Dear Editor,

We are very grateful for the careful reading and thoughtful comments provided by the editors and reviewers on the previous manuscript version of our study titled “The association between interleukin-6 gene -174G/C single nucleotide polymorphism and sepsis: An updated meta-analysis with trial sequential analysis”. The comments and suggestions are truly helpful for improving this manuscript. The revised manuscript and relevant materials have been uploaded.

In the revised manuscript, we have carefully addressed all the reviewers’ comments. We attempted to improve the preciseness of the study and made necessary corrections. The manuscript was revised by a fluent English speaker to improve the readability and the format was adjusted according to the editorial formatting comments.
Notably, we have to mention two issues. Firstly, we observed a mistake in our previous version during the process of review. That is wrong data of the number of cases and controls were taken from the study by Schluter B, which is shown in Table 1 with trace of change. We’ve corrected this mistake and comprehensively conducted analysis based on correct data. Secondly, the study by Abdel-Hady were not included in our previous version because the full text was not available. However, we finally acquired the full text through http://paper.pubmed.cn, a website of document sharing. After carefully screening the full text, we decided to include it in our meta-analysis.

We hope that all these efforts as advised by the editors and reviewers might make a different to the manuscript on the basis of the previous version. All revisions were highlighted in color blue and language editing were shown in form of track change. We also provided a point-by-point response to the reviewers’ comments as follows.

Thank you again for your kind attention and we hope that the revisions are acceptable and that our responses adequately address the comments.

Sincerely,

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Responds to the reviewer’s comments:

Reviewer reports:

Zorana Dobrijevic, Ph.D. (Reviewer 1): The study used appropriate methodology and the results were consistent with the objectives of the study. Since this meta-analysis is relatively well performed, it only requires minor corrections, together with English editing.

Comments and the suggestions for authors are the following:

1. The HWE deviations were assessed, but it should be clarified if studies with significant deviations in controls were considered inadequate for the data synthesis (mainly because of possible genotyping errors), or why was this test conducted if they were still included in the meta-analysis. This issue was discussed in the Discussion section, but should be mentioned also in Results, even though the results of omitting studies with HWE deviations were not shown.

Respond:

Thank you for your kindly suggestions. In order to the maximize the sample size, we included studies with HWE deviations in overall analysis. However, the analysis after excluding those studies were also carried out and proved that it does not influence the final result. We mentioned this issue in Results section (Page 7, Line 1-2) and Discussion section (Page 10, Line 58-61) and the result is shown in Table 2.

2. In the Results section, the search results should be more extensively described with the reference to the flow chart. Also, authors could name the studies excluded after the full-text review in order to allow readers to check the reasons for exclusion.

Respond:

Thank you for your kindly suggestions. We have described more information of search results in Results section (Page 6, Line 25-40). The excluded studies after the full-text review were also mentioned in Results section (Page 6, Line 29-31).
3. The authors should consider changing the term "race" with a more suitable one.

Respond:

Thank you for your kindly suggestions. We substituted “the European ancestry” or “the Caucasians” for the term “the Caucasian race”.

4. The terminology in interpreting association the results of association tests should be matched with other case-control studies and meta-analyses (i.e., using "to be associated with", or "to be correlated with", "to increase the risk" instead of "to have a link with…". The same goes for "allele contrast", which could be replaced with "allelic model", or "comparison of allele frequencies", etc).

Respond:

Thank you for your kindly suggestions. We have amended the expression according to your recommendations.

5. It would be more appropriate to present the numerical results of Egger's test instead of graphical representations, since only two plots were shown.

Respond:

Thank you for your kindly suggestions. We have provided the numerical results of Egger’s test in this version, as well as the graphs. The numerical results were shown in Table 2 and graphs were shown in supplementary figures (see additional file 2).
6. Some results from supplementary data were introduced in the Discussion section, which is inappropriate. These results are not necessary for discussion, provided that the exclusion of two studies included in the previous meta-analysis is properly discussed and well-argued.

Respond:

Thank you for your kindly suggestions. We have moved some contents of this part from Discussion section to Results section (Page 7, Line 54-58) and detail was discussed in Page 10, Line 27-34. The result was shown in Table S4 (see additional file 1).

7. Information about study design in terms of recruitment of controls could be useful (HB vs PB design, if any with PB) and adequate for subgroup meta-analysis.

Respond:

Thank you for your kindly suggestions. We have carefully checked the recruitment of controls of each included study. The information of controls were shown in Table 1. We’ve conducted subgroup analysis with specific control types such as healthy controls and Mendelian population. Other factors in terms of recruitment of controls, as far as we concerned, were not adequate for subgroup analysis.

8. Differences between biological aspects of sepsis in neonates, infants and adults could be more widely explained and useful in interpreting the obtained data.

Respond:

Thank you for your kindly suggestions. We have discussed more about this issue in Discussion section (Page 9, Line 23-46).
Sajad Ahmad Dar (Reviewer 2): Technical Comments to the Author:

The authors conducted a meta-analysis to find out the association between interleukin-6 gene -174G/C single nucleotide polymorphism and sepsis risk and mortality. While meta-analysis is a powerful approach to identify susceptibility of genes for disease association, the study conducted by Chen et al. has several limitations affecting the quality and reliability of the study.

1. The clear description about the selection steps and number of the studies is missing. The authors at places are mentioning that 26 studies (19 +7) were analyzed, while at other places 23 studies are mentioned, creating confusion.

Respond:

Thank you. We would like to clarify this issue.

In the previous version which you’ve reviewed, 16 studies merely discussed the risk of sepsis, 4 studies merely discussed the mortality of sepsis. Also, 3 studies discussed both the risk and mortality of sepsis. Thus, totally we got 23 studies (16+4+3). Thus, 19 (16+3) studies were included in the analysis of the risk and 7 studies (4+3) were included in the analysis of the risk.

In this revised version, we added one studies. The searching results changed accordingly. Also, we described this issue more clearly to avoid confusion.

The searching process and results were shown in Results section (Page 6, Line 25-44) and Figure 1.
2. Authors stated that there is no publication bias in this study, but it is apparent that there is no association of IL-6 -174G/C SNP with sepsis in most of the included studies. Thus, it is obvious that the meta-analysis will not get any association between this polymorphic substitution and sepsis, as revealed by previously published meta-analyses!

Respond:

Thank you for your kindly suggestion. We carefully reviewed our data and found a mistake that is we collected the wrong data from the study by Schluter B. We’ve corrected this mistake and re-analyzed the meta-analysis. The results showed that there was no association between IL-6 -174G/C SNP and sepsis, which is consistent with the previous study. Also, we conducted egger's test in every aspect of meta-analysis(Table 2). The statistical result showed no publication bias existed.

3. Without assessing the quality of the included studies (Selection of cases; comparability of cases and controls; and the Exposure of the subjects), the selection method of the studies for analyzing the IL-6 -174G/C SNP association with sepsis is ambiguous. The results of meta-analysis depend on the proper selection of the primary research based on their results. Therefore, the conclusions of this meta-analysis are too premature to appreciate in absence of quality assessment of studies.

Respond:

Thank you for your kindly suggestion. In this revised version, we conducted quality assessment according to Newcastle-Ottawa Scale. The results of quality assessment were shown in Table S1(see additional file 1).
4. Meta-analysis may result in type I errors. Low methodological quality of the studies, publication bias, and small study bias etc may result in unreliable p-values. Therefore, analyses like Trial Sequential Analysis (TSA), that combines an information size calculation (cumulated sample sizes of all included trials) for a meta-analysis with the threshold of statistical significance, needs to be performed. TSA is a tool for quantifying the statistical reliability of data in the cumulative meta-analysis adjusting significance levels for sparse data and repetitive testing on accumulating data.

Respond:

Thank you for this kindly suggestions. TSA is really a useful tool to assess the reliability of the results of meta-analysis. We conducted TSA according to your suggestion. The results and graphs of TSA are shown in Figure 3 and supplementary figures(see additional file 2).

5. English language needs a lot of improvement.

Respond:

Thank you for this kindly suggestion. We have asked help from Prof. Yiqun Chen, an English teacher of Nanjing University of Aeronautics and Astronautics to help us edit the language usage of the manuscript. Trace of correction were shown in the manuscript.