PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Association of genetic polymorphisms of CYP2E1, NAT2, GST, and SLCO1B1 with the risk of anti-tuberculosis drug-induced liver injury: a systematic review and meta-analysis
AUTHORS	Yang, Seungwon; Hwang, Se Jung; Park, Jung Yun; Chung, Eun Kyoung; Lee, Jangik I.

VERSION 1 - REVIEW

REVIEWER	Supharat Suvichapanich Department of Biochemistry, Faculty of Pharmacy, Mahidol University, Thailand
REVIEW RETURNED	25-Dec-2018

GENERAL COMMENTS	Yang et al. studied associations of NAT2, CYP2E1, GST, and SLCO1B1 with the risk of anti-tuberculosis drug induced liver injury (ATDILI) using systematic review and meta-analysis. The novelty of this manuscript is to investigate an association between SLCO1B1 and ATDILI. Unfortunately, they found no association between both of them. However, they identified the significant increased risk of ATDILI with CYP2E1 RsaI/PstI c1/c1, NAT2 slow acetylator and GSTM1 null which are similar to previous meta-analysis. This meta-analysis is well design. However, the manuscript has some major and minor points to improve before consider for an acceptance. Major revisions 1. Did the authors consider only tolerant controls or also include healthy control? Could the authors clarify this point in your inclusion/exclusion criteria? 2. If the authors focus on the study on tuberculosis patients, Hardy-Weinberg equilibrium (HWE) should not strictly be taken into account because they are not healthy control. It is normal that HWE was not achieved. In addition, many included studies might not investigate HWE so it is not reasonable to do this subgroup analysis. 3. Subgroup analysis based on the diagnosis criteria is meaningless and lead to misinterpretation for the reader. It will be useful if it is subgroup of mild, moderate to severe liver injury.

	4. Genotyping method is another topic that the authors can discuss as the source of heterogeneity due to sensitivity of each method instead of doing subgroup analysis which also has no meaning as well. 5. The authors should discuss more detail about gene-ethnicity interaction such as in term of allele/genotype frequencies. The current context seems too subjective. Minor revisions 1. Please state a version of Review Manager Software. 2. Please state a statistic test that authors used for analysis of total effect in a method section (Mantel-Haenszel test or inverse variance) 3. "SLCO1B1 encodes…" on page 17 should be italic because it is gene's name. 4. In table S6 should be "PCR-RFLP" instead of "PCR-RELP"

REVIEWER	Li Zhong College of Life Sciences, Shaanxi Normal University, Xi'an, China
REVIEW RETURNED	01-Jan-2019

GENERAL COMMENTS	In this paper, the authors investigated the association between genetic polymorphisms of NAT2, CYP2E1, GST and SLCO1B1 and the risk of anti-tuberculosis drug-induced liver injury (ATDILI). In brief, the project design and work flow was reasonable and this result was interesting. The result was also important for this disease and other related fields. My major concern was why the meta-analysis was performed under only one genomic model. The authors should be aware of that there are three genomic models (dominant, recessive and additive) for two alleles. Furthermore, the results in this paper were valueless since similar results were obtained for NAT2, CYP2E1, and GST in previous ATDILI publications and meta-analysis was not indispensable. Moreover, other functional experiments should be more meaningful. Therefore, I suggested the authors to perform the additional functional experiments on the role of genetic polymorphisms in ATDILI and resubmit the paper.

REVIEWER	B.Wilffert Unit of PharmacoTherapy, -Epidemiology & -Economics Groningen Research Institute of Pharmacy University of Groningen The Netherlands
REVIEW RETURNED	18-Jan-2019

GENERAL COMMENTS	The manuscript describes a very relevant and well-performed meta-analysis. I have only a small textual remark: p.15, l.23, medication instead of medications.

REVIEWER	Shaowen Tang Nanjing Medical University
REVIEW RETURNED	07-Feb-2019

GENERAL COMMENTS	In this study, the authors conducted a systematic review and meta-analysis to evaluate the role of genetic polymorphisms of CYP2E1, NAT2, GST, and SLCO1B1 in the risk of anti-tuberculosis drug-induced liver injury. I had some important concerns about the methodologies for this study. 1. A total of 53 eligible studies from 2001 to 2016 were finally included in this meta-analysis. Although the authors said in the manuscript, it is “to provide more updated, comprehensive, and compelling evidence”, and they searched the literatures from their inception to February 2018, there is no article included from 2017 to 2018. The latest article was published in 2016 (There is only one literature in 2015 and 2016, respectively.). Are the authors aware of any reason that investigation seemed to cease after 2016? As far as I know, there have been many similar original researches published during 2017-2018 (For example in NAT2, Chan, S. L., et al. PLoS One, 2017, 12(10): e0186200; Wattanapokayakit, S., et al. Int J Tuberc Lung Dis, 2016, 20(10): 1364-1369. For CYP2E1, Sun, Q., et al. Clin Drug Investig, 2017, 37(12): 1125-1136.). There are several similar systematic reviews (For example in NAT2, Suvichapanich, S., et al. Pharmacogenet Genomics, 2018, 28(7): 167-176. Zhang, M., et al. Br J Clin Pharmacol. 2018.). The authors thought that there was a limited number of studies evaluating the association between the risk of ATDILI and the SLCO1B1 genetic polymorphisms (n = 4). However, another study has been published in 2017 (Sun, Q., et al. Clin Drug Investig, 2017, 37(12): 1125-1136.). So, the authors should update the literature search, and revise all results. 2. The authors should describe the inclusion criteria clearly. 3. In the section of Literature search, the authors should list the entire set of search strategies of every database in the appendix. 4. The authors included the case-control or cohort studies. The type of original study needs to be listed in Table 1. Usually, different types of original research results cannot be directly merged, and the same type of researches should be merged firstly. 5. The type of data extracted by the author is Dichotomous, However, the forest plots were inconsistent with the results of the dichotomous data, especially being performed using the Review Manager Software. The author also needs to describe the software version number used. 6. The authors described that sensitivity analyses were conducted to assess the robustness of the results in the Methods section. However, the authors described some results in the Discussion section (Page 18, line 4-12). The authors should clarify which method is used for sensitivity analysis in the Methods section, and described some results in the Results section. 7. None of the funnel plots were substantially asymmetrical. An asymmetric inverted funnel-shape scatter plot was used to indicate the existence of potential publication bias. But besides funnel plot, the authors may consider Egger’s test. 8. Epidemiology of tuberculosis needs to be updated in the Introduction.

VERSION 1 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 1
Reviewer Name: Supharat Suvichapanich

Institution and Country: Department of Biochemistry, Faculty of Pharmacy, Mahidol University, Thailand

Please leave your comments for the authors below
Yang et al. studied associations of NAT2, CYP2E1, GST, and SLCO1B1 with the risk of anti-tuberculosis drug induced liver injury (ATDILI) using systematic review and meta-analysis. The novelty of this manuscript is to investigate an association between SLCO1B1 and ATDILI. Unfortunately, they found no association between both of them. However, they identified the significant increased risk of ATDILI with CYP2E1 RsaI/PstI c1/c1, NAT2 slow acetylator and GSTM1 null which are similar to previous meta-analysis.
This meta-analysis is well design. However, the manuscript has some major and minor points to improve before consider for an acceptance.
Major revisions

1. Did the authors consider only tolerant controls or also include healthy control? Could the authors clarify this point in your inclusion/exclusion criteria?

Thank you for your comment. We considered tolerant controls only in our meta-analysis. To address your comment, we further clarified the study inclusion and exclusion criteria for our meta-analysis as follows (Page 8, Lines 154-168):
“Studies were considered eligible if they met all of the following inclusion criteria: (1) studies with TB patients receiving anti-TB drug therapy; (2) studies with the control group of TB patients tolerant of anti-TB medications; (3) studies evaluating the association between the occurrence of ATDILI and genetic polymorphisms of CYP2E1, NAT2, GST, and SLCO1B1 388A>G and 521T>C; and (4) case-control or cohort observational studies. Excluded studies were as follows: (1) studies available only in the form of abstracts or meeting posters; (2) review or meta-analysis articles; (3) studies providing insufficient data necessary for the statistical data analysis; (4) studies in non-English language; (5) non-human studies including animal and in vitro studies; (6) studies with unpublished data; (7) studies providing insufficient information on genotyping methods; and (8) healthy controls.”

2. If the authors focus on the study on tuberculosis patients, Hardy-Weinberg equilibrium (HWE) should not strictly be taken into account because they are not healthy control. It is normal that HWE was not achieved. In addition, many included studies might not investigate HWE so it is not reasonable to do this subgroup analysis.

Thank you for your comment. To address your comment, the subgroup analysis results based on the achievement of HWE were deleted because only TB patients were included in our meta-analysis (Pages 11, Lines 205-206 in the methods section; Pages 13-14, Lines 256-258 and 272-274 in the results section, and data S4–S11 Tables).

3. Subgroup analysis based on the diagnosis criteria is meaningless and lead to misinterpretation for the reader. It will be useful if it is subgroup of mild, moderate to severe liver injury.

Thank you for your comment. Due to the limited information available in each study, % of mild,

moderate, or severe ATDILI cases could not be estimated. To address your comment, the subgroup analysis results based on the ATDILI diagnostic criteria were deleted (Page 11, Lines 205-206 in the methods section; Page 14, Lines 280-281 in the results section; Supplementary data S4–S11 Tables).

4. Genotyping method is another topic that the authors can discuss as the source of heterogeneity due to sensitivity of each method instead of doing subgroup analysis which also has no meaning as well.

Thank you for your comment. To address your comment, we identified differences in the genotyping method between studies as a source of heterogeneity in the results section as follows (Page 16, Lines 314-320):
“This high heterogeneity between studies may be due to substantial differences in ethnicity, anti-TB drug regimen, the genotyping method used, study design, and diagnostic criteria of ATDILI among the included studies (Table 1).”
Also, the subgroup analysis results based on the genotyping method were deleted according to your comments (Page 11, Lines 205-206; Supplementary data S4–S11 Tables).

5. The authors should discuss more detail about gene-ethnicity interaction such as in term of allele/genotype frequencies. The current context seems too subjective.

Thank you for your comment. Gene-ethnicity interactions are now further elucidated in our revised discussion section by reporting the frequency of the genotypes associated with an increased risk of ATDILI in various ethnic groups, which was highly variable among ethnic groups. Besides ethnicities, other risk factors for ATDILI reported in previous studies were related to the ethnic groups with a higher risk of ATDILI in our subgroup analyses (e.g., lower body mass index significantly associated with the ATDILI risk and specific ethnic groups generally have a lower body mass index compared to other ethnicities). Overall, to address your comment, we included the following discussion regarding gene-ethnicity interactions with respect to the CYP2E1 RsaI/PstI c1/c1 genotype, the NAT2 slow acetylator genotype, and the GSTM1 null genotype (Page 18, Lines 355-369 ; Page 19, Lines 383-388; Page 20, Lines 407-414):
“Our subgroup analysis showed a significantly increased risk of ATDILI in the CYP2E1 RsaI/PstI c1/c1 genotype carriers of East Asian ethnicity (S9 Table), suggesting a potential gene-ethnicity interaction 1. A previous study identified age, female sex, white race, non-Hispanic ethnicity, lower body mass index, elevated plasma aspartate transaminase concentrations at baseline, and nine months of daily INH use as risk factors for ATDILI. Considering their race, ethnicity, and relatively lower body mass index compared to other ethnicities, East Asians may be at an increased risk of ATDILI. As the CYP2E1 RsaI/PstI c1 allele frequency is relatively low in this population (79.8% vs. 88.5% to 99.8% in other ethnicities), the ethnicity itself might play an important role in developing hepatotoxicity through gene-ethnicity interaction. Furthermore, the relatively high frequency of c2 allele in this population might serve as a good control to estimate the effect of c1 allele on the risk of ATDILI; the rarity of this minor allele in other ethnicities could make it difficult to evaluate the association between the ATDILI risk and this genetic polymorphism.”
“The frequencies of NAT2 slow acetylator alleles are highly variable between ethnic groups, ranging from 32% in Koreans to 76% in Caucasians. Despite this large inter-ethnic variability in the NAT2 polymorphic allele frequency, the NAT2 slow acetylator genotype consistently and significantly increased the risk of ATDILI across all ethnicities, suggesting the critical role of NAT2 polymorphism in the development of ATDILI.”
“Our subgroup analysis showed a significantly increased risk of ATDILI in the GSTM1 null genotype carriers of Indian ethnicity; although not statistically significant, the risk of ATDILI was relatively high in the East Asian population with the GSTM1 null genotype (S11 Table). Considering the substantial difference in the GSTM1 null allele frequency between Indians (29.6%) and East Asians (52.1%), a

potential gene-ethnicity interaction may exist based on their race, ethnicity, and body size as aforementioned. Other characteristics than the GSTM1 polymorphism in these ethnicities may play a more important role in the development of ATDILI."

Minor revisions
1. Please state a version of Review Manager Software.

Thank you for your comment. To address your comment, we added the version number 5.3 of Review Manager Software in our revised manuscript as follows (Page 11, Line 215):
"Statistical analyses were performed using Review Manager Software version 5.3 (Cochrane Collaboration, London, UK)."

2. Please state a statistic test that authors used for analysis of total effect in a method section (Mantel-Haenszel test or inverse variance)

Thank you for your comment. To address your comment, we specified the statistic test used for our analysis of total effect in the method section as follows (Page 10 , Lines 193-195):
"The Mantel-Haenszel or DerSimonian-Laird method based on fixed- or random-effects models, respectively, were used depending on the presence of heterogeneity 2 3."

3. "SLCO1B1 encodes…" on page 17 should be italic because it is gene's name.

Thank you for your comment. To address your comment, we revised our manuscript accordingly as follows (Page 20, Line 420):
"SLCO1B1 encodes organic anion transporting polypeptide 1B1 (OATP1B1) which is a major influx drug transporter responsible for the hepatic uptake of various endogenous and exogenous substances including RIF 4."

4. In table S6 should be "PCR-RFLP" instead of "PCR-RELP"

Thank you for your comment. To address your comment, we revised our manuscript accordingly in the Supplementary data S5 Table.

Reviewer: 2
Reviewer Name: Li Zhong

Institution and Country: College of Life Sciences, Shaanxi Normal University, Xi'an, China

Please leave your comments for the authors below
In this paper, the authors investigated the association between genetic polymorphisms of NAT2, CYP2E1, GST and SLCO1B1 and the risk of anti-tuberculosis drug-induced liver injury (ATDILI). In brief, the project design and work flow was reasonable and this result was interesting. The result was also important for this disease and other related fields.

1. My major concern was why the meta-analysis was performed under only one genomic model. The authors should be aware of that there are three genomic models (dominant, recessive and additive) for two alleles.

Thank you for your comment. We are truly aware of the three genomic models including dominant, recessive, and additive models that can be tested for two alleles as you mentioned. Unfortunately, it was not practical to test all three genomic models for CYP2E1, NAT2, and GSTs because technically, we compared patients with different genotype-based phenotypes, i.e., slow acetylator vs.

fast/intermediate acetylator and null vs. non-null GSTs. Multiple allelic variants may represent the same phenotype (e.g., NAT2*5B, *6A, and *7B all classified as slow acetylator genotypes), and the genetic model selection can be varied depending on the specific allelic variant. Therefore, the genetic models used in previous original and meta-analysis studies were adopted for these polymorphic genes in our current study. In contrast, we evaluated specific allelic variants for SLCO1B1 (i.e., 388A>G and 521T>C), so all three genomic models of dominant, recessive, and additive models were assessed for SLCO1B1 genetic polymorphisms. Overall, to address your comment, our rationale and the related study limitation regarding the use of only one genomic model for CYP2E1, NAT2, and GSTs as well as the updated results for SLCO1B1 using all three genetic models for two alleles are included in our revised manuscript as follows (Page 10, Line 187-193; Page 22, Line 459-468 ; and Table 2 in Page 42):
“The genetic risk models for NAT2, CYP2E1, GSTM1, GSTT1, and GSTM1/GSTT1 have been studied in previous studies. Based on these previous studies, the most clinically significant and plausible model for each polymorphic gene was selected. Due to the relative paucity of data suggesting the most clinically relevant genetic model for SLCO1B1 388A>G and 521T>C polymorphisms, all three genomic models including dominant, recessive, and additive models were evaluated.”
“In addition, only one genetic model was used for CYP2E1, NAT2, and GST when evaluating the association between genetic polymorphisms of these genes and the risk of ATDILI. Although we acknowledge dominant, recessive, and additive genomic models can be used for two alleles, it could not be applied to our meta-analysis because we compared patients with different genotype-based phenotype, i.e., slow acetylator vs. fast/intermediate acetylator and null vs. non-null GSTs. Multiple allelic variants or allele subgroups may represent the same phenotype (e.g., NAT2*5B, *6A, and *7B all represent slow acetylator genotypes), and the genetic model selection can be varied depending on the specific allelic variant. Therefore, the genetic models used in previous original and meta-analysis studies were adopted for these polymorphic genes in our current study.”

2. Furthermore, the results in this paper were valueless since similar results were obtained for NAT2, CYP2E1, and GST in previous ATDILI publications and meta-analysis was not indispensable. Moreover, other functional experiments should be more meaningful. Therefore, I suggested the authors to perform the additional functional experiments on the role of genetic polymorphisms in ATDILI and resubmit the paper.

Thank you for your comment. We cannot agree more with you in terms of the value of additional functional experiments on the role of genetic polymorphisms in ATDILI. Especially, as our subgroup analysis results suggested, it may be of great interest to identify ethnicity-specific genotypes significantly associated with ATDILI in each of the various ethnic groups. However, the objective of this current study was to evaluate the association between the risk of ATDILI and genetic polymorphisms of CYP2E1, NAT2, GST, and SLCO1B1 in tuberculosis patients through providing more updated, comprehensive, and compelling evidence. As shown in our forest plots (Figures 2 to 4) as well as the study characteristic summary table (Table 1), numerous studies reported conflicting results regarding the association between the risk of ATDILI and a specific genotype of CYP2E1, NAT2, GSTs, and SLCO1B1. This may be due to the substantial inter-study differences in sample sizes and/or characteristics of the patient populations primarily included in these studies. According to Walker and colleagues. the main objectives of a meta-analysis are to: 1) summarize and integrate results from a number of individual studies; 2) analyze differences in the results among studies; 3) overcome small sample sizes of individual studies to detect effects of interest, and analyze end points that require larger sample sizes; 4) increase precision in estimating effects; 5) evaluate effects in subsets of patients; 6) determine if new studies are needed to further investigate an issue; 7) generate new hypotheses for future studies. Through the meta-analysis, we overcame small sample sizes of the individual studies included in our current study, and summarized and integrated results from a number of individual previous studies. In addition, through our subgroup analyses, we

identified the needs for future studies to identify ethnicity-specific genes associated with the risk of ATDILI. In this context, meta-analysis was an appropriate design to evaluate the association between the risk of ATDILI and genetic polymorphisms of CYP2E1, NAT2, GST, and SLCO1B1 as in our current study, and thus, our current meta-analysis fills a knowledge gap and provides valuable insight into the genetic predisposition to ATDILI.

Reviewer: 3
Reviewer Name: B.Wilffert

Institution and Country: Unit of PharmacoTherapy, -Epidemiology & -Economics, Groningen Research Institute of Pharmacy, University of Groningen, The Netherlands

Please leave your comments for the authors below
The manuscript describes a very relevant and well-performed meta-analysis.
I have only a small textual remark: p.15, l.23, medication instead of medications.

Thank you for your comment. To address your comment, we revised our manuscript accordingly as follows (Page 18, Line 371-373):
“This is consistent with previous study findings because hepatotoxicity commonly occurs with anti-TB drugs such as INH and RIF and thus, use of more than one hepatotoxic anti-TB medication increases the risk of ATDILI 5.”

Reviewer: 4
Reviewer Name: Shaowen Tang

Please leave your comments for the authors below
In this study, the authors conducted a systematic review and meta-analysis to evaluate the role of genetic polymorphisms of CYP2E1, NAT2, GST, and SLCO1B1 in the risk of anti-tuberculosis drug-induced liver injury. I had some important concerns about the methodologies for this study.

1. A total of 53 eligible studies from 2001 to 2016 were finally included in this meta-analysis. Although the authors said in the manuscript, it is “to provide more updated, comprehensive, and compelling evidence”, and they searched the literatures from their inception to February 2018, there is no article included from 2017 to 2018. The latest article was published in 2016 (There is only one literature in 2015 and 2016, respectively.). Are the authors aware of any reason that investigation seemed to cease after 2016? As far as I know, there have been many similar original researches published during 2017-2018
(For example in NAT2, Chan, S. L., et al. PLoS One, 2017, 12(10): e0186200; Wattanapokayakit, S., et al. Int J Tuberc Lung Dis, 2016, 20(10): 1364-1369. For CYP2E1, Sun, Q., et al. Clin Drug Investig, 2017, 37(12): 1125-1136.). There are several similar systematic reviews (For example in NAT2, Suvichapanich, S., et al. Pharmacogenet Genomics, 2018, 28(7): 167-176. Zhang, M., et al. Br J Clin Pharmacol. 2018.). The authors thought that there was a limited number of studies evaluating the association between the risk of ATDILI and the SLCO1B1 genetic polymorphisms (n = 4). However, another study has been published in 2017 (Sun, Q., et al. Clin Drug Investig, 2017, 37(12): 1125-1136.). So, the authors should update the literature search, and revise all results.

Thank you for your comment. To address your comment, we have reviewed the manuscripts you mentioned and updated our literature search results accordingly. One additional study for NAT2 was identified and included in our meta-analysis (Wattanapokayakit S et al. Int J Tuberc Lung Dis 2016;20(10):1364-1369). Other studies mentioned above were meta-analyses with the exception of the Sun Q et al. study for SLCO1B1. As stated in the study selection criteria of our manuscript, we included case-control or cohort studies only to perform meta-analysis; therefore, other meta-analysis

studies previously published as original research were not included in our current analysis. In our database search, we did identify these meta-analysis studies; however, they were removed in the study selection process based on our study inclusion/exclusion criteria. The SLCO1B1 study conducted by Sun Q and colleagues did not evaluate 388A>G and 521T>C genetic polymorphisms which were specifically examined in our current meta-analysis; the number of original studies accumulated was insufficient to conduct a meta-analysis for other SLCO1B1 genetic polymorphisms. Therefore, this study for SLCO1B1 performed by Sun Q and colleagues was excluded in our study selection process because the genetic polymorphisms of our interest were not evaluated in this study. Overall, we updated our meta-analysis result for NAT2 by including one additional study by Wattanapokayakit S and colleagues; results for other genes (i.e., CYP2E1, GST, and SLCO1B1) stayed the same.
Updated results are available in Table 1, Figure 1, Figure 3, Supplementary S4 Table, and Supplementary S9 Table. Results for the NAT2 meta-analysis were also revised in the main text as follows (Pages 14, Lines 265-268):
"Using the random-effects model, the pooled estimates of all included studies (n = 35) showed a significant association between the risk of ATDILI and the NAT2 polymorphism (OR for the slow acetylator genotype = 3.30, 95% CI 2.65–4.11, P < 0.00001; I2 = 54%, Pheterogeneity < 0.0001) (Figure 3)."

2. The authors should describe the inclusion criteria clearly.

Thank you for your comment. To address your comment, we further clarified the study inclusion and exclusion criteria for our meta-analysis as follows (Pages 8-9 , Lines 154-168):
"Studies were considered eligible if they met all of the following inclusion criteria: (1) studies with TB patients receiving anti-TB drug therapy; (2) studies with the control group of TB patients tolerant of anti-TB medications; (3) studies evaluating the association between the occurrence of ATDILI and genetic polymorphisms of CYP2E1, NAT2, GST, and SLCO1B1 388A>G and 521T>C; and (4) case-control or cohort observational studies. Excluded studies were as follows: (1) studies available only in the form of abstracts or meeting posters; (2) review or meta-analysis articles; (3) studies providing insufficient data necessary for the statistical data analysis; (4) studies in non-English language; (5) non-human studies including animal and in vitro studies; (6) studies with unpublished data; (7) studies providing insufficient information on genotyping methods; and (8) healthy controls."

3. In the section of Literature search, the authors should list the entire set of search strategies of every database in the appendix.

Thank you for your comment. To address your comment, the detailed search strategies for each electronic database used in this analysis are presented in Supplementary data S2 Table. This information is also presented in our revised main text as follows (Page 8, Lines 149-150):
"The detailed search strategies for each electronic database used in this analysis are presented in S2 Table."

4. The authors included the case-control or cohort studies. The type of original study needs to be listed in Table 1. Usually, different types of original research results cannot be directly merged, and the same type of researches should be merged firstly

Thank you for your comment. Although we acknowledge case-control and cohort studies are two different types of original research, these two study designs were combined for our primary meta-analysis to sufficiently increase the statistical power (Table 1 and Supplementary data S9 to S11 Tables). Instead, subgroup analyses were performed based on the study design by separately conducting meta-analyses with case-control studies and cohort studies, respectively. These results are shown in Supplementary data S9 to S11 Tables. Our subgroup analyses showed the

heterogeneity I2 value for either study design alone was not always less than that for all studies combined together, probably suggesting other sources of heterogeneity than the study design may contribute more to the heterogeneity in our meta-analysis. Overall, to address your comment, we included the type of study design in Table 1. Additional subgroup analyses based on the study design were conducted to compare results between the meta-analysis using all studies combined together and that using the studies of the same type of study design only (Supplementary data S9 to S11 Tables).

5. The type of data extracted by the author is Dichotomous, However, the forest plots were inconsistent with the results of the dichotomous data, especially being performed using the Review Manager Software. The author also needs to describe the software version number used.

Thank you for your comment. To address your comment, we have reviewed our data analysis procedure and results, and confirmed no problem in our data analysis process. Actually, our forest plots appear to be similar to those reported in other previous meta-analyses based on dichotomous data (Sheng YJ et al. Infection, Genetics and Evolution 2014;24:34-40), especially the one using the Review Manager Software. To clarify the software version used in our study, we added the version number 5.3 of Review Manager Software in our revised manuscript as follows (Page 11 , Line 215).
“Statistical analyses were performed using Review Manager Software version 5.3 (Cochrane Collaboration, London, UK).”

6. The authors described that sensitivity analyses were conducted to assess the robustness of the results in the Methods section. However, the authors described some results in the Discussion section (Page 18, line 4-12). The authors should clarify which method is used for sensitivity analysis in the Methods section, and described some results in the Results section.

Thank you for your comment. We clarified our sensitivity analysis method as the leave-one-out method in the Methods section, and results of the sensitivity analysis are presented in the Results section as follows (Page 11, Lines 208-212; Page 16, Lines 310-323):
“Sensitivity analysis was conducted to assess the robustness of the results and to identify the source of heterogeneity using the leave-one-out method. In each analysis, one study was deleted, and with the one study left out, the meta-analysis was performed; this process was repeated until every study had been deleted from our included study pool for each tested polymorphic gene.”
“Our primary analysis results showed significantly high heterogeneity between studies for CYP2E1 RsaI/PstI (I^2 = 60%, $P < 0.0001$), NAT2 (I^2 = 54%, $P < 0.0001$), GSTM1/GSTT1 (I^2 = 59%, $P = 0.006$), and SLCO1B1 521T>C (dominant genetic model: I^2 = 66%, $P = 0.03$) polymorphisms. This high heterogeneity between studies may be due to substantial differences in ethnicity, anti-TB drug regimen, the genotyping method used, study design, and diagnostic criteria of ATDILI among the included studies (Table 1). Through the sensitivity analyses, outlier studies were identified as the major source of heterogeneity. After removing these outlier studies, heterogeneity was substantially reduced (I^2 = 60% to 42% for CYP2E1 RsaI/PstI 6, I^2 = 54% to 34% for NAT2 7 8, I^2 = 59% to 0% for GSTM1/GSTT1 9 10, and I^2 = 66 % to 0% for SLCO1B1 521T>C dominant genetic model 11). The overall results for the association between the risk of ATDILI and these genetic polymorphisms after excluding the outlier studies stayed the same as those from our primary analysis results.”

7. None of the funnel plots were substantially asymmetrical. An asymmetric inverted funnel-shape scatter plot was used to indicate the existence of potential publication bias. But besides funnel plot, the authors may consider Egger’s test.

Thank you for your comment. To address your comment, we revised our manuscript as follows (Pages 12-13, Lines 242-243):
“None of the funnel plots showed an asymmetric inverted funnel shape, indicating the absence of

potential publication bias."
In terms of Egger's test, we did consider performing this test; however, unfortunately, the Review Manager Software does not have a function to conduct this test. As our funnel plots did not show the existence of potential publication bias, Egger's test results may not be as critical. Thank you for your understanding in advance!

8. Epidemiology of tuberculosis needs to be updated in the Introduction.

Thank you for your comment. We reviewed the most recent global tuberculosis report published by the World Health Organization and updated the tuberculosis epidemiology accordingly (Page 6, Lines 93-94):
"It poses a major public health threat globally with approximately 1.3 million deaths and 10 million new cases in 2017 12."

VERSION 2 – REVIEW

REVIEWER	Shaowen Tang Nanjing Medical University
REVIEW RETURNED	22-Apr-2019

GENERAL COMMENTS	Thanks to the authors for the significant efforts in improving the manuscript, and all the questions have been settled satisfactorily. There is a small error in S2 Table (Search strategies) that needs to be modified. What does #51 here mean in PubMed search strategy?

REVIEWER	Supharat Suvichapanich Department of Biochemistry, Faculty of Pharmacy, Mahidol University
REVIEW RETURNED	07-May-2019

GENERAL COMMENTS	Yang et al. studied associations of NAT2, CYP2E1, GST, and SLCO1B1 with the risk of anti-tuberculosis drug induced liver injury (ATDILI) using systematic review and meta-analysis. The authors fulfilled all reviewer's requests. I would suggest this manuscript an acceptance to publish in this journal.

VERSION 2 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 4
Reviewer Name: Shaowen Tang

Institution and Country: Nanjing Medical University

Please state any competing interests or state ‘None declared’: None declared

Please leave your comments for the authors below
Thanks to the authors for the significant efforts in improving the manuscript, and all the questions have been settled satisfactorily. There is a small error in S2 Table (Search strategies) that needs to be modified. What does #51 here mean in PubMed search strategy?

Response: Thank you for your comment. To address your comment, we have corrected “#51” to “drug metaboli#er*)” in S2 Table.

Reviewer: 1
Reviewer Name: Supharat Suvichapanich

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Please state any competing interests or state ‘None declared’: None declared

Please leave your comments for the authors below
Yang et al. studied associations of NAT2, CYP2E1, GST, and SLCO1B1 with the risk of anti-tuberculosis drug induced liver injury (ATDILI) using systematic review and meta-analysis.

The authors fulfilled all reviewer's requests. I would suggest this manuscript an acceptance to publish in this journal.

Response: Thank you for your insightful review.