



Association between *TXNRD1* polymorphisms and anti-tuberculosis drug-induced hepatotoxicity in a prospective study

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ABSTRACT. Anti-tuberculosis drug-induced hepatotoxicity (ATDH) is a serious adverse reaction to anti-tuberculosis (TB) treatment. Thioredoxin reductase 1 (TXNRD1), encoded by the *TXNRD1* gene, is an important enzyme involved in oxidant challenge. TXNRD1 plays a key role in regulating cell growth and transformation, and protects cells against oxidative damage. We investigated the association between *TXNRD1* polymorphisms and ATDH susceptibility. In this prospective study, 280 newly diagnosed TB patients were followed-up for 3 months after beginning anti-TB therapy. Tag single-nucleotide polymorphisms (tag-SNPs) of *TXNRD1* were selected using Haploview 4.2 based on

the HapMap database of the Chinese Han in Beijing (CHB) panel. Genotyping was performed using the MassARRAY platform. Of the 280 patients enrolled in this study, 33 were lost to follow-up, 24 had ATDH, and 223 were free from ATDH. After adjusting for sex, age, smoking status, and body mass index, there were no significant differences in the allele and genotype frequency distributions of *TXNRD1* SNPs between the ATDH and non-ATDH groups (all $P > 0.05$). The haplotype analysis showed that haplotype TCAGCC was associated with an increased risk of ATDH susceptibility [$P = 0.024$, OR (95%CI) = 6.273 (1.023-38.485)]. Further stratified analyses showed that the haplotype TCAGCC was associated with ATDH susceptibility in female subjects [$P = 0.036$, OR (95%CI) = 5.711 (0.917-35.560)] and non-smokers [$P = 0.029$, OR (95%CI) = 6.008 (0.971-37.158)]. Our results suggest that *TXNRD1* variants may favor ATDH susceptibility in females and non-smokers. Further studies are required to verify this association.

Key words: Thioredoxin reductase 1; *TXNRD1*; Polymorphism; Anti-tuberculosis drug-induced hepatotoxicity; ATDH

INTRODUCTION

Tuberculosis (TB) is a threat to people's health and has been recognized as a worldwide health problem, with 13.0 million prevalent TB cases, 9.6 million new TB cases, and 1.3 million deaths in 2014 (World Health Organization, 2015). For controlling the TB epidemic, a standard anti-TB treatment protocol including isoniazid, rifampin, pyrazinamide, ethambutol, and/or streptomycin for 6-9 months has been recommended (World Health Organization, 2002). However, these anti-TB drugs usually have some adverse drug reactions (ADRs) (Chen et al., 2015). One of the most common and serious ADRs is anti-tuberculosis drug-induced hepatotoxicity (ATDH), which can lead to non-adherence, drug resistance, or treatment failure (Kaona et al., 2004). Therefore, a better understanding of the risk factors of ATDH development would be helpful. Previous studies have shown that the risk factors can be classified as non-genetic and genetic factors (Chen et al., 2015). Non-genetic factors include female sex, race, and environmental factors such as alcohol intake (Pande et al., 1996; Lee et al., 2002; Marra et al., 2007; de Castro et al., 2010). The genetic factors are involved in drug metabolism, transport, and immune and antioxidant responses, and include N-acetyltransferase 2 (*NAT2*), BTB and CNC homology 1 (*BACH1*), tumor necrosis factor- α (*TNF*) and adenosine triphosphate binding cassette B1 (*ABCB1*) (Yimer et al., 2011; Kim et al., 2012; Nanashima et al., 2012; Du et al., 2013).

Thioredoxin reductase 1 (TXNRD1), encoded by the *TXNRD1* gene, is an important NADPH-dependent enzyme with broad substrate specificity related to oxidant challenge (Kwon et al., 2012). TXNRD1 plays a key role in regulating cell growth and transformation, and protecting cells against oxidative damage (Kwon et al., 2012). Previous studies have found that there is a strong association between oxidative stress and liver disease, and the TXNRD1-mediated antioxidant function protects the liver from oxidative injury (Minami et al., 2005; Zidek et al., 2007; Cederbaum et al., 2009). Kwon et al. (2012) reported that the genetic variations in *TXNRD1* favor the development of drug-induced liver injury (DILI).

However, there has been no study on the association between *TXNRD1* polymorphisms and ATDH susceptibility. Therefore, in this study we aimed to investigate the associations between *TXNRD1* polymorphisms and ATDH among the Chinese Han population.

MATERIAL AND METHODS

Study subjects

In this study, 280 newly diagnosed TB patients were recruited before receiving anti-TB therapy between August 2012 and March 2015 in the West China Hospital of Sichuan University. All TB patients were diagnosed by specialist physicians based on respiratory symptoms, typical infiltrate findings on chest radiographs, bacteriological evidence (sputum smear or culture), or histopathological evidence (ATS, 2000). Patients with hepatitis B virus (HBV), hepatitis C virus (HCV), or human immunodeficiency virus (HIV), those who drank habitually, had liver disease or abnormal liver function before using anti-TB drugs, those who were concurrently using hepatotoxic drugs, and those lost to follow-up, were excluded.

All subjects took rifampicin 600 mg (or 450 mg if body weight was <50 kg), isoniazid 300 mg, ethambutol 750 mg, and pyrazinamide 1500 mg every day for 2 months. After 2 months of treatment, pyrazinamide was discontinued and the other three drugs were continued for a total of 6 months.

We performed liver function tests routinely at 2 weeks, 1 month, 2 months, and 3 months from the start of treatment, or whenever the subjects displayed any notable manifestations of liver dysfunction, such as loss of appetite or jaundice. We adopted diagnostic criteria for ATDH based on the International Consensus Meeting, as follows: 1) an increase of over two fold above the upper limit of normal (ULN) in the level of alanine aminotransferase (ALT) with/without \geq two times the ULN in the level of aspartate aminotransferase (AST) and total bilirubin during the anti-TB therapy period (Bénichou, 1990); and 2) the result of causality assessment was certain, probable, or possible (World Health Organization, 2005; Corsini and Bortolini, 2013).

Ethics statement

Each subject was required to provide written informed consent. The Ethics Committee of the West China Hospital of Sichuan University, China, provided authorization for our study.

Selection/genotyping of *TXNRD1* single-nucleotide polymorphisms (SNPs)

TXNRD1 is located on chromosome 12. SNPs were selected from 3000 base pairs downstream to 3000 base pairs upstream of the *TXNRD1* gene. According to the HapMap Chinese Han in Beijing (CHB) database (<http://hapmap.ncbi.nlm.nih.gov>), with minor allele frequency (MAF) \geq 0.05 and $r^2 \geq$ 0.80, we chose six tag single-nucleotide polymorphisms (tag-SNPs) of *TXNRD1* (rs10735393, rs4964287, rs4595619, rs4246270, rs4246271, and rs11611385) for further genotyping.

Peripheral venous blood samples were collected from all patients in ethylenediaminetetraacetic acid (EDTA) tubes (BD Vacutainers, Franklin Lakes, NJ, USA). Genomic DNA was extracted using a Genomic DNA Extraction kit (Axygen Scientific

Inc., Union City, CA, USA). Selected SNPs of *TXNRDI* were genotyped by the Sequenom MassARRAY iPLEX platform using polymerase chain reaction (PCR) and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry.

Statistical analysis

SPSS software and the SHEsis system (<http://analysis.Bio-x.cn/SHEsisMain.htm>) were adopted for statistical analysis. The SNPs and distributions of demographic characteristics between the ATDH and non-ATDH groups were calculated by the chi-squared test (categorical data) or the *t*-test (numerical data). Linkage disequilibrium (LD) between SNPs and haplotype analyses were examined using the SHEsis program. Agreement with the Hardy-Weinberg equilibrium (HWE) was assessed using the chi-squared test. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated by logistic regression analysis adjusting for confounding factors including sex, age, smoking status, and body mass index (BMI). P values < 0.05 were considered statistically significant.

RESULTS

Demographic and clinical characteristics of study subjects

Of the 280 subjects initially recruited to this study, we excluded 33 subjects: 19 were lost to follow-up; 12 had used other hepatotoxic medications, had complications from diseases influencing liver function, or were unable to provide samples for liver function tests in follow-up; and 2 individuals failed to respond to first-line anti-TB treatment and switched to second-line anti-TB therapy before completing 3 months of therapy. The remaining 247 TB patients completed 3 months of first-line anti-TB treatment and follow-up. Of those 247 subjects, 24 (9.8%) patients with levels of ALT/AST/total bilirubin more than twice the ULN were enrolled into the ATDH group, and the remaining 223 (90.2%) individuals were put in the non-ATDH group. The demographic characteristics of the study population are listed in Table 1. No significant differences were found in gender, age, smoking status, or BMI between the ATDH and non-ATDH groups.

Table 1. Characteristics of patients in the anti-tuberculosis drug-induced hepatotoxicity (ATDH) and non-ATDH groups.

Characteristic	Patients with ATDH (N = 24)	Patients without ATDH (N = 223)	P value
Gender (male/female)	8/16	90/133	0.502
Age (years) ^a	32.79 ± 11.96	38.91 ± 16.00	0.070
Weight (kg) ^a	54.86 ± 8.69	54.67 ± 10.30	0.932
BMI (kg/m ²) ^a	21.58 ± 2.93	20.53 ± 3.74	0.217
Smoking history	6 (25.0%)	63 (28.3%)	0.717
Baseline value			
ALT (U/L) ^b	23 (15-36)	17 (11-24)	0.095
AST (U/L) ^b	22 (20-34)	22 (18-27)	0.147
Total bilirubin (μM) ^b	11.4 (8.4-13.5)	10.2 (7.6-14.1)	0.950
During treatment (peak value)			
ALT (U/L) ^b	172 (111-255)	24 (17-34)	< 0.0001
AST (U/L) ^b	83 (69-201)	26 (20-34)	< 0.0001
Total bilirubin (μM) ^b	12.8 (7.7-19.3)	9.1 (6.2-12.6)	0.073

BMI = body mass index; ALT = alanine aminotransferase; AST = aspartate aminotransferase. ^aValues are reported as mean ± SD (range). ^bValues are reported as median (inter-quartile range).

Association between TXNRD1 SNPs and ATDH

Six tag-SNPs (rs10735393, rs4964287, rs4595619, rs4246270, rs4246271, and rs11611385) of TXNRD1 were selected and successfully genotyped in our study. The genotypic frequencies of these SNPs in the ATDH and the non-ATDH groups are shown in Table 2. As shown in Table 2, we analyzed the data with three different genetic models: allelic, codominant, and dominant. After adjusting for confounding factors including gender, age, smoking status, and BMI, there were no statistically significant differences (all P > 0.05) between the two groups. Using SHEsis software, the linkage disequilibrium (LD) across the SNPs was calculated with both r² and D' (Figure 1). We performed further haplotype analysis by excluding the rare haplotypes (lowest frequency threshold = 0.03) (Table 3). The analysis results showed that the TCAGCC haplotype increased the risk of ATDH susceptibility [P = 0.024, OR (95%CI) = 6.273 (1.023-38.485)].

Table 2. Association between TXNRD1 single-nucleotide polymorphisms (SNPs) and anti-tuberculosis drug-induced hepatotoxicity (ATDH).

SNPs	Groups	Subjects with genotype, N (%)			Allelic model		Codominant model		Dominant model	
		00	01	11	OR (95%CI) ^a	P ^a value	OR (95%CI) ^a	P ^a value	OR (95%CI) ^a	P ^a value
rs10735393	non-ATDH	153 (69.9%)	56 (25.6%)	10 (4.6%)						
T>G	ATDH	15 (62.5%)	8 (33.3%)	1 (4.2%)	1.408 (0.651-3.045)	0.385	1.872 (0.704-4.982)	0.209	1.708 (0.667-4.374)	0.264
rs4964287	non-ATDH	129 (57.8%)	75 (33.6%)	19 (8.5%)						
C>T	ATDH	15 (62.5%)	8 (33.3%)	1 (4.2%)	0.765 (0.334-1.752)	0.527	0.805 (0.285-2.272)	0.681	0.753 (0.281-2.017)	0.573
rs4595619	non-ATDH	78 (35.6%)	95 (43.4%)	46 (21.0%)						
G>A	ATDH	7 (29.2%)	12 (50.0%)	5 (20.8%)	1.174 (0.611-2.257)	0.630	1.236 (0.426-3.589)	0.697	1.278 (0.482-3.389)	0.622
rs4246270	non-ATDH	158 (71.5%)	55 (24.9%)	8 (3.6%)						
A>G	ATDH	15 (62.5%)	8 (33.3%)	1 (4.2%)	1.425 (0.637-3.186)	0.388	1.602 (0.589-4.356)	0.355	1.579 (0.604-4.130)	0.352
rs4246271	non-ATDH	157 (71.4%)	55 (25.0%)	8 (3.6%)						
G>C	ATDH	15 (62.5%)	8 (33.3%)	1 (4.2%)	1.412 (0.631-3.156)	0.401	1.586 (0.583-4.313)	0.366	1.562 (0.597-4.088)	0.363
rs11611385	non-ATDH	146 (66.1%)	60 (27.1%)	15 (6.8%)						
C>T	ATDH	15 (62.5%)	8 (33.3%)	1 (4.2%)	1.011 (0.437-2.341)	0.980	1.152 (0.403-3.296)	0.792	1.074 (0.397-2.908)	0.888

OR = odds ratio; CI = confidence interval; 0 = common allele; 1 = minor allele. ^aAdjusted for age, gender, body mass index (BMI), and smoking in a logistic regression model. For OR calculations, each group was compared with the non-ATDH group.

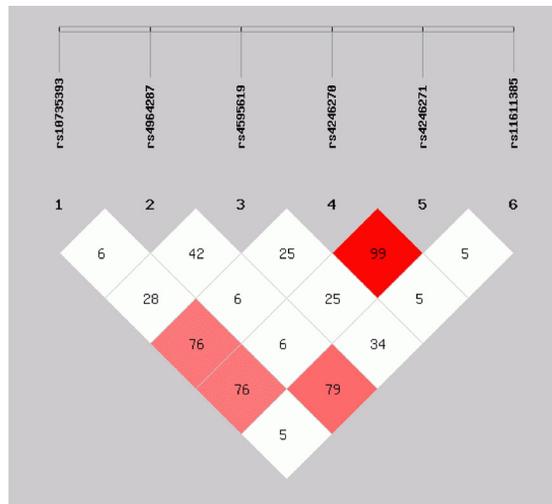


Figure 1. Linkage disequilibrium (LD) of TXNRD1 single nucleotide polymorphisms. The r² values are displayed on the squares.

Table 3. Frequencies of haplotypes consisting of *TXNRDI* single-nucleotide polymorphisms (SNPs).

Haplotypes	Stratification variables	Cases, N (%)	Controls, N (%)	OR (95%CI)	P value
GCAAGC	All	2.0 (4.2%)	9.0 (2.1%)	2.068 (0.434-9.860)	0.352
	Female	2.0 (6.3%)	4.0 (1.5%)	4.267 (0.750-24.287)	0.076
	Non-smoker	2.0 (5.6%)	6.0 (1.9%)	2.990 (0.581-15.401)	0.17
GCAGCC	All	8.0 (16.7%)	65.99 (15.1%)	1.124 (0.504-2.510)	0.775
	Female	6.0 (18.7%)	41.0 (15.8%)	1.233 (0.478-3.182)	0.665
	Non-smoker	6.0 (16.7%)	49.0 (15.7%)	1.070 (0.423-2.706)	0.887
TCAGCC	All	2.0 (4.2%)	3.0 (0.7%)	6.273 (1.023-38.485)	0.024
	Female	2.0 (6.3%)	3.0 (1.2%)	5.711 (0.917-35.560)	0.036
	Non-smoker	2.0 (5.6%)	3.0 (1.0%)	6.008 (0.971-37.158)	0.029
TCGAGC	All	26.0 (54.2%)	251.0 (57.3%)	0.876 (0.481-1.593)	0.664
	Female	15.0 (46.9%)	149.0 (57.3%)	0.657 (0.315-1.373)	0.262
	Non-smoker	18.0 (50.0%)	179.0 (57.4%)	0.737 (0.369-1.471)	0.386
TTAAGT	All	10.0 (20.8%)	90.0 (20.5%)	1.015 (0.487-2.114)	0.969
	Female	7.0 (21.9%)	52.0 (20.0%)	1.120 (0.459-2.732)	0.803
	Non-smoker	8.0 (22.2%)	62.0 (19.9%)	1.147 (0.499-2.641)	0.746

TB = tuberculosis; OR = odds ratio; CI = confidence interval.

Comparison of the association between *TXNRDI* SNPs and ATDH stratified by gender

Previous studies have shown that gender is important in the pharmacokinetics of drugs (Tanaka and Hisawa, 1999; Morris et al., 2003). Therefore, we further investigated the association between *TXNRDI* SNPs and ATDH susceptibility stratified by gender (Table 4). After adjusting for confounding factors including gender, age, smoking status, and BMI, there were no significant differences between the two groups in the male and female subgroups. We also performed haplotype analysis in the two subgroups. There was a statistically significant difference in females but not in males. As shown in Table 3, the OR for the TCAGCC haplotype as a risk factor in females was 5.711 (95%CI = 0.917-35.560, P = 0.036).

Comparison of the association between *TXNRDI* SNPs and ATDH stratified by smoking status

In the subgroup analysis stratified by gender, we found that the TCAGCC haplotype demonstrated an increased risk of ATDH susceptibility in female subjects. Furthermore, among the 149 female patients in our study, 145 (97.3%) individuals were non-smokers. Therefore, to fully explore whether there was a relationship between *TXNRDI* SNPs and ATDH in non-smokers, we further analyzed the *TXNRDI* data stratified by smoking status (Table 4). No statistically significant differences were found between the ATDH and non-ATDH groups in the smoking and non-smoking subgroups. Further haplotype analysis showed that the TCAGCC haplotype increased the risk of ATDH in non-smokers but not in smokers [P = 0.029, OR (95%CI) = 6.008 (0.971-37.158)].

DISCUSSION

ATDH is one of the most common and serious ADRs (Shang et al., 2011). Individuals show obvious differences in ADRs. Therefore, a better understanding of the predisposing factors of ATDH susceptibility is crucial for guiding individualized treatment. Studies have shown that the predisposing factors of susceptibility to ATDH mainly comprise environmental and genetic factors, especially the latter (Chen et al., 2015). These genetic factors, such as

Table 4. Association between *TXNRD1* single-nucleotide polymorphisms (SNPs) and anti-tuberculosis drug-induced hepatotoxicity (ATDH) stratified by gender and smoking status.

SNPs	Stratification variables	Groups	Subjects with genotype, N (%)			Allelic model		Codominant model		Dominant model	
			00	01	11	OR (95%CI) ^a	P ^a value	OR (95%CI) ^a	P ^a value	OR (95%CI) ^a	P ^a value
rs10735393 T>G	Female	non-ATDH	92 (70.8%)	31 (23.8%)	7 (5.4%)						
		ATDH	9 (56.2%)	6 (37.5)	1 (6.2%)	1.604 (0.653-3.938)	0.303	2.260 (0.684-7.472)	0.181	2.064 (0.659-6.465)	0.214
		Male	non-ATDH	61 (68.5%)	25 (28.1%)	3 (3.4%)					
	Smoker	non-ATDH	4 (6.75%)	2 (25.0%)	0 (0)	0.882 (0.175-4.457)	0.880	1.215 (0.197-7.491)	0.834	1.043 (0.171-6.347)	0.964
		ATDH	44 (69.8%)	18 (28.6%)	1 (1.6%)						
		Non-smoker	non-ATDH	4 (66.7%)	2 (33.3%)	0 (0)	1.280 (0.209-7.828)	0.790	2.487 (0.253-24.485)	0.435	1.944 (0.210-17.988)
rs4964287 C>T	Female	non-ATDH	109 (69.9%)	38 (24.4%)	9 (5.8%)						
		ATDH	11 (61.1%)	6 (33.3%)	1 (5.6%)	1.390 (0.582-3.320)	0.459	1.837 (0.592-5.705)	0.293	1.677 (0.572-4.918)	0.346
		Male	non-ATDH	78 (58.6%)	45 (33.8%)	10 (7.5)					
	Smoker	non-ATDH	10 (62.5%)	5 (31.2%)	1 (6.2%)	1.091 (0.405-2.938)	0.863	0.930 (0.256-3.371)	0.911	0.995 (0.297-3.332)	0.994
		ATDH	51 (56.7%)	30 (33.3%)	9 (10.0%)						
		Non-smoker	non-ATDH	5 (62.5%)	3 (37.5%)	0 (0)	0.561 (0.114-2.751)	0.476	0.824 (0.135-5.024)	0.833	0.653 (0.107-3.972)
rs4595619 G>A	Female	non-ATDH	35 (54.7%)	22 (34.4%)	7 (10.9%)						
		ATDH	4 (66.7%)	2 (33.3%)	0 (0)	0.484 (0.051-4.546)	0.525	0.662 (0.055-7.973)	0.745	0.533 (0.044-6.468)	0.622
		Male	non-ATDH	94 (59.1%)	53 (33.3%)	12 (7.5%)					
	Smoker	non-ATDH	11 (61.1%)	6 (33.3%)	1 (5.6%)	1.074 (1.432-2.668)	0.877	1.040 (1.326-3.319)	0.947	1.056 (0.350-3.184)	0.923
		ATDH	48 (36.9%)	53 (40.8%)	29 (23.3%)						
		Non-smoker	non-ATDH	4 (25.0%)	7 (43.8%)	5 (31.2%)	1.699 (0.761-3.796)	0.196	1.155 (0.271-4.925)	0.845	1.638 (0.465-5.774)
rs4246270 A>G	Female	non-ATDH	30 (33.7%)	42 (47.2%)	17 (19.1%)						
		ATDH	3 (37.5%)	5 (62.5%)	0 (0)	0.659 (0.186-2.338)	0.518	1.952 (0.275-13.857)	0.504	1.119 (0.183-6.861)	0.903
		Male	non-ATDH	21 (33.3%)	30 (47.6%)	12 (19.0%)					
	Smoker	non-ATDH	2 (33.3%)	4 (66.7%)	0 (0)	0.833 (0.166-4.174)	0.824	2.142 (0.173-26.469)	0.553	1.696 (0.134-21.514)	0.684
		ATDH	57 (36.5%)	65 (41.7%)	34 (21.8%)						
		Non-smoker	non-ATDH	5 (27.8%)	8 (44.4%)	5 (27.8%)	1.479 (0.701-3.119)	0.304	1.229 (0.337-4.487)	0.755	1.494 (0.476-4.688)
rs4246271 G>C	Female	non-ATDH	93 (70.5%)	32 (24.2%)	7 (5.3%)						
		ATDH	9 (56.2)	6 (37.5%)	1 (6.2%)	1.402 (0.553-3.556)	0.477	1.668 (0.494-5.630)	0.410	1.607 (0.509-5.075)	0.419
		Male	non-ATDH	65 (73.0%)	23 (25.8%)	1 (1.1%)					
	Smoker	non-ATDH	6 (75.0%)	2 (25.0%)	0 (0)	1.387 (0.259-7.418)	0.702	1.504 (0.226-10.010)	0.673	1.504 (0.226-10.010)	0.673
		ATDH	4 (66.7%)	2 (33.3%)	0 (0)	1.956 (0.290-13.196)	0.491	2.651 (0.237-29.706)	0.429	2.651 (0.237-29.706)	0.429
		Non-smoker	non-ATDH	112 (70.9%)	38 (24.1%)	8 (5.1%)					
rs11611385 C>T	Female	non-ATDH	11 (61.1%)	6 (33.3%)	1 (5.6%)	1.290 (0.517-3.217)	0.585	1.441 (0.447-4.652)	0.541	1.414 (0.467-4.283)	0.540
		ATDH	92 (70.2%)	32 (24.4%)	7 (5.3%)						
		Male	non-ATDH	9 (56.2%)	6 (37.5%)	1 (6.2%)	1.383 (0.545-3.509)	0.494	1.642 (0.486-5.544)	0.424	1.582 (0.501-4.997)
	Smoker	non-ATDH	65 (73.0%)	23 (25.8%)	1 (1.1%)						
		ATDH	6 (75.0%)	2 (25.0%)	0 (0)	1.387 (0.259-7.418)	0.702	1.504 (0.226-10.010)	0.673	1.504 (0.226-10.010)	0.673
		Non-smoker	non-ATDH	46 (73.0%)	17 (27.0%)	0 (0)	1.956 (0.290-13.196)	0.491	2.651 (0.237-29.706)	0.429	2.651 (0.237-29.706)
rs11611385 C>T	Female	non-ATDH	111 (70.7%)	38 (24.2%)	8 (5.1%)						
		ATDH	11 (61.1%)	6 (33.3%)	1 (5.6%)	1.274 (0.511-3.178)	0.603	1.421 (0.440-4.589)	0.557	1.394 (0.460-4.224)	0.557
		Male	non-ATDH	88 (66.7%)	36 (27.3%)	8 (6.1%)					
	Smoker	non-ATDH	10 (62.5%)	5 (31.2%)	1 (6.2%)	1.548 (0.560-4.281)	0.399	1.421 (0.382-5.280)	0.600	1.528 (0.445-5.248)	0.500
		ATDH	58 (65.2%)	24 (27.0%)	7 (7.9%)						
		Non-smoker	non-ATDH	5 (62.5%)	3 (37.5%)	0 (0)	0.706 (0.143-3.485)	0.669	1.081 (0.178-6.566)	0.933	0.867 (0.143-5.254)
rs11611385 C>T	Female	non-ATDH	40 (63.5%)	18 (28.6%)	5 (7.9%)						
		ATDH	4 (66.7%)	2 (33.3%)	0 (0)	0.646 (0.068-6.135)	0.704	0.889 (0.075-10.521)	0.925	0.746 (0.063-8.878)	0.816
		Male	non-ATDH	106 (67.1%)	42 (26.6%)	10 (6.3%)					
	Smoker	non-ATDH	11 (61.1%)	6 (33.3%)	1 (5.6%)	1.450 (0.572-3.675)	0.433	1.512 (0.465-4.918)	0.492	1.541 (0.500-4.744)	0.451
		ATDH									
		Non-smoker	non-ATDH								

OR = odds ratio; CI = confidence interval; 0 = common allele; 1 = minor frequency allele. ^aAdjusted for age, gender, and smoking in a logistic regression model. For OR calculations, each group was compared with the control group.

NAT2, *BACH1*, *TNF*, and *ABCB1*, are involved in the pharmacokinetics of drugs including metabolism, transport, and immune and antioxidant responses (Yimer et al., 2011; Kim et al., 2012; Nanashima et al., 2012; Du et al., 2013). Studies have shown that *TXNRD1*-mediated antioxidant function protects the liver against oxidative injury, and the genetic variations in *TXNRD1* are related to DILI (Minami et al., 2005; Zidek et al., 2007; Kwon et al., 2012). However, there have not been any reports on the association between *TXNRD1* polymorphisms and ATDH susceptibility. Therefore, the aim of our study was to explore the genetic associations between *TXNRD1* polymorphisms and ATDH susceptibility.

Six tag-SNPs (rs10735393, rs4964287, rs4595619, rs4246270, rs4246271, and rs11611385) were selected for this research. Our findings showed that there were no statistically significant differences in the allele and genotype frequency distributions of *TXNRD1* SNPs between the ATDH and non-ATDH groups (all P > 0.05). In further haplotype analysis, the TCAGCC haplotype showed an increased risk of ATDH susceptibility. Previous studies have shown that gender plays an important role in the pharmacokinetics of drugs (Tanaka and Hisawa, 1999; Morris et al., 2003). Therefore, we further investigated the association between *TXNRD1* SNPs and ATDH susceptibility stratified by gender. We found that only the

TCAGCC haplotype demonstrated a statistically significant difference in the risk of susceptible to ATDH among female individuals. No statistically significant differences were found in the male subgroup analysis. The vast majority of females included in our study were non-smokers. Therefore, we speculated that *TXNRD1* may be associated with ATDH susceptibility in non-smokers. Thus, we further analyzed the *TXNRD1* data stratified by smoking status. There was a statistically significant difference in the non-smokers, but not in the smokers. The TCAGCC haplotype exhibited a risk of ATDH susceptibility in the non-smoking subgroup. Our results suggest that *TXNRD1* variants may favor ATDH susceptibility in female non-smokers. Previous studies have shown that ATDH is more common among females (Lee et al., 2002; Marra et al., 2007). Kwon et al. (2012) found that there were associations between the genetic variations of *TXNRD1* and DILI. Other studies have shown that *TXNRD1*-mediated antioxidant function protects the liver against oxidative injury (Minami et al., 2005; Zidek et al., 2007). Thus, our results are in accordance with previous findings from the literature.

The strengths of our study are as follows. First, this is the first study on the association between *TXNRD1* SNPs and ATDH susceptibility. The TCAGCC haplotype showed an increased risk of susceptible to ATDH. This result provides evidence of the association between *TXNRD1* SNPs and ATDH susceptibility in the Chinese Han population. Furthermore, there was a different correlation in the female subgroup. Therefore, it can be speculated that there is an association between *TXNRD1* and non-smoking. Almost all the female subjects were non-smokers. The statistically significant difference is more obvious in the non-smokers than in the female subgroup analysis. Therefore, there may be a female non-smoking effect, with non-smoking having a greater influence. Second, our study was a prospective study and was less affected by certain biases such as recall bias.

The limitations of our study are as follows. First, the study population was limited to Chinese Han subjects. Relevant research that includes other nationalities is needed to fully explore the association between *TXNRD1* SNPs and ATDH. Second, the sample size was small, particularly the ATDH group, partially owing to the strict inclusion criteria. Thus, larger sample studies should be performed to verify the findings.

In conclusion, our results indicated that the TCAGCC haplotype is associated with increased risk of ATDH susceptibility. Our study is the first prospective study of *TXNRD1* SNPs and ATDH susceptibility. The results suggest that *TXNRD1* variants may favor the pathogenesis of ATDH in Chinese Han individuals. Large-scale studies on different ethnic populations are required to verify the findings.

Conflicts of interest

The authors declare no conflict of interest.

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