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Association of ABCB6 Polymorphisms and Serum Levels with Susceptibility to Antituberculosis Drug-induced Hepatotoxicity

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ABSTRACT

Background & Aims: The occurrence of anti-tuberculosis drug-induced hepatotoxicity (ATDH) deserves our attention. To explore the role of ATP-binding cassette B6 (ABCB6) polymorphisms and serum levels in the risk of antituberculosis drug-induced hepatotoxicity (ATDH).
Methods: A 1:4 matched case–control study was conducted. Four single nucleotide polymorphisms (SNPs) and serum ABCB6 levels were analyzed, and false discovery rate (FDR) was performed to correct for multiple comparison.
Results: 224 ATDH cases and 896 controls were included. SNP rs1109867 in *ABCB6* gene had a decreased risk of ATDH under dominant models (FDR corrected p-value = 0.027). Furthermore, serum *ABCB6* levels at three different times in the ATDH cases were higher than those in the controls.
Conclusions: SNP rs1109867 in *ABCB6* gene may contribute to the susceptibility to ATDH among patients receiving anti-TB treatment.

Key words: antituberculosis drug-induced hepatotoxicity – ATP-binding cassette B6 – ABCB6 – genetic polymorphisms – case – control study.

Abbreviations: ABCB6: ATP-binding cassette B6; ALAS1: aminolevulinic acid synthase-1; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ATDH: anti-tuberculosis drug-induced hepatotoxicity; CI: confidence interval; CYP450: cytochrome P450; DBil: direct bilirubin; ELISA: enzyme-linked immunosorbent assay; EMB: ethambutol; FDR: false discovery rate; FECH: ferrochelatase; HWE: Hardy–Weinberg equilibrium; INH: isoniazid; IQRs: interquartile ranges; MAF: minor allele frequency; OD: optical density; ORs: odds ratios; PPIX: protoporphyrin IX; PZA: pyrazinamide; RIF: rifampin; TB: tuberculosis; TBil: total bilirubin; TFB: transcription factor binding; ULN: upper limit of normal; SCZ: schizophrenia.

INTRODUCTION

Tuberculosis (TB), caused by the bacillus *Mycobacterium tuberculosis*, has been a persistent global health challenge throughout history. It was the world's second leading cause of death from a single infectious agent, after coronavirus disease (COVID-19), and caused almost twice as many deaths as HIV/AIDS in 2022 [1]. Worldwide, an estimated 10.6 million (95% UI: 9.9-11.4 million) cases emerged, with China occupying a prominent third spot behind India and Indonesia, contributing 7.1% of the world's TB burden [1]. To curb TB dissemination, timely and effective anti-TB treatment is paramount. First-line anti-TB drugs, isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and ethambutol (EMB) formulate a 2-month intensive phase, followed by a 4-month continuation with INH and RIF [2, 3]. This scheme has a positive therapeutic effect and is well tolerated by the great majority of patients. Nonetheless, these drugs elicit a spectrum of adverse reactions; one of the well-known toxic effects is hepatotoxicity, also called anti-TB drug-induced hepatotoxicity (ATDH) [4]. The development of ATDH may affect treatment adherence, prompt interruptions, and foster relapse, drug resistance, and therapeutic failure [5]. The incidence of ATDH ranges from 2-28% during standard TB treatment [6, 7], based on the study populations, various definitions, and treatment regimens. ATDH has the potential to lead to liver failure and death, therefore, the mechanism and predisposing factors of

ATDH must be revealed to mitigate liver injury and complete the anti-TB treatment smoothly.

Anti-TB drug-induced hepatotoxicity emergence intertwines with multiple factors, with a recent focus on gene polymorphisms modulating protoporphyrin IX (PPIX) metabolism [8-12]. PPIX is a heterocyclic organic compound consisting of four pyrrole rings, and is the final intermediate in the haem biosynthetic pathway, and its homeostasis is tightly regulated during haem synthesis. PPIX imbalance sparks hepatic damage, as PPIX buildup in human porphyrias triggers skin photosensitivity, biliary stones, hepatobiliary injury, and including, liver failure [13]. Murine studies have revealed that INH/RIF combined treatments downregulated bile acid transporters, fostering PPIX/bile acids accumulation, liver dysfunction, and ATDH onset [14]. Furthermore, ferrochelatase (FECH) inhibition and aminolevulinic acid synthase-1 (ALAS1) induction might synergistically augment PPIX accumulation, exacerbating INH/RIF-mediated liver injury [8]. Since the INH/RIF combination is generally applied during the entire course of initial anti-TB treatment as a standard strategy, the prospect of ATDH is unavoidable.

ATP-binding cassette (ABC) transporters were vital in diverse bioprocesses, such as haem biosynthesis, iron-sulfur cluster formation, antigen presentation, and insulin secretion [15]. The ATP-binding cassette B6 (ABCB6), also known as MTABC3 and P-glycoprotein related protein is a homodimeric ABC B subfamily transporter containing 842 amino acid residues and 11 transmembrane helices [16] and functions as a mitochondrial porphyrin transporter. Some xenobiotics and/or their metabolites inhibit FECH and cause PPIX accumulation, while certain xenobiotics require cytochrome P450 (CYP450) to produce metabolites that are FECH inhibitors [13]. It has been shown that ABCB6 deficiency suppresses CYP450 expression in mouse and human hepatocytes [17]. Furthermore, ABCB6 upregulation triggers p53 activation via PPIX, facilitated by its p53 response element, and elevates cellular PPIX levels upon overexpression [18]. ABCB6 transports coproporphyrinogen III, a PPIX precursor, to mitochondria [19]. Therefore, genetic variations in ABCB6 can modulate mitochondrial PPIX levels. ABCB6 may regulate the haem biosynthetic pathway together with the plasma membrane porphyrin transporter ABCG2 under normal physiological and pathological conditions [18]. All of these researches suggested that ABCB6 is closely related to the anabolism of PPIX. Hence, ABCB6 polymorphisms may modulate PPIX synthesis and accumulation, which in turn affects the susceptibility to ATDH.

For further exploration, an individual matched case-control study was conducted to explore the susceptibility of ABCB6 genetic polymorphisms with the risk of ATDH using the cohort of anti-TB treatment patients founded and followed according to the home-based anti-TB treatment adverse reactions study [20] in the Chinese Han population of Jiangsu Province.

METHODS

Study Population

All anti-TB treatment patients who were newly diagnosed as active pulmonary TB were recruited from four hospitals from Jiangsu Province from May 2017 to July 2021. All patients received the standard anti-TB treatment according to the World Health Organization (WHO) guidelines [21], and signed the informed consent form before participating in the study. A combination of active self-recorded diaries and passive scheduled laboratory tests was used to enhance the identification of liver injury during the treatment. Records of patients have been described in detail in our previous article [22]. All TB patients were recruited from the Han population and followed up until the completion of treatment. Patients with abnormal alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), or total bilirubin (TBil) levels at baseline, as well as those with tumors, mental diseases, disabling diseases or other diseases that may cause liver dysfunction, were excluded from the present study. In addition, patients without sufficient residual blood samples for subsequent genotyping were also excluded.

Patients who exhibited abnormalities in liver function tests during anti-TB treatment were screened, and ATDH cases were defined as those with ALT >3 ULN (upper limit of normal), with/without TBil > 2 ULN or simultaneous increases AST, TBil and ALP, with at least one of these >2 ULN. The ULN values used in our study were 40 U/L for ALT, 40 U/L for AST, 130 U/L for ALP and 21 μmol/L for TBil, respectively. According to the criteria of the Chinese Society of Hepatology[23], the clinical patterns of hepatotoxicity were classified as hepatocellular (ALT ≥ 3 ULN and R ≥ 5), cholestatic (ALP ≥2 ULN and R ≤ 2) and hepatocellular-cholestatic mixed (ALT ≥ 3 ULN, ALP ≥ 2 ULN and 2 < R < 5). R = (actual ALT/ALT ULN)/(actual ALP/ALP ULN). Additionally, based on the updated Roussel Uclaf Causality Assessment Method (RUCAM), only those judged to be highly probable (score of 9 or more), probable (6-8), and possible (3-5) [24] could be identified as ATDH cases.

Patients who fulfilled the criteria for ATDH were assigned to the case group, whereas those who did not were considered candidate controls. For each ATDH case, four controls were randomly selected and matched for age (within 5 years), gender, and treatment history.

Biochemical Tests and Blood Samples Collections

Before anti-TB treatment, designated hospitals usually ask patients to perform routine biochemical tests and recommend regular liver function tests. Post-test, the remaining blood sample was stored in a - 80 °C freezer by the laboratory physician. Our research team regularly visited four designated hospitals, retrieved liver function indices [including ALT, AST, ALP, TBil and direct bilirubin (DBil)] and treatment records of TB patients via hospital systems in Jiangsu province. Additionally, we administered an enrollment questionnaire and procured remaining blood samples for further laboratory analysis.

Candidate SNP Selection and Genotyping

The genetic polymorphism data of the full sequence of *ABCB6* were obtained by performing thorough searches on the dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/). Candidate SNPs located in *ABCB6* genomic regions were selected using the Haploview 4.2 (Broad Institute, Cambridge, MA, USA) software based on the following

criteria. First, the minor allele frequency (MAF) was greater than 10% in the Chinese Han population (CHB). Second, an r^2 for linkage disequilibrium was greater than 0.8. Third, SNPs related to the toxicity of other drugs already described in the literature were selected preferentially. Finally, four SNPs in the ABCB6 gene were selected for genotyping (Supplementary file, Table SI). Genomic DNA was extracted from peripheral blood samples using the FastPure ® DNA Blood Mini Kit (Vazyme, Nanjing, China). DNA samples were stored at -20^{0}C until detection. The quality and quantity of DNA were estimated using the Nanodrop 2000 UV-Vis Spectrophotometer (Thermo Fisher Scientific Inc., USA). All assays were performed in 5 μL reactions using TaqMan Genotyping Master-Mix on 96-well plates. The primers and probes for each SNP were shown in Supllementary file (Table SII). To ensure the quality of genotyping, technicians were blinded to the status of cases and controls, and more than 10% of the samples were genotyped in duplicate, achieving an accuracy rate of 100%.

Detection of Serum ABCB6 Concentrations

The serum samples were selected from three consecutive different periods: baseline, and two times after initial anti-TB treatment, and only patients with three serum samples among the above ATDH cases and controls were included in further serological analysis (an unmatched case–control study). An enzyme-linked immunosorbent assay (ELISA) kit (Mlbio, Shanghai) was used to detect the level of serum ABCB6 in both groups following the manufacturer's instructions. Briefly, 50 μl of the standard solution is accurately dispensed into ELISA plate wells, while test wells receive 40 μl diluent + 10 μl sample. Plate is sealed, incubated at 37°C for 30 min. Concurrently, a 30x wash solution is prepared and kept ready. Post-incubation, film is removed, wells are washed 5 times with wash solution, and plate is dried. Next, 50 μl of enzyme-conjugated reagent (excluding blank wells) is added, followed by re-incubation and washing. Chromogens A & B (50 μl each) are sequentially pipetted, mixed, and incubated at 37°C for 10 min in dark for color development. Finally, add 50 μl of stop solution to terminate the reaction. Zero is calibrated using blank control, and optical density (OD) at 450 nm is sequentially measured. For each sample, the concentration of ABCB6 was obtained according to a standard curve constructed using the standard density as the horizontal axis and the OD values as the vertical axis.

Statistical Analysis

The ATDH cases and the controls were compared using univariate analysis, in which continuous variables were examined using the student's t test or a nonparametric test, whereas categorical variables were tested using a conditional logistic regression model. Continuous variables were expressed as means ± standard deviations or medians with interquartile ranges (IQRs), whereas categorized variables were expressed as numbers and percentages. Hardy–Weinberg equilibrium (HWE) was tested for all the SNPs in controls subjects using a goodness-of-fit chi-square test. A multivariate conditional logistic regression model was chosen after adjusting for drinking history, smoking history, liver disease history and hepatoprotectant use to find the association between genotypes and the risk of ATDH. Odds ratios (ORs) and 95% confidence intervals (CIs) were used as measures of association. The effect of SNPs on the risk of ATDH was comprehensively evaluated based on allele and genotypic frequencies, as well as in the distributions of the dominant, recessive and additive genetic models. Subsequently, the potential correlations between ABCB6 SNPs and ATDH were examined in the subgroup of patients who presented with different clinical patterns of hepatotoxicity[25]. Furthermore, false discovery rate (FDR) methods were employed to perform correction for multiple tests. Finally, the difference in serum levels among different genotypes was explored using the Kruskal-Wallis H test. Besides, the Wilcoxon rank-sum test was used to analyze the differences in serum levels of each liver function index between the ATDH cases and the controls at the three time points, and the Mann-Kendall trend test method was used to analyze the time trend. The analyses were performed using R software (version 4.3.1). All p-values were two-tailed, and a p-value less than 0.05 were considered to indicate statistical significance.

Ethical Statement

Participant recruitment was approved by the Ethics Committee of Nanjing Medical University (Protocol No: [2018]579, Approval date: 22.02.2018). All investigations were conducted in accordance with the guidelines of the 1975 Declaration of Helsinki.

RESULTS

Clinical Characteristics of TB Patients

The present study encompassed 1120 TB patients (224 ATDH cases and 896 controls). Of the 224 ATDH cases, 73 cases (32.6%) were judged as possible, 124 (55.4%) as probable, and 27 (12.0%) as highly probable, according to the updated RUCAM. Furthermore, a total of 147 cases (65.6%) were classified as hepatocellular hepatotoxicity, 25 (11.2%) as cholestatic, and 52 (23.2%) as mixed. The baseline demographic and clinical characteristics of the ATDH cases and matched controls were detailed in Table I. There were no significant differences between the two groups regarding demographic parameters, including gender, age, hepatoprotectant use, drinking history, and baseline values of liver biochemical parameters (p>0.05). However, significant differences were observed in smoking and liver disease history, where the proportion of cases with a history of smoking or liver disease was higher than in the control group (p=0.041 and p=0.019, respectively). Furthermore, during treatment, the peak values of AST, ALT, ALP, TBil, and DBil were significantly greater in the ATDH cases compared to the controls (p < 0.0001).

Genotype Analysis

Four SNPs were selected and genotyped successfully for 99.9% of patients. For all SNPs, no difference in allele distributions was found between the ATDH cases and controls (all p>0.05, Supplementary file, Table SIII). However, patients carrying the GT/TT genotype of rs1109867 in the *ABCB6* gene had a decreased risk of ATDH (dominant model, adjusted OR=0.442, 95%CI: 0.237-0.826, p=0.010, Table II), and significant differences were also found after FDR correction (adjusted p=0.03). There was no statistical difference in the

Table I. Characteristics of patients in ATDH cases and controls

Characteristics	ATDH cases (N=224)	Controls (N=896)	p
Sex (male/female)	164/60	656/240	-
Age (year)[a]	50.1±17.7	50.7±18.2	0.656[c]
Hepatoprotectant (%)	115(51.3)	469(52.3)	0.756[d]
Liver diseases history (%)	49(21.9)	139(18.9)	0.019[d]
Smoking history (%)	29(12.9)	78(8.7)	0.04[d]
Drinking history (%)	10(4.5)	39(4.4)	0.941
Baseline value			
ALT (U/L)[b]	15.0(9.0-21.7)	13.7(9.0-20.0)	0.408[e]
AST (U/L)[b]	21.0(15.0-25.0)	20.0(16.0-24.0)	0.391[e]
TBil (μmol/L)[b]	10.1(7.9-12.9)	10.0(7.5-13.2)	0.816[e]
DBil (μmol/L)[b]	3.4(2.3-4.0)	3.3(2.3-4.4)	0.439[e]
ALP (U/L)[b]	83.0(67.4-90.0)	81.1(63.7-93.0)	0.220[e]
During treatment (peak value)			
ALT (U/L)[b]	173.0(132.5-278.8)	23.0(15.0-32.0)	<0.0001[e]
AST (U/L)[b]	119.0(70.5-200.0)	29.0(23.0-39.0)	<0.0001[e]
TBil (μmol/L)[b]	19.4(14.1-25.1)	14.0(10.5-19.0)	<0.0001[e]
DBil (μmol/L)[b]	7.3(5.1-10.9)	4.7(3.4-6.6)	<0.0001[e]
ALP (U/L)[b]	121.0(87.0-148.0)	91.1(76.0-114.8)	<0.0001[e]

ATDH: antituberculosis drug-induced hepatotoxicity; ALT: alanine transaminase; AST: aspartate transaminase; TBil: total bilirubin; DBil: direct bilirubin; ALP: alkaline phosphatase. Normal range: ALT < 40U/L; AST < 40U/L; TBil < 21μmol/L; DBil < 6.9μmol/L; ALP < 130U/L. [a] Values are presented as mean ± standard deviations. [b] Values are presented as median (interquartile range). [c] Independent-sample t-test. [d] Conditional logistic regression model analysis. [e] Wilcoxon rank-sum test.

distribution of other genotypes between the ATDH cases and controls.

Subgroup Analysis

Further subgroup analysis was conducted based on the clinical pattern of hepatotoxicity, and the results confirmed that patients carrying the GT/TT genotype of rs1109867 in the *ABCB6* gene had a decreased risk of hepatocellular hepatotoxicity (adjusted OR=0.362, 95%CI: 0.168-0.780, p=0.009, Table III), and the significant association remained after FDR correction (adjusted p=0.027).

Genetic Polymorphism and Liver Function Indicators

The correlations between the SNP rs1109867 and liver function indicators were further analyzed, and significant differences were observed in the peak value of TBil and ALP among patients with the three genotypes (p<0.001, p=0.002, respectively, Table IV). Subgroup analysis based on the clinical pattern of hepatotoxicity also confirmed similar findings. Patients with the GT genotype had the lowest serum TBil (median = 13.6, IQR: 10.2-17.8, p<0.001), and patients with the TT genotype had the lowest serum ALP (median = 88.0, IQR: 72.0-117.0, p=0.006) (Table IV).

Table II. Genotypes distribution of *ABCB6* gene in two groups and the risks of ATDH

SNPs	Groups	Subjects with genotype, N (%)			Dominant model		Recessive model		Additive model	
		00	01	11	OR (95% CI) [a]	p	OR (95% CI)[a]	p	OR (95% CI)[a]	p
rs1109866 (T>C)	Controls	654(73.0)	220(24.6)	22(2.4)	1.000		1.000		1.000	
	ATDH cases	162(72.3)	54(24.1)	8(3.6)	1.075(0.761-1.517)	0.682	1.518(0.663-3.477)	0.323	1.104(0.826-1.474)	0.504
rs1109867 (G>T)	Controls	97(10.8)	393(43.9)	406(45.3)	1.000		1.000		1.000	
	ATDH cases	35(15.6)	87(38.8)	102(45.5)	0.442(0.237-0.826)	0.010#	1.057(0.580-1.926)	0.857	0.720(0.476-1.087)	0.118
rs3731885 (G>A)	Controls	647(72.2)	185(20.6)	64(7.1)	1.000		1.000		1.000	
	ATDH cases	160(71.4)	43(19.2)	21(9.4)	1.056(0.752-1.483)	0.753	1.371(0.811-2.319)	0.239	1.096(0.861-1.395)	0.457
rs3755047 (G>A)	Controls	669(74.7)	220(24.6)	7(0.7)	1.000		1.000		1.000	
	ATDH cases	181(80.8)	41(18.3)	2(9.0)	0.759(0.527-1.094)	0.139	0.988(0.375-2.607)	0.981	0.814(0.595-1.115)	0.199

ABCB6: ATP-binding cassette B6; ATDH: antituberculosis drug-induced hepatotoxicity; SNP: single nucleotide polymorphism; OR: odds ratio; 95%CI: 95% confidence interval; 0 = common allele; 1 = minor allele. [a] Conditional logistic regression model analysis and adjusted for smoking history, drinking history, liver disease history and hepatoprotectant use. #FDR-corrected p-values (p-value = 0.030).

Table III. Subgroup analysis among different clinical patterns of hepatotoxicity with different genetic models and the risks of ATDH

SNPs	Model	Hepatocellular		Cholestatic		Mixed	
		OR (95% CI)[a]	p[a]	OR (95% CI)[a]	p[a]	OR (95% CI)[a]	p[a]
rs1109866(T>C)							
TT	Dom	1.054(0.684-1.623)	0.813	0.621(0.187-2.055)	0.435	1.269(0.644-2.502)	0.491
CT	Rec	2.420(0.922-6.354)	0.073	-	0.999	0.386(0.045-3.343)	0.388
CC	Add	1.155(0.808-1.651)	0.429	0.612(0.192-1.952)	0.406	1.078(0.611-1.900)	0.798
rs1109867(G>T)							
GG	Dom	0.362(0.168-0.780)	0.009[#]	0.936(0.054-16.160)	0.964	0.580(0.165-2.042)	0.396
GT	Rec	1.115(0.496-2.507)	0.792	0.737(0.199-2.732)	0.648	1.379(0.398-4.775)	0.612
TT	Add	0.641(0.376-1.093)	0.102	0.789(0.258-2.408)	0.677	0.898(0.381-2.118)	0.806
rs3731885(G>A)							
GG	Dom	1.002(0.657-1.528)	0.991	0.784(0.245-2.508)	0.682	1.437(0.724-2.851)	0.300
GA	Rec	1.510(0.792-2.878)	0.210	1.376(0.183-10.378)	0.757	1.114(0.389-3.188)	0.841
AA	Add	1.089(0.806-1.472)	0.578	0.930(0.407-2.126)	0.863	1.219(0.760-1.955)	0.412
rs3755047(G>A)							
GG	Dom	0.755(0.476-1.197)	0.231	0.597(0.182-1.965)	0.396	0.867(0.421-1.788)	0.700
GA	Rec	2.082(0.619-7.007)	0.236	-	0.999	0.422(0.071-2.518)	0.344
AA	Add	0.860(0.574-1.288)	0.465	0.570(0.191-1.698)	0.313	0.823(0.458-1.479)	0.514

For abbreviations see Table II. Dom, dominant model; Rec, recessive model; Add, additive model. [a] Conditional logistic regression model analysis and adjusted for smoking history, drinking history, liver disease history and hepatoprotectant use. [#] DR-corrected p-values (p-value = 0.027).

Serum ABCB6 Levels Between ATDH Cases and Controls

Serum ABCB6 levels were measured in 250 subjects (including 58 ATDH cases and 192 controls, the baseline characteristics were showed in Table SIV) at three different time points to further investigate the correlation between ABCB6 serum levels and the risk of ATDH. There was no statistical difference in the time of two tests after initial treatment between the ATDH cases and the controls (p=0.605, p=0.756, respectively, Table V). The average ABCB6 levels at three time points in the ATDH cases were higher than those in the controls (258.7 pg/ml, 275.8 pg/ml, 335.0 pg/ml in the ATDH cases; 235.1 pg/ml, 234.6 pg/ml, 235.6 pg/ml in the controls, respectively), and the corresponding statistical test p-values were 0.021, <0.001, <0.001, respectively, Table V). However, the increasing trend of the serum ABCB6 levels in the ATDH cases was not statistically significant (p=0.296, Fig. 1). Additionally, at the third liver function test in the ATDH cases, patients with GG genotypes of SNP rs1109867 had higher

Table IV. Distribution of serum liver function at the peak value of SNP rs1109867 in ABCB6 gene under different clinical patterns of hepatotoxicity

Serum liver function	SNP rs1109867 genotypes	All patients		Hepatocellular		Cholestatic		Mixed	
		Median (IQR)	p*	Median (IQR)	p*	Median (IQR)	p*	Median (IQR)	p*
ALT	GG	30.0(15.0-126.5)	0.306	30.6(15.8-158.5)	0.220	24.5(12.2-43.0)	0.784	27.0(15.0-46.5)	0.941
	GT	25.0(17.0-41.3)		25.0(16.9-42.0)		23.2(15.8-34.2)		26.0(18.8-47.0)	
	TT	27.0(16.8-82.5)		27.0(18.0-85.0)		24.0(14.50-38.5)		26.0(15.5-65.5)	
AST	GG	32.0(23.1-72.8)	0.917	32.0(23.0-92.5)	0.387	35.5(23.3-61.3)	0.578	31.0(25.7-50.5)	0.427
	GT	32.0(25.0-46.4)		31.2(24.0-44.7)		30.2(24.5-41.0)		34.0(27.0-53.6)	
	TT	32.0(24.0-63.0)		33.0(24.0-70.0)		29.0(20.0-52.0)		31.0(24.0-60.5)	
TBil	GG	14.5(10.2-19.7)	<0.001	13.7(10.0-20.3)	<0.001	15.6(12.0-19. 5)	0.482	15.4(10.8-17.4)	0.649
	GT	13.9(10.5-19.3)		13.6(10.2-17.8)		12.8(12.0-19.5)		15.1(11.2-20.5)	
	TT	16.1(12.1-21.4)		16.3(12.3-21.8)		15.6(11.1-20.0)		15.4(11.8-21.1)	
DBil	GG	5.7(4.0-7.6)	0.274	5.7(4.0-7.9)	0.394	6.2(4.4-10.1)	0.533	5.4(3.5-7.2)	0.346
	GT	5.1(3.5-7.5)		5.0(3.5-7.2)		5.2(3.6-7.0)		5.6(3.6-8.0)	
	TT	5.0(3.4-7.0)		5.0(3.5-7.3)		4.7(3.3-4.7)		5.0(3.8-6.7)	
ALP	GG	101.9(83.1-123.3)	0.002	99.5(83.4-122.0)	0.006	93.0(82.7-148.8)	0.728	109.3(82.9-123.8)	0.134
	GT	97.5(81.0-119.6)		96.0(79.3-115.8)		103.6(87.1-158.5)		99.1(81.8-129.0)	
	TT	90.0(73.0-121.0)		88.0(72.0-117.0)		113.0(84.0-132.5)		89.0(77.0-117.5)	

For abbreviations see Tables I and II. IQR, interquartile range. For normal values see Table I. *Kruskal-Wallis H test.

Table V. Differences in serum ABCB6 levels between the two groups

ABCB6 level	ATDH cases (n=58)	Controls (n=192)	p*
Days from baseline test to first test after anti-TB treatment (days)[a]	25.0(12.3-48.0)	23.5(10.0-38.5)	0.605
Days from baseline test to second test after anti-TB treatment (days)[a]	63.0(33.5-114.5)	62.0(34.0-105.5)	0.756
Baseline ABCB6 level (pg/ml)[a]	258.7(222.5-326.5)	235.1(195.6-295.6)	0.021
ABCB6 level at first test after anti-TB treatment (pg/ml)[a]	275.8(247.3-371.9)	234.6(198.6-302.2)	<0.001
ABCB6 level at second test after anti-TB treatment (pg/ml)[a]	335.0(275.4-478.4)	235.6(190.5-303.5)	<0.001

For abreviations see Table II. [a] Values are represented by the median (interquartile range). * Wilcoxon rank-sum test.

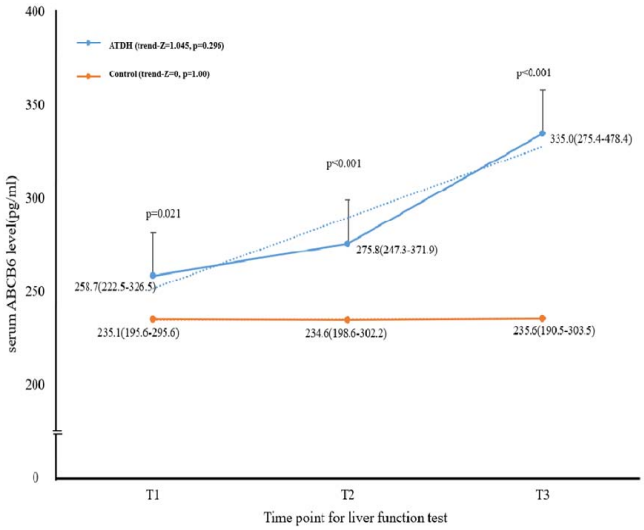

Fig. 1. Changes in serum ABCB6 level at three time points for liver function test in two groups. (The results are expressed as median (IQR). Statistically significant difference ($p < 0.05$) within each group at different time points. In ATDH cases, T1: the initial of anti-tuberculosis treatment (baseline); T2: the first occurrence of abnormal liver function test; T3: the peak of liver function test. In controls, T1: the initial of anti-tuberculosis treatment (baseline); T2 and T3: The second and third routine liver function tests, respectively). (ABCB6, ATP-binding cassette B6; ATDH, antituberculosis drug-induced hepatotoxicity).

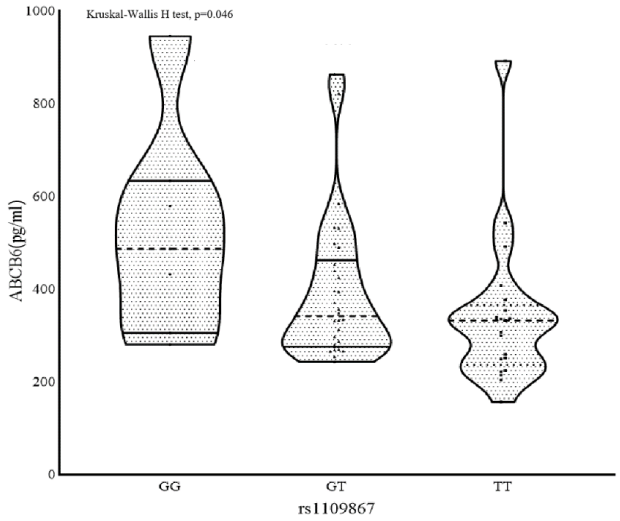

Fig. 2. Violin plot of serum levels of ABCB6 in the different rs1109867 genotypes at the peak of liver function test in ATDH cases. (ABCB6, ATP-binding cassette B6; ATDH, antituberculosis drug-induced hepatotoxicity).

serum ABCB6 levels than those with other two genotypes (GG vs. GT vs. TT: 486.76 pg/ml vs. 341.41 pg/ml vs. 332.58 pg/ml, p=0.046, respectively, Fig. 2).

DISCUSSION

A case-control study was performed to investigate the association between *ABCB6* gene polymorphisms, serum ABCB6 levels and ATDH. Our results showed that patients carrying the GT/TT genotype of rs1109867 in the *ABCB6* gene had a decreased risk of ATDH (dominant model, adjusted OR=0.442, 95%CI: 0.237-0.826, p=0.010); significant differences were also found in patients with hepatocellular hepatotoxicity (adjusted OR=0.362, 95%CI: 0.168-0.780, p=0.009), and these differences persisted after FDR correction. Besides, significant differences were observed in the peak values of TBil and ALP among patients with different rs1109867 genotypes. Furthermore, serum ABCB6 levels varied significantly between different rs1109867 genotypes. To date, this study is the first to explore the relationships between ABCB6 gene polymorphisms, serum ABCB6 levels, and the risk of ATDH. The results indicated that the genetic variant (SNP rs1109867) in the *ABCB6* gene may decrease the susceptibility to ATDH.

The human *ABCB6* gene on chromosome 2q35 comprises 19 exons, spanning approximately 9187 bases (https://www.ncbi.nlm.nih.gov/gene). The protein encoded by this gene is a member of the heavy metal importer subfamily and plays a role in porphyrin transport. ABC transporters, omnipresent in humans, orchestrate vital substrate transfer, defend against toxins, balance fluids, and eliminate waste [26]. Dysregulation and genetic mutations of ABC transporters elicit diverse pathologies, such as oncogenic multidrug resistance or cystic fibrosis [26]. The ABCB6 gene serves as the molecular basis for the Langereis (Lan) blood group antigen and may be related to many genetic diseases such as familial pseudohyperkalemia [27] and dyschromatosis universalis hereditarian [28]. The research uncovered an

open, hydrophobic, positively charged niche in ABCB6 that enhances porphyrin attachment, exemplified by PPIX, a tetrapyrrole-based substrate with anionic carboxylates [29, 30]. Contrarily, a separate investigation failed to link ABCB6 genotype with acute hepatic or erythropoietic porphyria severity, disputing its role as a PPIX exporter [31]. Few studies have been reported on gene polymorphisms of *ABCB6*, whether it is rs1109867 or other SNPs. A case-control study has investigated whether the SNPs, including rs1109867, in *ABCB6* contribute to the risk of schizophrenia (SCZ) in a Han Chinese population; however, no association between SCZ and the SNP rs1109867 of ABCB6 was observed (p = 0.38)[32]. Analysis of Encyclopedia of DNA Elements data as implemented in the online tool RegulomeDB (http://regulomedb.org/) indicated that rs1109867 may influence the transcription factor binding (TFB) site and deoxyribonuclease (DNase) peak. The SNP rs1109867 is located in the functional regions of promoter histone marks and enhancer histone marks, DNase, proteins bound and may change the motifs of TFB according to the HaploReg database (http://pubs.broadinstitute.org/mammals/haploreg/ haploreg.php). Furthermore, the RNAfold Web Server (http://rna.tbi.univie.ac.at//cgi-bin/RNAWebSuite/RNAfold.cgi), applied to detect mRNA secondary structures, showed that there is a difference in the lowest free energy between the rs1109867-G and -T allele (−44.0 kcal/mol vs. −43.90 kcal/mol). The local structural changes were shown clearly in Supplementary file, Fig. S1, where structural alterations may affect the function.

The previous study has shown that ABCB6 may influence the expression and activity of CYP450 in the liver [17], and meta-analysis has uncovered an association between CYP450 polymorphisms and the risk of ATDH [33]. In addition, there are studies showing that the *ABCB6* gene could be identified as a prognosis and therapy marker for hepatocellular carcinoma patients [34, 35]. The present study also indicated that both *ABCB6* gene polymorphism and serum ABCB6 levels may affect the development of ATDH. However, the findings from the eQTL online database contradicted the results of this study. Online eQTL analysis was conducted using the GTEx portal, examining gene expression. The results showed no significant differences in the expression levels of the three genotypes of SNP rs1109867 among the 208 liver samples (p=0.71). This may be because eQTL analysis was based on the detection of gene expression at the transcription level (mRNA expression level), while this study was based on serum protein levels at the translational level. Due to the multi-level and complexity of gene expression regulation, these two levels may not be completely consistent[36]. Moreover, the detection time during the anti-TB treatment also plays a certain role. Specifically, when the liver function indicators reach their peak, the serum ABCB6 protein also attain a high level, whereas the mRNA level may have already been degraded. This results in a high protein level accompanied by a low gene expression level [37, 38]. Therefore, the precise biological significance of SNP rs1109867 in the ABCB6 gene requires further clarification through functional studies.

The present study was the first to uncover the relationship between ABCB6 genetic polymorphisms, serum ABCB6 levels and ATDH in China, with a relatively large sample size. Each ATDH case was individually matched and adjusted for the covariates, minimizing potential confounding factors that may be related to ATDH. FDR correction was used for multiple comparisons, reducing the false-positive results. However, there were some limitations that cannot be ignored. The sample size for ABCB6 serological analysis was relatively small, because this study requires three consecutive serum samples, including baseline and two after initial treatment, so that the changing trend of ABCB6 can be observed. Additionally, patients from only four hospitals in Jiangsu province were included, which may not be representative of the entire Chinese Han population.

In summary, based on this case-control study, genetic polymorphisms of rs1109867 in the ABCB6 may contribute to the susceptibility to ATDH, and the serum ABCB6 levels in ATDH cases during anti-TB treatment were significantly higher than those in controls. However, further studies in larger and more diverse populations are necessary to validate these relationships.

CONCLUSION

This study is the first to explore the relationship between ABCB6 gene polymorphisms and serum levels and ATDH. Based on the 1:4 matched case-control study, the SNP rs1109867 in the *ABCB6* gene may contribute to susceptibility to ATDH among Eastern Chinese Han patients. Larger studies are needed to validate these findings.

Conflicts of interest: None to declare.

Authors' contribution: R.C. contributed to the conception, design, data collection, analysis and drafting the article. J.C. contributed to the conception, design, analysis, data interpretation. H.P., L.L., X.H., M.Z., and H.Y. helped in the data acquisition and laboratory analysis. S.T. critically revised the manuscript and approved the final version of the manuscript. All authors approved the final version to be published and agreed to be accountable for all aspects of the work

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