Author's response to reviews

Title: Associations between interleukin-1 gene polymorphisms and sepsis risk: a meta-analysis

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Author's response to reviews: see over
Dear Editors and Reviewers:

Thank you very much for concerning our manuscript (1214409578105331) entitled "Associations between interleukin-1 gene polymorphisms and sepsis risk: a meta-analysis". We have carefully read the comments and would like to appreciate you for the insightful and helpful critiques. We have revised all the questions in our revised manuscript one by one. We hope that the manuscript is considerably strengthened. Below please find our point-by-point response to the comments.

Response to reviewer 1:

1. Forest plots should be used to compare study results with those from the meta analyses.

   Thank you very much for the suggestion, we have added forest plots of each polymorphism under any genetic model in Figure 2, 3 and supplemental Figure 1-5, which may be helpful in the intuitive interpretation of the individual or pooled ORs.

2. The subset analyses introduce multiple testing issues which have not been addressed.

   According to the original data of all included studies, we performed meta-analysis of five SNPs with dominant, recessive and allelic genetic models and further stratified analysis. However, we did not make multiple comparison adjustment for
insufficient data. There might be false positive results for not doing multiple
comparison adjustment of different polymorphisms, which could increase the
probability of type I error.

3. There is a lack of discussion on the different definitions of sepsis.

Thank you for pointing out, we have added the discussion that whether the effect
of polymorphisms differed depending on the way in which sepsis was described
(sepsis, severe sepsis, or septic shock) according to the published consensus
definitions for sepsis in Page 16.

4. Figures 2, 3 and 4 are not suitable for publication.

Yes, we have revised the funnel plots and corresponding figure legends, which
were renamed as Figure 4, 5, 6.

5. The results description should be simplified with the addition of graphical
comparisons.

In response to the reviewer’s comment, we have simplified the description of
result (Result section: page9-11) with the addition of forest plots (Figure 2, 3 and
supplemental Figure 1-5).

Response to reviewer 2:

1. I would recommend the authors to thoroughly edit the manuscript to improve
the quality of English.

Thank you very much for the comments, we have carefully checked and revised the manuscript.

2. **Eligibility of articles for the meta-analysis was not based on the quality of the research, for example an appropriate source of controls in case-control studies.**

   I reckon this is one of the major limitations of the manuscript and I encourage the authors to address it. I am not familiar with the Newcastle-Ottawa method for quality assessment, but the authors state that studies with a score of 7 or higher are considered of high quality, and then include in the meta-analysis studies with lower scores. The authors should please correct or justify this inconsistency.

   Thank you for pointing out this question. We have revised the description of quality score in result section (Page 9) and performed further stratified analyses for all polymorphisms based on quality score in corresponding results section (divided into ≥7 and <7) (Table 2). In addition, we addressed this issue in limitations of discussion section (Page 18 Sixth).

3. **The threshold for significance has been set by the authors as equal to 0.05.**

   Please avoid expressions as "slight significant association" or similar for results where p-value is larger than 0.05. If you reckon the result is anyhow noteworthy, please describe the apparent effect of the polymorphism but clearly
stating that it did not reach statistical significance.

Regarding to the statistical analysis, we have made changes for the description of the pooled results in manuscript (corresponding abstract and result section: page 2, 9, and 11).

4. I would suggest to use a random effect method whether or not heterogeneity exists, so that results can be interpreted in an homogenous way (estimates from random and fixed effect methods have different meanings).

The reviewer raised good comments. We have reanalyzed the pooled OR using random-effects model whether or not heterogeneity exists considering the small number of included studies for every polymorphism (Table 2), which might increase the confidence of pooled results. In addition, the pooled results from random-effects model were not different compared with those from fixed-effects model.

5. It would be helpful to include forest plots showing ORs and their 95% CI for the individual studies, so that heterogeneity can be visually assessed. To add useful information, the size of boxes for ORs should be proportional to the weight of the study in the meta-analysis.

Thank you very much for the suggestion, we have added forest plots showing ORs and their 95% CI of each polymorphism under all genetic models in Figure 2, 3 and supplemental Figure 1-5. In addition, heterogeneity could be also visually
assessed.

6. **Please further investigate and describe the potential sources of heterogeneity. In particular, the relevance of the different genetic background of the populations included in the analysis should be stressed. Furthermore, one important source of heterogeneity might well be the different source of controls. Please also conduct meta-regression with covariates for all polymorphisms and show the results (perhaps as supplementary material).**

Thank you for the comments on the potential sources of heterogeneity. Meta-regression with covariates containing ethnicity, sepsis severity, sources of controls, sample size for IL-1RN VNTR was performed and the results were shown in supplementary Figures S6-8. Moreover, we revised the description of heterogeneity analysis in result section (Page 12) and addressed it in discussion section (Page 17). However, some potential factors such as pathogen type, age, sex ratio were not performed due to limitations of the data which also restricted our ability to detect possible sources of heterogeneity, which was added in limitations (Page 18 Fourth)

7. **Please show the results of sensitivity analysis (also as supplementary material).**

Thanks for your question. The results of sensitivity analysis were added as supplementary material (Table S2-6) and the corresponding changes were supplied in result section (sensitivity analysis: Page 12-13).
8. Please briefly describe sepsis in introduction. Is it also important that more references are given to support the authors' statements (e.g. the role of IL-1α, IL-1β and IL-1ra in sepsis).

We have addressed this issue and added more substantial evidence for the role of IL-1 in sepsis in the introduction section (Introduction section: paragraph 1-3, page 4-5).

9. More references should be given to support the authors' statements in discussion (e.g. on the role of IL-1#).

OK, thank you for this question. We have rewritten the discussion section and added more references to address our results of meta-analysis (Discussion section: page 14-16).

Response to reviewer 3:

1. The authors perform both total (including all studies) and subgroup (separated by ethnicity and sepsis severity) analyses. It would seem like the ethnicity subgrouping definition that would reduce the most heterogeneity caused by differences in ethnicity would be to separate the studies into three categories: “Asian”, “Caucasian”, and “Other” (African American, Hispanic, Jewish/Arabic). Additional analysis according to the subgrouping described above should be performed in order to determine if new associations are revealed and to ascertain whether this subgrouping scheme leads to a decrease
in between study heterogeneity estimates. If interesting results/decreased heterogeneity are shown to be the result, then this could be presented in supplement perhaps?

We appreciate the reviewer very much for the detailed explanations about the ethnic categories for subgroup analysis. According to the reviewer’s suggestions, we have made some revision of subgroup analysis based ethnicity in the Table 2 and corresponding result section (page 9-11).

2. Here the authors state that “For studies including subjects of different populations, data were extracted separately”, but numerous places elsewhere in the manuscript they mention that there are 19 studies in their meta-analysis. This is misleading. Since the Johnson study contained both Caucasian and Black subjects, they were extracted separately (Johnson-1, Johnson-2) in accordance with the author’s listed methods and were treated as two independent studies, effectively bringing the number of studies included in the meta-analysis to 20 and not 19, which may be a bit confusing to readers. An extension to the last sentence that states that after the data were extracted separately, they were treated as independent studies (as in the case of Johnson-1 and Johnson-2) would make things more clear. Also this should be addressed elsewhere in the manuscript where the author’s mention “19” studies.

Thank you for pointing out this question, we have revised the description. Two studies containing Caucasian and Black subjects were included in one article by
Johnson et al [14] and it was considered as two independent studies (as in the case of Johnson-1 and Johnson-2) in the following data analysis. (Result section: page 8).

3. The author’s do not show any information about Hardy-Weinberg equilibrium tests being performed in the control samples of each of the individual studies. Where possible, these tests should be performed as deviation from equilibrium could indicate a source of bias in there analyses. Please perform these calculations and present them (perhaps as a part of table 1 after the last column).

We have addressed this issue and added the information of Hardy-Weinberg equilibrium of each study in Table 1 and result section (page 9).

4. The authors state that a score of 7 or greater indicates high quality, but then they state in the results section (paragraph 1) that quality scores ranging from 5 to 9 indicate high quality. These two statements are not in agreement. If 7 stars indicate high quality, than do scores below 7 indicate low quality, and if so then why were studies with quality scores below 7 included in analysis? The authors do not state explicitly what their quality score “cut-off” value is, if this value is 5, which is below the value of 7 which indicates good quality, what is the justification for this?

Thank you for pointing out, we have revised the description of quality score in
result section (Page 9) and performed further stratified analyses for all polymorphisms based on quality score in corresponding results section (divided into ≥7 and <7) (Table 2). In addition, we have added the tabulation of the quality scores for all included studies in supplemental Table 1.

5. The author’s cite Higgins 2003, when describing the tests that they performed to assess heterogeneity. However, the author’s mention using a Q test (assumedly the standard Cochran Q test), which is not the method developed by Higgins et al in the paper they cite. Higgins et al, present the calculation of inconsistency (I²) as a superior method to calculate the amount of heterogeneity as it measures the amount of variation across studies that is truly due to heterogeneity rather than chance. This would be a superior measure of heterogeneity in this case as the standard Cochran Q test is known to perform poorly in meta-analyses where the total number of studies is low. This fact is somewhat minimized by using a cut-off of p<0.10 (which seems to be the standard practice, which the authors have employed). But since the authors reference Higgins here a statement about I² should be included, also the calculated I² values and associated p-values should be presented with the Q values where the authors discuss heterogeneity. If this reference was included in error and the authors did not in fact use the Higgins et al method then this needs to be corrected in this paragraph as well as in the reference section.

Thank you for pointing out, we have performed heterogeneity test using both
Cochrane Q test and $I^2$ statistic, and added the information in method section (page 7) and Table 2.

6. In Table 1, in the IL-1B-511A/G section – Watanabe 2005 study. The authors present that for the Watanabe 2005 study there are 39 (19 case, 20 controls) individuals with at least one copy of the B allele, and 74 individuals (49 case, 25 controls) individuals who are homozygous for the major allele. Upon review of the Watanabe et al study, the authors (Watanabe et al) present in Table 3 (line 3) and in Table 4 (column 4, lines 1 and 2) data that states that there are 39 individuals who are homozygous for the major allele (*2-) and 74 individuals who are heterozygous or homozygous for the minor allele (*2+). Watanabe et al define the less frequent (minor allele) as the *2 allele on page 1183 of their published paper. This information indicates that the authors of this manuscript (Zhang et al) have flipped the genotype counts in Table 1 for the Watanabe study, and the correct genotype distribution should be: Cases: AA=19, AB+BB=49, and Controls: AA=20, AB+BB=25 (B allele=*2 allele, so *2- would be individuals without the B allele so AA individuals, *2+ would be individuals with the B allele so AB and BB individuals). Because of the difference in allele coding between Watanabe 2005 and this manuscript, interpretation between the two articles is challenging, and it is possible that I have misinterpreted something when moving between the two papers. The authors of this manuscript should check the Watanabe 2005 paper and their analyses and
confirm that the genotype distributions are correct. If it is determined that there is a discrepancy, then the source of the error must be determined. There is the possibility that the error is merely a typo when formulating the table and the analyses was in fact correct. There is also the possibility that the analysis was performed using the wrong genotype counts, in which case reanalysis would be necessary.

Thank you very much for pointing out. We carefully check each data extracted from included studies, revised this error and reanalyzed the results for IL-1B-511 (Table 1).

7. Table 1, IL-1B-31C/T – Shalhub 2009, IL-1RN VNTR L/2 – Sole-Violan 2010. Upon inspection of the two datasets, it does not seem appropriate to include a study that contains individuals with sepsis (any form) in both the case and control populations; because this study would not be evaluating the effect of polymorphism genotype on sepsis risk, but the effect of that genotype on sepsis severity only. The authors should provide justification for inclusion of the study the way that it was analyzed or reanalyze the study to address sepsis risk and recalculate any associated pooled odds ratios. The results section should be revised after reanalysis.

We appreciate the reviewer very much for the detailed review of included studies. After careful checking each study, we removed the two studies (Watanabe 2005 and Shalhub 2009), which did not provide detailed information to classify sepsis and non-sepsis patients, and reanalyzed the pooled results (corresponding result
8. **Note about Sole-Violan and Watanabe datasets** As noted above, the Sole-Violan and Watanabe datasets do not appropriately assess sepsis risk as presented in this manuscript. However they do appropriately assess sepsis severity (individuals who develop SS and/or SSH vs. those who develop less severe sepsis). If the authors wanted to pursue this they could perhaps include in their main aims that they wish to assess sepsis risk and severity and include these two studies in a separate analysis of sepsis severity? This is just a suggestion for an additional exploratory analysis if the authors are in fact interested in evaluating sepsis severity as well as sepsis risk, but if sepsis risk is to remain the main focus of this manuscript then this suggestion need not be addressed.

The reviewer raised good suggestions. We really agree with the reviewer’s opinions. However, we did not explore this issue due to limited information from the two studies, which should be a new topic for further study and the limitation of this manuscript (Discussion section: page 18 finally).

9. **Results Section, Heterogeneity analysis subsection, Paragraph 2** I applaud the authors for further examining heterogeneity in this study through the use of meta-regression. I would however, like to have been given more information on how the meta-regression was conducted. For instance, was a random or fixed effects model used for the meta-regression? Was each covariate analyzed
independently or were they all included in one regression model? Also, source of control subjects is a possible cause of heterogeneity in meta-analyses. Since the authors have access to this information, source of controls should be included as a covariate/possible heterogeneity source in the meta-regression analysis. Also there is no mention of the meta-regression that was performed in the materials and methods section of the manuscript and perhaps there should be?

Thank you for the comment about meta-regression. We have conducted meta-regression for IL-1RN VNTR with all covariates containing ethnicity, sepsis severity, sources of controls, sample size in one random-effect model using REML method (Methods section: page 8) and shown the results in supplementary Figures (Figure S6-8). Moreover, we revised the description of meta-regression in result sections (Page 12).

10. The authors do not present any forest plots to visualize the individual odds ratios (or pooled odds ratios) that they present in table 2. Forest plots allow readers to see the directionality and effect size of the individual studies which is helpful in the intuitive interpretation of the pooled odds ratios. These plots should be created and included in the manuscript. As the plots are mentioned in the incorrect Figure legends (for figures 2,3,4) it seems like the authors have indeed created these plots but may have accidentally omitted them from this submission. Please address this.
Thank you very much for the suggestion, we have added forest plots of each polymorphism under any genetic model in Figure 2, 3 and supplemental Figures (Figure S1-5). Moreover, the incorrect Figure legends of funnel plots were revised (corresponding Figure legends: Figure 4, 5, 6).

Thank you again for your review and re-consideration!

Sincerely yours,

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