

NAT2, *CYP2C9*, *CYP2C19*, and *CYP2E1* genetic polymorphisms in anti-TB drug-induced maculopapular eruption

Sang-Heon Kim · Sang-Hoon Kim · Ho Joo Yoon ·
Dong Ho Shin · Sung Soo Park · Youn-Seup Kim ·
Jae-Seuk Park · Young Koo Jee

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Abstract

Purpose It has been suggested that drug-metabolizing enzymes might play important roles in the development of anti-tuberculosis drug (ATD)-induced maculopapular eruption (MPE), as in ATD-induced hepatitis. We investigated the associations between the genetic polymorphisms of drug-metabolizing enzymes and ATD-induced MPE.

Methods We enrolled 62 patients with ATD-induced MPE (mean age 47.2±19.0, male 59.7%) and 159 patients without any adverse reactions to ATD (mean age 42.8±17.6, male 65.4%), among patients with pulmonary tuberculosis (TB) and/or TB pleuritis and treated with first-line anti-TB medications, including isoniazid, rifampin, ethambutol, and pyrazinamide. We compared the

genotype distributions of single nucleotide polymorphisms and haplotypes in four drug-metabolizing enzymes (N-acetyltransferase 2 (*NAT2*), cytochrome P450 (*CYP*) 2C9, *CYP2C19*, and *CYP2E1*) among patients with ATD-induced MPE and patients tolerant to ATD using a multivariate logistic regression analysis. These analyses were made without identification of the responsible ATD. **Results** -1565 C>T of *CYP2C9* showed a significant association with ATD-induced MPE ($P=0.022$, OR=0.23, 95% CI 0.07–0.78), with a lower frequency of genotypes carrying minor alleles (CT or TT) in the case group than in the controls. Additionally, W212X of *CYP2C19* was significantly associated with the risk of ATD-induced MPE ($P=0.042$, OR=0.27, 95% CI 0.09–0.82). In an analysis of the *CYP2C19*–*CYP2C9* haplotypes (-1418 C>T_W212X_-1565 C>T_-1188 C>T), ht3[T-A-T-C] showed a significant association with the development of ATD-induced MPE ($P=0.012$, OR=0.13, 95% CI 0.03–0.57). No significant associations between the other genetic polymorphisms and ATD-induced MPE were observed. **Conclusions** *CYP2C19* and *CYP2C9* genetic polymorphisms are significantly associated with the risk of developing ATD-induced MPE, and the genetic variants in *NAT2* and *CYP2E1* are not closely related to the development of this adverse reaction.

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S.-H. Kim · H. J. Yoon · D. H. Shin · S. S. Park
Department of Internal Medicine,
Hanyang University College of Medicine,
Haengdang-dong 17,
Seongdong-gu, Seoul, Korea

S.-H. Kim
Department of Internal Medicine,
Eulji University School of Medicine,
Hagye-dong 280-1,
Nowon-gu, Seoul, Korea

Y.-S. Kim · J.-S. Park · Y. K. Jee (✉)
Department of Internal Medicine,
Dankook University College of Medicine,
Anseo-dong San 16-5,
Cheonan, Korea
e-mail: ykjee@dankook.ac.kr

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Introduction

Cutaneous adverse drug reactions, such as maculopapular eruptions (MPE), are common and major adverse reactions

induced by first-line anti-tuberculosis drugs (ATD) [1]. The incidence of ATD-induced skin rashes is 0.1–5.7% in patients receiving first-line ATD such as isoniazid, rifampin, ethambutol, and pyrazinamide. Although mild symptoms can be self-limiting and tolerated with or without symptomatic treatment, moderate to severe skin rashes require a discontinuation of medication, which often delays scheduled tuberculosis (TB) treatment [2]. Of the ATD-induced serious side effects requiring discontinuation of medication, the incidence of ATD-induced rash was highest (0.25 events per 100 person-months of treatment) among various adverse events such as hepatitis and gastrointestinal side effects [3].

Anti-tuberculosis drugs-induced skin reactions appear most frequently as MPE [4]. Although some demographic and clinical factors, such as old age, female sex, malnutrition, chronic liver diseases and HIV infection, have been related to the risk of ATD-induced adverse reactions [3, 5, 6], the mechanisms of ATD-induced MPE are not well understood. Drug-induced MPE appears to be mediated by a delayed drug-hypersensitivity reaction in which drug-specific T cells are activated in a major histocompatibility complex-dependent manner [7]. Drugs are not immunogenic in most cases; however, reactive metabolites generated by drug metabolizing enzymes can bind to a high-molecular-weight protein and initiate an immune response [8]. Therefore, it can be hypothesized that drug-metabolizing enzyme polymorphisms are associated with the development of drug-induced MPE. Genetic polymorphisms in cytochrome P450 (CYP) 2 C9 have been associated with cutaneous adverse reactions induced by diphenylhydantoin [9].

Isoniazid metabolites, such as hydrazine, induce liver injury [10], and the metabolisms of isoniazid and the formation of isoniazid metabolites are genetically determined [11]. The association between ATD-induced hepatitis and drug-metabolizing enzyme polymorphisms has been extensively investigated [12]. Genetic polymorphisms in drug-metabolizing enzymes, including N-acetyltransferase 2 (NAT2) [13–15], CYP2E1 [16, 17], glutathione S-transferase M1 (GSTM1) [18], and glutathione S-transferase T1 (GSTT1) [19], are significantly associated with the development of ATD-induced hepatitis. However, the relationship of these genetic polymorphisms to the development of ATD-induced MPE has not been explored except for GSTT1 and GSTM1 null mutations, which revealed no significant association with ATD-induced MPE [20].

In this study, we investigated the associations between drug-metabolizing enzyme gene polymorphisms and ATD-induced MPE. Among numerous phase I and phase II enzymes, we selected NAT2, CYP2C9, CYP2C19, and CYP2E1 because these enzymes are involved in ATD

metabolism and are implicated in adverse drug reactions induced by various drugs including ATD [6, 21].

Materials and methods

Participants

We enrolled patients with ATD-induced MPE and patients without any adverse reactions to ATD, among patients newly diagnosed with pulmonary TB and/or TB pleuritis and treated with first-line anti-TB medications such as isoniazid, rifampin, ethambutol, and pyrazinamide. The case and control subjects were recorded in the Adverse Drug Reaction Pharmacogenomic Research Group database of Korea, in which cases of adverse reactions induced by various drugs were collected from seven university hospitals in Korea (Dankook University Hospital, Eulji University Hospital, Hanyang University Hospital, Hallym University Hospital, Seoul National University Hospital, Ajou University Hospital, and Seoul National University Bundang Hospital) from July 2003 to October 2008. This study was approved by the institutional review boards of each participating hospital. Written informed consent was obtained from all enrolled subjects.

Before treatment, each patient underwent a liver function test, complete blood cell count analysis, and hepatitis B or C viral marker study. The presence of HIV infection was not examined in our study population, because the prevalence of HIV infection was very low even among the patients with TB in Korea [22]. All the patients with tuberculosis were treated with first-line ATD according to treatment guidelines from the American Thoracic Society (New York, NY, USA), Centers for Disease Control and Prevention (Atlanta, GA, USA), and the Infectious Diseases Society of America (Arlington, VA, USA) [1]. The treatment consisted of an initial phase of 2 months and a subsequent continuation phase of 4 or more months. During the initial phase, four drugs were administered including isoniazid (300–400 mg daily), rifampicin (450–600 mg daily), ethambutol (600–800 mg daily), and pyrazinamide (1,000–1,500 mg daily). Doses of each drug were adjusted based on the body weight of the patient. In the following continuation phase, only pyrazinamide was discontinued, while the other three drugs were continued. Apart from four drugs, none of the other ATD, including streptomycin, was used for the treatment of TB. Patients were seen at regular intervals and questioned about symptoms regarding adverse reactions to anti-TB drugs. In addition, an unscheduled visit occurred if a new symptom appeared. ATD-induced MPE was defined as the development of MPE after receiving first-line ATD and the disappearance of MPE after discontinuing ATD because of MPE. Skin lesion diagnoses

were made by allergists or dermatologists at each hospital. In all the patients with MPE, skin lesions developed within 1 month of the initiation of treatment. The exclusion criteria were:

1. Patients with skin diseases before treatment
2. Chronic renal failure and chronic liver diseases affecting drug metabolism
3. Chronic alcoholism
4. Other chronic medical conditions requiring medication
5. Non-adherence to the treatment

We selected age- and gender-matched control subjects from among patients with TB who did not show any ATD-related adverse reactions during the treatment period and who agreed to participate in this study. No significant difference in demographic parameters, such as age, gender, height and weight, or body mass index was found between case and control subjects (Table 1).

Selection of SNPs and genotyping

DNA was extracted from peripheral blood of the study subjects using the Genomic PUREGENE® DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA). We selected the *NAT2*, *CYP2C9*, *CYP2C19*, and *CYP2E1* SNPs for evaluation in this case-controlled analysis, as described previously [14]. Briefly, we evaluated the SNP frequencies in 43 healthy Korean subjects. Priorities were given to 2-kb SNPs in the 5'-upstream region of the promoter and nonsynonymous coding SNPs in the exons from genetic polymorphisms reported in the public SNP database (NCBI dbSNP; www.ncbi.nlm.nih.gov/snp), although non-coding SNPs might have an impact on protein expression and function. Then, we selected SNPs for genotyping in our study population among the informative SNPs with minor allele frequencies greater than 0.02 and r^2 values >0.8 after determining linkage disequilibrium (LD) patterns (Supplementary Table 1). The selected SNPs were genotyped with the high throughput single base-pair extension method (SNP-IT™ assay) using a SNPstream 25 K system, which was customized to automatically genotype DNA samples in 384-well plates and provide a colorimetric readout (Orchid Biosciences, Princeton, NJ, USA), as described previously [23]. After examining Lewontin's D' ($|D'|$) and the LD

coefficient r^2 between all pairs of biallelic loci, the *CYP2C19* and *CYP2C9* haplotypes and their frequencies were estimated using Haploview version 3.32 (<http://www.broad.mit.edu/mpg/haploview/>).

Statistical analysis

We compared genotype frequencies of SNPs and haplotypes between patients with ATD-induced MPE and ATD-tolerant controls using a multivariate logistic regression analysis adjusted for age and gender. The Hardy–Weinberg equilibrium was tested using Chi-squared tests. All statistical analyses were performed using SAS (version 9.13; SAS Institute, Cary, NC, USA), and P values <0.05 were regarded as statistically significant. Furthermore, we evaluated statistical significance by correcting P values for multiple comparisons to account for the observed alleles (numbers of SNPs or haplotypes) using the Bonferroni method.

Results

Polymorphisms in patients with ATD-induced MPE and controls

The SNP genotype frequencies of four genes in the case and control groups and the results of the analysis for associations are presented in Table 2. No significant difference in genotype frequencies was observed between patients with ATD-induced MPE and ATD-tolerant controls in the analysis of four selected *NAT2* SNPs (−9796A>T and −9601A>G in the promoter and R197Q and G286E in the exons).

Of three *CYP2C9* SNPs, −1565 C>T showed a significant association with ATD-induced MPE, with lower frequencies of genotypes carrying a minor allele (CT or TT) in the case group compared with controls (4.9% vs 18.5%, corrected P value [P_c]=0.022, OR=0.23, 95% CI 0.07–0.78). The genotype frequencies of the other two *CYP2C9* SNPs (−1188 C>T and I359L) were not different between case and control subjects. Another significant association was found in W212X of *CYP2C19*. A significantly smaller number of patients with ATD-induced MPE

Table 1 Study subject characteristics

Characteristics	ATD-induced MPE ($N=62$)	ATD-tolerant ($N=159$)	P value
Male gender, % (n)	59.7 (37)	65.4 (104)	NS
Age, years	47.2±19.0	42.8±17.6	NS
Height, cm	162.6±10.0	165.3±8.9	NS
Weight, kg	58.1±11.1	58.0±10.2	NS
Body mass index, kg/m ²	21.4±4.7	21.2±3.1	NS

Data are shown as the means ± SDs, except for gender

ATD, anti-tuberculosis drug; MPE, maculopapular eruption; NS, not significant

Table 2 Genotype frequencies of the *NAT2*, *CYP2C9*, *CYP2C19* and *CYP2E1* SNPs

SNP	Genotype	ATD-induced MPE, <i>N</i> =62 (%)	ATD-tolerant, <i>N</i> =159 (%)	<i>P</i> _c value*	OR (95% CI)
<i>NAT2</i>					
-9796 T>A	TT	39 (62.9)	103 (64.8)	NS	1.15 (0.62–2.14)
	TA+AA	23 (37.1)	56 (35.2)		
-9601A>G	AA	18 (29.5)	34 (21.4)	NS	0.61 (0.31–1.20)
	AG+GG	43 (70.5)	125 (78.6)		
R197Q	GG	40 (69.0)	102 (68.0)	NS	1.05 (0.54–2.05)
	GA+AA	18 (31.0)	48 (32.0)		
G286E	GG	39 (66.1)	116 (76.3)	NS	1.67 (0.86–3.23)
	GA+AA	20 (33.9)	36 (23.7)		
<i>CYP2C9</i>					
-1565 C>T	CC	58 (95.1)	128 (81.5)	0.022	0.23 (0.07–0.78)
	CT+TT	3 (4.9)	29 (18.5)		
-1188 C>T	TT	19 (31.7)	51 (32.7)	NS	1.05 (0.55–1.99)
	TC+CC	41 (68.3)	105 (67.3)		
I359L	AA	54 (90.0)	142 (92.2)	NS	1.32 (0.47–3.68)
	AC+CC	6 (10.0)	12 (7.8)		
<i>CYP2C19</i>					
-1418 C>T	CC	49 (83.1)	115 (73.7)	NS	0.57 (0.27–1.23)
	CT+TT	10 (16.9)	41 (26.3)		
W212X	GG	57 (93.4)	127 (80.9)	0.042	0.27 (0.09–0.82)
	GA+AA	4 (6.6)	30 (19.1)		
<i>CYP2E1</i>					
-1055 C>T	CC	40 (66.7)	97 (63.4)	NS	0.89 (0.47–1.68)
	CT+TT	20 (33.3)	56 (36.6)		
-352A>G	AA	43 (70.5)	110 (71.0)	NS	1.07 (0.55–2.08)
	AG+GG	18 (29.4)	45 (29.0)		
-333A>T	TT	27 (44.3)	64 (41.6)	NS	0.95 (0.52–1.74)
	TA+AA	34 (55.7)	90 (58.4)		

ATD, anti-TB drug; MPE, maculopapular eruption; OR, odds ratio; CI, confidence interval; NS, not significant; SNP, single nucleotide polymorphism

*The *P*_c values were adjusted using Bonferroni's correction for multiple comparisons

had minor allele-containing genotypes (GA or AA) than controls (6.6% vs 19.1%, *P*_c=0.042, OR=0.27, 95% CI 0.09–0.82). The distribution of the other SNP of *CYP2C19*, -1418 C>T, was similar in case and control subjects. We found no significant association between each of the three *CYP2E1* SNPs (1055 C>T, -352A>G, and -333A>T) and ATD-induced MPE.

Haplotypes of *CYP2C19*-*CYP2C9* in patients with ATD-induced MPE and controls

Because *CYP2C9* and *CYP2C19* are located end to end on chromosome 10q24, and the SNPs of the two genes showed a significant LD, we investigated whether the *CYP2C9* and *CYP2C19* haplotypes were associated with ATD-induced MPE. Of three *CYP2C19*-*CYP2C9* haplotypes (-1418 C>T_W212X_-1565 C>T_-1188 C>T) with frequencies

higher than 0.05, ht3[T-A-T-C], carrying both significantly associated SNPs, *CYP2C19* W212X and *CYP2C9* -1565 C>T, showed a significant association with the development of ATD-induced MPE (*P*_c=0.012, OR=0.13, 95% CI 0.03–0.57; Table 3). Ht3[T-A-T-C] was present in only 0.4% of the ATD-induced MPE group, whereas 18.7% of the ATD-tolerant controls were positive for ht3[T-A-T-C]. In the *NAT2* and *CYP2E1* haplotype analysis, no significant association between the haplotypes of each gene and ATD-induced MPE was observed (Table 4).

Discussion

To the best of our knowledge, this was the first study to investigate the associations between drug-metabolizing enzyme gene polymorphisms and ATD-induced MPE,

Table 3 *CYP2C9* and *CYP2C19* haplotype frequencies

Haplotype ^a	Genotype	ATD-induced MPE, <i>N</i> =62 (%)	ATD-tolerant, <i>N</i> =159 (%)	<i>P</i> c value*	OR (95% CI)
ht1[C-G-C-T]	Present	49 (83.0)	126 (81.3)	NS	1.14 (0.51–2.56)
	Absent	10 (17.0)	29 (18.7)		
ht2[C-G-C-C]	Present	33 (55.9)	71 (45.8)	NS	1.68 (0.90–3.12)
	Absent	26 (44.1)	84 (54.2)		
ht3[T-A-T-C]	Present	2 (0.4)	29 (18.7)	0.012	0.13 (0.03–0.57)
	Absent	57 (96.6)	126 (81.3)		

ATD, anti-TB drug; MPE, maculopapular eruption; OR, odds ratio; CI, confidence interval; ht, haplotype; NS, not significant

*The *P*c values were adjusted using Bonferroni's correction for multiple comparisons

^aHaplotypes (*CYP2C19* -1418 C>T_ W212X_ *CYP2C9* -1565 C>T_ -1188 C>T) with frequencies >0.05 are listed in descending order

which is one of the most common adverse reactions induced by ATD. Among the SNPs of four drug-metabolizing enzymes (*NAT2*, *CYP2C9*, *CYP2C19*, and *CYP2E1*), *CYP2C9* -1565 C>T and *CYP2C19* W212X showed a significant association with ATD-induced MPE. Furthermore, the *CYP2C19*–*CYP2C9* haplotype [T-A-T-C] was significantly associated with the risk of developing ATD-induced MPE. However, SNPs in *NAT2* [13–15] and *CYP2E1* [16, 17], which have been reported to be significantly associated with ATD hepatitis, did not show significant associations with ATD-induced MPE.

CYP2C9 is involved in the metabolism of up to 10% of therapeutic agents, mostly nonsteroidal anti-inflammatory drugs, oral anti-coagulants, sulfonyleurea compounds, and angiotensin receptor antagonists. Metabolism of isoniazid

and rifampin is partly mediated by *CYP2C9* [21]. Of more than 30 known *CYP2C9* polymorphisms, *CYP2C9**2 (R144C) and *3 (I359L) affect the metabolism and treatment response of various drugs [24]. In our analysis, *CYP2C9**2 was not found in Koreans, in accordance with previous reports in Asians and Koreans [25], and the *CYP2C9**3 genotype frequency, which was 0.111 in healthy Koreans (Supplementary Table 1) and 0.042 in the study population, did not show an association with ATD-induced MPE. Interestingly, -1565 C>T in the promoter area was significantly associated with the risk of developing MPE while taking ATD medication. Contrary to the very low frequencies of -1565 C>T in Caucasians [26], -1565 C>T was present in 9.5% of healthy Koreans and 7.8% of the study population in this study, and genotypes

Table 4 *NAT2* and *CYP2E1* haplotype frequencies

Haplotype	Genotype	ATD-induced MPE, <i>N</i> =62 (%)	ATD-tolerant, <i>N</i> =159 (%)	<i>P</i> value	OR (95% CI)
<i>NAT2</i> ^a					
ht1[G-G-G]	Present	39 (68.4)	119 (81.5)	NS	0.47 (0.23–0.96)
	Absent	18 (31.6)	27 (18.5)		
ht2[A-A-G]	Present	18 (31.6)	47 (32.2)	NS	1.06 (0.54–2.09)
	Absent	39 (68.4)	99 (67.8)		
ht3[A-G-G]	Present	18 (31.6)	53 (36.3)	NS	0.80 (0.41–1.54)
	Absent	39 (68.4)	93 (63.7)		
ht4[A-G-A]	Present	20 (35.1)	35 (24.0)	NS	0.80 (0.41–1.54)
	Absent	37 (64.9)	111 (76.0)		
<i>CYP2E1</i> ^b					
ht1[C-A-T]	Present	50 (83.3)	130 (86.1)	NS	0.79 (0.35–1.80)
	Absent	10 (16.7)	21 (13.9)		
ht2[T-A-A]	Present	19 (31.7)	53 (35.1)	NS	0.88 (0.46–1.68)
	Absent	41 (68.3)	98 (64.9)		
ht3[C-G-A]	Present	17 (28.3)	41 (27.2)	NS	1.11 (0.56–2.18)
	Absent	43 (71.7)	110 (72.8)		

ATD, anti-TB drug; MPE, maculopapular eruption; OR, odds ratio; CI, confidence interval; ht, haplotype; NS not significant

^a *NAT2* haplotypes (-9601A>G_R197Q_ G286E) with frequencies >0.05 are listed in descending order

^b *CYP2E1* haplotypes (-1055 C>T_ -352A>G_ -333A>T) with frequencies >0.05 are listed in descending order

carrying the variant allele (−1565 T) were much lower in patients with ATD-induced MPE compared with ATD-tolerant controls. These findings suggest a protective role for −1565 T in the development of MPE induced by ATD. Although the functional role of −1565 C>T in enzyme activities has not yet been determined and was not evaluated in the current study, it can be hypothesized that −1565 C>T also has an impact on CYP2C9 expression and enzymatic activity. In ATD-induced hepatitis, variant genotypes in the promoter of *NAT2* affected gene expression and the production of reactive metabolites [14]. Another possible explanation for the association between −1565 C>T and ATD-induced MPE is that this SNP is in close LD with IVS3 −65 G>C and IVS4 −15A>G [26]; therefore, metabolic activity could be influenced by −1565 promoter activity and mRNA stabilizing activity. Because a close LD was found between *CYP2C9* −1565 C>T and *CYP2C19* W212X and the *CYP2C9* and *CYP2C19* haplotype [T-A-T-C] showed significant association with ATD-induced MPE, we can speculate that the statistical association of *CYP2C9* −1565 C>T is due to the effect of varied *CYP2C19* W212X enzymatic activity. However, the functional role of *CYP2C9* −1565 C>T should be evaluated in future studies.

In addition to *CYP2C9* −1565 C>T, we found a significant association with *CYP2C19* W212X (*CYP2C19**3 in the nomenclature) in ATD-induced MPE. *CYP2C19* is involved in the metabolism of isoniazid and rifampin [21]. W212X is associated with a poor metabolizer phenotype and is mainly found in Asians with a frequency of 0.06–0.10 [27]. Therefore, it can be speculated that genetic variation in *CYP2C19* W212X decreases enzymatic activity and affects the formation of metabolites, which can initiate an immune response. To test this hypothesis, enzymatic activity or a measurement of metabolites in peripheral blood and/or in skin should be evaluated.

Unlike previous studies of ATD-induced hepatitis, our study found no association between *NAT2* polymorphisms and ATD-induced MPE implying a different pathogenesis for these complications [13–15, 28]. An increasing body of evidence confirms that associations between genotypes and drug-induced hypersensitivity reactions are phenotype-specific [29]. For example, HLA-B*1502 is strongly associated with Stevens–Johnson syndrome induced by carbamazepine [30]. However, this association was not found in other carbamazepine-induced cutaneous adverse reactions, such as MPE and hypersensitivity syndrome [31]. Similarly, our findings of an inconsistency in the association between *NAT2* polymorphisms and phenotypes of ATD-induced hypersensitivity reactions [14] suggest that associations between *NAT2* polymorphisms and ATD-induced adverse reactions are also phenotype-specific. Moreover, contrary to the association between *CYP2E1* polymorphisms

and ATD-induced hepatitis [16, 17], no significant relationship was observed between *CYP2E1* polymorphisms and ATD-induced MPE. These findings also imply that the association between *CYP2E1* polymorphisms and ATD-induced adverse reactions is phenotype-specific.

A few limitations of this study should be addressed. First, the sample size is small in keeping with the relatively low incidence of ATD-induced MPE; nonetheless, the use of a multi-center design enabled the inclusion of more patients than in previous similar studies. The statistical significance of the SNP results presented in this study proved valid even after the application of Bonferroni correction for multiple comparisons. Second, we enrolled the patients with ATD-induced MPE without identification of the responsible drug. Therefore, it is possible that a significant association between one specific ATD and the related enzyme gene polymorphisms might be masked by the cases with the other forms of ATD-induced MPE. However, it is practically impossible to identify all the culprit drugs in every case. Given this limitation in the clinical practice, the previous genetic association studies on ATD-induced adverse reactions have not specified the single culprit ATD-induced ADR [13–16, 18–20]. Nevertheless, we found some significant genetic markers related to the development of ATD-induced MPE. Because combination chemotherapy is the recommended treatment for TB, our results could assist the identification of high-risk patients before treatment initiation.

In summary, the results showed a significant association of *CYP2C9* −1565 C>T, *CYP2C19* W212X, and a haplotype of *CYP2C9*–*CYP2C19* [T-A-T-C] with the development of ATD-induced MPE. No significant association between the *NAT2* and *CYP2E1* polymorphisms and ATD-induced MPE was observed. We believe that our results contribute to the understanding of the pathogenesis of ATD-induced MPE and may contribute to the development of predictive genetic testing to identify patients at high risk of this complication.

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Conflict of interest None of the authors has any conflict of interest.

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