang, W.H. (2015) Correlation of lymphovascular invasion with clinicopathological factors in invasive breast cancer: a meta-analysis. *Int. J. Clin. Exp. Med.* **8**, 17789–17795

25   Tsai, M.S., Chang, S.Y., Lin, S.W., Kuo, C.H., Sun, H.Y., Wu, B.R. et al. (2017) Treatment response to unboosted atazanavir in combination with tenofovir disoproxil fumarate and lamivudine in human immunodeficiency virus-1-infected patients who have achieved virological suppression: A therapeutic drug monitoring and pharmacogenetic study. *J. Microbiol. Immunol. Infect.* **50**, 789–797, https://doi.org/10.1016/j.jmii.2015.12.012

26   Anderson, P.L., Aquilante, C.L., Gardner, E.M., Predhomme, J., McDaneld, P., Bushman, L.R. et al. (2009) Atazanavir pharmacokinetics in genetically determined CYP3A5 expressors versus non-expressors. *J. Antimicrob. Chemother.* **64**, 1071–1079, https://doi.org/10.1093/jac/dkp317

27   Cicconi, P., Bini, T., Barassi, A., Casana, M., Turri, O., Pateri, F. et al. (2011) Detrimental effect of atazanavir plasma concentrations on total serum bilirubin levels in the presence of UGT1A1 polymorphisms. *J. Acquir. Immune Defic. Syndr.* **56**, e96–97, https://doi.org/10.1097/QAI.0b013e318203e7e7

28   Morello, J., Alvarez, E., Cuenca, L., Vispo, E., Gonzalez-Lahoz, J., Soriano, V. et al. (2011) Short communication: use of serum bilirubin levels as surrogate marker of early virological response to atazanavir-based antiretroviral therapy. *AIDS Res. Hum. Retroviruses* **27**, 1043–1045, https://doi.org/10.1089/aid.2011.0019

29   Javelle, E., Oliver, M., Savini, H., Aubry, C., Badens, C. and Simon, F. (2012) Severe atazanavir-associated hyperbilirubinemia revealing Canton G6PD deficiency in an Asian HIV-infected patient. *AIDS* **26**, 249–251, https://doi.org/10.1097/QAD.0b013e32834e1d33

30   Culley, C.L., Kiang, T.K., Gilchrist, S.E. and Ensom, M.H. (2013) Effect of the UGT1A1*28 allele on unconjugated hyperbilirubinemia in HIV-positive patients receiving Atazanavir: a systematic review. *Ann. Pharmacother.* **47**, 561–572, https://doi.org/10.1345/aph.1R550

31   Panagopoulos, P., Paraskevis, D., Katsarolis, I., Sypsa, V., Detsika, M., Protopapas, K. et al. (2014) High prevalence of the UGT1A1*28 variant in HIV-infected individuals in Greece. *Int. J. STD AIDS* **25**, 860–865, https://doi.org/10.1177/0956462414523259

32 Rotger, M., Taffe, P., Bleiber, G., Gunthard, H.F., Furrer, H., Vernazza, P. et al. (2005) Gilbert syndrome and the development of antiretroviral therapy-associated hyperbilirubinemia. *J. Infect. Dis.* **192**, 1381–1386, https://doi.org/10.1086/466531

33 Ribaudo, H.J., Daar, E.S., Tierney, C., Morse, G.D., Mollan, K., Sax, P.E. et al. (2013) Impact of UGT1A1 Gilbert variant on discontinuation of ritonavir-boosted atazanavir in AIDS Clinical Trials Group Study A5202. *J. Infect. Dis.* **207**, 420–425, https://doi.org/10.1093/infdis/jis690

34 Ferraris, L., Vigano, O., Peri, A., Tarkowski, M., Milani, G., Bonora, S. et al. (2012) Switching to unboosted atazanavir reduces bilirubin and triglycerides without compromising treatment efficacy in UGT1A1*28 polymorphism carriers. *J. Antimicrob. Chemother.* **67**, 2236–2242, https://doi.org/10.1093/jac/dks175

35 Park, W.B., Choe, P.G., Song, K.H., Jeon, J.H., Park, S.W., Kim, H.B. et al. (2010) Genetic factors influencing severe atazanavir-associated hyperbilirubinemia in a population with low UDP-glucuronosyltransferase 1A1*28 allele frequency. *Clin. Infect. Dis.* **51**, 101–106, https://doi.org/10.1086/653427

36 Lankisch, T.O., Moebius, U., Wehmeier, M., Behrens, G., Manns, M.P., Schmidt, R.E. et al. (2006) Gilbert's disease and atazanavir: from phenotype to UDP-glucuronosyltransferase haplotype. *Hepatology* **44**, 1324–1332, https://doi.org/10.1002/hep.21361

37 Fellay, J., Boubaker, K., Ledergerber, B., Bernasconi, E., Furrer, H., Battegay, M. et al. (2001) Prevalence of adverse events associated with potent antiretroviral treatment: Swiss HIV Cohort Study. *Lancet* **358**, 1322–1327, https://doi.org/10.1016/S0140-6736(01)06413-3

38 Antunes, F. (2017) Atazanavir sulfate + cobicistat for the treatment of HIV infection. *Expert Rev. Anti Infect. Ther.* **15**, 569–576, https://doi.org/10.1080/14787210.2017.1323634

39 Kiang, T.K., Ensom, M.H. and Chang, T.K. (2005) UDP-glucuronosyltransferases and clinical drug-drug interactions. *Pharmacol. Ther.* **106**, 97–132, https://doi.org/10.1016/j.pharmthera.2004.10.013

40 Bosma, P.J., Seppen, J., Goldhoorn, B., Bakker, C., Oude Elferink, R.P., Chowdhury, J.R. et al. (1994) Bilirubin UDP-glucuronosyltransferase 1 is the only relevant bilirubin glucuronidating isoform in man. *J. Biol. Chem.* **269**, 17960–17964

41 Tukey, R.H. and Strassburg, C.P. (2000) Human UDP-glucuronosyltransferases: metabolism, expression, and disease. *Annu. Rev. Pharmacol. Toxicol.* **40**, 581–616, https://doi.org/10.1146/annurev.pharmtox.40.1.581

42 Schauer, R., Stangl, M., Lang, T., Zimmermann, A., Chouker, A., Gerbes, A.L. et al. (2003) Treatment of Crigler-Najjar type 1 disease: relevance of early liver transplantation. *J. Pediatr. Surg.* **38**, 1227–1231, https://doi.org/10.1016/S0022-3468(03)00273-2

43 Radominska-Pandya, A., Czernik, P.J., Little, J.M., Battaglia, E. and Mackenzie, P.I. (1999) Structural and functional studies of UDP-glucuronosyltransferases. *Drug Metab. Rev.* **31**, 817–899, https://doi.org/10.1081/DMR-100101944

44 Marques, S.C. and Ikediobi, O.N. (2010) The clinical application of UGT1A1 pharmacogenetic testing: gene-environment interactions. *Hum. Genomics* **4**, 238–249

45 Bosma, P.J., Chowdhury, J.R., Bakker, C., Gantla, S., de Boer, A., Oostra, B.A. et al. (1995) The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. *N. Engl. J. Med.* **333**, 1171–1175, https://doi.org/10.1056/NEJM199511023331802

46 Beutler, E., Gelbart, T. and Demina, A. (1998) Racial variability in the UDP-glucuronosyltransferase 1 (UGT1A1) promoter: a balanced polymorphism for regulation of bilirubin metabolism? *Proc. Natl. Acad. Sci. U.S.A.* **95**, 8170–8174, https://doi.org/10.1073/pnas.95.14.8170

47 Memon, N., Weinberger, B.I., Hegyi, T. and Aleksunes, L.M. (2016) Inherited disorders of bilirubin clearance. *Pediatr. Res.* **79**, 378–386, https://doi.org/10.1038/pr.2015.247

48 Horsfall, L.J., Zeitlyn, D., Tarekegn, A., Bekele, E., Thomas, M.G., Bradman, N. et al. (2011) Prevalence of clinically relevant UGT1A alleles and haplotypes in African populations. *Ann. Hum. Genet.* **75**, 236–246, https://doi.org/10.1111/j.1469-1809.2010.00638.x

49 Sanna, S., Busonero, F., Maschio, A., McArdle, P.F., Usala, G., Dei, M. et al. (2009) Common variants in the SLCO1B3 locus are associated with bilirubin levels and unconjugated hyperbilirubinemia. *Hum. Mol. Genet.* **18**, 2711–2718, https://doi.org/10.1093/hmg/ddp203

50 Johnson, A.D., Kavousi, M., Smith, A.V., Chen, M.H., Dehghan, A., Aspelund, T. et al. (2009) Genome-wide association meta-analysis for total serum bilirubin levels. *Hum. Mol. Genet.* **18**, 2700–2710, https://doi.org/10.1093/hmg/ddp202