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*Letter to the Editor*

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# Association of *UGT2B7* polymorphisms with risk of induced liver injury by anti-tuberculosis drugs in Chinese Han

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**Abstract**

Anti-tuberculosis drug-induced liver injury (ATLI) is common during the treatment of tuberculosis (TB). As an important enzyme in the metabolism of many drugs, UGT2B7 (uridine diphosphate glucuronyl transferase 2B7) was associated with drug-induced liver disorder. This study investigated the association between the polymorphisms of *UGT2B7* and ATLI in Chinese Han. Totally, 280 newly diagnosed TB patients had been followed up for 3 months after the prescription of anti-TB therapy. Tag-single-nucleotide polymorphism (tag-SNPs) (rs10028494 and rs7668282) were genotyped with the MassARRAY platform. The associations between tag-SNPs and ATLI risk were analyzed by logistic regression analysis adjusting for confounding factors. In this prospective study, 33 patients were lost to follow-up, and 24 patients were diagnosed with ATLI and considered as the case group. The remaining 223 subjects without ATLI were considered as the control group. No significant association was observed in allele and genotype frequencies of *UGT2B7* between the two groups. This study is the first attempt to investigate the association of genetic polymorphisms of *UGT2B7* with ATLI in Chinese Han. There is no significant association between *UGT2B7* polymorphisms and ATLI in Chinese Han.

**Keywords**

anti-tuberculosis drug-induced liver injury, polymorphism, tuberculosis, uridine diphosphate glucuronyl transferase 2B7

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## Introduction

Tuberculosis (TB) is a chronic infectious disease spreading in the world. World Health Organization (WHO) reported 10.4 million new TB cases in 2015.[1] Anti-tuberculosis drug-induced liver injury (ATLI) is a common adverse effect of anti-TB treatment. Severe ATLI may lead to anti-TB treatment suspension, even hepatic failure. The incidences of ATLI vary in various studies. Chen et al.[2] have concluded that the rates of ATLI reported in the previous studies are from 0.1% to 27.7%, while the incidence is 2.55% in China.[3] The rate of isoniazid (INH) hepatitis in oriental males is 14 times that of Black men.[4] The genetic susceptibility is associated with the risk of ATLI.

In human body, more than 90% of drugs dependent on hepatic clearance are metabolized by enzymes of uridine diphosphate glucuronyl transferase (UGT)

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and cytochromes P450 (CYP).[5] UGT plays a critical role in drug detoxification and elimination through catalyzing a glucuronidation reaction in the phase II metabolism.[6] Rifabutin and rifampicin may inhibit several human UGTs in vitro.[7] Rifabutin was reported to inhibit UGT1A1, UGT1A9, UGT2B7, and so on in human liver microsome.[7] Rifampicin had the ability to induce the activity of UGT2B7, especially in the TB and HIV co-infected patients.[8]

Both UGT1A and UGT2B7 are the main enzymes in the UGT family. The genotypes of *UGT1A1**27 and *UGT1A1**28, *UGT1A6*-19T/G, *UGT1A6*-308C/A, and *UGT1A6*-541A/G were reported to be associated with ATLI.[9,10] However, the study referring to the association between *UGT2B7* and ATLI was rare. Thus, we investigated the association of *UGT2B7* polymorphisms with ATLI in the Chinese Han population.

## Materials and methods

### *Study population*

A total of 280 Chinese Han TB patients were enrolled from the West China hospital of Sichuan University from 2012 to 2015 in this prospective study. TB patients were diagnosed by two experienced TB professors according to clinical manifestation, chest images including X-ray/computed tomography (CT), and positive smear/culture/TB-DNA polymerase chain reaction results. The regimen prescribed to patients was 2HRZE/4HR, which consists of INH (H) (300 mg/day), rifampicin (R) (450–600 mg/day, depending on weight), pyrazinamide (Z) (1500 mg/day), and ethambutol (E) (750 mg/day) for 2 months, followed by INH and rifampicin for 4 months. Liver function was monitored once a month routinely, and the participants were required to test liver function immediately if symptoms including hepatalgia, nausea, vomiting, loss of appetite, fever, and jaundice appeared. According to the Chinese expert recommendation, ATLI is defined as an elevated alanine transaminase (ALT) or direct bilirubin more than two times of upper limit of normal range (ULN), or elevated aspartate transaminase (AST), alkaline phosphatase (ALP), and total bilirubin simultaneously and at least one of the above three markers greater than two times of ULN. Patients with any liver diseases, combined utilization of other hepatotoxic drugs during this study, abnormal liver function test results before anti-TB treatment, and cancer or other diseases may affect liver function were excluded.

Informed consent was obtained from all individual participants included in the study. The study was approved by the ethical committee of the West China Hospital of Sichuan University.

### *Tag-single-nucleotide polymorphism selection and genotyping*

Tag-single-nucleotide polymorphism (Tag-SNPs), with minor allele frequency (MAF) $\geqslant 0.05$, located within the region 3000 base pairs upstream and 300 base pairs downstream of *UGT2B7* were downloaded from the Chinese Han in Beijing database of HapMap (http://hapmap.ncbi.nlm.nih.gov/index.html.en, HapMap Data Rel 27 Phase II + III, on NCBI B36 assembly, dbSNP b126). Data were analyzed by Haploview 4.2 software (http://www.broadinstitute.org/), using the Tagger pairwise method ($r^2 \geqslant 0.80$). Final selections were two tag-SNPs (rs10028494 and rs7668282).

Genomic DNA was extracted from the peripheral venous blood samples using a genomic DNA purification kit (Axygen Scientific Inc., Union City, CA, USA). Selected SNPs were genotyped by Sequenom MassARRAY iPLEX platform that utilizes matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. The probes and primers were designed using the SpectroDESIGNER software (Sequenom, Sequenom Inc., San Diego, CA, USA). Approximately 5% of random samples were repeatedly genotyped with a concordance rate of 100%.

### *Data analysis*

Continuous and categorical variables were analyzed using U test and chi-square test, respectively. Hardy–Weinberg equilibrium among the controls was tested using chi-square test. Genotype distributions with different genetic models were examined using multivariate logistic regression analysis, adjusting for age, sex, body mass index (BMI), and smoking history. Linkage disequilibrium was calculated using SHEsis online software (http://analysis.bio-x.cn). Power calculation under different genetic models were performed ($\alpha = 0.05$) using Power and Sample Size Calculation Software (http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize) and Quanto software

**Table 1.** Characteristics of patients with and without ATLI.

| Characteristic | Case (N = 24) | Control (N = 223) | *P* value |
|---|---|---|---|
| Sex (male/female) | 8/16 | 90/133 | 0.502 |
| Age (years) | 32.79 ± 11.96 | 38.91 ± 16.00 | 0.070 |
| BMI (kg/m$^2$) | 21.58 ± 2.93 | 20.53 ± 3.74 | 0.217 |
| Smoking history | 6 (25.0%) | 63 (28.3%) | 0.717 |
| Baseline value | | | |
| AST (U/L) | 22 (20–34) | 22 (18–27) | 0.147 |
| ALT (U/L) | 23 (15–36) | 17 (11–24) | 0.095 |
| Total bilirubin (μmol/L) | 11.4 (8.4–13.5) | 10.2 (7.6–14.1) | 0.950 |
| During treatment | | | |
| AST (U/L) | 83 (69–201) | 26 (20–34) | <0.0001 |
| ALT (U/L) | 172 (111–255) | 24 (17–34) | <0.0001 |
| Total bilirubin (μmol/L) | 12.8 (7.7–19.3) | 9.1 (6.2–12.6) | 0.073 |

ATLI: anti-tuberculosis drug-induced liver injury; BMI: body mass index; AST: aspartate transaminase; ALT: alanine transaminase.

**Table 2.** *UGT2B7* polymorphisms in patients with and without ATLI.

| SNPs | Case group N (%) | Control group N (%) | *P** | Genetic model | OR[a] (95% CI) | *P*[a] |
|---|---|---|---|---|---|---|
| rs10028494 (A > C) | | | | | | |
| A | 35 (72.9) | 323 (73.1) | | Dominant | 1.238 (0.565–2.716) | 0.594 |
| C | 13 (27.1) | 119 (26.9) | 0.981 | Recessive | 0.591 (0.099–3.537) | 0.565 |
| AA | 12 (50.0) | 121 (54.8) | | Additive | 0.591 (0.099–3.537) | 0.804 |
| CA | 11 (45.8) | 81 (36.7) | | | | |
| CC | 1 (4.2) | 19 (8.5) | 0.578 | | | |
| rs7668282 (T > C) | | | | | | |
| T | 45 (93.8) | 417 (93.5) | | Dominant | 0.788 (0.194–3.198) | 0.738 |
| C | 3 (6.2) | 29 (6.5) | 0.946 | Recessive | – | – |
| TT | 21 (87.5) | 194 (87.0) | | Additive | 0.788 (0.194–3.198) | 0.738 |
| CT | 3 (12.5) | 29 (13.0) | 0.944 | | | |
| CC | 0 | 0 | | | | |

ATLI: anti-tuberculosis drug-induced liver injury; SNP: single-nucleotide polymorphism; OR: odds ratio; CI: confidence interval; BMI: body mass index.
[a]Adjusted for sex, age, BMI, and smoking history with logistic regression.
*P-values were computed by 2×3 or 2×2 $\chi^2$-test.

(Version 1.2.4). Statistical tests were performed using the Statistical Package for the Social Sciences (SPSS, SPSS Inc., Chicago, IL, USA), version 17.0. A $P < 0.05$ was considered to be statistically significant.

## Results

### *Subject characteristics*

In this prospective study, 280 patients with TB were enrolled. In the period of following up, 19 patients were lost to contact and 14 patients were excluded because of poor compliance. Then, a total of 247 TB patients who completed 3 months of anti-TB therapies and follow-up were researched. Among which 24 (9.7%) patients with ATLI and 223 (90.3%) patients with anti-tuberculosis drugs tolerance were observed. There were no significant differences in age, sex, BMI, and smoking history between two groups (Table 1).

### *SNP analysis*

The genotype distributions of rs10028494 and rs7668282 at *UGT2B7* in the controls were all in Hardy-Weinberg equilibrium (HWE). The allele and genotype frequencies of cases and controls showed no difference in Table 2. There was no significant association in different genetic models of both tag-SNPs after adjusting for compound factors including age, BMI, and smoking history between the two groups (Table 2).

**Table 3.** Power of the study at current sample size with different minor allele frequencies (MAF) and relative risks (RR) under different genetic models.

| SNP | MAF (%) | Genetic model | Power | | |
|---|---|---|---|---|---|
| | | | RR = 2 | RR = 3 | RR = 4 |
| rs10028494 (A > C) | 26.9 | Allelic | 0.61 | 0.95 | 0.99 |
| | | Dominant | 0.36 | 0.69 | 0.86 |
| | | Recessive | 0.16 | 0.38 | 0.58 |
| | | Additive | 0.57 | 0.93 | 0.99 |
| rs7668282 (T > C) | 6.5 | Allelic | 0.35 | 0.70 | 0.88 |
| | | Dominant | 0.23 | 0.53 | 0.75 |
| | | Recessive | – | – | – |
| | | Additive | 0.26 | 0.59 | 0.81 |

SNP: single-nucleotide polymorphism.

### *Power calculation*

With the sample size of 24 and 223 subjects in the ATLI group and non-ATLI group, respectively, the power of the study to detect relative risks (RR) of 2.0, 3.0, and 4.0 using the two tag-SNPs studied under different genetic models are listed in Table 3. The study has reasonable power (>80%) to detect genetic factors with RR 3.0 and above under the allelic or additive models in rs10028494 and RR 4.0 and above in rs7668282.

## Discussion

The susceptible genes of ATLI involved in drug metabolism or immunological reaction. Inter-individual variability exists in *UGT* tissue expression and activity, which is determined by gene transcription level.[11] *UGT2B7*, located on chromosome 4q13, participates in biotransformation of cholic acid, fatty acids, and the metabolism of many drugs, including anti-carcinogens, non-steroidal anti-inflammatory drugs, and anti-TB drugs.[12]

The polymorphisms of *UGT2B7* take part in diseases by altering the metabolism of substrates. Breast cancer patients with *UGT2B7* (268Tyr/Tyr) genotype benefitted most from epirubicin-based chemotherapy due to the effect of *UGT2B7* on epirubicin elimination.[13] *UGT2B7**2 was implicated in diclofenac-related hepatotoxicity, due to the higher glucuronidation activity.[14] *UGT2B7* was associated with the concentration and clearance of antiretroviral drugs in the combination regimen including rifampicin for TB/HIV patients.[8] Up to now, the research about the association of genetic polymorphism of *UGT2B7* with the development of ATLI was paucity. Only Yimer et al.[15] reported no relationship between *UGT2B7*-372G>A genotype and drug-induced liver injury (DILI) in TB-HIV co-infected patients in Ethiopia.

This study showed the first data about the association of *UGT2B7* and ATLI in Chinese Han. However, we failed to find any positive results. Maybe the small sample size is one of the limitations. Meanwhile, we didn't consider the effects of gene–gene and gene–environment interactions.

In conclusion, no significant association of common polymorphisms of *UGT2B7* between the case and control groups suggests *UGT2B7* may not play important roles in the development of ATLI in Chinese Han. Further study is needed with larger sample size and in different ethnic populations.

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### Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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