INTRODUCTION

Since the 21st century, the incidence of tuberculosis in China has been reduced to 50% at the beginning of this century, but there are still 1 million new tuberculosis patients every year.1-3 At present, the standard drugs for anti-tuberculosis treatment are isoniazid, rifampicin, pyrazinamide combined with ethambutol, or streptomycin. Among them, isoniazid, rifampicin, and pyrazinamide have
potential hepatotoxicity, and the incidence of adverse liver reactions is about 2.55%–11.9%, which may lead to liver failure and death in severe cases. Anti-tuberculosis drug-induced liver injury (ADLI) is one of the important reasons for drug failure and subsequent drug resistance of tuberculosis. However, ADLI has the characteristics of obvious individual differences and unpredictability, which brings great health hazards and economic losses to patients and increases the medical burden.

Non-genetic factors such as gender, age, alcohol consumption, history of liver disease, concomitant infection, and nutritional status are risk factors for ADLI, but their clinical prediction is limited. There are two possible mechanisms of ADLI. First, abnormal metabolism of drugs leads to accumulation of toxic products, resulting in intracellular oxidative stress response and changes in mitochondrial permeability, and then leading to apoptosis or necrosis of liver cells. Second, drugs cause immune regulation and inflammatory responses in the liver.

Drug metabolism enzymes (DME) play a significant role in drug detoxification and activation, which exert important effect on drug efficacy and sensitivity to toxicity. Anti-tuberculosis drugs are mainly metabolized by two kinds of DMEs including phase I enzymes and phase II enzymes. Cytochrome P450 2S1 (CYP2S1) is a family monoxygenase, which plays an important role in the metabolism of various substances including Anti-tuberculosis drugs. The rs338599 loci have been most important and most widely studied of CYP2S1 gene polymorphism. In the present study, we would like to explore whether CYP2S1 rs338599 polymorphism at the respective gene loci confers risk to ADLI and provide evidence of being used as novel marker for ADLI risk prediction.

2.2 | Epidemiological investigation

During the study period, all tuberculosis patients’ general condition and basic information should be recorded in detail through an epidemiological investigation. The epidemiological survey consisted of age, sex, body mass index (BMI), educational qualifications, marital status, profession, smoking status, alcohol consumption, medical history, and liver function. The survey method standard for smoking and drinking was recommended by World Health Organization (1984). Objective indicators should be used as far as possible, and two pre-surveys should be conducted before the formal survey performed. During the study period, patients in the non-ADLI group were followed up for recent regular liver function tests.

2.3 | CYP2S1 gene polymorphism

Three milliter of fasting venous blood was collected from all tuberculosis patients, which was immediately centrifuged and stored at −80°C for detection. DNA extraction was performed using human peripheral blood genomic DNA Extraction and Purification Kit (Zhongke Leiming Biotechnology Co., LTD). The selection SNP loci were based on searching bioinformatics databases and previous literatures. The genotypes of CYP2S1 gene polymorphism were performed using the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method. The representative PCR-amplified DNA samples were examined by DNA sequencing in order to confirm the genotyping results. The detailed primer sequence for the present polymorphism was as follows: Forward: 5′-CTCCTGATCTCAGGTTCTGAAGG-3′, Reverse: 5′-CAGGGGTAGTCTCGGTTGA-3′.

2.4 | Statistical analysis

The SPSS 19.0 was applied for whole analysis, the measurement data were represented by (X ± S), and the comparison of the means of two independent samples was performed by t test. The counting data were expressed as percentage (%) and was performed by chi-square test. Hardy–Weinberg equilibrium (HWE) among case group and control group were checked with the goodness-of-fit chi-square test. Odds ratios (OR) and 95% confidence intervals (CI) were counted to evaluate the relationship between risk factors and ADLI by performing binary logistic regression. Both age and sex were adjusted to assess the relative risk. p < 0.05 indicated the difference was statistically significant.

3 | RESULTS

3.1 | General information of tuberculosis patients

No difference was detected in age, sex, smoking status, profession, education level, marital status, alcohol consumption, or using liver-protecting drugs (p > 0.05). The detailed information is shown
in Table 1. Furthermore, no difference was detected in five anti-tuberculosis drugs. The detailed information is shown in Table 2.

### 3.2 CYP2S1 gene polymorphism

Polymerase chain reaction-restriction fragment length polymorphism method showed that compared with non-ADLI group, GG genotype and G allele were significantly higher in ADLI group ($p < 0.05$). The detailed information is shown in Table 3.

### 4 DISCUSSION

Drug-induced liver injury is one of the most common and serious adverse drug reactions, which is caused by drug hypersensitivity or their metabolites during drug-use process. Drug-induced liver injury leads to 3%–5% jaundice, and it is also the main cause of acute liver failure, which can lead to death in severe cases. Currently, more than 1,100 drugs are known to have potential hepatotoxicity, including Chinese herbal medicines, anti-tuberculosis drugs, anti-infective drugs, antipyretic analgesics, and anti-tumor drugs. In Chinese population, liver injuries caused by anti-tuberculosis drugs are mainly isoniazid, rifampicin, and pyrazinamide, which accounts for 21.56% of all drug-induced liver injuries.5

Human CYP2S1 gene is 13 kb in length, located on chromosome 19q. 13.2, including 9 exons, encoding 504 amino acid proteins with molecular weight of about 55.8 kDa, with 8 single-nucleotide polymorphisms (SNPs) loci. CYP2S1 mRNA expression level is low in liver, but high in extrahepatic tissues such as respiratory and digestive systems. In addition, CYP2S1 mRNA and protein are also expressed in human skin. CYP2S1 plays an important physiological role in the metabolism of dodecanoids, and may play a role in inflammatory response, tumor, and other pathological processes. Domestically and foreign studies have found that one-phase DME CYP2E1, two-phase DME NAT2, GSTM1, three-phase drug transporter ABCB1, immune regulatory genes HLA-DQA1, and HLA-DQB1 were significantly correlated with liver injury induced
by anti-tuberculosis drugs. These studies examined the contribution of genetic factors to liver injury induced by anti-tuberculosis drugs. As far as we know, this is the first study, which investigates the CYP2S1 rs338599 polymorphism in ADLI risk. Our results indicated that CYP2S1 rs338599 polymorphism conferred decreases risk to ADLI. The tuberculosis patients who had GG genotype or G allele were not susceptible to ADLI. In other words, CYP2S1 rs338599 polymorphism had protective effect on ADLI.

In the present study, there were two potential limitations must be acknowledged. Firstly, all subjects were enrolled from only one hospital and all patients came from Hebei population. China is a country with vast territory and 56 ethnic groups. In addition, race, ethnicity, and region have important and profound influence on gene polymorphism. The current results do not necessarily apply elsewhere. Secondly, current study only investigated only one SNP. It would be interesting and meaningful to research more SNP loci of CYP2S1 gene to learn about their associations with ADLI risk. Therefore, current results must be treated and interpreted with caution due to objectives and limitations.

## 5 CONCLUSION

Our results indicated that CYP2S1 rs338599 polymorphism conferred reduced risk to ADLI. The tuberculosis patients who had GG genotype or G allele were not susceptible to ADLI. CYP2S1 rs338599 polymorphism may be a novel marker for ADLI risk prediction.

### AUTHOR CONTRIBUTIONS
Ying Qi conceived study design. Liyuan Wang conceived the content concept. Liyuan Wang and Hui Wang performed the data collection, extraction, analyzed the data, interpreted, and reviewed the data and drafts. Ying Qi reviewed the final draft. All authors were involved in literature search, writing the article, and had final approval of the submitted and published versions; contributed to data analysis, drafting, or revising the article; have agreed on the journal to which the article will be submitted; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

### ACKNOWLEDGEMENT
We sincerely acknowledge the staff in Clinical Laboratories Department at Affiliated Hospital of Hebei University for their support.

### CONFLICT OF INTEREST
The authors declare that they have no competing interests.

### DATA AVAILABILITY STATEMENT
All data generated or analyzed during this study are included in this published article.

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### TABLE 3 Comparison of genotype and allele between ADLI group and non-ADLI group

| CYP2S1 rs338599 | Non-ADLI group (N = 112) | ADLI group (N = 50) |
|-----------------|--------------------------|---------------------|
|                 | n | Percentage (%) | n | Percentage (%) | OR (95% CI)<sup>a</sup> | p<sup>a</sup> |
| GG              | 65 | 58.0          | 19 | 38.0  | 0.31(0.13-0.73) | 0.006  |
| GC              | 30 | 26.8          | 15 | 30.0  | 0.53(0.21-1.34) | 0.180  |
| CC              | 17 | 15.2          | 16 | 32.0  | 1.00<sup>REF</sup> |       |
| G               | 160| 71.4          | 53 | 54.8  | 0.45(0.28-0.73) | 0.001  |
| C               | 64 | 28.6          | 47 | 45.2  | 1.00<sup>REF</sup> |       |

Abbreviations: ADLI, anti-tuberculosis drug-induced liver injury; CI, confidential index; OR, odds ratio; SNP, single-nucleotide polymorphism.

<sup>a</sup>Adjusted for sex and age by logistic regression model.
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How to cite this article: Wang L, Wang H, Qi Y. CYP2S1 rs338599 polymorphism confers reduced risk to anti-tuberculosis drug-induced liver injury and may be a novel marker for its risk prediction. J Clin Lab Anal. 2022;36:e24478. doi:10.1002/jcla.24478