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 revised 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 and the reviewers for the insightful and helpful critiques. We have thoroughly revised all the questions again one by one. We hope that the revision is considerably strengthened. Below please find our point-by-point response to the comments.

1. As pointed out by the three reviewers, the quality of English needs to be further improved (both grammar and spelling) and I would recommend that you request assistance from an English speaking copy editor before submitting your paper again.

We would like to appreciate you and the reviewers for pointing out, we have carefully corrected the spelling and grammatical errors, and revised the manuscript.

2. The multiple testing issues of the subset analyses have not been addressed

Thank you for pointing out. According to your suggestion, we performed multiple testing in subset analyses using Benjamini-Hochberg (BH) step-up correction method, which control the false discovery rate (FDR) (Methods section and corresponding results section).
3. As pointed out in my first review, eligibility of articles for the meta-analysis was not based on an appropriate source of controls in case-control studies, and this issue has not been fully addressed by the authors. From Table 1, it is apparent that the included studies used several different sources of controls, most of which were not selected from the general (healthy) population but from hospital patients. When in a study cases and controls are not drawn from the same population selection bias might occur and results are not fully reliable. The authors addressed the issue of the different source of controls by including this factor in meta-regression. Beside the reasons given above, this seems not sufficient to me as meta-regression was conducted for one SNP only.

We appreciate the reviewer very much for the detailed explanations about the different source of controls. In this issue, we have not performed stratified analysis based on the source of controls due to small number of studies in each subgroup, which might increase the probability of type I error and bias the pooled results. The limitation was added into discussion sections (Page 18).

4. It is not clear to me why studies which used healthy controls (as David 2010, Emonts 2010, Balding 2003, Garcia Segarra 2007, Ma 2002 and Fang 1999) score 0 in the 3rd column of the quality score table, which refers to community controls, while studies using hospital controls score 1.

First of all, we would like to say that we are very sorry to make an incorrect quality score. According to the Newcastle-Ottawa quality assessment scale (NOS) (Reference
28), we performed quality score for each study included into the meta-analysis again and revised the related result section (Page 9-11).

5. The authors should please try to further clarify the presentation of results throughout the text.

Thank you very much for pointing out. We have addressed the reviewer’s suggestions in the revision.

6. Meta-regression and analysis of publication bias have been conducted for IL-1RN VNTR only, stating that for the other polymorphisms they were not performed due to the small number of studies (n<10). Could you please explain why, and give a justification for this in the methods section.

According to previous study (Reference 30), meta-regression was more effective when the number of studies is more than or equal to 10, which was added into methods section (Page 8).

7. Figure legends that describe what the symbols in the figures represent are essential. Currently, none of the figure legends for Figures 2-6 describe the symbols presented in the corresponding figures; instead they seem to be more Figure Titles rather than Figure Legends. The authors need to add information to the figure legends so that readers will know exactly what these images mean in terms of presentation of the applicable results. Please address this.
According to your suggestion, we have added information to the figure legends for Figure 2-6 and supplemental Figure 1-5.

8. *The results for the VNTR polymorphism in Table 2 should reflect the analysis that excludes the Bessler study (a notation in the table can be added to indicate this). Additionally, the results section that discusses this variant should be rewritten to reflect the outcome from the analysis excluding Bessler for the allelic model and the statements about “borderline significance” in the model including Bessler should be deleted. A sentence stating that models deviating from HWE in controls were not included in allelic model test should also be added to the methods section. Please address this.*

We appreciate the reviewer very much for the detailed explanations about the HWE in control population. We revised the result of IL-1RN VNTR after removing the Bessler study (Page 11-12, Table 2, Figure 3).

9. *In this section and elsewhere in the manuscript, the authors repeatedly state that a “borderline significant” association with sepsis risk was found in the Asian subset. The association between VNTR and sepsis risk in the Asian subset is in fact non-significant (p>0.05). Additionally, this subset only contains two studies, one of which is of low quality (quality score < 7). Presenting these results as “borderline significant” and highlighting them in the discussion, conclusion, and Table 2 is extremely misleading as evidence for an actual*
association in this subset is highly questionable at best. If the author’s wish to highlight the result in this subset it would be more appropriate to say that they “observed a similar trend” in Asian patients as that seen in the overall analysis. Statements made in the discussion section that cite a significant association in the Asian subset and interpret this as an indication that previous studies may have shown discrepancies due to differing environmental and genetic backgrounds should be revised; while diverse genetic background and environmental factors may have played a role in the discrepancies for VNTR seen in earlier studies this fact is not supported by a “significant association with risk of sepsis in overall comparison and Asians” as the authors state. Please address this and extensively revise or provide justification for these statements.

Regarding to the statistical analysis, we have made changes for the description of the pooled results in manuscript.

10. The authors state that the IL1b+3594 polymorphism has been reported to influence the production of IL-1 beta protein and cite Pociot et al, as a reference. Upon further review, Pociot et al clearly state there is an allele dosage effect for this polymorphism on protein production. The authors should clearly state which allele of IL1B+3594 causes increased protein production in response to LPS-stimulation. The authors go on to say that their finding (TT individuals were less susceptible to sepsis) was inconsistent with higher IL-1B levels, but
they do not explicitly tell readers why this is inconsistent here. Does the T allele associate with high IL-1B levels? Do high IL-1B levels associate with increased susceptibility to sepsis? The authors mention early in the manuscript (Introduction) that excessive IL-1 production is linked to increased risk of sepsis, SIRS, septic shock, etc. but since they are explicitly discussing a result that is contrary to what would be expected, readers should be briefly reminded in this paragraph why exactly this result is interesting. Finally, the last sentence of this paragraph mentions that the pooled results were affected by a single study, but the authors do not say which study (this should be mentioned here). Please revise this paragraph to improve clarity.

Thank you for pointing out, we have revised this paragraph in revision (Page 14)

Thank you again for your review and re-consideration!

Sincerely yours,

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