# *GSTM1* and *GSTT1* genetic polymorphisms and their association with antituberculosis drug-induced liver injury

NOPPADOL CHANHOM[1], WANVISA UDOMSINPRASERT[1], USA CHAIKLEDKAEW[2], SURAKAMETH MAHASIRIMONGKOL[3], SUKANYA WATTANAPOKAYAKIT[3] and JIRAPHUN JITTIKOON[1]

Departments of [1]Biochemistry and [2]Pharmacy, Faculty of Pharmacy, Mahidol University, Bangkok 10400; [3]Genomic Medicine Centre, Division of Genomic Medicine and Innovation Support, Department of Medical Sciences, Ministry of Public Health, Nonthaburi 11000, Thailand

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**Abstract.** Antituberculosis (anti-TB) drugs are the most common cause of drug-induced liver injury (DILI). There are numerous studies revealing the associations between the polymorphisms of pharmacogenes and the risk of anti-TB DILI (ATDILI). In the present study, relevant studies regarding the pharmacogenes associated with ATDILI were systematically searched in PubMed and Scopus. A total of 24 genes associated with ATDILI were reported on and the top five reported genes in terms of frequency were revealed to be N-acetyltransferase 2, cytochrome P450 family 2 subfamily E member 1, glutathione S-transferases [glutathione S-transferase mu 1 (*GSTM1*) and glutathione S-transferase theta 1 (*GSTT1*)] and solute carrier organic anion transporter family member 1B1. As ATDILI may be the result of direct and indirect interactions, the encoded proteins were further analysed using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) to observe the protein-protein interactions and the associations amongst these proteins. The results suggested that only *GSTT1* and *GSTM1* were central proteins associated with all the other analysed proteins. Therefore, the association between *GSTT1* or *GSTM1* and the risk of developing ATDILI were further analysed. The results revealed that a *GSTM1* deletion genotype was significantly associated with risk of ATDILI [odds ratio (OR), 1.28; 95% confidence interval (CI), 1.08-1.51; P=0.004], whereas the *GSTT1* deletion genotype and *GSTM1*/*GSTT1* dual-deletion genotype were not significantly associated with risk of ATDILI. Subgroup analysis based on ethnicity was performed and the results demonstrated a significant association between *GSTM1* and ATDILI in South Asian individuals (OR, 1.48; 95% CI, 1.12-1.95; P=0.005), which has not been reported previously, to the best of our knowledge. In conclusion, *GSTM1* was associated with ATDILI in South Asian individuals.

*Correspondence to:* Dr Jiraphun Jittikoon, Department of Biochemistry, Faculty of Pharmacy, Mahidol University, 447 Sri-Ayuthaya Road, Rajathevi, Bangkok 10400, Thailand
E-mail: jiraphun.jit@mahidol.ac.th

*Abbreviations:* anti-TB, antituberculosis; ATDILI, antituberculosis drug-induced liver injury; BMI, body mass index; CI, confidence interval; DILI, drug-induced liver injury; GST, glutathione S-transferase; OR, odds ratio; PPI, protein-protein interaction; ROS, reactive oxygen species; TB, tuberculosis

*Key words:* drug-induced liver injury, glutathione S-transferase, tuberculosis, hepatotoxicity, systematic review, meta-analysis, adverse effect, genetic polymorphisms

## Introduction

Antituberculosis drug-induced liver injury (ATDILI) is one of the most common adverse reactions to drugs used to treat tuberculosis (TB), frequently resulting in the discontinued or interrupted use of drugs, and thus contributing to the socio-economic burden of the disease (1). The majority of patients with TB with DILI develop irreversible liver failure and eventually require a liver transplant due to a poorly defined pathogenesis and delayed diagnosis (2). Therefore, an improved understanding of the causes underlying hepatotoxicity induced by anti-TB drugs may result in the identification of novel markers and novel therapeutic targets for preventing and slowing the progression of DILI. Although the exact aetiology of DILI is not completely understood, it is considered a multifactorial disease which stems from a range of risk factors, including drugs used for treatment of TB and the dose, duration, hepatic metabolism and lipophilicity of those drugs, and other factors including sex, age and metabolism (2). Genetics has been proposed as a critical contributor to the pathogenesis of DILI (3). Thus, a focus has been placed on the potential influence of genetic factors in the development of ATDILI, in which a variety of genetic polymorphisms including in N-acetyltransferase 2 (*NAT2*), cytochrome P450 family 2 subfamily E member 1 (*CYP2E1*) and glutathione S-transferases [glutathione S-transferase mu 1 (*GSTM1*) and glutathione S-transferase theta 1 (*GSTT1*)] have been reported to be associated with an increased risk of ATDILI (4).

Of the various genes known to be associated with ATDILI, *GST*s are gaining increasing interest as potential mediators of hepatotoxicity. *GST*s are essential phase II metabolizing enzymes for detoxification, which are responsible for mitigating the cellular damage resulting from oxidative stress via

conjugating glutathione to substrates including reactive oxygen species (ROS) in response to liver injury, and have been implicated in hepatotoxicity (5). Thus, they exert a protective effect against cellular damage, and one study revealed that gene deletions caused by the homozygous null mutations of *GSTM1* and *GSTT1*, which are two major *GST*s involved in the isoniazid metabolism pathway, were significantly associated with an elevated risk of DILI in patients with TB (6). A number of clinical studies have demonstrated a potential significant association between *GSTM1* or *GSTT1* and susceptibility to ATDILI and DILI (7-21); however, the results obtained from these studies are inconsistent. For example, Rana *et al* (14) revealed that *GSTM1* was associated with an increased risk of ATDILI; however, in a study by Chatterjee *et al* (7), an association was not observed. A meta-analysis is a valuable tool for deriving meaningful conclusions from data and may help resolve inconsistencies in research, and may thus assist in clarifying the association between polymorphisms of *GSTM1* or *GSTT1* and ATDILI.

Numerous meta-analyses have revealed an association between *GSTM1* and *GSTT1* null genotypes and susceptibility to ATDILI (6,22-24); nevertheless, there remain important gaps in our knowledge. Firstly, previous meta-analyses have included publications with confounding factors, including patients with hepatitis virus infection or human immunodeficiency virus (HIV) (23,25). Furthermore, since a large number of studies have been published, it is necessary to perform an updated meta-analysis to assess the association of genetic polymorphisms with ATDILI. Additionally, although genetic data derived from numerous meta-analyses are based on a large multi-ethnic population, the association between polymorphisms and individuals of South Asian descent require further validation. To address the aforementioned limitations of previous meta-analyses, the present meta-analysis was designed with a more stringent selection criteria to verify the precise associations between *GSTM1* and *GSTT1* with susceptibility to ATDILI. Therefore, the aim of the present analysis was to summarize and analyse the body of available data regarding the pharmacogenomics associated with ATDILI using a systematic review and meta-analysis approach, along with network analysis in order to gain insight into the molecular interactions between these pharmacogenes, their genetic polymorphisms and association with a susceptibility to ATDILI based on genetics and ethnicity.

## Materials and methods

*Identification of genes associated with ATDILI*. The PubMed (https://www.ncbi.nlm.nih.gov/pubmed) and Scopus (https://www.scopus.com/) databases were searched using the following terms: 'drug-induced liver injury' OR 'hepatitis' OR 'drug-induced hepatitis' OR 'drug-induced hepatotoxicity' OR 'hepatotoxicity' OR 'liver injury' AND 'pharmacogenomic OR pharmacogenetic*' OR 'genetic polymorphism' OR 'polymorphism*' AND 'antituberculosis OR antitubercular' OR 'tuberculosis treatment'. Original articles examining the association between genetic polymorphisms and ATDILI with a cut-off date of October 30, 2018, were gathered. The frequencies of each gene associated with ATDILI were counted and the top five most frequent genes were and analysed using the STRING online software (26). STRING integrated and ranked protein/gene associations were benchmarked based on reference data which consisted of experimental and predicted interactions between each protein. The interaction network was considered relevant if the confidence score was >0.7 (26). In the protein-protein interaction (PPI) network, each node represented a protein, and each edge indicated a physical or theoretical interaction between two proteins in the network.

*Search strategy for literature on GSTM1 and GSTT1*. Searches for original publications were performed in PubMed, Scopus and Web of Science (https://www.webofknowledge.com/) databases with a cut-off date of December 25, 2018. The process was performed using the PICO strategy (27). The PICO in this study was set as follows: Population (P), tuberculosis patient; Intervention (I), *GSTM1* or *GSTT1* polymorphisms; Comparator (C), gene deletion and wild type; and Outcomes (O), liver injury or hepatotoxicity. The search strategies were constructed by combining search terms using Boolean operators, including 'OR' within the same domain and 'AND' amongst domains (28).

*Study selection for meta-analysis of GSTM1 and GSTT1*. For inclusion of a study, it had to satisfy the specific inclusion and exclusion criteria. The inclusion criteria were: i) case-control or cohort study investigating the association between *GSTM1* or *GSTT1* and susceptibility to ATDILI susceptibility; and ii) study participants were patients with TB receiving isoniazid, rifampicin, pyrazinamide or ethambutol. The exclusion criteria were: i) case report, secondary or tertiary publications including reviews, systematic review and meta-analysis; ii) articles not available in English; iii) studies using healthy volunteers as the control group; iv) studies using a duplicated set of subjects and/or data; and v) studies which included participants with confounding factors of hepatotoxicity, including those co-infected with hepatitis virus or human immunodeficiency virus, excessive consumption of alcoholic beverages or concomitant administration of other potential hepatotoxic medication.

*Assessment of the quality of the study*. To assess the quality of each study, two reviewers independently evaluated the quality of the publications according to the Newcastle-Ottawa scale (29). The score from the Newcastle-Ottawa scale ranges from 0-9. Publications were included and designated as 'pass' if the publication assessment score was >5.

*Data extraction for meta-analysis*. Data from all the eligible studies were extracted into a data sheet. The following data were extracted: First author, publication year, study design, ethnicity, sample size, sex, age, body mass index (BMI), observed medication and the number of cases/controls for each *GST* (*M1*, *T1* and *M1*/*T1*) genotype with their odds ratio (OR) and 95% confidence intervals (CI). If any disagreement between data extraction results by two reviewers were revealed, it was resolved by discussion and consensus with a third reviewer.

*Statistical analysis*. The extracted data such as age and BMI are presented as the mean. Meta-analysis of the effects of

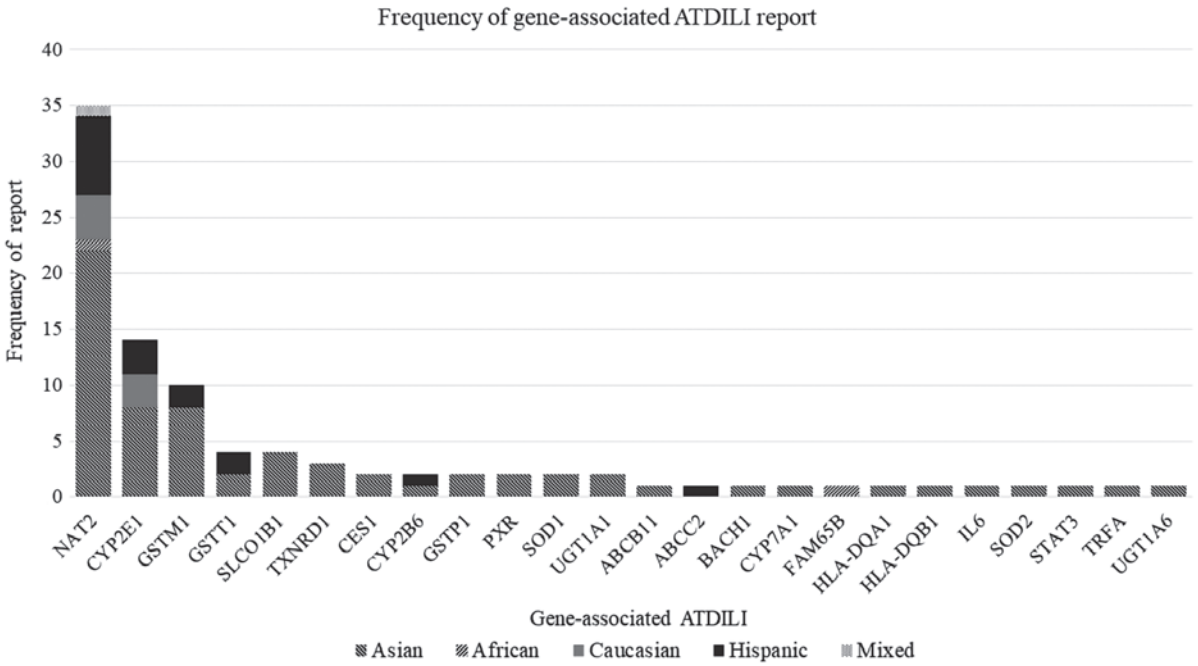


Figure 1. Frequency of reports on each gene-associated ATDILI in PubMed and Scopus databases. The reports were searched up to October 30, 2018 and separated according to ethnicity. ATDILI, antituberculosis drug-induced liver injury.

*GSTM1* and *GSTT1* genes were performed using Review Manager Version 5.3 (Nordic Cochrane Centre, Copenhagen, Denmark). The Mantel-Haenszel fixed-effects model was used for the overall calculation of the OR, 95% CI and P-value calculations. All data are represented as estimated OR with 95% CI. Subgroup analyses were performed by stratifying patients according to ethnicity as follows: East Asian, South Asian, South East Asian, European and South American. $P<0.05$ was considered to indicate a statistically significant difference. Heterogeneity among studies was estimated using a $\chi^2$ test or if the $I^2$ value was >50% (30). Subgroup analysis was utilized for the meta-analysis where a $\chi^2$ P-value <0.05 or an $I^2$ value >50% was considered significant (31). Finally, publication bias was investigated using a funnel plot.

## Results

*Genes associated with ATDILI*. A total of 973 studies were obtained from PubMed and Scopus databases based on the aforementioned search criteria. Of these, 91 were duplicates and excluded. Subsequent to refining the data using the exclusion criteria, 788 publications were excluded as follows: irrelevant (n=695); studies in non-human models (n=54); secondary publications (n=30); and studies where healthy volunteers were used as the control group (n=9). Therefore, 94 publications were included in this analysis. However, only 77 publications identified a statistically significant gene associated with ATDILI. There were 94 reports of genes in the 77 publications. The top five most frequently reported genes were *NAT2* (35 reports), *CYP2E1* (14 reports), *GSTM1* (10 reports), *GSTT1* (4 reports) and solute carrier organic anion transporter family member 1B1 (*SLCO1B1*; 4 reports) (Fig. 1). The PPI analysis of these five genes revealed interactions amongst *GSTM1*, *GSTT1*, *CYP2E1* and *NAT2*. Interestingly,

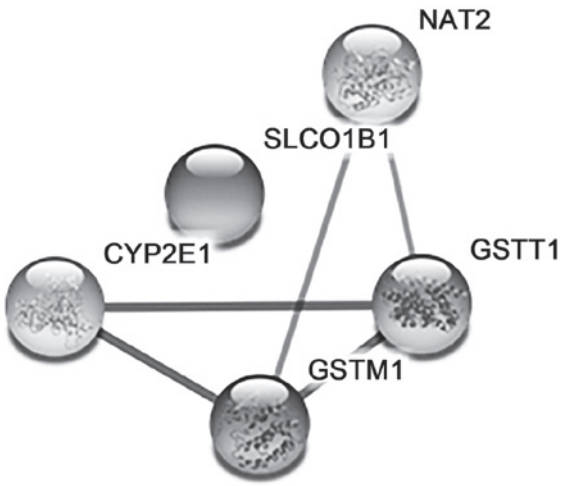


Figure 2. Protein-protein interaction network of NAT2, CYP2E1, GSTM1, GSTT1 and SLCO1B1 with an interaction score of >0.7. Each node represents each protein in this pathway. There are three types of edge. The yellow edge represents the interaction by textmining, while the cyan egde demonstrates the interaction in curated databases, and the black edge represents co-expression. NAT2, N-acetyltransferase 2; CYP2E1, cytochrome P450 family 2 subfamily E member 1; GSTM1, glutathione S-transferase mu 1; GSTT1, glutathione S-transferase θ1; SLCO1B1, solute carrier organic anion transporter family member 1B1.

according to STRING analysis, associations between *GSTM1* or *GSTT1* with *CYP2E1* or *NAT2* enzymes were observed in the PPI network (Fig. 2).

*Study selection for meta-analysis of the association between GSTM1 or GSTT1 with ATDILI*. The selection process of the included studies is illustrated in Fig. 3. Initially, a total of 278 publications were used, and 15 publications were selected with 905 cases of ATDILI from a total of 3,785 patients with

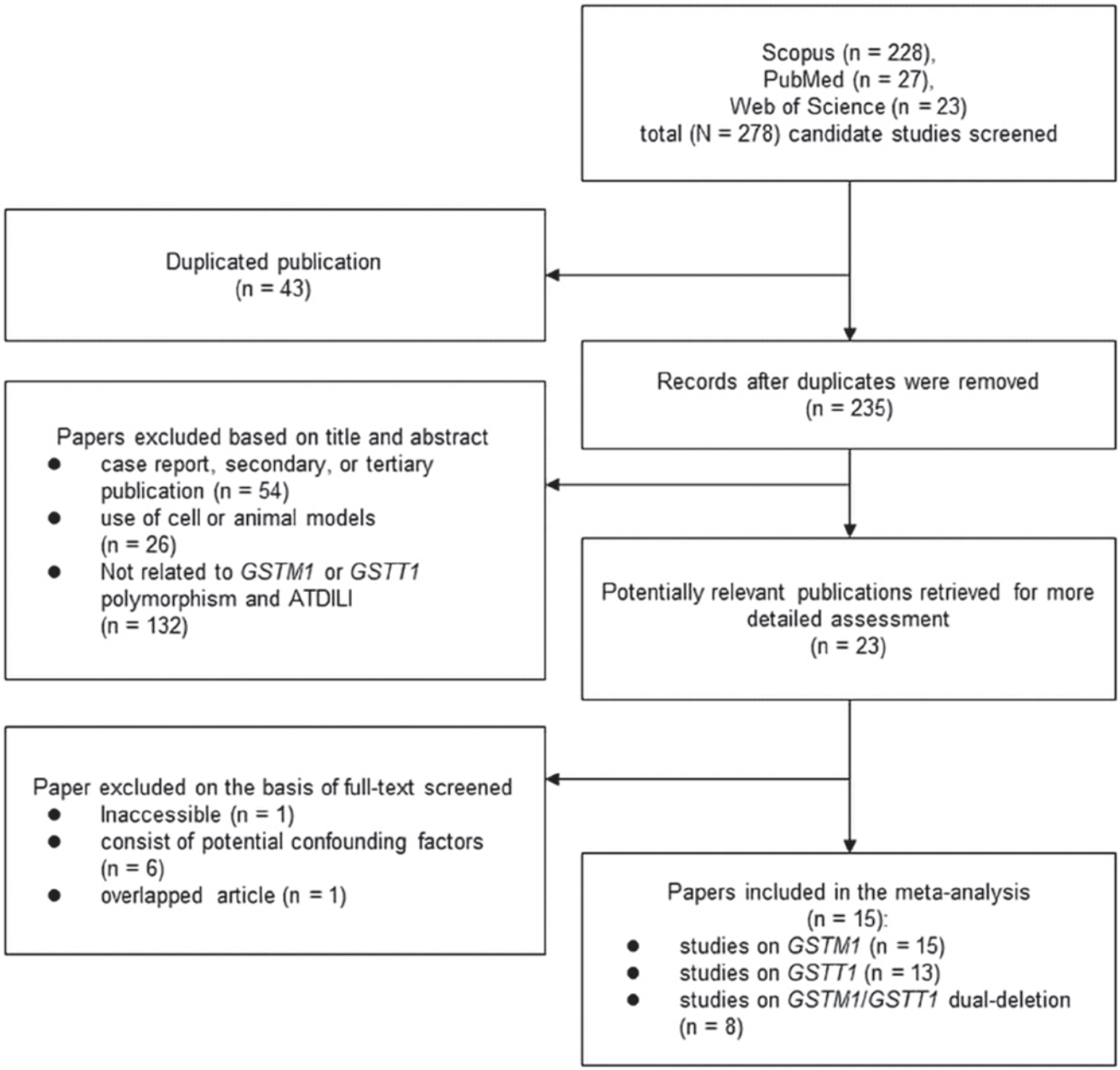


Figure 3. Flow diagram for the present meta-analysis. A total 278 publications were retrieved from databases up to December 25, 2018. A total of 15 studies were selected as eligible publications subsequent to screening and quality assessment process. *GSTM1*, glutathione S-transferase $\mu$1; *GSTT1*, glutathione S-transferase θ1; ATDILI, antituberculosis drug-induced liver injury.

TB. All included publications were qualified according to the Newcastle-Ottawa scale for case-control and cohort studies.

*Study characteristics of the meta-analyses*. The studies used were published between 2001 and 2018 and are presented in Table I. The majority of the studies were performed on East Asian (9,12,18,20,21) and South Asian (7,8,10,14-17) patients. Other studies were performed on South East Asian (13), South American (19) and European patients (11).

*Meta-analysis of results*. The associations between *GSTM1*, *GSTT1* and *GSTM1*/*GSTT1* dual-deletions with the susceptibility of ATDILI in patients with TB are summarized in Figs. 4-6. The results suggested that *GSTM1* was significantly associated with a susceptibility of ATDILI with an OR of 1.28 (95% CI, 1.08-1.51; P<0.05; Fig. 4). However, there was no significant difference between the cases and controls on the influence of the dual gene deletions, the deletion of *GSTT1* (Fig. 5) or *GSTM1*/*GSTT1* (Fig. 6).

*Meta-analysis sensitivity, heterogeneity and publication bias analyses*. Sensitivity analysis was performed by removing a single study at a time to assess the effect of each study on the overall estimate. The results demonstrated that the meta-analysis of the *GSTM1* deletion genotype passed the sensitivity analysis. Heterogeneity was not observed in the *GSTM1* deletion genotype studies (data not shown). Subgroup analysis was used to explain the heterogeneity by subgrouping the studies according to ethnicity as follows; East Asian, South Asian and South East Asian (Fig. 7). Publication bias

Table I. Main characteristics of the eligible publications in the present updated meta-analysis.

| Author, year | Ethnicity | Study design | Sample size case/control | | Sex (male/female) | | | Age (mean) | | BMI (mean) | | (Refs.) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Case | Control | Total | Case | Control | Case | Control | Case | Control | |
| Perwitasari *et al* 2018 | South East Asian | Cohort | 136 | 71 | 207 | NA | NA | NA | NA | NA | NA | (13) |
| Xiang *et al* 2014 | East Asian | Cohort | 89 | 1,858 | 1,947 | 60/29 | 1,012/846 | 37 | 46 | 20.3 | 21.4 | (21) |
| Singla *et al* 2014 | South Asian | Cohort | 17 | 391 | 408 | 11/7 | 241/150 | 48 | 33 | NA | NA | (17) |
| Sharma *et al* 2014 | South Asian | Cohort | 113 | 201 | 314 | 63/50 | 145/56 | 28 | 35 | 17.1 | 17.1 | (16) |
| Rana *et al* 2014 | South Asian | Case-control | 50 | 245 | 295 | 33/22 | 152/93 | 44 | 42 | 19.23 | 23.1 | (14) |
| Liu *et al* 2014 | East Asian | Case-control | 20 | 143 | 163 | 12/8 | 85/58 | 4 | 6 | NA | NA | (12) |
| Gupta *et al* 2013 | South Asian | Case-control | 50 | 246 | 296 | 24/26 | 139/107 | 37 | 37 | 21.0 | 21.0 | (8) |
| Tang *et al* 2012 | East Asian | Case-control | 89 | 356 | 445 | 65/24 | 260/96 | 44 | 44 | 19.5 | 19.4 | (18) |
| Teixeira *et al* 2011 | South American | Case-control | 26 | 141 | 167 | 16/10 | 74/67 | 48 | 43 | NA | NA | (19) |
| Wang *et al* 2010 | East Asian | Case-control | 104 | 111 | 215 | 70/34 | 75/36 | 49 | 45 | 19.8 | 20.2 | (20) |
| Kim *et al* 2010 | East Asian | Case-control | 57 | 190 | 247 | 34/27 | 129/61 | 47 | 42 | NA | NA | (10) |
| Chatterjee *et al* 2010 | South Asian | Case-control | 51 | 100 | 151 | 25/26 | 63/37 | 37 | 33 | 16.2 | 17.5 | (7) |
| Leiro *et al* 2008 | European | Case-control | 35 | 60 | 95 | 14/21 | 25/35 | 34 | 31 | 23.1 | 22.1 | (11) |
| Huang *et al* 2007 | East Asian | Case-control | 63 | 63 | 126 | 42/22 | NA | 62 | NA | NA | NA | (9) |
| Roy *et al* 2001 | South Asian | Case-control | 33 | 33 | 66 | 15/18 | NA | 30 | NA | NA | NA | (15) |

NA, Not Available; BMI, body mass index.

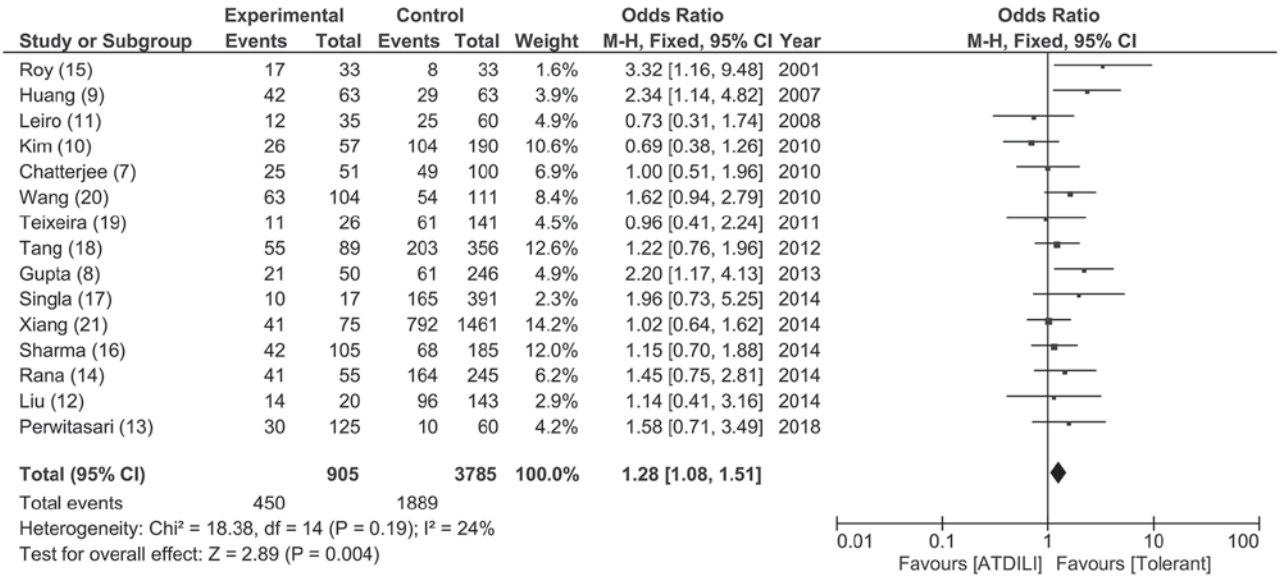


Figure 4. Forest plot comparison between *GSTM1* deletion and *GSTM1* presence and the susceptibility of ATDILI from 15 publications. CI, confidential interval; M-H, Mantel-Haenszel; ATDILI, antituberculosis drug-induced liver injury; *GSTM1*, glutathione S-transferase $\mu$1.

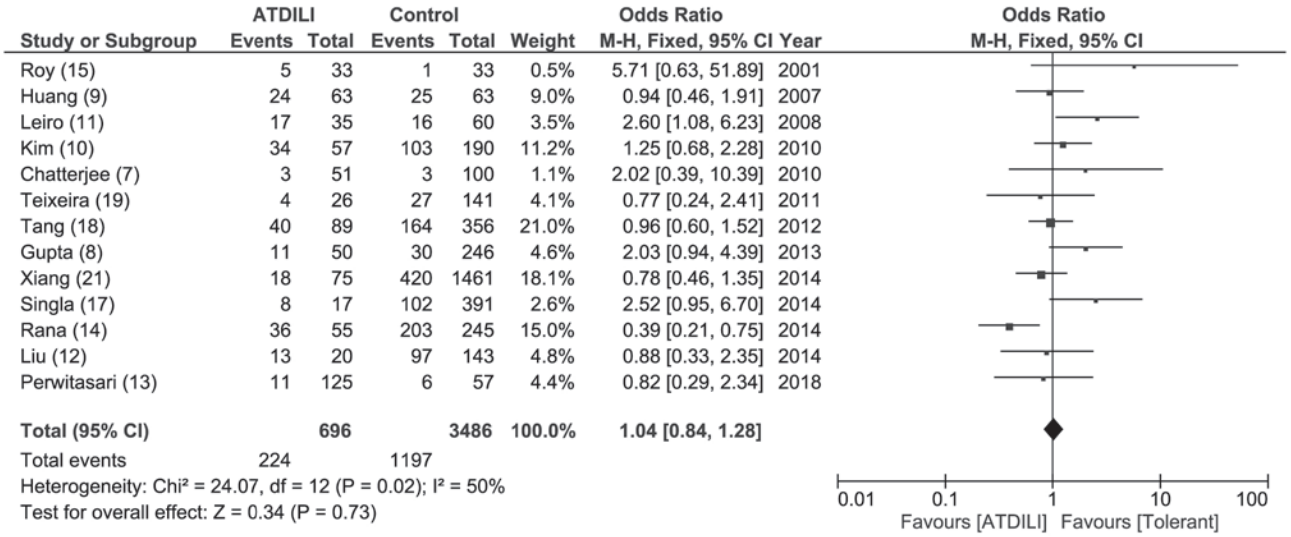


Figure 5. Forest plot comparison between *GSTT1* deletion and *GSTT1* presence and the susceptibility of ATDILI from 13 publications. CI, confidential interval; M-H, Mantel-Haenszel; ATDILI, antituberculosis drug-induced liver injury; *GSTT1*, glutathione S-transferase θ 1.

was investigated using a funnel plot. In the funnel plots, a condensed plot presented at the top of the 95% CI triangle indicated a publication bias for studies with a large number of enrolled patients. However, the plots were symmetrical between each of the sides within the triangle which demonstrated that publication bias was not revealed to be a negative or positive result.

*Results of subgroup meta-analysis*. The subgroup analyses based on the ethnicity of patients with TB were performed for the *GSTM1* gene deletion (Fig. 7). The results demonstrated that the *GSTM1* deletion was significantly associated with ATDILI in South Asian patients with an OR of 1.48 (95% CI, 1.12-1.95; P<0.01). On the contrary, there was no significant association between *GSTM1* with ATDILI in any of the other ethnicities.

## Discussion

Despite extensive research efforts, current understanding of the mechanisms which regulate the progression of hepatotoxicity progression during the treatment of TB remains unclear. As such, the majority of the patients with hepatotoxicity induced by anti-TB drugs will develop end-stage liver injury due to a lack of reliable and specific biomarkers for ATDILI (32). Genetic variations associated with ATDILI have been extensively studied, and may be used as genetic biomarkers for identifying patients who may be at increased risk of ATDILI, prior to the prescription of therapeutics which may aggravate the risk (33). In the present analysis, previous studies on the association of genetic variations in patients with ATDILI were analysed and the top five most frequently reported genes were *NAT2*, *CYP2E1*, *GSTM1*, *GSTT1* and *SLCO1B1*.

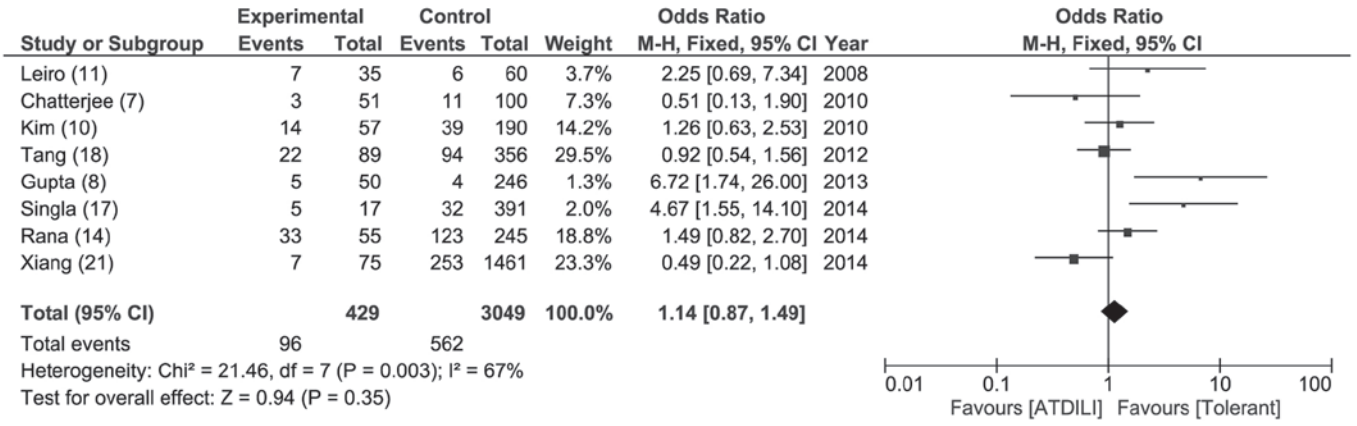


Figure 6. Forest plot comparison between *GSTM1*/*GSTT1* dual-deletion and presence and the susceptibility of ATDILI from 8 publications. CI, confidential interval; M-H, Mantel-Haenszel; ATDILI, antituberculosis drug-induced liver injury; *GSTM1*, glutathione S-transferase $\mu$ 1; *GSTT1*, glutathione S-transferase θ 1.

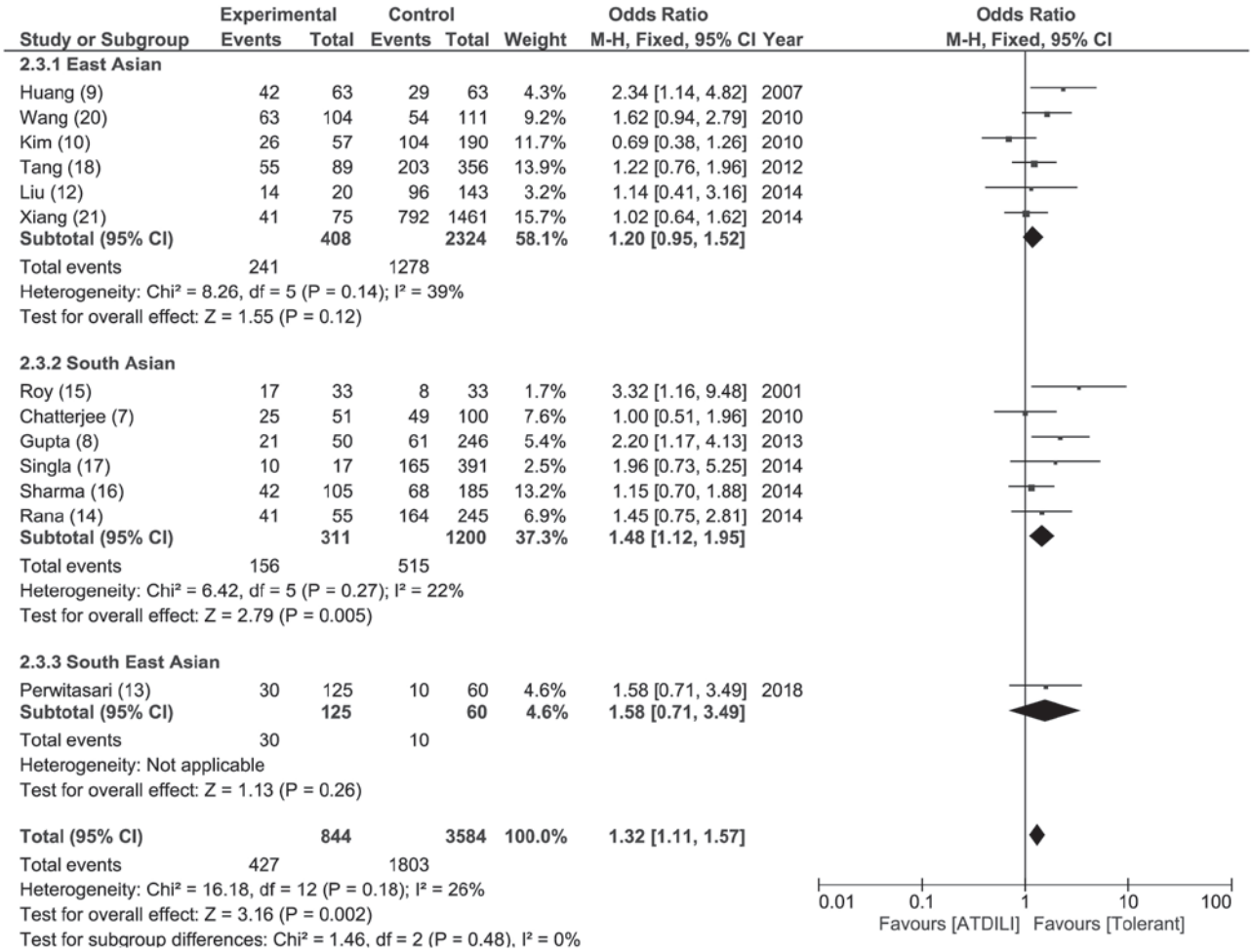


Figure 7. Asian ethnicity subgroup analysis forest plot comparison between *GSTM1* deletion and normal and the susceptibility of ATDILI from 13 publications and 3 Asian ethnicities. CI, confidential interval; M-H, Mantel-Haenszel; ATDILI, antituberculosis drug-induced liver injury; *GSTM1*, glutathione S-transferase $\mu$1.

In addition, PPI network analysis of these genes illustrated that there were direct links between *NAT2*, *CYP2E1*, *GSTM1* and *GSTT1* enzymes, and *GST*s were indicated as serving as a key molecule in this PPI network. Supporting this hypothesis, alternative results from a PPI network delineated that *GSTM1* was functionally associated with *CYP2E1* and *GSTT1* in drug metabolism in Kyoto Encyclopedia of Genes and Genomes pathway analysis (34) and was frequently reported alongside the *NAT2* enzyme in numerous studies (8,14,17). Based on the metabolism pathway of isoniazid, *NAT2*, *CYP2E1*, *GSTM1* and *GSTT1* enzymes functionally associate with each other to metabolize isoniazid, and *GSTM1* and *GSTT1* enzymes

function to detoxify the toxic metabolites formed following the metabolism of isoniazid by *NAT2* and *CYP2E1* (35). Based on the data on the genes associated with ATDILI *NAT2, CYP2E1, GSTM1* and *GSTT1*, polymorphisms of these genes may together contribute to the risk of ATDILI. Thus, further investigation is required to determine the effects of polymorphisms of these genes, and how they may contribute to the risk of ATDILI. To the best of our knowledge, there are no publications which have investigated the influence of the multi-genetic contribution on the risk of ATDILI. Therefore, the present study was not able to perform a meta-analysis to demonstrate the association of the multi-gene effect on the risk of ATDILI. A meta-analysis was performed to verify the association between polymorphisms of *GSTM1* and *GSTT1* and ATDILI risk. There was an association between a *GSTM1* null genotype with an increased risk of ATDILI in patients with TB. This result supports the hypothesis that genetic variation in genes encoding proteins responsible for managing oxidation may increase the risk of ATDILI, and genetic polymorphisms of *GSTM1* and *GSTT1* may serve as novel genetic markers for predicting which patients with TB are at a higher risk of ATDILI.

As the *GST* enzymes are fundamental for the elimination of ROS through the conjugation of glutathione to substrates including xenobiotics and ROS (4), it is not surprising that they exert protective effects on numerous different types of cells from the oxidative stress induced by anti-TB drugs, and an absence of *GST* activity leaves the liver more susceptible to ATDILI (2). The two major *GST* enzymes which conjugate isoniazid metabolites are *GSTM1* and *GSTT1*, and an absence of their activity caused by homozygous null mutations has been implicated in liver injury owing to a lack of protection from oxidant species (5). Supporting this, the results of the present meta-analysis revealed that a null *GSTM1* genotype was significantly associated with a higher risk of ATDILI ($P<0.05$), in agreement with a number of previous meta-analyses (5,6,21-23). Previously, *GSTM1* was reported to be significantly correlated with a susceptibility to develop ATDILI only in East Asian individuals, although in the present analysis, an association between *GSTM1* polymorphisms and South Asian individuals was observed. The reason for this inconsistency is unknown, but may be attributed to the lesser number of included publications due to the more stringent inclusion and exclusion criteria. Therefore, further investigation is required to determine the association between *GSTM1* deletion and ATIDILI risk in East Asian, South East Asian, Caucasian and African individuals.

It has been demonstrated that the homozygous null mutation of *GSTT1* may result in the loss of detoxification activity of hepatotoxic reactive metabolites in hepatocytes (36). Thus, it seems plausible that a *GSTT1* null genotype may result in an increased risk of ATDILI. Nonetheless, in the present study, no significant association between a null *GSTT1* genotype and ATDILI was observed, consistent with previous meta-analyses (6,22-24). A potential explanation for this result may be due to the small sample sizes. In the present study, although 905 ATDILI cases and 2,880 controls were pooled, the number of subjects was still insufficient. Tang *et al* (6) suggested that a cohort of >10,000 patients is required to determine the significance of a polymorphism when the risk of a polymorphism is moderate (37). Alternatively, Bao *et al* (4) illustrated that there are multiple factors, including genetic and environmental factors, which influence the pathogenesis of ATDILI. In the present analysis, confounding environmental factors including viral hepatitis, HIV infection and alcohol consumption were excluded, but other factors including age, sex and BMI were included, which may have thus resulted in an inaccurate association. Altogether, it was not possible to ascertain whether a null *GSTT1* genotype was associated with the risk of ATDILI, for which a multi-centre genetic association study with larger sample sizes and well-characterized subjects is required.

Whether combined *GSTM1* and *GSTT1* null genotypes are associated with an elevated risk of ATDILI remains yet to be determined. Lucena *et al* (38) focused on investigating the potential association between a combination of *GSTM1* and *GSTT1* polymorphisms with DILI and demonstrated a significant association between *GSTM1* and *GSTT1* dual-null polymorphisms and an increased risk of ATDILI. In the present analysis, no significant association between *GSTM1*/*GSTT1* dual-null genotypes and ATDILI risk was observed. This contrasting result may be attributable to differences in the pathophysiology of hepatoxicity, ethnicity and the type of drugs prescribed between studies. Supporting the observation that there was no association between *GSTM1*/*GSTT1* dual-null genotypes and ATDILI risk, Ginsberg *et al* (39) investigated the genotypic frequency and distribution of *GSTM1* and *GSTT1* null genotypes in a wide spectrum of the human populations, and they revealed that *GSTM1* or *GSTT1* null genotypes were highly prevalent in Asian patients compared with *GSTM1*/*GSTT1* dual-null polymorphisms.

If there is a direct link between a null *GSTM1* genotype and an increased risk of ATDILI in patients with TB, it may be hypothesized that a homozygous deletion of *GSTM1* results in a loss of GSTM1 enzyme activity contributing to the pathogenesis of hepatoxicity influenced by anti-TB drugs. Hepatotoxicity induced by anti-TB drugs, particularly isoniazid, is mediated through toxic intermediaries, including hydrazine and acetyldiazine, in addition to ROS (40,41). An increase in ROS production results in increased oxidative stress, which in turn induces organ failure, particularly of the liver (40), in which *NAT2* and *CYP2E1* have been revealed to be responsible for a biotransformation pathway of isoniazid-induced increased ROS generation (34). In a *NAT2*- and *CYP2E1*-mediated detoxification of an isoniazid-mediated increase in ROS levels, *GST* enzymes are known to serve a protective function against oxidative stress-induced cellular injury (4), suggesting that *GST*s may serve a function in the detoxification of anti-TB drugs. This hypothesis is supported by the results from the PPI network analysis, in which a functional association between *GST*s with *NAT2* and *CYP2E1* was observed, and the centrality of *GST* enzymes in the interactions between molecules was established. Although results from the present meta-analysis along with PPI network analysis provide support for an association between *GSTM1* deletion and an increased risk of ATDILI in patients with TB, the function of genetic variations of *GST*s genes and the risk of ATDILI remains yet to be determined, and additional research is required to determine their function in the detoxification of oxidative intermediates which results from the metabolism of anti-TB drugs.

There are certain limitations in the present study. The major limitation of the present study is that it only illustrated the association of ATDILI with *GSTT1* polymorphisms using the previous case-control and cohort study results; however, the exact function of *GSTT1* in the pathogenesis of ATDILI was unable to be determined. To address the cause and effect association, further experimental studies using *in vivo* models need to be performed. Another limitation is that the present study only included publications which were published in the English language, thus a language bias was unavoidable. In addition, pooling data from different types of study designs, including case-controlled and cohort studies, may provide a higher number of studies compared with previous meta-analyses; however, these may contribute to significant heterogeneity. On the other hand, the strength of the present study is that all publications with confounding factors were excluded to better mitigate the effect of the confounding factors, providing more reliable results.

In conclusion, the meta-analysis revealed that a *GSTM1* null genotype was significantly associated with the susceptibility of ATDILI, particularly in South Asian individuals. However, there was no association between a null *GSTT1* or a dual-null *GSTM1*/*GSTT1* genotype and risk of ATDILI. STRING analysis revealed that *GST*s interact with other proteins associated with ATDILI, including *NAT2* and *CYP2E1*, and thus may exert a protective function. Additional studies are required with larger sample sizes, well-characterized subjects and various ethnicities including South East Asian, Caucasian and African individuals to draw a more precise conclusion and support the use of these genetic markers for predicting the risk of ATDILI in patients with TB.

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**Availability of data and materials**

The data analysed and generated during the current study are available from the corresponding author on reasonable request.

**Authors' contributions**

JJ, UC, SM and SW conceived and designed the study. NC and WU performed literature review. NC retrieved the data. JJ and NC reviewed the assessment of the quality of the study. NC performed the meta-analyses. NC, JJ, and WU wrote the manuscript. All authors edited and revised the manuscript. All authors approved the final version of the manuscript.

**Ethics approval and consent to participate**

Not applicable.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**References**

1. Tostmann A, Boeree MJ, Aarnoutse RE, de Lange WC, van der Ven AJ and Dekhuijzen R: Antituberculosis drug-induced hepatotoxicity: Concise up-to-date review. J Gastroenterol Hepatol 23: 192-202, 2008.
2. Ramappa V and Aithal GP: Hepatotoxicity related to anti-tuberculosis drugs: Mechanisms and management. J Clin Exp Hepatol 3: 37-49, 2013.
3. Clare KE, Miller MH and Dillon JF: Genetic factors influencing drug-induced liver injury: Do they have a role in prevention and diagnosis? Curr Hepatol Rep 16: 258-264, 2017.
4. Bao Y, Ma X, Rasmussen TP and Zhong XB: Genetic variations associated with anti-tuberculosis drug-induced liver injury. Curr Pharmacol Rep 4: 171-181, 2018.
5. Eaton DL and Bammler TK: Concise review of the glutathione S-transferases and their significance to toxicology. Toxicol Sci 49: 156-164, 1999.
6. Tang N, Deng R, Wang Y, Lin M, Li H, Qiu Y, Hong M and Zhou G: *GSTM1* and *GSTT1* null polymorphisms and susceptibility to anti-tuberculosis drug-induced liver injury: A meta-analysis. Int J Tuberc Lung Dis 17: 17-25, 2013.
7. Chatterjee S, Lyle N, Mandal A and Kundu S: *GSTT1* and *GSTM1* gene deletions are not associated with hepatotoxicity caused by antitubercular drugs. J Clin Pharm Ther 35: 465-470, 2010.
8. Gupta VH, Singh M, Amarapurkar DN, Sasi P, Joshi JM, Baijal R, H R PK, Amarapurkar AD, Joshi K and Wangikar PP: Association of *GST* null genotypes with anti-tuberculosis drug induced hepatotoxicity in western indian population. Ann Hepatol 12: 959-965, 2013.
9. Huang YS, Su WJ, Huang YH, Chen CY, Chang FY, Lin HC and Lee SD: Genetic polymorphisms of manganese superoxide dismutase, NAD(P)H: Quinone oxidoreductase, glutathione S-transferase M1 and T1, and the susceptibility to drug-induced liver injury. J Hepatol 47: 128-134, 2007.
10. Kim SH, Kim SH, Yoon HJ, Shin DH, Park SS, Kim YS, Park JS and Jee YK: *GSTT1* and *GSTM1* null mutations and adverse reactions induced by antituberculosis drugs in koreans. Tuberculosis (Edinb) 90: 39-43, 2010.
11. Leiro V, Fernandez-Villar A, Valverde D, Constenla L, Vázquez R, Piñeiro L and González-Quintela A: Influence of glutathione S-transferase M1 and T1 homozygous null mutations on the risk of antituberculosis drug-induced hepatotoxicity in a Caucasian population. Liver Int 28: 835-839, 2008.
12. Liu F, Jiao AX, Wu XR, Zhao W, Yin QQ, Qi H, Jiao WW, Xiao J, Sun L, Shen C, *et al*: Impact of glutathione S-transferase M1 and T1 on anti-tuberculosis drug-induced hepatotoxicity in Chinese pediatric patients. PLoS One 9: e115410, 2014.
13. Perwitasari DA, Darmawan E, Mulyani UA, Vlies PV, Alffenaar JC, Atthobar J and Wilffert B: Polymorphisms of *NAT2*, *CYP2E1*, *GST*, and HLA related to drug-induced liver injury in indonesian tuberculosis patients. Int J Mycobacteriology 7: 380-386, 2018.
14. Rana SV, Sharma SK, Ola RP, Kamboj JK, Malik A, Morya RK and Sinha SK: N-Acetyltransferase 2, cytochrome P4502E1 and glutathione S-transferase genotypes in antitubercular treatment-induced hepatotoxicity in North Indians. J Clin Pharm Ther 39: 91-96, 2014.

15. Roy B, Chowdhury A, Kundu S, Santra A, Dey B, Chakraborty M and Majumder PP: Increased risk of antituberculosis drug-induced hepatotoxicity in individuals with glutathione S-transferase M1 'null' mutation. J Gastroenterol Hepatol 16: 1033-1037, 2001.
16. Sharma SK, Jha BK, Sharma A, Sreenivas V, Upadhyay V, Jaisinghani C, Singla R, Mishra HK and Soneja M: Genetic polymorphisms of *CYP2E1* and *GSTM1* loci and susceptibility to anti-tuberculosis drug-induced hepatotoxicity. Int J Tuberc Lung Dis 18: 588-593, 2014.
17. Singla N, Gupta D, Birbian N and Singh J: Association of *NAT2*, *GST* and *CYP2E1* polymorphisms and anti-tuberculosis drug-induced hepatotoxicity. Tuberculosis (Edinb) 94: 293-298, 2014.
18. Tang SW, Lv XZ, Zhang Y, Wu SS, Yang ZR, Xia YY, Tu DH, Deng PY, Ma Y, Chen DF and Zhan SY: *CYP2E1*, *GSTM1* and *GSTT1* genetic polymorphisms and susceptibility to antituberculosis drug-induced hepatotoxicity: A nested case-control study. J Clin Pharm Ther 37: 588-593, 2012.
19. Teixeira RL, Morato RG, Cabello PH, Muniz LM, Moreira Ada S, Kritski AL, Mello FC, Suffys PN, Miranda AB and Santos AR: Genetic polymorphisms of NAT2, CYP2E1 and GST enzymes and the occurrence of antituberculosis drug-induced hepatitis in Brazilian TB patients. Mem Inst Oswaldo Cruz 106: 716-724, 2011.
20. Wang T, Yu HT, Wang W, Pan YY, He LX and Wang ZY: Genetic polymorphisms of cytochrome P450 and glutathione S-transferase associated with antituberculosis drug-induced hepatotoxicity in Chinese tuberculosis patients. J Int Med Res 38: 977-986, 2010.
21. Xiang Y, Ma L, Wu W, Liu W, Li Y, Zhu X, Wang Q, Ma J, Cao M, Wang Q, *et al*: The incidence of liver injury in uyghur patients treated for TB in xinjiang uyghur autonomous region, China, and its association with hepatic enzyme polymorphisms NAT2, CYP2E1, GSTM1 and GSTT1. PLoS One 9: e85905, 2014.
22. Cai Y, Yi J, Zhou C and Shen X: Pharmacogenetic study of drug-metabolising enzyme polymorphisms on the risk of anti-tuberculosis drug-induced liver injury: A meta-analysis. PLoS One 7: e47769, 2012.
23. Li C, Long J, Hu X and Zhou Y: *GSTM1* and *GSTT1* genetic polymorphisms and risk of anti-tuberculosis drug-induced hepatotoxicity: An updated meta-analysis. Eur J Clin Microbiol Infect Dis 32: 859-868, 2013.
24. Sun F, Chen Y, Xiang Y and Zhan S: Drug-metabolising enzyme polymorphisms and predisposition to anti-tuberculosis drug-induced liver injury: A meta-analysis. Int J Tuberc Lung Dis 12: 994-1002, 2008.
25. Cai L, Cai MH, Wang MY, Xu YF, Chen WZ, Qin SY, Wan CL and He L: Meta-analysis-based preliminary exploration of the connection between ATDILI and schizophrenia by *GSTM1/T1* gene polymorphisms. PLoS One 10: e0128643, 2015.
26. Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, Simonovic M, Roth A, Santos A, Tsafou KP, *et al*: STRING v10: Protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res 43: D447-D452, 2015.
27. Aslam S and Emmanuel P: Formulating a researchable question: A critical step for facilitating good clinical research. Indian J Sex Transm Dis AIDS 31: 47-50, 2010.
28. Grewal A, Kataria H and Dhawan I: Literature search for research planning and identification of research problem. Indian J Anaesth 60: 635-639, 2016.
29. Wells G, Shea B, O'Connell D, Robertson J, Peterson J, Welch V, Losos M and Tugwell P: The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Ottawa Hospital Research Institute, 2019.
30. Higgins JP and Thompson SG: Quantifying heterogeneity in a meta-analysis. Stat Med 21: 1539-1558, 2002.
31. Higgins JP, Thompson SG, Deeks JJ and Altman DG: Measuring inconsistency in meta-analyses. BMJ 327: 557-560, 2003.
32. McGill MR and Jaeschke H: Biomarkers of drug-induced liver injury: Progress and utility in research, medicine, and regulation. Expert Rev Mol Diagn 18: 797-807, 2018.
33. Saukkonen JJ, Cohn DL, Jasmer RM, Schenker S, Jereb JA, Nolan CM, Peloquin CA, Gordin FM, Nunes D, Strader DB, *et al*: An official ATS statement: Hepatotoxicity of antituberculosis therapy. Am J Respir Crit Care Med 174: 935-952, 2006.
34. Kanehisa M and Goto S: KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res 28: 27-30, 2000.
35. Wang P, Pradhan K, Zhong XB and Ma X: Isoniazid metabolism and hepatotoxicity. Acta Pharm Sin B 6: 384-392, 2016.
36. Roy PD, Majumder M and Roy B: Pharmacogenomics of anti-TB drugs-related hepatotoxicity. Pharmacogenomics 9: 311-321, 2008.
37. Ioannidis JP, Trikalinos TA and Khoury MJ: Implications of small effect sizes of individual genetic variants on the design and interpretation of genetic association studies of complex diseases. Am J Epidemiol 164: 609-614, 2006.
38. Lucena MI, Andrade RJ, Martínez C, Ulzurrun E, García-Martín E, Borraz Y, Fernández MC, Romero-Gomez M, Castiella A, Planas R, *et al*: Glutathione S-transferase m1 and t1 null genotypes increase susceptibility to idiosyncratic drug-induced liver injury. Hepatology 48: 588-596, 2008.
39. Ginsberg G, Smolenski S, Hattis D, Guyton KZ, Johns DO and Sonawane B: Genetic polymorphism in glutathione transferases (*GST*): Population distribution of *GSTM1*, *T1*, and *P1* conjugating activity. J Toxicol Environ Health B Crit Rev 12: 389-439, 2009.
40. Chowdhury A, Santra A, Bhattacharjee K, Ghatak S, Saha DR and Dhali GK: Mitochondrial oxidative stress and permeability transition in isoniazid and rifampicin induced liver injury in mice. J Hepatol 45: 117-126, 2006.
41. Zhai Q, Lu SR, Lin Y, Yang QL and Yu B: Oxidative stress potentiated by diallylsulfide, a selective CYP2E1 inhibitor, in isoniazid toxic effect on rat primary hepatocytes. Toxicol Lett 183: 95-98, 2008.