Author’s response to reviews

Title: Genetic variants in Forkhead box O1 associated with predisposition to sepsis in a Chinese Han population

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Author’s response to reviews:

Dear Editors and Reviewers:

Thank you for your letter and for the reviewers’ comments concerning our manuscript entitled “Genetic variants in Forkhead box O1 associated with predisposition to sepsis in a Chinese Han population” (ID: INFD-D-18-02255). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have made correction which we hope meet with approval. Revised portion are marked in red in the paper. The main corrections in the paper and the responds to the reviewer’s comments are as following:

Responds to Rafael Roesler (Reviewer 1):

Q1. BACKGROUND

Compared to the number of studies that focused on the process of inflammatory response, there are fewer studies that focused on genetic predisposition, but there is a representative number.

Answer : Thank you very much for your suggestion. The previous sentences were “Most of the previous studies on the pathogenesis of sepsis focused on the process of the inflammation response. Few studies have focused on the genetic predisposition to sepsis”. We have changed into “Compared to the number of studies that focused on
the process of inflammatory response, there are fewer studies that focused on genetic predisposition, but there is a representative number” and added few representative references.

Q2. ETHICS

The authors state that "the methods used this study were carried out in accordance with the approved guidelines". Which guidelines? It is important to state that the study was performed accordingly to Helsinki declaration.

It is always recomendable to include the committee's reference number.

Answer: Thank you very much for your suggestion. The guideline meant the instruction for authors which may confuse readers, so we have deleted this sentence. We have added the committee’s reference number into our manuscript.

Q3. METHODS

1. Identify time period in which the study was conducted. This is important to evaluate the adequacy of the sepsis definition adopted.

ANSWER: Thank you very much for your remind. This study was indeed conducted before the new definition of sepsis published, so we checked these sepsis patients by new definition, and changed the reference.

2. Provide general characterisrics of the population studied (age, gender, severity...)

ANSWER: We had provided general caracteristics of the population shown in table 1 and table 2.

3. By normal controls, I believe the authors mean healthy controls, which is a more adequate terminology. If so, why not using critical care patients without sepsis as controls, and match more parameters, as, for example, infection? I believe this limitation should be explicited in discussion.

ANSWER : Thank you very much for your valuable suggestion. We have added this limitation into our discussion section, and these sentences were “Second, normal controls used in this study were all healthy controls instead of critical care patients without sepsis, so many parameters like infection were not matched”.

4. In the whole exome sequencing, taking the number of subjects included (n=18, 10 septic patients and 8 controls), it is possible that some SNP were found only in sepsis patients by chance? In validation, only the selected SNPs evaluated in SNP selection were tested. Is it possible that other SNP that were not evaluated were also different between these two groups? I believe this limitation should be better acknowledged in discussion.
Answer: Thanks for your suggestion. We have made correction according to the Reviewer’s comments. The limitation has been added into our discussion section and these sentences are “Third, only a few SNPs were selected from whole exome sequencing for validation and other SNP which were also different between these two groups were not evaluated.”

5. To compare serum levels of FOXO1, a parametric test should be employed.

Answer: Thank you for your reminding. We indeed used a parametric test to compare serum levels of FOXO1, but we didn’t indicate in our manuscript. We have added this sentence into our statistical analysis section.

Q4. CONCLUSIONS

The authors state that "the rs2721068 and rs17446614 SNPs were shown for the first time to correlate to the genetic predisposition to sepsis, and they were also the first SNPs in the FOXO1 gene to be associated with the incidence of sepsis". To associate the presence of a gene with the INCIDENCE of sepsis, a cohort study would be necessary. In this case, it is only possible to state that the gene is associated with sepsis, without imply casualty. Also, the authors state that "the rs2721068 in dominant model and rs17446614 in recessive model were correlated with the genetic predisposition to sepsis" and again, this would only be possible to infer though a cohort study.

Answer: Thank you very much. According to your suggestion, we have changed these sentences into “The rs2721068 and rs17446614 SNPs were shown for the first time to correlate to the genetic predisposition to sepsis, and they were also the first SNPs in the FOXO1 gene to be associated with sepsis.” and “the rs2721068 in dominant model and rs17446614 in recessive model were correlated to sepsis”.

Q5. REFERENCES

The journal adopts Vancouver style. Review the use of bold.

Answer: Thank you. We have changed the reference style as you suggested.

Responds to Evangelos Giamarellos-Bourboulis (Reviewer 2):

Evangelos Giamarellos-Bourboulis (Reviewer 2): This is a study on sepsis genetic predisposition with a novel study design i.e. WES of selected cases followed by SNP analysis in a validation cohort. I have some major concerns for this submission:

Q1. The authors need to explain the criteria of selection of the 10 sepsis patients for WES. Why did they not select extreme phenotypes? How do they explain that PCT levels in survivors and
non-survivors of these depicted cases were similar? This is contradictory to what is reported by others.

Answer: Thank you very much for your suggestion. We were very sorry we didn’t fully understand your question. In order to exclude SNPs related to the severity of infection, the survivors and non-survivors recruited into this study were matched by APACHE II score, SOFA score, PCT and CRP levels. We have added this explanation in the first paragraph of the results section.

Q2. Where p-values adjusted for multiple comparisons? The reported p-values of Tables 5 and 6 are close to 0.05.

Answer: In Table 5, p-value was adjusted using the Benjamini-Hochberg method.

Q3. The authors need to explain the decrease of FOXO1 protein in sepsis. Is this an effect of sepsis-induced immunosuppression? Why levels do not differ between AA/AG and GG genotypes within the sepsis subgroup? This makes the genetic finding not translated to protein significance.

Answer: One of our previous studies showed that FOXO1 was a target gene of miR-223 and miR-15a/16, which are involved in the pathway of lymphocyte apoptosis during sepsis. miR-223 and miR-15a/16 can cause the increase of lymphocyte apoptosis during sepsis. So, as a target gene, foxo1 levels were lower in normal group. The two genotype of foxo1 levels were significant different between sepsis and normal controls, but there were no significant differences between the two genotype in the two groups. Thank you again.

We tried our best to improve the manuscript and made some changes in the manuscript. These changes will not influence the content and framework of the paper. And here we did not list the changes but marked in red in revised paper.

We appreciate for Editors/Reviewers’ warm work earnestly, and hope that the correction will meet with approval.

Once again, thank you very much for your comments and suggestions.