Association of ABCG2 polymorphisms with susceptibility to anti-tuberculosis drug-induced hepatotoxicity in the Chinese population

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ABSTRACT

1. The accumulation of endogenous hepatotoxin protoporphyrin IX (PPIX) in the liver was proposed to be a novel mechanism of anti-tuberculosis drug-induced hepatotoxicity (ATDH). ATP-binding cassette transporter G2 (ABCG2) plays an important role in modulating PPIX concentrations. This study aimed to explore the role of ABCG2 genetic polymorphisms in the risk of ATDH in Chinese patients.

2. A 1:4 matched case–control study was performed among 202 ATDH cases and 808 controls. Conditional logistic regression model was used to estimate the association between genotypes and the risk of ATDH by odds ratios (ORs) with 95% confidence intervals (CIs).

3. Male patients with CC genotype of rs2622605 had an increased risk of ATDH (adjusted OR = 1.615, 95% CI: 1.119–2.332, p = 0.001). The peak value of alkaline phosphatase (ALP) was significantly higher in male patients with CC genotype of rs2622605 than in those with TT + TC genotype during antituberculosis treatment (102.0 U/L vs. 98.0 U/L, p = 0.029).

4. This is the first attempt to evaluate the association between ABCG2 genetic variants and the risk of ATDH. Based on the 1:4 matched case–control study, the polymorphism at rs2622605 in the ABCG2 gene may be associated with the susceptibility to ATDH in Chinese male patients.

Introduction

Tuberculosis is a global disease, found in every country in the world and remains a major public health issue. In 2020, an estimated 9.9 million people fell ill with tuberculosis globally (Hasan et al. 2021). Tuberculosis is curable and preventable, and approximately 85% of tuberculosis patients can be successfully treated with standard anti-tuberculosis treatment of daily isoniazid (INH), rifampin (RMP), pyrazinamide (PZA), and ethambutol (EMB) in the first two months, and daily INH and RMP for the subsequent four months (Ben Amar et al. 2015). However, long-term with multiple drugs may lead to different adverse events, which could result in discontinued or interrupted treatment, increased risks of drug resistance, extended treatment course, treatment failure, relapse, and even death (Castro et al. 2015). One of the common adverse events is anti-tuberculosis drug-induced hepatotoxicity (ATDH) (Tostmann et al. 2008). The incidence of ATDH during standard multidrug treatment has been reported between 2 and 28% (Tostmann et al. 2008), which varies widely depending upon the characteristics of the study, drug regimens involved, and threshold used to define hepatotoxicity. Although the overall incidence of ATDH may be decreasing, it still remains a medical issue not to be underestimated (Ramappa and Aithal 2013).

Although anti-tuberculosis drugs have been used for decades worldwide, the pathogenesis underlying hepatotoxicity is not completely clear (Ramappa and Aithal 2013). Scientists from all over the world have conducted extensive research. Recently, animal experiment indicated that cotreatment with INH and RMP caused accumulation of protoporphyrin IX (PPIX) in the liver through pregnane X receptor (PXR)-mediated alteration of the haem biosynthesis pathway (Li, Lu, et al. 2013; He et al. 2017). PPIX, one of the endogenous hepatotoxin, is ubiquitously present in all living cells in small amounts as a precursor of haem (Sachar, Anderson, et al. 2016). INH and RMP could enhance the transcriptional activation of the aminolevulinic synthase-1 (ALAS1) gene, while ALAS1 is the rate-limiting enzyme in the production of haem in the liver (Fraser et al. 2003). INH was also confirmed to downregulate the expression of ferrochelatase (FECH), the enzyme that catalyses PPIX and Fe^{2+} to synthetise haem (Sachar, Li, et al. 2016). Furthermore, treatment with INH increases ALAS1 activity in the mouse liver (Sachar, Li, et al. 2016). Large amounts of PPIX are toxic to the liver and can cause liver injury (Sachar, Anderson, et al. 2016). Therefore, FECH downregulation and ALAS1 induction may exert a synergistic effect on PPIX accumulation (He et al. 2017), which helps to elucidate the potential mechanism of ATDH. The schematic summary of the proposed mechanism of...
INH/RMP-induced PPIX accumulation is shown in Figure 1 (Li, Lu, et al. 2013; Sachar, Li, et al. 2016; He et al. 2017). ATP-binding cassette transporter G2 (ABCG2; also known as breast cancer resistance protein, BCRP) predominantly localises to the plasma membrane, which plays a physiological role in an organism’s self-defence mechanism (Doyle et al. 1998), and the elimination of some toxic substances or harmful agents (Nakanishi and Ross 2012). Accumulating evidence indicates that mitochondrial ABCG2 plays a particularly important role in regulating the ALA-mediated PPIX levels through PPIX export from mitochondria to the cytosol (Kobuchi et al. 2012), and from the cytoplasm to the extracellular lumen (Kitajima et al. 2019). Both mitochondrial and cytosolic fractions of ABCG2-deficient hepatocytes have higher levels of PPIX than fractions isolated from wild-type hepatocytes (Lin et al. 2013). ABCG2 expression is elevated in foetal liver cell line (L-02) after INH/RMP treatment, which indicates that L-02 cells also have a protective response to PPIX accumulation (He et al. 2017). Therefore, ABCG2 plays an important role in regulating the modulation of PPIX concentrations under normal physiological and pathological conditions (Krishnamurthy and Schuetz 2011). It is tempting to speculate that genetic variants of ABCG2 may affect the synthesis and accumulation of PPIX, which in turn affect the susceptibility to ATDH.

Since 2016, we established an anti-tuberculosis treatment cohort among the Eastern Chinese population in Jiangsu Province according to the home-based anti-tuberculosis treatment adverse reactions (HATTAR) study (Yang et al. 2019). Using this cohort, an individual-matched case–control study was conducted to evaluate the role of ABCG2 genetic variants in the risk of ATDH in China.

Methods

Patients and clinical criteria

The study was approved by the Ethics Committee of Nanjing Medical University. Between May 2016 and December 2019, anti-tuberculosis treatment patients were recruited from the outpatient department of four designated infectious hospitals in Jiangsu Province, and patients who met the following criteria were included in this study: (1) Tuberculosis patients ready to receive standard anti-tuberculosis treatment according to the WHO guidelines (Zha and Nahid 2019); (2) Tuberculosis patients belonged to Chinese Han population (CHB); and (3) signed the informed consent form. Liver function test (LFT) was performed before anti-tuberculosis treatment. In the first 2 months of anti-tuberculosis treatment, patients received scheduled outpatient follow-up and LFTs every 2 weeks at designated hospitals according to the local free-tuberculosis service policy. Whenever patients had symptoms of suspected hepatitis (such as anorexia, nausea, vomiting, malaise, or tea-coloured urine), they could go to the designated hospital and receive LFTs at that time (Chen et al. 2019; Yang et al. 2019). Even without any symptoms of drug-induced hepatitis, patients were advised to have LFTs every two weeks. The residual blood sample for LFT of each patient was preserved. All recruited tuberculosis patients followed up until their completion treatment. Tuberculosis patients with abnormal liver functions including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TBil) levels at baseline, or with mental illness and serious diseases (such as tumours) were excluded from our study.

ATDH were defined in accordance with the criterion from the Chinese Society of Hepatology (Yu et al. 2017), namely ALT \( \geq 3 \) ULN (upper limit of normal) or ALP \( \geq 2 \) ULN. Additionally, the results of causality assessment were possible (3–5 score), probable (6–8 score) or highly probable (>8 score) based on the updated RUCAM score system (Danan and Teschke 2015). Patients who fulfilled the criterion of ATDH were assigned to the case group, and each case was matched with four normal serum liver function controls by age at baseline (±5 years), sex, treatment history (categorised as primary treatment and retreatment tuberculosis patients).
SNP selection and genotyping

The residual blood sample from the LFTs was used for genomic DNA extraction. All eligible single nucleotide polymorphisms (SNPs) in the ABCG2 gene regions, which included the 2 kb upstream and downstream of the genes, were downloaded from the CHB of the 1000 Genomes Project database. SNPs were selected by Haploview version 4.2 (Broad Institute, Cambridge, MA) software based on the following criteria: (1) minor allele frequency (MAF) ≥10% and (2) $r^2$ of pairwise linkage disequilibrium ≥ 0.8. Additionally, the HaploReg version 4.1 (https://pubs.broad-institute.org/mammals/haploreg/haploreg.php) was referenced to select potentially functional SNPs as much as possible. Finally, seven SNPs with specific functional significance in ABCG2 gene were determined for the genotyping using TaqMan allelic discrimination technology (Table 1, Table S1). Genotyping was performed by blinding the case or control status. Over 10% of the samples were randomly genotyped in duplicate using the same assay with the accuracy rate of 100%.

Statistical analysis

Baseline and clinical characteristics between the case and control group were compared using t test or nonparametric test for continuous variables, and conditional logistic regression model for categorised variables. Hardy–Weinberg equilibrium (HWE) among the controls was tested using a goodness-of-fit chi-square test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by the multivariate conditional logistic regression model, and adjusted for smoking, drinking, liver disease, and hepatoprotective drugs use. Three genetic models (dominant, recessive, and additive models) and subgroup analysis were performed to comprehensively analyse the effect of SNPs and the risk of ATDH. The genetic variants that could affect different gene expression were identified via online expression quantitative trait loci (eQTL) analysis from GETx-Portal (https://www.gtexportal.org/home/). R software for Windows version 4.0.1 was used to perform all the analyses. A two-tailed $p$ value < 0.05 was considered statistically significant.

Results

Baseline and clinical characteristics

A total of 1010 tuberculosis patients (202 ATDH cases and 808 controls) were included in the study. Among 202 ATDH cases, there were 146 males and 56 females. Fifty-eight cases (28.7%) were judged as possible (3–5 score), 116 cases (57.4%) as probable (6–8 score), and 28 cases (13.9%) as highly probable (>8 score). The baseline characteristics of the cases and matched controls are summarised in Table 2. There were no significant differences between the two groups regarding demographic parameters, including age, smoking, or drinking history, and baseline values of liver biochemical parameters ($p > 0.05$). However, the proportion of hepatoprotective drugs use was higher in the control group than in the case group ($p = 0.005$), while the proportion of liver disease history in the case group was higher than that in the control group ($p = 0.039$). During the treatment, the peak values of alanine transaminase (ALT), aspartate transaminase (AST), ALP, TBil, and direct bilirubin (DBil) of ATDH cases were significantly higher than controls ($p < 0.001$).

ABCG2 genetic polymorphisms in cases and controls

Six SNPs (rs4693924, rs2054576, rs2231137, rs2622605, rs3114020, and rs2622606) of the ABCG2 gene were in HWE in the controls, except the SNP rs2231148. The distributions of genotypes for the selected SNPs in the ABCG2 gene are shown in Table S2. However, there was no significant difference in the overall genotypic distribution under different genetic models for each of the SNPs between ATDH cases and controls ($p > 0.05$). Further subgroup analysis was performed among different sex. Male patients with the CC genotype at SNP rs2622605 in the ABCG2 gene had an increased risk of ATDH (adjusted OR = 1.615, 95% CI: 1.119–2.332, $p = 0.011$), and marginally significant association were also found under additive model ($p = 0.049$) (Table 3).

Serum liver indicators in different genotypes

As shown in Table 4, significant differences in male patients were identified regarding the peak value of ALP during treatment between the TT + TC genotype and CC genotype of rs2622605 (recessive model) (98.0 U/L vs. 102.0 U/L, $p = 0.029$). The same indicator was also found to be marginally significant different among TT genotype, TC genotype, and CC genotype of rs2622605 (additive model) (100.0 U/L vs. 97.9 U/L vs. 102.0 U/L, $p = 0.048$).

Table 1. Information of seven SNPs in ABCG2 gene.

| SNPs       | Position\(\text{a}\) | Location | Alleles   | MAF(%)\(\text{b}\) | HWE $p$ value\(\text{c}\) |
|------------|----------------------|----------|-----------|-------------------|--------------------------|
| rs4693924  | chr4:88102072        | Intron variant | G>A     | 26.2              | 0.489                    |
| rs2231148  | chr4:88107326        | Intron variant | T>A     | 17.0              | <0.001                   |
| rs2054576  | chr4:88107623        | Intron variant | A>G     | 26.2              | 1.000                    |
| rs2231137  | chr4:88139962        | Missense variant | C>T   | 30.6              | 0.154                    |
| rs2622605  | chr4:88158234        | Intron variant | T>C     | 33.0              | 0.107                    |
| rs3114020  | chr4:88162514        | Intron variant | T>C     | 32.5              | 0.277                    |
| rs2622606  | chr4:88163229        | Intron variant | T>A     | 18.0              | 0.336                    |

SNPs: single nucleotide polymorphisms; ABCG2: ATP-binding cassette transporter G2; MAF: minor allele frequency; HWE: Hardy–Weinberg equilibrium
\(\text{a}\)SNP position in NCBI dbSNP of GRCh38 (http://www.ncbi.nlm.nih.gov/projects/SNP).
\(\text{b}\)MAF for Han Chinese in Beijing in the Hapmap database.
\(\text{c}\)HWE $p$ value in the controls.
Table 2. Characteristics of patients among ATDH cases and controls.

| Characteristic                        | ATDH cases (N = 202) | Controls (N = 808) | p Value |
|--------------------------------------|----------------------|--------------------|---------|
| Sex (male/female)                    | 146/56               | 584/224            | –       |
| Treatment history (primary/re-treatment) | 167/35               | 668/140            | –       |
| Age (year)                           | 49.4 ± 17.9          | 50.3 ± 18.1        | 0.536   |
| Hepatoprotective drug (use/not use)  | 96/106               | 455/353            | 0.005   |
| Liver diseases history (yes/no)      | 44/158               | 129/679            | 0.039   |
| Smoking history (yes/no)             | 28/174               | 87/721             | 0.169   |
| Drinking history (yes/no)            | 7/195                | 38/770             | 0.434   |
| Baseline value                        |                      |                    |         |
| ALT (U/L)                             | 15.0 (9.6–21.0)      | 14.0 (9.0–19.0)    | 0.094   |
| AST (U/L)                             | 22.0 (17.0–25.0)     | 20.0 (16.0–25.0)   | 0.052   |
| TBil (umol/L)                         | 10.2 (7.5–13.1)      | 9.8 (7.2–12.6)     | 0.284   |
| DBil (umol/L)                         | 3.5 (2.4–4.2)        | 3.3 (2.3–4.2)      | 0.656   |
| ALP (U/L)                             | 83.0 (68.0–90.0)     | 83.0 (68.0–96.9)   | 0.656   |
| During treatment (peak value)         |                      |                    |         |
| ALT (U/L)                             | 166.3 (127.4–258.3)  | 23.0 (15.0–33.0)   | <0.001  |
| AST (U/L)                             | 131.5 (86.3–205.8)   | 29.3 (23.0–38.0)   | <0.001  |
| TBil (umol/L)                         | 20.0 (14.9–25.9)     | 13.3 (9.9–18.0)    | <0.001  |
| DBil (umol/L)                         | 7.7 (5.5–11.6)       | 4.7 (3.3–6.6)      | <0.001  |
| ALP (U/L)                             | 122.8 (90.6–155.5)   | 95.0 (78.0–115.1)  | <0.001  |

ATDH: anti-tuberculosis drug-induced hepatotoxicity; ALT: alanine transaminase; AST: aspartate transaminase; TBil: total bilirubin; DBil: direct bilirubin; ALP: alkaline phosphatase.

 Normal range: ALT <40 U/L; AST <40 U/L; TBil <21 umol/L; DBil <6.9umol/L; ALP <130 U/L.

 aValues are presented as mean ± standard deviations.

 bValues are presented as median (interquartile range).

 cIndependent-sample t-test.

dConditional logistic regression model analysis.

eWilcoxon rank-sum test.

Table 3. Subgroup analysis by sex with different genetic models and the risks of ATDH.

| SNPs   | Model | Male (N = 730) | OR (95% CI)a | p Value | Female (N = 280) | OR (95% CI)a | p Value |
|--------|-------|---------------|--------------|---------|------------------|--------------|---------|
| rs2054576 (A>G) |       |               |              |         |                  |              |         |
| AA     | Dom   | 1.201 (0.827–1.734) | 0.337 | 0.959 (0.524–1.756) | 0.363 | 1.480 (0.564–3.883) | 0.426 |
| AG     | Rec   | 1.071 (0.546–2.099) | 0.843 | 1.058 (0.671–1.669) | 0.807 |
| GG     | Rec   | 1.136 (0.847–1.523) | 0.394 | 1.605 (0.871–2.960) | 0.129 |
| rs2231137 (C>T)  |       |               |              |         |                  |              |         |
| CC     | Dom   | 0.920 (0.636–1.332) | 0.660 | 1.605 (0.871–2.960) | 0.129 |
| CT     | Rec   | 1.127 (0.616–2.062) | 0.699 | 0.454 (0.132–1.564) | 0.211 |
| TT     | Add   | 0.977 (0.735–1.298) | 0.873 | 1.143 (0.727–1.796) | 0.564 |
| rs2231148 (T>A)  |       |               |              |         |                  |              |         |
| TT     | Dom   | 0.978 (0.413–2.314) | 0.959 | 3.013 (0.379–23.936) | 0.297 |
| TA     | Rec   | 1.150 (0.787–1.681) | 0.471 | 0.573 (0.306–1.074) | 0.082 |
| AA     | Add   | 1.103 (0.797–1.527) | 0.553 | 0.757 (0.457–1.254) | 0.279 |
| rs2622605 (T>C)  |       |               |              |         |                  |              |         |
| TT     | Dom   | 1.019 (0.557–1.861) | 0.952 | 1.322 (0.530–3.296) | 0.550 |
| TC     | Rec   | 1.615 (1.119–2.332) | 0.011 | 0.736 (0.407–1.331) | 0.310 |
| CC     | Add   | 1.339 (1.002–1.790) | 0.049 | 0.911 (0.603–1.378) | 0.659 |
| rs2622606 (T>A)  |       |               |              |         |                  |              |         |
| TT     | Dom   | 0.976 (0.662–1.439) | 0.902 | 1.428 (0.599–3.406) | 0.422 |
| TA     | Rec   | 1.465 (0.654–3.280) | 0.354 | 1.232 (0.523–2.902) | 0.633 |
| AA     | Add   | 1.040 (0.756–1.431) | 0.810 | 1.348 (0.562–3.229) | 0.504 |
| rs3114020 (T>C)  |       |               |              |         |                  |              |         |
| TT     | Dom   | 1.135 (0.628–2.053) | 0.675 | 0.994 (0.412–2.394) | 0.989 |
| TC     | Rec   | 1.188 (0.820–1.721) | 0.363 | 0.693 (0.381–1.261) | 0.231 |
| CC     | Add   | 1.132 (0.857–1.496) | 0.383 | 0.831 (0.549–1.257) | 0.380 |
| rs4693924 (G>A)  |       |               |              |         |                  |              |         |
| GG     | Dom   | 1.228 (0.842–1.789) | 0.286 | 1.386 (0.745–2.577) | 0.303 |
| GA     | Rec   | 0.807 (0.380–1.713) | 0.577 | 1.650 (0.633–4.300) | 0.306 |
| AA     | Add   | 1.099 (0.816–1.480) | 0.537 | 1.337 (0.845–2.116) | 0.215 |

ATDH: anti-tuberculosis drug-induced hepatotoxicity; SNPs: single nucleotide polymorphisms; OR: Odds ratio; 95% CI: 95% confidence interval; Dom: dominant model; Rec: recessive model; Add: additive model.

dConditional logistic regression model analysis and adjusted for smoking history, drinking history, liver disease history, and hepatoprotective drugs use.

Online eQTL analysis

From the GTEx portal, further online eQTL analysis of SNP rs2622605 was conducted with the gene expression in 208 liver samples, and there were significant differences in the expression levels of the three genotypes in liver samples (p = 0.015, Figure 2).

Discussion

This study showed that male patients carrying the CC geno- type of rs2622605 in the ABCG2 gene had an increased risk of ATDH (adjusted OR = 1.615, 95% CI: 1.119–2.332, p = 0.011), and marginally significant association were also found under additive model (p = 0.049). Additionally, there
Table 4. Distribution of serum liver indicators in male patients with different genetic model of SNP rs2622605.

| Serum liver indicators (peak value) | Recessive model | Additive model |
|-------------------------------------|-----------------|---------------|
|                                     | TT + TC (N = 435) | CC (N = 295) | p Value$^a$ | TT (N = 78) | TC (N = 357) | CC (N = 295) | p Value$^a$ |
| ALT$^a$                             | 27.0 (18.2–47.0) | 27.0 (17.0–82.5) | 0.892 | 32 (19.0–81.5) | 27.0 (18.0–43.0) | 27.0 (17.0–82.5) | 0.744 |
| AST$^a$                             | 32.4 (24.9–48.3) | 33.2 (26.0–65.4) | 0.121 | 33.7 (26.5–65.7) | 32.0 (24.5–45.0) | 33.2 (26.0–65.4) | 0.828 |
| TBI$^a$                             | 14.7 (11.1–20.0) | 14.7 (10.6–20.8) | 0.823 | 14.8 (11.3–21.2) | 14.7 (11.1–19.6) | 14.7 (10.6–20.8) | 0.386 |
| DBII$^a$                            | 5.5 (4.0–7.8) | 5.2 (3.5–7.5) | 0.445 | 5.5 (3.9–8.0) | 5.6 (4.0–7.7) | 5.2 (3.5–7.5) | 0.087 |
| ALP$^a$                             | 98.0 (82.0–119.1) | 102.0 (82.0–130.0) | 0.029 | 100.0 (83.2–119.0) | 97.9 (81.8–119.2) | 102.0 (82.0–130.0) | 0.048 |

ALT: alanine transaminase; AST: aspartate transaminase; TBIL: total bilirubin; DBIL: direct bilirubin; ALP: alkaline phosphatase.

Normal range: ALT $< 40$ U/L, AST $< 40$ U/L, TBIL $< 21$ μmol/L, DBIL $< 6.9$ μmol/L, ALP $< 130$ U/L.

$^a$Values are presented as median (interquartile range).

$^b$Kruskal–Wallis H test.

Table 4. Distribution of serum liver indicators in male patients with different genetic model of SNP rs2622605.

Figure 2. Expression Quantitative Trait Loci (eQTL) violin plot of ABCG2 gene expression at SNP rs2622605 in 208 liver samples. According to the eQTL violin plots, the vertical axis is the level of gene expression and horizontal axis is three different genotypes of SNP rs2622605. The result revealed that there were significant differences in the expression levels of the three genotypes in liver samples ($p = 0.015$). ABCG2: ATP-binding cassette transporter G2; SNP: single nucleotide polymorphism.

was a significant difference in the peak value of ALP in male patients with different genotypes of rs2622605 (TT + TC vs. CC, 98.0 U/L vs. 102.0 U/L, $p = 0.029$; TT vs. TC vs. CC, 100.0 U/L vs. 97.9 U/L vs. 102.0 U/L, $p = 0.048$). Further eQTL gene expression analysis provided information supporting the functional role of SNP rs2622605 in liver tissues, which indicated that the polymorphism at rs2622605 in the ABCG2 gene may cause variations in gene expression. To date, this study was the first to explore the relationship between ABCG2 genetic polymorphisms and the risk of ATDH, and the subgroup analysis showed that genetic variant (SNP rs2622605) in the ABCG2 gene may increase susceptibility to ATDH in male anti-tuberculosis treatment patients, which further illustrated that ABCG2 may contribute to the development of ATDH.

The human ABCG2 gene on chromosome 4q22 comprises 21 exons, spanning approximately 141,000 bases and containing 38,287 SNPs (https://www.ncbi.nlm.nih.gov/genome). SNP rs2622605 is located in intron 2 of the ABCG2 gene, and few studies on this SNP have been reported. In China, one genome-wide association study identified a significant correlation between SNP rs2622605 and plasma uric acid levels in extremely obese and normal weight individuals ($p = 0.0026$) (Li, Jiao, et al. 2013). Functional annotation using online ENCODE data (http://regulomedb.org/) indicated that SNP rs2622605 may influence chromatin structure and histone modifications, which may serve as a regulatory variant in the development of lung cancer (Sun et al. 2017). The eQTL analysis also revealed that the liver expression levels of the ABCG2 gene in TC genotypes were higher than those in other genotypes ($p = 0.015$). The mRNA centroid secondary structures of ABCG2 gene were analysed using the RNAfold Web Server (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi) (Mathews et al. 2016), and the results indicated that a significant difference was found in the lowest free energy between rs2622605 -T and -C alleles (−0.58 kcal/mol vs. −7.10 kcal/mol) in the mRNA centroid secondary structures of ABCG2 gene (Figure S1). In this study, a significant association between SNP rs2622605 in the ABCG2 gene and the risk of ATDH was only found in male patients (adjusted OR $= 1.615$, 95% CI: 1.119–2.332, $p = 0.011$). Although conclusions from different studies were inconsistent regarding whether sex was related to the occurrence of ATDH in China (male sex as a risk factor (Zhang et al. 2015; Zhong et al. 2021) or female sex as a risk factor (Zhao et al. 2020)), one previous study showed that erythrocyte PPIX levels increased during childhood and youth to reach a stable level in adults, although, on average, the PPIX level was higher in men (mean of 56 μmol/L, range: 6–139) than in women (mean of 38 μmol/L, range: 6–82) (Heerfordt et al. 2020). Additionally, the results from human ABCG2 expression in a limited series of livers of male and female humans showed that ABCG2 expression was consistently higher in men compared with women (Merino et al. 2005). Interestingly, ABCG2 expression in livers with the variant C421A (rs2231142) allele was significantly lower than that in the wild-type livers ($p < 0.002$) (Prasad et al. 2013). So, all of these results suggested that men may be more prone to liver injury than women when there is a genetic variant in SNP rs2622605 due to higher PPIX levels.

However, eQTL analysis revealed that the ABCG2 gene expression level of the TC genotype was the highest, followed by the CC genotype, and the lowest was the TT genotype (Figure 2). The expression level of the CC genotype was the lowest among the three genotypes. This discrepancy could have several reasons. First, this study only indicated
that male patients with CC genotype of rs2622605 had an increased risk of ATDH. The 208 liver samples for online eQTL analysis of the gene expression were from 146 men and 62 women. Second, the subjects of this study were all anti-tuberculosis treatment patients, while the subjects of the online eQTL analysis were all healthy individuals. Third, the statistically significant finding in this study is primarily based on the recessive model of SNP rs2622605 in male patients, and the gene expression level of CC genotype was also lower than that of TC genotype. Furthermore, according to the HapMap database, SNP rs2622605 was in high linkage disequilibrium with another potentially functional polymorphism (rs3114020, $r^2=1.00$) (Sun et al. 2017). In the CHB, rs3114020 TC or TT in ABCG2 could actually increase the risk of gout in the dominant model (OR = 1.58, 95% CI: 1.00–2.51, $p=0.048$) (Jiri et al. 2016). Therefore, the exact biological significance of SNP rs2622605 in the ABCG2 gene needs further clarification by functional studies.

To the best of our knowledge, this is the first study attempting to evaluate the association between ABCG2 genetic polymorphisms and the risk of ATDH in the Chinese population, and it contributes to the further understanding of potential mechanism of ATDH. Additionally, this is a 1:4 matched case–control study with a relatively large sample size in China. In order to reduce the misclassification of diagnosis, all suspected hepatitis patients were assessed using the RUCAM score system. However, two limitations cannot be ignored. First, there was a significant departure from the HWE in rs2231148, which maybe because the selected subjects were all tuberculosis patients. Second, we did not test for hepatitis C virus (HCV) infection, although the prevalence of HCV infection is very low in China.

Based on the 1:4 matched case–control study, the polymorphism at rs2622605 in the ABCG2 gene may be related to ATDH in Chinese male anti-tuberculosis treatment patients. Studies in larger, varied populations are required to validate our findings.

**Disclosure statement**

The authors declare no conflict of interest.

**Data availability statement**

The data sets used and/or analysed during this study are available from the corresponding author on reasonable request.

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