

# Antituberculosis Drug-Induced Adverse Events in the Liver, Kidneys, and Blood: Clinical Profiles and Pharmacogenetic Predictors

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Antituberculosis drug-induced adverse drug reactions (ATD-ADRs) are increasing globally, and it is key to identify candidate ATD-ADRs loci for clinical management. We prospectively enrolled 1,235 highly suspicious tuberculosis (TB) inpatients to investigate the profiles and genetic risk factors of ATD-ADRs in the liver, kidneys, and blood. Overall, 644 subjects were eligible and genotyped for seven polymorphisms in drug-metabolizing enzymes and transporter genes. Clinical follow-up and blood analysis were performed regularly. We found that a notable rate of ATD-ADRs (incidence: 16.5%, drug intervention rate: 10.4%), mainly involving hepatotoxicity (10.6%) and leukopenia (3.3%) in western China. *CYP2D6* rs1135840 and *NUDT15* rs116855232 increased the risks of hepatotoxicity and leukopenia with an odds ratio of 2.52 and 4.97, respectively. Both variants showed excellent negative predictive values (93.7% and 98.1%, respectively) but moderate sensitivities (72.7% and 52.4%, respectively). These data provide new insight into ATD-ADRs in the Chinese population and may offer future leads for diagnosis and treatment.

## Study Highlights

### WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Antituberculosis drug-induced adverse drug reactions (ATD-ADRs) are becoming increasingly common globally. Nevertheless, there is currently a paucity of information regarding the characteristics and risk factors underlying common and severe ATD-ADRs, which are worthwhile to explore in clinical settings.

### WHAT QUESTION DID THIS STUDY ADDRESS?

The study investigated the overall profiles and genetic risk factors of ATD-ADRs in the liver, kidneys, and blood of a large prospective tuberculosis cohort in western China.

### WHAT THIS STUDY ADDS TO OUR KNOWLEDGE

This study describes notable profiles of ATD-ADRs in the liver, kidneys, and blood in patients from western China, with a significant impact on subsequent therapeutic orientation. This study also provides a novel evidence that *CYP2D6* rs1135840 and *NUDT15* rs116855232 are susceptibility loci for the development of hepatotoxicity and leukopenia, respectively.

### HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE

Rs1135840 and rs116855232 are potential pharmacogenetic markers for hepatotoxicity and leukopenia in TB patients. Regular blood monitoring and pharmacogenetic analyses are useful for identifying patients at elevated risk of ATD-ADRs during individualized TB treatment.

Tuberculosis (TB) remains a major global health concern.<sup>1</sup> Although short-course strategies have been successfully implemented, TB management remains associated with serious issues, such as an increasing prevalence of multidrug-resistant *Mycobacterium tuberculosis* (MTB), disease relapse in elderly and immunocompromised patients, and the surge in antituberculosis drug-induced adverse drug reactions (ATD-ADRs). Long-term antituberculosis (anti-TB) combination regimens could lead to various types and levels of adverse drug reactions (ADRs), which

could subsequently lead to therapy modification or discontinuation, the emergence of drug resistance,<sup>2</sup> and even treatment failure or mortality.<sup>3</sup> Antituberculosis drug-induced hepatotoxicity (ATDH) is the most prevalent and serious ADR, with occurrence rates of 4.8–36% in different areas, and could cause liver failure and mortality in up to 15% and 67% of patients, respectively.<sup>4–7</sup> In addition to the leading ATD-ADRs of hepatotoxicity, there are other severe ATD-ADRs, such as nephrotoxicity and hematotoxicity, that are less frequent but are still lasting or

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Received 27 July 2017; accepted 23 October 2017; advance online publication 23 November 2017. doi:10.1002/cpt.924

fatal. These ADRs also pose considerable challenges to clinicians but have not yet been well elucidated.<sup>5,8</sup> More in-depth knowledge of the characteristics and associated risk factors of ATD-ADRs is urgently needed to predict and prevent ADRs, particularly among vulnerable populations.

Genomic variants are increasingly recognized as pivotal factors influencing the interindividual variability of drug effectiveness or the risk for ADRs.<sup>9,10</sup> The interrogation of genetic variants and their predisposition to ADRs has developed into clinical pharmacogenomics (PGx), which is widely used to screen patients who are at risk for ADRs from therapies for cancer and other diseases. PGx has particular value in determining an individual's combined medications.<sup>10–14</sup> A preemptive PGx-oriented approach has been proposed to achieve the goal of personalized medicine for many diseases and has also demonstrated significant potential in anti-TB treatment.<sup>13,14</sup> Recently, N-acetyl transferase 2 (*NAT2*) genotype testing has been proposed for isoniazid (INH) daily dose optimization by the Pharmacogenomics Knowledgebase (pharmaGKB) database<sup>15</sup> and the China Precision Medicine Clinical Research and Application Association.<sup>16</sup> *NAT2* slow acetylator genotypes are designated biomarkers for the development of ATDH.<sup>17</sup> Jung *et al.* and Azuma *et al.*<sup>18,19</sup> demonstrated that an *NAT2* genotype-guided regimen achieved therapeutic concentrations of INH, significantly prevented the occurrence of ATDH, and reduced early treatment failure from 38% to 15%, emphasizing the significance of pharmacogenetically oriented therapy in TB clinical practice. In addition to *NAT2*, polymorphisms in other metabolizing enzyme genes, such as cytochrome P450 *2E1* and glutathione S-transferase *M1*, and certain *HLA* alleles have been reported to affect patients' susceptibility to ATDH;<sup>20</sup> however, robust and definite conclusions cannot be drawn due to controversial results from previous studies. The roles of other genes involved in drug disposition and drug response signaling pathways, such as drug transporter genes, are still seldom investigated. In addition, little is known about the genetic factors of nephrotoxicity and hematotoxicity during anti-TB chemotherapy. There is currently a paucity of information regarding the genetic risk markers underlying common or severe ATD-ADRs, which should be explored in clinical settings.

China ranks third among all high-TB endemic countries, accounting for 8.6% of the global tuberculosis incidence,<sup>1</sup> and most TB cases are reported in central and western China.<sup>21</sup> Thus, identifying the features and genetic risk factors of ADRs during anti-TB chemotherapy in the Chinese TB population is necessary. The objective of this prospective study was to determine the profiles of ATD-ADRs in the liver, kidneys, and blood of patients in western China and to explore candidate genetic biomarkers to predict common ADRs in TB management.

## RESULTS

### Patient characteristics

The baseline characteristics of the TB patients are summarized in **Table 1**. A total of 644 active TB patients was ultimately eligible, of whom 61.03% were men and 84.47% were Han Chinese. The average age was 42.25 years, and the mean body mass index (BMI) was 19.2 kg/m<sup>2</sup>. Male patients were older and had higher

smoking and drinking status than female patients. Four-fifths of the TB patients had involved pulmonary tuberculosis (PTB), and 51.87% had previously received ATDs. Most patients (92.7%) suffered from local infectious symptoms, such as cough and sputum upon admission, followed by fever (52.64%) and poor appetite (37.58%). Most patients with chest images had infiltration and effusion (40.22%) or fibrosis and calcification (39.11%). The positive rates for TB-DNA and an interferon gamma release assay (TB-IGRA), which were 54.28% and 61.64% respectively, were considerably higher than those of MTB cultures and microscope smears (41.23% and 30.51%). Significant differences were observed in the proportion of PTB cases and the positive smear results between males and females. Quantitative laboratory traits of the participants at baseline, peak, and valley during anti-TB therapy are also shown in **Supplementary Table S1**. Overall, most laboratory indicators in the ADR patients differed between the patients with and without ADRs. The patients with ADRs had a worsening presentation in laboratory examinations during anti-TB therapy, while the presentation of those without ADRs did not change significantly.

### Profiles of ATD-ADRs in the liver, blood, and kidneys

As shown in **Table 2** and **Table S2**, the overall incidence rate of ADRs among the patients was 16.5% (106/644), and the main ATD-ADRs included ATDH (10.6%) and leukopenia (3.3%). Sixty-eight patients were considered cases of ATDH, all of which presented alterations in hepatic enzymes and 21 of which developed symptomatic hepatitis. ATDH cases were graded as mild (48.5%, 33/68), moderate (27.9%, 19/68), and severe (23.6%, 16/68) (**Table S3**), and age and gender were similar between the three groups ( $P = 0.723$  and  $0.895$ , respectively). Thirteen patients experienced uncommon hyperbilirubinemia (2.0%). Among hematological ADRs, leukopenia was common (3.3%, 21/644), followed by thrombocytopenia (2.5%, 16/644), whereas no cases of anemia were observed. Regarding ADRs in the kidneys, acute (AKI) or chronic (CKD) were rare—only 10 and 12 patients suffered from AKI or CKD, respectively. Females were at a greater risk of developing leukopenia and had a reduced risk for AKI ( $P = 0.002$ ,  $0.037$ , respectively), but given the limited number of samples, these results should be interpreted with particular caution.

Although most of these 106 ADR events were mild (36.8%) and moderate (53.8%), the severe events, which accounted for 9.4% of the cases, should not be ignored (**Table S2**). A total of 67 ADR patients required therapeutic intervention, with ATDH and leukopenia being the most important reasons for intervention. ATDs were withdrawn or replaced from 43.4% of the ADR patients (46/106) and were then rechallenged later in most cases, with other drugs administered to 23.6% of the patients (25/106) to relieve symptoms. On eight occasions, severe hepatitis resulted in hospitalization, and INH, rifampicin (RIF), and pyrazinamide (PZA) were discontinued in two instances (one concurrent severe ATDH and AKI, one concomitant ATDH and leukopenia) and were not restarted in either.

**Table 1** The clinical features of TB patients

Clinical features	All patients (n = 644)	Gender		P
		Male (n = 393)	Female (n = 251)	
Ethnicity (Han/Others)	544/100	333/60	211/40	0.365
Age, mean ± SD (years)	42.25 ± 19.56	43.64 ± 20.42	40.08 ± 17.97	0.021
BMI (kg/m <sup>2</sup> )	19.20 ± 4.04	19.30 ± 5.32	18.42 ± 4.88	0.244
Smoking, Yes / (Ever + No)	254 (39.44)	242 (61.58)	12 (4.78)	< 0.001
Drinking, Yes / (Ever + No)	302 (46.89)	271 (68.96)	31 (12.35)	< 0.001
TB status, n (%)				0.495
<i>De novo</i>	310 (48.13)	194 (49.36)	116 (46.22)	
Retreated	334 (51.87)	199 (50.64)	135 (63.78)	
TB subtype, n (%)				0.039
PTB	329 (51.09)	216 (54.96)	113 (45.02)	
EPTB	101 (15.68)	54 (13.74)	47 (18.73)	
PTB & EPTB	214 (33.23)	123 (31.30)	91 (36.25)	
General symptoms, n (%)				
Fever	339 (52.64)	212 (53.94)	127 (50.60)	0.407
Weight loss	223 (34.63)	144 (36.64)	79 (31.47)	0.179
Night sweat	188 (29.19)	114 (29.01)	74 (29.48)	0.897
Poor appetite	242 (37.58)	154 (39.19)	88 (35.06)	0.292
Fatigue	174 (27.02)	112 (28.50)	62 (24.70)	0.290
Local infectious symptoms, n (%)				0.063
Presence	597 (92.70)	358 (91.09)	239 (95.22)	
Absence	47 (7.30)	35 (8.91)	12 (4.78)	
Chest image of PTB (n = 271), n (%)				0.697
Normal	1 (0.37)	1 (0.54)	—	
Infiltration and effusion	109 (40.22)	74 (40.22)	35 (40.23)	
Caseation and cavitation	55 (20.30)	40 (21.74)	15 (17.24)	
Fibrosis and calcification	106 (39.11)	69 (37.50)	37 (42.53)	
Microbiological results				
Positive culture, n (%)	47/114 (41.23)	32/71 (45.07)	15/41 (34.88)	0.248
Positive smear, n (%)	198/593 (30.51)	136/362 (37.57)	62/231 (26.84)	0.007
Positive TB-DNA, n (%)	222/409 (54.28)	140/248 (56.45)	82/161 (50.93)	0.274
Positive TB-IGRA, n (%)	98/159 (61.64)	68/106 (64.15)	32/53 (60.38)	0.818

TB, tuberculosis; PTB, pulmonary tuberculosis; EPTB, extrapulmonary tuberculosis.

### Associations and effect evaluations of candidate single nucleotide polymorphisms (SNPs) on ATD-ADRs

**SNP distributions in the TB cases.** Genetic variants and their characteristics are listed in **Table S4**. Seven successfully genotyped SNPs conformed to Hardy–Weinberg equilibrium ( $P > 0.05$  for all). Compared with male patients, female patients had a higher proportion of the mutant T allele-based genotype in *NUDT15* rs116855232 ( $P = 0.029$ ) (**Table S5**). There were no nominally

significant differences in age and gender among the different genotypes for other SNPs (all  $P > 0.05$ ).

**Potential pharmacogenomic predictors of ATD-ADRs.** Details regarding the correlations among the genotypes and ADRs are presented in **Table 3**. Rs1135840 and rs116855232 were potential pharmacogenetic markers for identifying individuals at elevated risk of hepatotoxicity and leukopenia during anti-TB

**Table 2** The characteristics of adverse drug reactions from TB patients

Adverse drug reactions	All TB patients	Age		Age-based		Gender	
		Mean $\pm$ SD	<i>P</i>	Old /Young	<i>P</i>	M/F	<i>P</i>
Thrombocytopenia							
Presence	16	42.81 $\pm$ 20.94	0.908	6/10	0.964	11/5	0.521
Absence	628	42.24 $\pm$ 19.55		232/396		382/246	
Leukopenia							
Presence	21	45.81 $\pm$ 21.05	0.397	8/13	0.912	<b>6/15</b>	<b>0.002</b>
Absence	623	42.13 $\pm$ 19.52		230/393		<b>387/236</b>	
Hyperbilirubinemia							
Presence	13	43.46 $\pm$ 18.14	0.822	3/10	0.295	10/3	0.235
Absence	631	42.23 $\pm$ 19.61		235/396		383/248	
ATDH							
Presence	68	42.66 $\pm$ 18.94	0.855	24/44	0.764	42/26	0.895
Absence	576	42.20 $\pm$ 19.65		214/362		351/225	
AKI							
Presence	10	<b>55.70 <math>\pm</math> 25.21</b>	<b>0.028</b>	5/5	0.397	<b>9/1</b>	<b>0.037</b>
Absence	634	<b>42.04 <math>\pm</math> 19.41</b>		233/401		<b>384/250</b>	
CKD							
Presence	12	<b>54.25 <math>\pm</math> 22.48</b>	<b>0.032</b>	6/6	0.345	8/4	0.773
Absence	632	<b>42.02 <math>\pm</math> 19.45</b>		400/232		385/247	

Old  $\geq$ 50 years and Young <50 years; M/F, Male/Female; ATDH, antituberculosis drug-induced hepatotoxicity; AKI, acute kidney injury; CKD, chronic kidney damage. Significant associations are denoted in bold.

therapy. TB patients with the GG genotype of rs1135840 experienced a higher risk (2.52-fold) of ATDH than those with an A allele-containing genotype (adjusted  $P = 0.009$ ). Patients with rs1135840 GG showed a marginally higher alanine aminotransferase (ALT) value ( $P = 0.042$ , **Table S6**), but rs1135840 was not associated with ATDH severity. Regarding rs116855232, T allele carriers were more likely to suffer from leukopenia, exhibiting a significantly increased risk (odds ratio (OR) = 4.97; 95% confidence interval (CI) = 2.06–11.97, adjusted  $P = 0.003$ , **Table 3**). Sixteen of the 644 TB patients were identified with a homozygous TT mutation in rs116855232, and the TT genotype-related risk of leukopenia was markedly higher in leukopenia (OR (95% CI) = 7.82 (2.05–29.87), adjusted  $P = 0.018$ ). No significant differences among the frequencies of the other five SNPs were obtained between the ADR cases and the controls (all  $P > 0.05$  before correction).

**Performances of significant SNP screening for ATDH and leukopenia.** We evaluated the predictive capacities of the significant SNPs *CYP2D6* rs1135840 and *NUDT15* rs116855232 (**Table 4**). Because predictive biomarkers are used to exclude patients at risk for developing ADRs, negative predictive value (NPV) and sensitivity become the most important indicators in the analysis.<sup>30</sup> Rs1135840 had good capabilities for these parameters, with an NPV of 93.7% and a sensitivity of 72.7%. The performance of

rs116855232 in predicting ATD-induced leukopenia was moderate, with an NPV of 98.1% but a sensitivity of only 52.4%. Both SNPs showed poor positive predictive values (PPVs).

## DISCUSSION

Globally, ADR has been a major contributor toward anti-TB treatment discontinuation or failure, particularly in developing countries such as China. In the present study, ATD-ADRs noticeably and substantially impacted anti-TB treatment, with an incidence of 16.5% and an intervention rate of 10.4%; fortunately, most were reversible after timely interference, findings that were comparable to other studies from China.<sup>31–33</sup> In our study, ATDH, the most frequent and serious ATD-ADR, was moderate (10.6%) compared with the data in the literature on hospitalized patients (4.8–36%) because of different definitions used, heterogeneous ethnic origins, various sample sizes, and even potential confounding causes of hepatotoxicity. Advanced age and female gender are common risk factors for the occurrence and severity of ATDH; however, significance was not achieved in the present study. The indicators of an unhealthy lifestyle, such as drinking, smoking, and staying up late, are more frequent in younger generations than in the elderly, which may increase younger individuals' predisposition to hepatotoxicity.

Leukopenia and thrombocytopenia are increasing and unfavorable among Chinese TB patients. Despite the threat that they

Table 3 SNP associated with adverse drug reactions from TB patients

Genotypes	Thrombocytopenia (n = 16)		Leukopenia (n = 21)		Hyperbilirubinemia (n = 13)		ATDH (n = 68)		AKI (n = 10)		CKD (n = 12)	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
<b>ABCB1 rs1045642</b>												
AG+GG (n = 538)	0.85 (0.24-3.04)	0.806	0.19 (0.34-4.11)	0.781	1.09 (0.24-4.97)	0.915	1.02 (0.52-2.02)	0.947	1.79 (0.22-14.25)	0.579	0.58 (0.16-2.19)	0.428
AA (n = 106)												
<b>ABCB1 rs1055302</b>												
CT+TT (n = 294)	1.20 (0.44-3.23)	0.724	1.61 (0.67-3.88)	0.282	2.73 (0.83-8.96)	0.085	1.07 (0.64-1.76)	0.806	1.19 (0.34-4.16)	0.781	0.85 (0.27-2.70)	0.780
CC (n = 350)												
<b>CYP2D6 rs1135840</b>												
GG (n = 344)	1.46 (0.52-4.06)	0.469	0.64 (0.27-1.54)	0.316	1.01 (0.34-3.04)	0.985	<b>2.52 (1.43-4.44)</b>	<b>0.009*</b>	0.86 (0.25-3.01)	0.819	0.61 (0.19-1.95)	0.403
CG+CC (n = 298)												
<b>CYP2C19 rs3758580</b>												
CT+TT (n = 335)	0.54 (0.20-1.51)	0.236	1.23 (0.51-2.97)	0.638	1.86 (0.55-6.24)	0.308	1.11 (0.67-1.84)	0.686	3.74 (0.79-17.77)	0.075	1.29 (0.41-4.12)	0.663
CC (n = 308)												
<b>CYP2C19 rs4244285</b>												
GG (n = 310)	1.82 (0.65-5.07)	0.244	0.80 (0.33-1.93)	0.623	0.67 (0.22-2.06)	0.481	0.89 (0.54-1.48)	0.657	0.27 (0.06-1.26)	0.073	0.77 (0.24-2.44)	0.651
AG+AA (n = 334)												
<b>CYP2C19 rs4986894</b>												
TT (n = 310)	1.82 (0.65-5.07)	0.244	0.80 (0.33-1.93)	0.623	0.67 (0.22-2.06)	0.481	0.89 (0.54-1.48)	0.657	0.27 (0.06-1.26)	0.073	0.77 (0.24-2.44)	0.651
CT+CC (n = 334)												
<b>NUDT15 rs116855232</b>												
CT+TT (n = 124)	1.41 (0.45-4.45)	0.568	<b>4.97 (2.06-11.97)</b>	<b>0.003*</b>	0.75 (0.17-3.47)	0.713	0.89 (0.46-1.71)	0.73	1.82 (0.46-7.13)	0.290	1.41 (0.38-5.28)	0.709
CC (n = 520)												

P value has been adjusted for age, gender and BMI.

Considering the low frequencies of some minor genotypes, seven SNPs were stratified based on the dominant or recessive model.

Notably, the rs116855232 TT genotype-related risk is markedly higher (OR = 7.82, 95% CI = 2.05-29.87, adjusted P = 0.018) in leukopenia.

\*Significant associations denoted in bold were performed with Bonferroni corrections.

**Table 4 Performance of pharmacogenetic screening for ATDH and leukopenia from TB patients**

Phenotype	rs1135840*GG			Predictive parameters percent (95% CI)	Phenotype	rs116855232* TT+CT			Predictive parameters percent (95% CI)
	Pos	Neg	Total			Pos	Neg	Total	
ATDH				Sen: 72.7 (61.0-82.0) Spe: 48.6 (44.6-52.7) PPV: 14.0 (10.7-18.0) NPV: 93.7 (90.7-96.2) Accuracy: 51.1 (47.2-54.9)	Leukopenia				Sen: 52.4 (32.4-71.7) Spe: 81.9 (78.6-84.7) PPV: 8.9 (5.0-15.2) NPV: 98.1 (96.5-99.0) Accuracy: 80.9 (77.7-83.8)
Presence	48	18	66		Presence	11	10	21	
Absence	296	280	576		Absence	113	510	623	
Total	344	298	642		Total	124	520	644	

Pos, positive; Neg, negative; Sen, sensitivity; Spe, specificity; PPV, positive predictive value; NPV, negative predictive value.

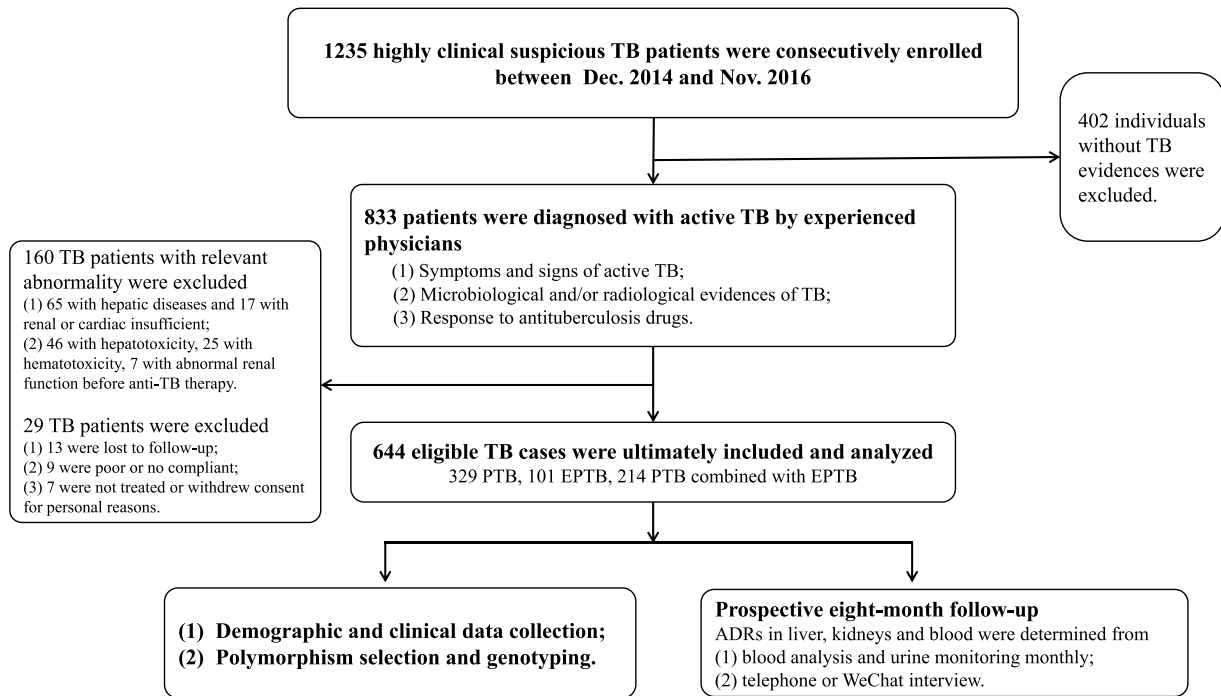
pose to patients, these events have been overlooked in studies from other regions of China.<sup>31–33</sup> A recent retrospective study included a relatively smaller sample of patients from northern China.<sup>33</sup> Two prospective reports,<sup>31,32</sup> which included outpatients and inpatients, came from the Centers for Disease Control and Prevention (CDC); however, since 2010 TB diagnosis and treatment have been transferred from local CDCs to specialized hospitals in China because of the better surveillance of ADRs in hospitals. Our prospective study included 644 TB inpatients from West China Hospital, the largest medical center in western China, and adopted a very strict ADR criterion and causality assessment. This is what differentiates the present study from other similar studies and makes the conclusions of the present study more persuasive and representative. Our findings also provide a basis for patient management, particularly in female patients, who develop hematological toxicity during TB therapy. The current study also evaluated other ADRs, including hyperbilirubinemia, AKI, and CKD, which were not fully understood in a few previous studies.<sup>34,35</sup> AKI and CKD caused by ATDs are rare but severe and have been reported concomitant with hepatitis in 10% of patients.<sup>7</sup> As an absolute number, such a rate is substantial and is a cause for concern in the high TB-burden regions of Asia and South Africa. This concomitance must be considered during the reintroduction to the drug or intermittent administration.

Notably, the present genetic analysis indicated that *CYP2D6* rs1135840 and *NUDT15* rs116855232 were potential pharmacogenomic predictors of developing ATDH and leukopenia. The *CYP2D6* gene encodes an important hepatic phase I drug-metabolizing enzyme involved in the metabolism of up to 25% of medications.<sup>36</sup> Lee *et al.* reported that rs1135840 GG is associated with higher norclozapine/clozapine serum drug levels.<sup>37</sup> Zhang *et al.* found that CpG hypermethylation of *CYP2D6* and rs1135840 increased the risk of ATDH in a small Chinese population.<sup>38</sup> We determined that the rs1135840 GG genotype was associated with an increased risk of ATDH, which was in accordance with Zhang *et al.*'s findings.<sup>38</sup> Previous significant SNPs that have been reported to be directly associated with ATDH are not in linkage disequilibrium with rs1135840 and are unlikely to be confounding factors for the association of rs1135840 with ATDH (Table S7). Located in exon 9 of the *CYP2D6* gene,

rs1135840 encodes a Ser/Thr missense substitution and is a part of the extremely common decreased-functional haplotype *CYP2D6*\*10. This variant could result in interindividual variability of *CYP2D6* enzymatic activity or expression. Previous studies and our results suggest that the *CYP2D6* gene may play a role in participating in the metabolism of ATDs or forming covalent adducts with ATDs, similar to the effect of some other general CYP enzymes on ATDs<sup>39,40</sup>; however, this possibility should be verified in functional assays.

The *NUDT15* enzyme catalyzes the hydrolysis of oxidatively damaged nucleotides to prevent them from being incorporated into DNA, thereby reducing DNA damage and the risk of apoptosis.<sup>41,42</sup> *NUDT15* rs116855232 C>T (p.Arg139Cys), which is characteristic of Asian and Native American populations, was observed in 10.8% of our study population and seemed to make a significant contribution to the development of leukopenia. In TB patients, the presence of the *NUDT15*\*T allele would confer a 4.97-fold increased risk for leukopenia, and the TT genotype-related risk is much higher (7.82-fold). A well-established association of *NUDT15* C>T with thiopurine-related leukopenia has been confirmed among different populations, endowing the homozygous TT with a remarkable up to 35-fold increase in leukopenia risk.<sup>41–44</sup> Functional experiments have demonstrated that the loss-of-function mutation *NUDT15* (rs116855232 TT) causes increased cell apoptosis and decreased cell viability, as well as low tolerance to 6-MP treatment.<sup>41</sup> In clinical practice in China, prior to azathioprine/6-mercaptopurine initiation, patients are currently required to test *NUDT15* rs116855232 C>T in advance, and a dose reduction of at least 50% is recommended for those with homozygous *NUDT15* variants. These findings compellingly point to *NUDT15* rs116855232 as a pharmacogenetic marker for drug-induced leukopenia, particularly in patients with Asian or Native American ancestry.

Genetic risk assessment is useful to exclude ADRs for their high NPV.<sup>30</sup> The NPVs of 93.7% and 98.1% obtained in our study are significant as screening tests, although well-designed prospective studies are warranted to confirm and generalize as appropriate. The PPVs are low for identifying persons with ATDH and leukopenia, which is likely due to the relatively high frequency of the risk genotypes in the study population (~53% for *CYP2D6* and 19% for *NUDT15*) compared with the low



**Figure 1** Flow chart of the study population. PTB, pulmonary tuberculosis; EPTB, extrapulmonary tuberculosis; anti-TB, antituberculosis.

incidence of these ADRs (~10.6% for ATDH and 3.3% for leukopenia).<sup>30</sup> These results also reflect that gene determinants are not sufficient to trigger ATD-ADRs, and combinatorial indicators including genetic risk variants and nongenetic risk factors may be more powerful in predicting ATD-ADRs.<sup>20</sup> Genetic assessment and regular blood monitoring are helpful to identify patients with a high ATD-ADR risk. A series of measures can be taken to prevent ATD-ADRs in these patients, including the use of lower initial doses, dose reduction, and coadministration of protective agents. Prompt withdrawal of the offending medication and regimen changes remain the most critical strategy applied in clinical practice.<sup>45</sup>

Our study has several limitations. First, given the small number of patients with ADRs, multicenter studies are warranted to confirm the findings. Second, a combination of drug-related factors, such as drug dosage, therapeutic drug monitoring, and genetic and nongenetic risk factors, should be incorporated into algorithms to improve predictive capability over that of genetic tests alone. Third, we assessed only the ADRs induced by standard multidrug regimens, and the specific effects of single drugs on ADRs, as well as the effects of high or toxic doses of ATDs in patients with extensively drug-resistant tuberculosis and tuberculous meningitis should be investigated further. Furthermore, some patients took TB medicines for more than 8 months, which was longer than our follow-up period; consequently, ATD-ADRs among different courses of treatment still need to be studied intensively.

In summary, western China had a marked epidemic of ATD-ADRs, significantly impacting subsequent therapeutic orientation. Regular blood examinations are suggested to closely monitor adverse events. We identified *CYP2D6* rs1135840 and *NUDT15*

rs116855232 as genetic risk factors for the development of ATDH and leukopenia, highlighting the importance of pharmacogenetic analyses during individualized TB treatment.

## METHODS

### Study design and patient population

We consecutively recruited 1,235 highly suspicious TB patients at West China Hospital, western China, between December 2014 and November 2016. In total, 833 TB patients were confirmed by experienced respiratory physicians, based on typical TB symptoms, microbiological and/or radiological evidence of MTB, and response to ATDs. Patients with hepatitis, HIV infection, and renal, hematological, and cardiac diseases were excluded. Those with definite signs of aberrant liver and renal function and abnormal hematologic indicators before anti-TB treatment were also excluded. All patients underwent short-course chemotherapy consisting of at least oral INH (300–400 mg/day), RIF (450–600 mg/day), PZA (1,500–2,000 mg/day), and ethambutol (EMB, 750–900 mg/day) for the first 2 months, followed by INH, RIF, and EMB for at least 4 more months. If patients developed a definite ADR, their treatments were adjusted accordingly. Twenty-nine patients were poorly adherent to the treatment or dropped out of the 8-month follow-up. Ultimately, 644 TB patients were enrolled in the prospective study.

Demographic and clinical data of the patients were obtained from medical records. Blood samples were collected from each participant for genotyping. Pretherapeutic laboratory tests and chest radiographs were performed in the clinical lab of West China Hospital. After ATDs were started, blood work, including analysis of biochemical parameters and hematological blood counts, and urine analysis were performed twice a month during the first 2 months and monthly in subsequent follow-up appointments. The peak or valley values of laboratory indicators during the 8-month follow-up were recorded and used to assess ATD-ADRs. Patients were regularly questioned about symptoms regarding ATD-ADRs over the telephone or WeChat if possible. A flow diagram of the process used to enroll the participants is shown in **Figure 1**. This study was approved by the Committee on Human Research, Publications and

Ethics, West China Hospital, Sichuan University (China), and written informed consent was obtained from each participant.

### Definition of ATD-ADRs and their severity

ATD-ADRs in the liver, kidneys, and blood were strictly defined as grade 2 or 3 according to the National Institutes of Health and Common Toxicity Criteria for Adverse Events v. 4.0 (CTCAE v. 4.0)<sup>22</sup> unless stated otherwise. Causality assessments of ATD-ADRs were used to determine whether ADRs were present or not.<sup>23</sup>

**ADRs in liver.** (1) ATDH. Individuals who presented with ALT and aspartate aminotransferase (AST) levels  $\geq 3 \times$  the upper limit of normal (ULN) (120 IU/L) in the presence of hepatitis symptoms such as jaundice, nausea, vomiting, and abdominal pain and individuals presenting with ALT and AST levels  $\geq 5 \times$  the ULN (150 IU/L), with or without symptoms, were considered to have ATDH.<sup>22,24</sup> The severity of ATDH was classified as mild, moderate, or severe.<sup>22,25</sup> (2) Hyperbilirubinemia. An increase in total bilirubin  $\geq 1.5 \times$  the ULN (42  $\mu\text{mol/L}$ ) was defined as hyperbilirubinemia.<sup>22</sup>

**Renal impairment.** CKD was defined as the persistence of kidney injury (eGFR  $< 60$  ml/min /1.73 m<sup>2</sup> and presence of proteinuria) for more than 3 months.<sup>26</sup> AKI was determined based on the presence of severe kidney injury within 48 h according to the consensus<sup>26</sup> and the KDIGO AKI Guideline.<sup>27</sup>

**Hematotoxicity.** Anemia (hemoglobin concentration  $\leq 80 \times 10^{12}/\text{L}$ ), leukopenia (absolute counts  $\leq 2.0 \times 10^9/\text{L}$ ), and thrombocytopenia (absolute counts  $\leq 75 \times 10^9/\text{L}$ ) were considered as forms of hematological toxicity.<sup>22</sup>

**ATD-ADRs severity.** ATD-ADRs were graded as mild, moderate, and severe. ADRs were described as mild when they had little impact on TB management and when treatment was unnecessary. Moderate ADRs were designated when related symptoms and signs returned to normal upon the change/discontinuation of ATDs or when symptomatic treatment was initiated. Severe ADRs were those that led to prolonged hospital stays, required special care, or caused disability or death.<sup>28</sup> For patients with moderate and severe ADRs, in addition to symptomatic treatment, TB medications were stopped if necessary and blood analyses were performed weekly until the blood indicators returned to normal levels.

### Candidate polymorphism selection and genotyping

Key genes in the drug metabolic pathways and drug transport pathways, including *CYP2D6*, *CYP2C19*, *ABCB1*, and *NUDT15*, which were representative and highly polymorphic, were selected based on their biological functions and putative evidence for associations with ATD-ADRs. *CYP2D6* and *CYP2C19* encode two important hepatic phase I drug-metabolizing enzymes, and *ABCB1* encodes P-glycoprotein, a well-known drug transporter protein, and these genes may be involved in metabolism/transport of ATDs (see **Supplementary References 1–12**). Additionally, *NUDT15* encodes an important hydrolytic enzyme in the Nudix hydrolase superfamily. We selected candidate SNPs in these four genes by performing thorough searches of the dbSNP database and 1000 Genomes Project. SNPs were included if they were located in the promoter or exon region and had a minor allele frequency  $>0.05$  among the Han Chinese in Beijing and if they had been implicated in the toxicity or ADRs of other drugs but rarely in that of anti-TB drugs. Under the experimental conditions required for genotyping seven SNPs (*CYP2D6* rs1135840; *CYP2C19* rs3758580, rs4244285, rs4986894; *ABCB1* rs1045642, rs1055302; *NUDT15* rs116855232) were eventually included. Genomic DNA was extracted from peripheral blood and was genotyped using a commercial custom-by-design 48-Plex SNPscan Kit, as described previously,<sup>29</sup> which is based on patented SNP genotyping technology with a double-ligation and multiplex fluorescence polymerase chain reaction. For quality control purposes, 30 random samples were

genotyped in duplicate, with a concordance rate of 100%, and three samples with different genotypes for each SNP were randomly selected to confirm the genotyping results by direct sequencing.

### Statistical analysis

Univariate analysis was used to analyze categorical variables with the chi-square test and continuous variables were analyzed with the Mann–Whitney *U*-test. Associations between SNPs, TB characteristics, and ATD-ADRs were evaluated using the unconditional logistic regression after adjusting for age, gender, and BMI. ORs and 95% CIs were used as measures of associations. Bonferroni corrections were used to adjust significant associations. The above-mentioned analyses were performed using SPSS v. 22.0 (IBM, Armonk, NY). Performances of SNPs, including sensitivity, specificity, PPV, and NPV, were calculated according to the OpenEpi (Dean, A.G. *et al.* The OpenEpi Collection of Epidemiologic Calculators <[http://www.openepi.com/Menu/OE\\_Menu.html](http://www.openepi.com/Menu/OE_Menu.html)> Accessed 4 April 2013). Statistical significance was set at 0.05.

Additional Supporting Information may be found in the online version of this article.

### ACKNOWLEDGMENTS

Xuejiao Hu, Mei Zhang, and Hao Bai contributed equally to this work. This work was funded by grants from the National Natural Science Foundation of China (81672095) and the Projects of the Health and Family Plan in Sichuan Province (16ZD004). The authors also thank the Shanghai Genesky Bio-Tech Genetic Core Lab for providing assistance with genotyping techniques.

### CONFLICT OF INTEREST

The authors declare that they have no competing interests.

### AUTHOR CONTRIBUTIONS

X.H., M.Z., H.B., X.C., and Y.Z. wrote the article; B.Y. designed the research; X.H., H.B., L.W., Y.C., L.D., Z.Z., W.P., T.L., and J.S. performed the research; X.H., M.Z., Y.L., and X.L. analyzed the data; M.Z. and X.C. contributed new reagents/analytical tools.

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