Received: 22 May 2020 | Revised: 16 September 2020 | Accepted: 24 September 2020

DOI: 10.1002/jcla.23630

RESEARCH ARTICLE

WILEY

Collagen type XVIII alpha 1 chain (*COL18A1*) variants affect the risk of anti-tuberculosis drug-induced hepatotoxicity: A prospective study

Yuhui Cheng[1] | Lin Jiao[1,2] | Weixiu Li[1] | Jialing Wang[1] | Zhangyu Lin[1] | Hongli Lai[1] | Binwu Ying[1,2]

[1]West China School of Medicine, Sichuan University, Chengdu, China

[2]Department of Laboratory Medicine, West China Hospital, Sichuan University, Chengdu, China

Correspondence
Binwu Ying, No. 37, Guoxue Alley, Chengdu, Sichuan 610041, China.
Email: docbwy@126.com

Funding information
This work was supported by the National Science & Technology Pillar Program during the 13th Five-year Plan Period [Grant 2018ZX10715003].

Abstract

Background: The role of collagen type XVIII alpha 1 chain (*COL18A1*) in anti-tuberculosis drug-induced hepatotoxicity (ATDH) has not been reported. This study aimed to explore the association between of *COL18A1* variants and ATDH susceptibility.

Methods: A total of 746 patients were enrolled in our study from December 2016 to April 2018, and all subjects in the study signed an informed consent form. The custom-by-design 2x48-Plex SNPscanTM kit was used to genotype all selected 11 SNPs. Categorical variables were compared by chi-square (χ^2) or Fisher's exact test, while continuous variables were compared by Mann-Whitney's U test. Plink was utilized to analyze allelic and genotypic frequencies, and genetic models. Multivariate logistic regression analyses were used to adjust potential factors. The odds ratios (ORs) with corresponding 95% confidence intervals (CIs) were also calculated.

Results: Among patients with successfully genotyping, there were 114 cases and 612 controls. The mutant A allele of rs12483377 conferred the decreased risk of ATDH (OR = 0.13, 95%CI: 0.02–0.98, $P = 0.020$), and this significance still existed after adjusting age and gender ($P = 0.024$). The mutant homozygote AA genotype of rs12483377 was associated with decreased total protein levels ($P = 0.018$).

Conclusion: Our study first revealed that the A allele of *COL18A1* rs12483377 was associated with the decreased risk of ATDH in the Western Chinese Han population, providing new perspective for the molecular prediction, precise diagnosis, and individual treatment of ATDH.

KEYWORDS

anti-tuberculosis drug-induced hepatotoxicity, *COL18A1*, genetic polymorphisms, susceptibility

Yuhui Cheng and Lin Jiao, contributed equally to this work.

1 | INTRODUCTION

Anti-tuberculosis drug-induced hepatotoxicity (ATDH) is the most serious adverse drug reaction during the course of tuberculosis (TB) therapy.[1] ATDH is defined as the inflammation of hepatocytes caused by idiosyncratic reaction to the anti-TB drugs.[2] The following 4 mechanisms are considered as the pathogenesis of ATDH: I) the enzymes and pathways about drug metabolizing, such as glutathione S-transferase (GST) and *N*-acetyl transferase 2 (NAT2); II) the accumulation of bile acids, lipids, and heme metabolites; III) the toxicity mediated by immune system; IV) the increasing oxidant stress.[3-6] It is noted that ATDH can be curable in the early stage,[7] although this disease has high mortality (22.7%) and morbidity (28%) [8-10] and adverse effects on the anti-TB treatment efficiency.[11] However, the ambiguous diagnostic criteria and atypical symptoms interfere with early prediction and diagnosis of ATDH. Even worse, the delayed diagnosis of ATDH aggravates the severity of the disease and increases the disease burden.[12,13] Clearly, it is urgent to explore new biomarkers for diagnosis of ATDH. With the development of molecular detection methods, genetic factors are gradually well-recognized and considered as the crucial elements in the pathogenesis, prediction, diagnosis and treatment of many diseases.[14-16] Nowadays, a growing body of evidence proves that single nucleotide polymorphisms (SNPs), such as pregnane X receptor (*PXR*) rs7643645[17,18] and phase I cytochrome P450 enzyme (*CYP2E1*),[8] play important roles in the prediction, diagnosis, and treatment of ATDH. However, these SNPs are not applied in clinic due to limited predictive capacity or reliability. Thus, there is a promising future for exploring the association between more meaningful SNPs and ATDH to achieve precise prediction and treatment of ATDH.

Collagen type XVIII alpha 1 chain (*COL18A1*) is located on chromosome 21q22.3, encoding the alpha XVIII collagen. The product of alpha XVIII collagen, endostatin (EST), is mainly present in the liver sinusoidal and basement.[19] The close relationship between EST and liver diseases has been reported in many studies.[20-22] Many researchers find that EST can initiate the nicotinamide adenine dinucleotide phosphate oxidase (NOX) redox signaling cascade.[20,23,24] While the activation of NOX can generate reactive oxygen species (ROS) to increase oxidant stress which is one of the pathogenesis of ATDH as described before, and thus leads to the exacerbation of liver injury.[4,23,25] Moreover, Wnt/β-catenin signaling directs multiple liver cell processes and it is the essential signal for protecting hepatocyte from oxidative stress-induced cell deaths.[26] Moreover, it is has been published that EST can inhibit Wnt/β-catenin signaling through promoting the degradation of β-catenin.[27] Hence, we speculated that *COL18A1* plays a role in ATDH by involving in the Wnt/β-catenin signaling, oxidative stress, and other various ways.[28-30]

It is a pity that few studies have paid attention to explore the correlation between *COL18A1* and ATDH. Regarding the high burden of ATDH in Western China,[31] we conducted this prospective study to evaluate the association between *COL18A1* polymorphisms and the risk of ATDH in the Western Chinese Han population for the first time. We aimed to explore novel targets for the pathogenesis and personal treatment of ATDH patients.

2 | METHODS

2.1 | Study population

In this prospective study, 746 subjects were recruited from the West China Hospital of Sichuan University from December 2016 to April 2018 consecutively. All enrolled patients in the study were unrelated Han ethnicity.

The study was approved by the Ethics Committee of West China Hospital of Sichuan University (Reference No. 198; 2014), and written informed consents were obtained from all patients.

2.2 | Inclusion criteria and exclusion criteria

All recruited patients must meet the following criteria: I) Patients were newly diagnosed as TB patients by 2 experienced respiratory physicians based on National Institute for Heath and Clinical Excellence (NICE) guidelines [NG33]: Tuberculosis [32]; II) patients should have normal liver function before the anti-TB treatment. If patients who had (a) immunodeficiency diseases such as HIV; (b) liver dysfunction such as hepatitis B or C infection, fatty liver; (c) received other hepatotoxic drugs; (d) renal dysfunction; (e) other lung or liver disorders such as lung cancer and cirrhosis would be excluded.

Included subjects would be treated with the WHO standard 6-months anti-TB treatment regimens, consisting of isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), and ethambutol (EMB). Besides, enrolled subjects did not take any other drugs which would cause liver damage.

During the anti-TB therapy, patients would be tested liver function regularly to monitor their liver function and the baseline levels of laboratory indicators before anti-TB treatment were tested. ATDH was defined as follows [33]: (a) an increase in alanine aminotransferase (ALT) levels more than 2 times upper limit of normal (ULN); (b) an increase in ALT 2 times upper ULN combined a rise in aspartate aminotransferase (AST) or total bilirubin (TB) levels.

2.3 | SNP selection

The genetic data of *COL18A1* were obtained from 1000 Genomes database. All SNPs should meet the criteria that the minor allele frequency (MAF) was greater than 0.02. The tag SNPs selected by Haploview (version 4.1), the locations of SNPs and relevant reports[34-41] also needed to be considered. Eventually, 11 SNPs (rs2236455, rs114220916, rs9980080, rs2236467, rs13048803, rs2838942, rs9980525, rs3753019, rs2236483, rs12483377, rs7867) were chosen.

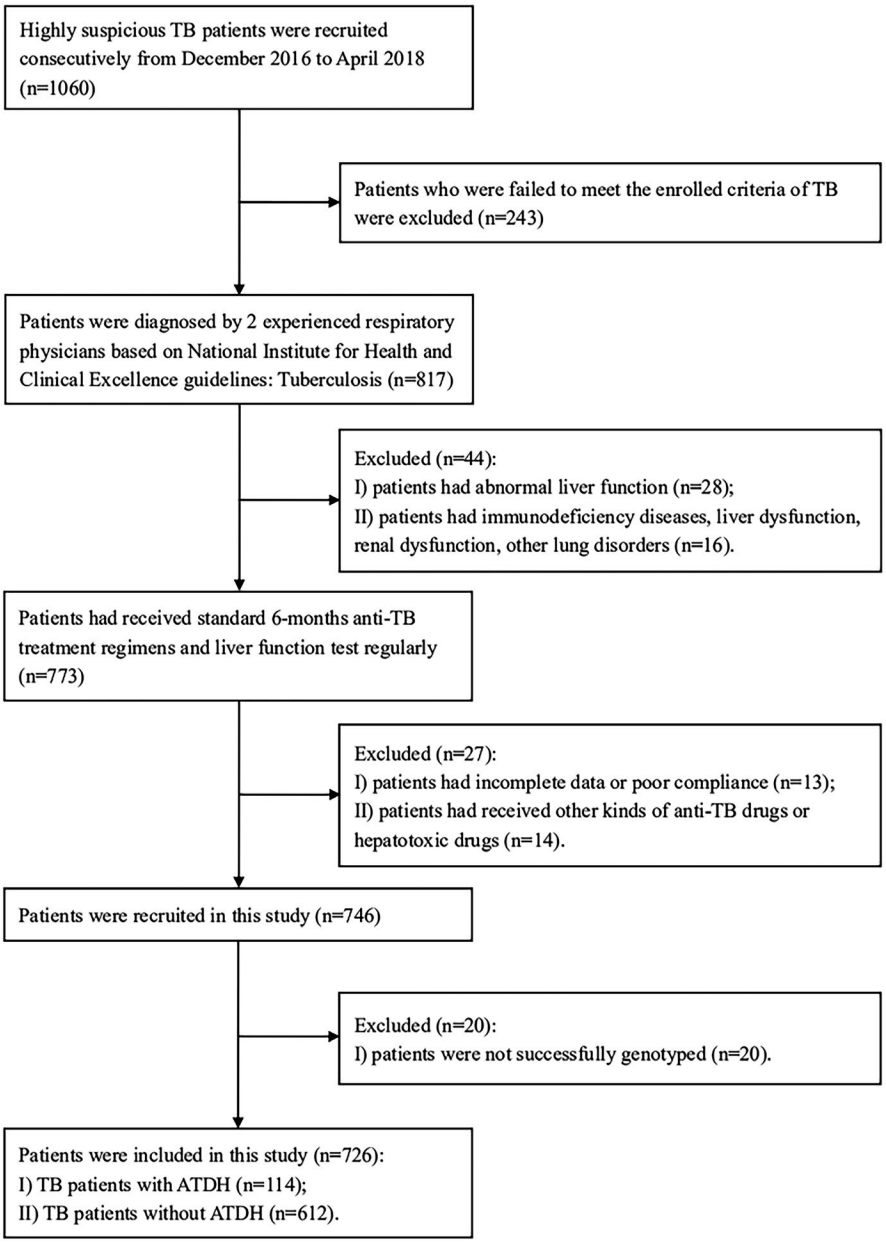

FIGURE 1 Flow diagram of selection of patients enrolled. Abbreviations: TB: tuberculosis; ATDH: anti-tuberculosis drug-induced hepatotoxicity

2.4 | Genotyping

Peripheral whole blood specimens were collected from each enrolled patient. All these samples were used to extract genomic deoxyribonucleic acid (DNA) via QLAamp® DNA Blood Mini Kit (Qiagen, Germany). Then, the custom-by-design 2x48-Plex SNPscanTM kit (Genesky Biotechnologies Inc, Shanghai, China) was utilized for genotyping all SNPs. All processes were carried out in accordance with the instructions.

2.5 | Statistical analysis

Categorical variables such as gender and drinking statuses were compared by chi-square (χ^2) or Fisher's exact test, whereas continuous variables such as age and serum ALT levels were compared by Mann-Whitney's U test. The Hardy-Weinberg equilibrium (HWE), allelic and genotypic frequencies, and genetic models (addictive, dominant and recessive model) were all performed by Plink (version 1.07). Multivariate logistic regression analyses were used to adjust potential impact factors via SPSS (IBM, Chicago, IL, USA; Version 22.0). Linkage disequilibrium (LD) and haplotype association were analyzed by both Haploview (The Broad Institute, Cambridge, MA, USA; Version 4.1) and online tool SNPstats (https://www.snpstats.net/preproc.php). Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for correlations. *P* valve ≤ 0.05 was considered to be statistically significant. Statistical Power was calculated by Power and Sample Size Program software. Ordinary one-way ANOVA was conducted by GraphPad Prism (version 8.0).

3 | RESULTS

3.1 | Study characteristics

A total of 746 TB patients were included in our study, while 726 patients were successfully genotyped with all selected SNPs (Figure 1). The incidence rate of ATDH was 15.70% (114/726). No differences in age ($P = 0.240$) and gender ($P = 0.752$) were found between the cases and the controls. Significant differences in the incidence of fever ($P = 0.007$), weight loss ($P = 0.036$), total bilirubin levels ($P = 0.003$), serum ALT levels ($P < 0.001$), serum AST levels ($P < 0.001$), uric acid levels ($P = 0.019$), alkaline phosphatase (ALP) levels ($P = 0.010$), and gamma glutamyl transpeptidase (GGT) levels ($P < 0.001$) were observed between these two groups. The demographic and clinical characteristics of all enrolled 726 patients are depicted in Table S1.

3.2 | Single selected SNP association with ATDH

All of the 11 SNPs genotypes from the controls did not deviate from the HWE. The mutant A allele frequency of rs12483377 was 0.46% and 3.29% in the cases and the controls, respectively. The mutant A allele conferred the decreased risk of ATDH (OR = 0.13, 95%CI: 0.02–0.98, $P = 0.020$). Logistic regression showed that this significance still existed after adjusting age and gender ($P = 0.024$), and the statistical power is 0.77 (Table 1). No case harbored AA genotype of rs12483377, while there were 2 patients with AA genotype in the controls. For the AG genotype of rs12483377, there was 1 and 35 subjects in the ATDH group and non-ATDH group, respectively. However, as shown in Table 2, comparable risk of ATDH was identified in these 3 genetic models of rs12483377. As for the genetic models of the other 10 selected SNPs, none of them reach the threshold value of statistical significance.

3.3 | Haplotype construction

With a threshold of pairwise r^2 value ≥ 0.80, 3 SNPs (rs114220916, rs9980080, and rs2236467) were in a LD block as well as 3 SNPs (rs2236467, rs13048803, and rs2838942), 5 SNPs (rs114220916, rs9980080, rs2236467, rs13048803, and rs2838942), 5 SNPs (rs9980080, rs2236467, rs13048803, rs2838942, and rs9980525) and 3 SNPs (rs2236483, rs12483377, and rs7867). However, none of the constructed haplotypes of *COL18A1* showed the significant associations with the risk of ATDH (Table 3). The LD plot of selected SNPs are presented in the Figure 2.

3.4 | The correlation within SNPs and laboratory indicators

For rs12483377, mutant homozygote AA genotype was associated with lower total protein ($P = 0.018$). However, no significant findings on the relationship between rs12483377 and other clinical characteristics were observed (Figure 3). The total protein levels under different genotypes were displayed in Table 4.

4 | DISCUSSION

In this present study, we investigated the relationship between *COL18A1* polymorphisms and the risk of ATDH in the Western Chinese Han population. We revealed that the mutant A allele of *CLO18A1* rs12483377 was associated with decreased risk of ATDH. Furthermore, the statistical significance of rs12483377 on total protein had been identified.

COL18A1, highly expressing in the liver (http://biogps.org/#goto=genereport&id=80781), is closely related to liver diseases.[25,42,43] Musso et al[44] have reported the relationship between *COL18A1* and liver fibrosis. Type XVIII collagen, encoded by *COL18A1*, can increase before and during the fibrotic stages of liver fibrosis.[45,46] The increased type XVIII collagen upregulates its own product, EST. EST is able to resist liver fibrosis by inhibiting the expression of TGF-β1 mRNA through RhoA/ROCK signal pathways in hepatic stellate cells (HSCs).[47,48] In addition, liver fibrosis refers to the progression of extracellular matrix excessive deposition, which is often promoted by the activation of HSCs. HSCs can transdifferentiate into cells which can secrete extracellular matrix.[49-51] Therefore, *COL18A1* is associated with liver fibrosis via regulating the expression of EST *COL18A1* is also related to hepatic carcinoma by EST.[52] EST can inhibit endogenous angiogenesis by suppressing the production of angiogenic factors, while angiogenesis is a common physiological and pathological process in liver cancer.[53,54] Besides, the relationship between *COL18A1* polymorphisms and liver cancer is also reported. Wu et al[36] have suggested that *COL18A1* rs7499, located in the 3'-UTR region, increases the risk of hepatocellular carcinoma in Chinese Han population by negatively working in the binding site for has-mir-328. Based on these relationships, both *COL18A1* and its product, EST, are considered as targets of liver cancer due to their function of restricting of endothelial proliferation and inhibiting the growth and metastasis of tumors.[55-57] Furthermore, Duncan et al[30] have revealed that type XVIII collagen is vital to preserve the integrity of liver during hepatotoxic injury through α1β1 integrin, integrin linked kinase and the Akt pathway. *COL18A1* is thus identified as a necessary survival factor of acute liver injury from carbon tetrachloride.

In our study, we firstly reported the relationship between *COL18A1* variants and ATDH susceptibility. We found that rs12483377 is associated with decreased risk of ATDH. It has been verified that the mutant A allele of rs2483377 could decrease the ability of EST to bind other molecules and the function to inhibit angiogenesis.[58] Considering the role of EST in the occurrence of ATDH as we described before, we deduced that rs12483377 may influence ATDH by regulating the expression of EST which participates in oxidative stress.[20,26,27] In addition, it is well-recognized

TABLE 1 The distributions of allelic and genotypic frequency between non-ATDH[a] and ATDH[b] for the selected 11 SNPs

			Allele					Genotype			
SNP	Allele	Group	1[b]/2[b]	OR (95% CI)	*P*	*P**	Power	11[b]/12[b]/22[b]	*P*	*P**	P_{HWE}
rs2236455	A>G	ATDH[a]	56/172	0.92 (0.67–1.29)	0.653	0.449		9/38/67	0.829	0.543	0.311
		Non-ATDH[a]	318/906					48/222/342			0.171
rs114220916	G>A	ATDH[a]	15/213	1.11 (0.63–2.00)	0.721	0.821		0/15/99	NA	0.752	1.000
		Non-ATDH[a]	73/1151					0/73/539			0.157
rs9980080	G>A	ATDH[a]	40/188	1.07 (0.74–1.56)	0.722	0.785		3/34/77	0.635	0.590	1.000
		Non-ATDH[a]	203/1021					22/159/431			0.143
rs2236467	G>A	ATDH[a]	16/212	1.09 (0.63–1.91)	0.752	0.980		0/16/98	NA	0.790	1.000
		Non-ATDH[a]	79/1145					0/79/533			0.166
rs13048803	G>A	ATDH[a]	39/189	1.10 (0.75–1.60)	0.635	0.705		2/35/77	0.763	0.578	0.521
		Non-ATDH[a]	194/1030					13/168/431			0.546
rs2838942	A>G	ATDH[a]	16/212	1.09 (0.63–1.91)	0.752	0.963		0/16/98	NA	0.792	1.000
		Non-ATDH[a]	79/1145					0/79/533			0.166
rs9980525	G>A	ATDH[a]	39/189	1.12 (0.77–1.64)	0.547	0.631		2/35/77	0.777	0.528	0.521
		Non-ATDH[a]	190/1034					11/168/533			0.284
rs3753019	G>A	ATDH[a]	108/120	1.11 (0.84–1.48)	0.456	0.455		27/54/33	0.460	0.869	0.578
		Non-ATDH[a]	547/677					115/317/180			0.253
rs2236483	G>A	ATDH[a]	93/135	0.82 (0.61–1.09)	0.168	0.199		18/57/39	0.322	0.364	0.846
		Non-ATDH[a]	560/664					134/292/186			0.330
rs12483377	G>A	ATDH[a]	1/227	**0.13 (0.02–0.98)**[c]	**0.020**	**0.024**	0.77	0/1/113	NA	0.055	1.000
		Non-ATDH[a]	39/1185					2/35/575			0.119
rs7867	A>G	ATDH[a]	98/130	0.86 (0.65–1.15)	0.32	0.262		23/52/39	0.592	0.298	0.450
		Non-ATDH[a]	570/654					139/292/181			0.330

Abbreviations: CI, Confidence interval; HWE, Hardy-Weinberg equilibrium; NA, Non-available; OR, Odd ratio; SNP, Single nucleotide polymorphisms.

[a]Non-ATDH and ATDH refer to patients without and with anti-tuberculosis drug-induced hepatotoxicity, respectively;

[b]"1": mutant allele; "2": wild allele; "11": mutant homozygote; "12": heterozygote; "22": wild homozygote;

[c]Statistically significant data will be highlighted in bold.

**P* value after adjusting the age and gender.

TABLE 2 Genetic models analyses of the selected 11 SNPs

SNP	Model	OR (95% CI)	*P*	$P^{*,a}$
rs2236455 (A>G)	Add	0.93 (0.68–1.28)	0.663	0.845
	Dom	0.89 (0.59–1.33)	0.568	0.538
	Rec	1.01 (0.48–2.12)	0.985	0.950
rs114220916 (G>A)	Add	1.12 (0.62–2.03)	0.712	NA
	Dom	1.12 (0.62–2.03)	0.712	0.752
	Rec	NA (NA– NA)	NA	NA
rs9980080 (G>A)	Add	1.07 (0.74–1.54)	0.728	0.752
	Dom	1.14 (0.75–1.76)	0.538	0.571
	Rec	0.72 (0.21–2.46)	0.606	0.590
rs2236467 (G>A)	Add	1.10 (0.62–1.97)	0.743	NA
	Dom	1.10 (0.62–1.97)	0.743	0.790
	Rec	NA (NA–NA)	NA	NA
rs13048803 (G>A)	Add	1.10 (0.75–1.62)	0.629	0.859
	Dom	1.14 (0.75–1.76)	0.538	0.571
	Rec	0.82 (0.18–3.70)	0.799	0.819
rs2838942 (A>G)	Add	1.10 (0.62–1.97)	0.743	NA
	Dom	1.10 (0.62–1.97)	0.743	0.792
	Rec	NA (NA–NA)	NA	NA
rs9980525 (G>A)	Add	1.13 (0.77–1.67)	0.537	0.998
	Dom	1.16 (0.76–1.79)	0.492	0.525
	Rec	0.98 (0.21–4.46)	0.975	0.938
rs3753019 (G>A)	Add	1.12 (0.84–1.49)	0.448	0.416
	Dom	1.02 (0.66–1.59)	0.920	0.951
	Rec	1.13 (0.83–2.16)	0.228	0.213
rs2236483 (G>A)	Add	0.82 (0.62–1.09)	0.174	0.140
	Dom	0.84 (0.55–1.23)	0.419	0.420
	Rec	0.67 (0.39–1.15)	0.143	0.132
rs12483377 (G>A)	Add	0.14 (0.02–1.04)	0.054	0.999
	Dom	0.14 (0.02–1.01)	0.051	0.055
	Rec	NA (NA–NA)	NA	0.999
rs7867 (A>G)	Add	0.87 (0.66–1.15)	0.330	0.337
	Dom	0.81 (0.53–1.23)	0.323	0.308
	Rec	0.86 (0.52–1.41)	0.551	0.522

Abbreviations: Add, Addictive model; CI, Confidence interval; Dom, Dominant model; NA, Non-available; OR, Odd ratio; Rec, Recessive model; SNP, Single nucleotide polymorphisms.

*P value after adjusting the age and gender.

that expression quantitative trait locus (eQTL) regulates the expression level of mRNA and protein specifically, and the expression level of mRNA/protein is proportional to the quantitative character.[59,60] Rs12483377 has 2 hits of cis eQTL hits (http://pubs.broadinstitute.org/mammals/haploreg/detail_v4.1.php?query=&id=rs12483377). It is reported that rs12483377 involved in regulating the expression of both *COL18A1* and solute carrier family 19 member 1 (*SLC19A1*).[61,62] Thus, we speculated that rs12483377 might reduce the expression of EST through eQTL, and thus functioned in the occurrence of ATDH.

Besides, in our study, there were significant differences in fever, weight loss, total bilirubin levels, serum ALT levels, serum AST levels, uric acid levels, ALP levels, and GGT levels between the case and control group. Fever and weight loss are the common symptoms of tuberculosis poisoning.[63] After Mycobacterium tuberculosis infected the body, it will produce toxins and metabolites, which can not only cause allergic reactions such as fever, fatigue, and so on, but also will stimulate the central nervous system, resulting in dysfunction of the autonomic nervous system which lead to night sweats.[63,64] Bilirubin, ALT, AST, ALP, and GGT are all associated with the liver metabolism.[65,66] As is stated

before, anti-TB treatment can result in liver injury through four mechanisms.[3] As for uric acid levels, all patients enrolled were treated with INH, RIF, PZA, and EMB, in which INH, PZA, EMB, and their metabolites could compete with uric acid for the organic acid excretion pathway, reducing the excretion of uric acid, thus causing the increase of uric acid y.[67] In this study, a statistical significance on the relationship between mutant homozygote AA and total protein was found. However, after reviewing the relevant literature, it is found that the current reports are

TABLE 3 Analysis of haplotypes assigned by *COL18A1* variants with the risk of ATDH[a]

Haplotype	OR (95% CI)	*P*	Frequency				
			ALL(n = 726)	ATDH*,a (n = 112)	Non-ATDH*,a (n = 614)	Frequency	Cumulative
Rs114220916- rs2236467	GGG	1.00 (NA–NA)	NA	0.77	0.76	0.78	0.77
	GAG	0.87 (0.59–1.29)	0.49	0.16	0.17	0.16	0.93
	AGA	0.79 (0.42–1.49)	0.47	0.05	0.06	0.05	0.99
Rs2236467- rs2838942	GGA	1.00 (NA–NA)	NA	0.77	0.76	0.78	0.77
	GAA	0.89 (0.60–1.31)	0.55	0.16	0.17	0.16	0.93
	AGG	0.87 (0.48–1.57)	0.65	0.06	0.07	0.06	1.00
Rs114220916- rs2838942	GGGGA	1.00 (NA–NA)	NA	0.77	0.76	0.78	0.77
	GAGAA	0.86 (0.58–1.28)	0.46	0.16	0.17	0.16	0.93
	AGAGG	0.79 (0.42–1.49)	0.46	0.05	0.06	0.05	0.99
Rs9980080- rs9980525	GGGAG	1.00 (NA–NA)	NA	0.77	0.76	0.78	0.77
	AGAAA	0.84 (0.56–1.26)	0.39	0.16	0.17	0.16	0.93
	GAGGG	0.82 (0.45–1.53)	0.54	0.06	0.07	0.06	0.99
Rs2236483- rs7867	GGA	1.00 (NA–NA)	NA	0.52	0.55	0.52	0.52
	AGG	1.14 (0.85–1.53)	0.38	0.41	0.39	0.41	0.93
	GGG	0.58 (0.27–1.25)	0.17	0.03	0.04	0.02	0.96
	AAG	6.69 (0.92–48.87)	0.06	0.03	0.00	0.03	0.98
	AGA	0.75 (0.24–2.31)	0.61	0.01	0.02	0.01	1.00

Abbreviations: Add, Addictive model; CI, Confidence interval; NA, Non-available; OR, Odd ratio.

[a]Non-ATDH and ATDH refer to patients without and with anti-tuberculosis drug-induced hepatotoxicity, respectively.

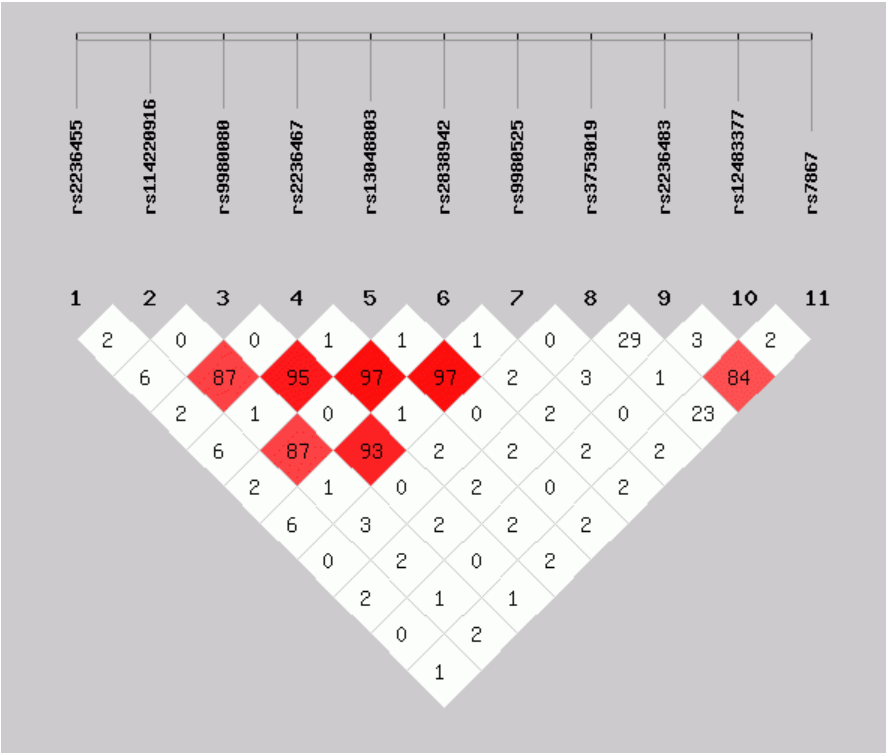

FIGURE 2 Linkage disequilibrium (LD) map of all single nucleotide polymorphisms (SNPs) in Collagen type XVIII alpha 1 chain (*COL18A1*). The threshold was set at pairwise $r^2 > 0.80$. The color of diamonds, paired with the percentages in diamonds, indicates the pairwise r^2 of all selected SNPs. Namely, the darker the color is, the higher the percentage is

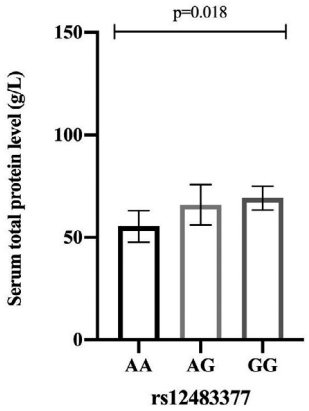

FIGURE 3 The association between rs12483377 and serum total protein level (g/L) among enrolled patients

TABLE 4 The correlation within rs12483377 and total protein

Genotype	Number	Median (percent 25%–75%)
AA	2	55.40 (51.55–59.25)
AG	36	69.10 (59.70–72.50)
GG	688	69.65 (63.90–75.30)

not enough to explain the two situations, so it may just be a statistical correlation and may not be of any clinical significance. Meanwhile, after reviewing the specific circumstances of these cases, the impact of the low number of homozygote AA on the analysis in this study can not be ruled out. Furthermore, this may also be an innovative finding, so the recruitment of people for the function verification and larger population verification and so on aiming for rs12483377 is under way.

4.1 | Strengths and limitations

We firstly investigated the relationship between *COL18A1* polymorphisms and ATDH susceptibility in Western Chinese Han population. Based on available evidence, we also speculated the potential mechanisms that how *COL18A1* polymorphisms affect ATDH susceptibility, contributing to the deep understanding of ATDH etiology to some extent. Besides, our finding is beneficial to explore more novel biomarkers of ATDH and decrease the burden brought by ATDH to some degree. Nevertheless, there were still some limitations in our study. The design of single center study restricts us to verify our findings in different ethnicities. Functional experiment about rs12483377 should have been further performed to validate our speculation. More high-quality studies with lager cohorts are warranted.

5 | CONCLUSION

Collectively, our study revealed that *CLO18A1* rs12483377 is related to the risk and specific characteristic of ATDH in the Western Chinese Han population, mining and further emphasizing the role of *CLO18A1* variants in ATDH.

CONFLICT OF INTEREST

Author Yuhui Cheng, Author Lin Jiao, Author Weixiu Li, Author Jialing Wang, Author Zhangyu Lin, Author Hongli Lai, Author Binwu Ying declare that they have no conflict of interest.

AUTHORS CONTRIBUTIONS

Research design: Binwu Ying. Data collection: Yuhui Cheng, Lin Jiao, Weixiu Li, Jialing Wang, Zhangyu Lin. Data analysis: Yuhui Cheng, Lin Jiao, Weixiu Li, Jialing Wang, Zhangyu Lin, Hongli Lai. Project administration: Binwu Ying. Writing-original draft: All authors. Writing-revision: All authors.

DATA AVAILABILITY STATEMENT

All data to this article can be found at the end of this manuscript.

ORCID

Yuhui Cheng https://orcid.org/0000-0001-9270-0781
Zhangyu Lin https://orcid.org/0000-0001-9240-9724
Hongli Lai https://orcid.org/0000-0002-7146-3518
Binwu Ying https://orcid.org/0000-0001-7828-1453

REFERENCES

1. Lee LN, Huang CT. Mitochondrial DNA variants in patients with liver injury due to anti-tuberculosis drugs. *J Clin Med*. 2019;8:1207.
2. Yang LX, Liu CY, Zhang LL, et al. Clinical characteristics of patients with drug-induced liver injury. *Chin Med J (Engl)*. 2017;130(2):160-164.
3. Bao Y, Ma X, Rasmussen TP, Zhong XB. Genetic variations associated with anti-tuberculosis drug-induced liver injury. *Curr Pharmacol Rep*. 2018;4(3):171-181.
4. Jia ZL, Cen J, Wang JB, et al. Mechanism of isoniazid-induced hepatotoxicity in zebrafish larvae: activation of ROS-mediated ERS, apoptosis and the Nrf2 pathway. *Chemosphere*. 2019;227:541-550.
5. Ramachandran A, Visschers RGJ, Duan L, Akakpo JY, Jaeschke H. Mitochondrial dysfunction as a mechanism of drug-induced hepatotoxicity: current understanding and future perspectives. *J Clin Transl Res*. 2018;4(1):75-100.
6. Kim JH, Nam WS, Kim SJ, et al. Mechanism investigation of rifampicin-induced liver injury using comparative toxicoproteomics in mice. *Int J Mol Sci*. 2017;18(7):1417.
7. Yew WW, Chang KC, Chan DP. Oxidative stress and first-line antituberculosis drug-induced hepatotoxicity. *Antimicrob Agents Chemother*. 2018;62(8):e02637-17.
8. Yang S, Hwang SJ, Park JY, Chung EK, Lee JI. Association of genetic polymorphisms of CYP2E1, NAT2, GST and SLCO1B1 with the risk of anti-tuberculosis drug-induced liver injury: a systematic review and meta-analysis. *BMJ Open*. 2019;9(8):e027940.
9. Yee D, Valiquette C, Pelletier M, Parisien I, Rocher I, Menzies D. Incidence of serious side effects from first-line antituberculosis drugs among patients treated for active tuberculosis. *Am J Respir Crit Care Med*. 2003;167(11):1472-1477.
10. Huai C, Wei Y, Li M, et al. Genome-wide analysis of DNA methylation and antituberculosis drug-induced liver injury in the han Chinese population. *Clin Pharmacol Ther*. 2019;106(6):1389-1397.

11. Bouazzi OE, Hammi S, Bourkadi JE, et al. First line anti-tuberculosis induced hepatotoxicity: incidence and risk factors. *Pan Afr Med J*. 2016;25:167.
12. Huang YS. Recent progress in genetic variation and risk of antituberculosis drug-induced liver injury. *J Chin Med Assoc*. 2014;77(4):169-173.
13. Zhang J, Zhao Z, Bai H, et al. Genetic polymorphisms in PXR and NF-kappaB1 influence susceptibility to anti-tuberculosis drug-induced liver injury. *PLoS One*. 2019;14(9):e0222033.
14. Chen S, Yang P, Chen SP, Wu C, Zhu QX. Association between genetic polymorphisms of NRF2, KEAP1, MAFF, MAFK and anti-tuberculosis drug-induced liver injury: a nested case-control study. *Sci Rep*. 2019;9(1):14311.
15. Zhang M, Wu SQ, He JQ. Are genetic variations in glutathione S-transferases involved in anti-tuberculosis drug-induced liver injury? A meta-analysis. *J Clin Pharm Ther*. 2019;44(6):844-857.
16. Khan S, Mandal RK, Elasbali AM, et al. Pharmacogenetic association between NAT2 gene polymorphisms and isoniazid induced hepatotoxicity: trial sequence meta-analysis as evidence. *Biosci Rep*. 2019;39(1):BSR20180845.
17. Lamba J, Lamba V, Strom S, Venkataramanan R, Schuetz E. Novel single nucleotide polymorphisms in the promoter and intron 1 of human pregnane X receptor/NR1I2 and their association with CYP3A4 expression. *Drug Metab Dispos*. 2008;36(1):169-181.
18. Wang JY, Tsai CH, Lee YL, et al. Gender-dimorphic impact of PXR genotype and haplotype on hepatotoxicity during antituberculosis treatment. *Medicine (Baltimore)*. 2015;94(24):e982.
19. Karsdal MA, Nielsen SH, Leeming DJ, et al. The good and the bad collagens of fibrosis - their role in signaling and organ function. *Adv Drug Deliv Rev*. 2017;121:43-56.
20. Boodhwani M, Nakai Y, Mieno S, et al. Hypercholesterolemia impairs the myocardial angiogenic response in a swine model of chronic ischemia: role of endostatin and oxidative stress. *Ann Thorac Surg*. 2006;81(2):634-641.
21. Yan M, Dongmei B, Jingjing Z, et al. Antitumor activities of Liver-targeting peptide modified Recombinant human Endostatin in BALB/c-nu mice with Hepatocellular carcinoma. *Sci Rep*. 2017;7(1):14074.
22. Bao Y, Feng WM, Tang CW, et al. Endostatin inhibits angiogenesis in hepatocellular carcinoma after transarterial chemoembolization. *Hepatogastroenterology*. 2012;59(117):1566-1568.
23. Jin S, Zhang Y, Yi F, Li PL. Critical role of lipid raft redox signaling platforms in endostatin-induced coronary endothelial dysfunction. *Arterioscler Thromb Vasc Biol*. 2008;28(3):485-490.
24. Kimura K, Shirabe K, Yoshizumi T, et al. Ischemia-reperfusion injury in fatty liver is mediated by activated NADPH Oxidase 2 in rats. *Transplantation*. 2016;100(4):791-800.
25. Chau YP, Lin SY, Chen JH, Tai MH. Endostatin induces autophagic cell death in EAhy926 human endothelial cells. *Histol Histopathol*. 2003;18(3):715-726.
26. Tao GZ, Lehwald N, Jang KY, et al. Wnt/beta-catenin signaling protects mouse liver against oxidative stress-induced apoptosis through the inhibition of forkhead transcription factor FoxO3. *J Biol Chem*. 2013;288(24):17214-17224.
27. Seppinen L, Pihlajaniemi T. The multiple functions of collagen XVIII in development and disease. *Matrix Biol*. 2011;30(2):83-92.
28. Park WB, Kim W, Lee KL, et al. Antituberculosis drug-induced liver injury in chronic hepatitis and cirrhosis. *J Infect*. 2010;61(4):323-329.
29. Abramavicius S, Velickiene D, Kadusevicius E. Methimazole-induced liver injury overshadowed by methylprednisolone pulse therapy: case report. *Medicine (Baltimore)*. 2017;96(39):e8159.
30. Duncan MB, Yang C, Tanjore H, et al. Type XVIII collagen is essential for survival during acute liver injury in mice. *Dis Model Mech*. 2013;6(4):942-951.
31. Mijiti P, Yuehua L, Feng X, et al. Prevalence of pulmonary tuberculosis in western China in 2010–11: a population-based, cross-sectional survey. *Lancet Glob Health*. 2016;4(7):e485-e494.
32. Turnbull L, Bell C, Child F. Tuberculosis (NICE clinical guideline 33). *Arch Dis Child Educ Pract Ed*. 2017;102(3):136-142.
33. Ho CC, Chen YC, Hu FC, et al. Safety of fluoroquinolone use in patients with hepatotoxicity induced by anti-tuberculosis regimens. *Clin Infect Dis*. 2009;48(11):1526-1533.
34. Locke AE, Dooley KJ, Tinker SW, et al. Variation in folate pathway genes contributes to risk of congenital heart defects among individuals with Down syndrome. *Genet Epidemiol*. 2010;34(6):613-623.
35. Castro-Giner F, Bustamante M, Ramon González J, et al. A pooling-based genome-wide analysis identifies new potential candidate genes for atopy in the European Community Respiratory Health Survey (ECRHS). *BMC Med Genet*. 2009;10:128.
36. Wu X, Wu J, Xin Z, et al. A 3' UTR SNP in COL18A1 is associated with susceptibility to HBV related hepatocellular carcinoma in Chinese: three independent case-control studies. *PLoS One*. 2012;7(3):e33855.
37. Yip SP, Leung KH, Fung WY, et al. A DNA pooling-based case-control study of myopia candidate genes COL11A1, COL18A1, FBN1, and PLOD1 in a Chinese population. *Mol Vis*. 2011;17:810-821.
38. Defago MD, Gu D, Hixson JE, et al. Common genetic variants in the endothelial system predict blood pressure response to sodium intake: the GenSalt study. *Am J Hypertens*. 2013;26(5):643-656.
39. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424.
40. Schalkwyk LC, Meaburn EL, Smith R, et al. Allelic skewing of DNA methylation is widespread across the genome. *Am J Hum Genet*. 2010;86(2):196-212.
41. Metayer C, Scélo G, Chokkalingam AP, et al. Genetic variants in the folate pathway and risk of childhood acute lymphoblastic leukemia. *Cancer Causes Control*. 2011;22(9):1243-1258.
42. Jia JD, Bauer M, Sedlaczek N, et al. Modulation of collagen XVIII/endostatin expression in lobular and biliary rat liver fibrogenesis. *J Hepatol*. 2001;35(3):386-391.
43. Hsieh MY, Lin ZY, Chuang WL. Serial serum VEGF-A, angiopoietin-2, and endostatin measurements in cirrhotic patients with hepatocellular carcinoma treated by transcatheter arterial chemoembolization. *Kaohsiung J Med Sci*. 2011;27(8):314-322.
44. Akhdar H, El Shamieh S, Musso O, et al. The rs3957357C>T SNP in GSTA1 is associated with a higher risk of occurrence of hepatocellular carcinoma in european individuals. *PLoS One*. 2016;11(12):e0167543.
45. Schuppan D, Cramer T, Bauer M, Strefeld T, Hahn EG, Herbst H. Hepatocytes as a source of collagen type XVIII endostatin. *Lancet*. 1998;352(9131):879-880.
46. Jia J, Bauer M, Boigk G, Ruehl M, Schuppan D, Wang B. Expression of collagen XVIII mRNA in rat liver fibrosis. *Zhonghua Gan Zang Bing Za Zhi*. 2000;8(5):274-275.
47. Ren H, Li Y, Chen Y, Wang L. Endostatin attenuates PDGF-BB- or TGF-beta1-induced HSCs activation via suppressing RhoA/ROCK1 signal pathways. *Drug Des Devel Ther*. 2019;13:285-290.
48. Chen J, Liu DG, Yang G, et al. Endostar, a novel human recombinant endostatin, attenuates liver fibrosis in CCl4-induced mice. *Exp Biol Med (Maywood)*. 2014;239(8):998-1006.
49. Cheng Q, Li C, Yang CF, et al. Methyl ferulic acid attenuates liver fibrosis and hepatic stellate cell activation through the TGF-beta1/Smad and NOX4/ROS pathways. *Chem Biol Interact*. 2019;299:131-139.

50. Friedman SL. Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. *J Biol Chem*. 2000;275(4):2247-2250.
51. Tsuchida T, Friedman SL. Mechanisms of hepatic stellate cell activation. *Nat Rev Gastroenterol Hepatol*. 2017;14(7):397-411.
52. Walia A, Yang JF, Huang YH, Rosenblatt MI, Chang JH, Azar DT. Endostatin's emerging roles in angiogenesis, lymphangiogenesis, disease, and clinical applications. *Biochim Biophys Acta*. 2015;1850(12):2422-2438.
53. Chamani R, Asghari SM, Alizadeh AM, et al. Engineering of a disulfide loop instead of a Zn binding loop restores the anti-proliferative, anti-angiogenic and anti-tumor activities of the N-terminal fragment of endostatin: Mechanistic and therapeutic insights. *Vascul Pharmacol*. 2015;72:73-82.
54. Li T, Kang G, Wang T, Huang H. Tumor angiogenesis and anti-angiogenic gene therapy for cancer. *Oncol Lett*. 2018;16(1):687-702.
55. Bao D, Jin X, Ma Y, Zhu J. Comparison of the structure and biological activities of wild-type and mutant liver-targeting peptide modified recombinant human endostatin (rES-CSP) in human hepatocellular carcinoma HepG2 cells. *Protein Pept Lett*. 2015;22(5):470-479.
56. Poluzzi C, Iozzo RV, Schaefer L. Endostatin and endorepellin: a common route of action for similar angiostatic cancer avengers. *Adv Drug Deliv Rev*. 2016;97:156-173.
57. Ding RL, Xie F, Hu Y, et al. Preparation of endostatin-loaded chitosan nanoparticles and evaluation of the antitumor effect of such nanoparticles on the Lewis lung cancer model. *Drug Deliv*. 2017;24(1):300-308.
58. Lurje G, Husain H, Power DG, et al. Genetic variations in angiogenesis pathway genes associated with clinical outcome in localized gastric adenocarcinoma. *Ann Oncol*. 2010;21(1):78-86.
59. Verta JP, Landry CR, MacKay J. Dissection of expression-quantitative trait locus and allele specificity using a haploid/diploid plant system - insights into compensatory evolution of transcriptional regulation within populations. *New Phytol*. 2016;211(1):159-171.
60. Tian J, Keller MP, Broman AT, et al. The dissection of expression quantitative trait locus hotspots. *Genetics*. 2016;202(4):1563-1574.
61. Fehrmann RS, Jansen RC, Veldink JH, et al. Trans-eQTLs reveal that independent genetic variants associated with a complex phenotype converge on intermediate genes, with a major role for the HLA. *PLoS Genet*. 2011;7(8):e1002197.
62. Westra HJ, et al. Systematic identification of trans eQTLs as putative drivers of known disease associations. *Nat Genet*. 2013;45(10):1238-1243.
63. Singer-Leshinsky S. Pulmonary tuberculosis: Improving diagnosis and management. *JAAPA*. 2016;29(2):20-25.
64. Fealey RD. Interoception and autonomic nervous system reflexes thermoregulation. *Handb Clin Neurol*. 2013;117:79-88.
65. Kietzmann T. Metabolic zonation of the liver: The oxygen gradient revisited. *Redox Biol*. 2017;11:622-630.
66. Reinke H, Asher G. Circadian clock control of liver metabolic functions. *Gastroenterology*. 2016;150(3):574-580.
67. Louthrenoo W, Hongsongkiat S, Kasitanon N, et al. Effect of antituberculous drugs on serum uric acid and urine uric acid excretion. *J Clin Rheumatol*. 2015;21(7):346-348.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Cheng Y, Jiao L, Li W, et al. *Collagen type XVIII alpha 1 chain (COL18A1)* variants affect the risk of anti-tuberculosis drug-induced hepatotoxicity: A prospective study. *J Clin Lab Anal*. 2021;35:e23630. https://doi.org/10.1002/jcla.23630