Reviewer's report

Title: Genetic variations in the TIRAP gene are associated with increased risk of sepsis-associated acute lung injury

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Reviewer: Carlos Flores

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Dear Editor,

The manuscript entitled “Genetic variations in the TIRAP gene are associated with increased risk of sepsis-associated acute lung injury” by Song and coworkers describes an association study of selected SNPs in TIRAP gene and acute lung injury (ALI) risk. A strong association was found for two SNPs and haplotypes when comparing ALI patients with either healthy controls or sepsis patients. This is a well conducted study using an appropriate case-control sample size, and with a reasonable hypothesis given the importance of TLR pathways in sepsis and ALI. The following are suggestions to improve the article and to adhere it to STREGA standards for reporting genetic association studies:

Major Compulsory Revisions.

1) Abstract (but also the main text). The study design is not cohort-based but a case-control. The authors should substitute the cohort designation by appropriate terms throughout the text.

2) Methods, study enrollment and design. I guess when authors refer to ALI they are actually referring to ALI + ARDS. If so, please, state that with a sentence in Methods. Additionally, it would be interesting to know how many of the ALI patients developed ARDS and if the authors recorded the number of samples with severe sepsis. If available, please provide percentages in Table 1. This should be stated in Table 1. Additionally, the authors should explore if ORs increase when focusing exclusively on ARDS patients instead of all ALI. This might give an idea of whether SNPs are more related to severity than to susceptibility.

3) Methods, study enrollment and design. It is not clear how healthy controls were recruited. To allow reproducibility, additional information needs to be provided: if sampling was hospital or population-based, if individuals were from the same location or from different parts of the country, etc…

4) Methods, SNP selection and genotyping, third paragraph. Please give more details of the genotyping method used and if error checks were assessed. Were sequences read by both strands? Was genotyping blind to disease status? Were genotyping calls performed simultaneously for the entire study or was it performed sample by sample? Was all genotyped performed in the same lab (state where)? Did the authors duplicate a fraction of the samples to monitor
5) Methods, Statistical analysis. The authors state the use of alleles to calculate the ORs and 95%CIs but report genotype ORs as well. Please, explain how allelic ORs were calculated and which inheritance model was used for genotype tests.

6) Methods, Statistical analysis. The sentence of association adjustments needs re-writing. How were covariates added to the logistic regression model? Please, declare the alpha that needed to consider the association significant after Bonferroni adjustment. Did the authors set the alpha based only on the 5 SNPs or the 5 SNPs x 2 comparisons?

7) Methods, Statistical analysis. Why did authors chose to test haplotype associations with PLINK? Why not continue using Haploview?

8) Results, Associations of the TIRAP gene SNPs with ALI risk. The Hardy-Weinberg equilibrium p-values should be provided in Table 2. This table should be defined as supplementary material as will be useful only for a limited number of readers.

9) Results, Associations of the TIRAP gene SNPs with ALI risk. The authors suggest that associations of rs595209 and rs8177375 might be independent given the r2 value. That might be the case, however, there may be interaction between the two sites as well. This should be explored as well.

10) Discussion, third paragraph, starting from the fourth sentence till the end of the paragraph. This is mostly a discussion about the possible functional roles of the associated SNPs. Given that these were tagSNPs, it is more likely that these are tagging something else more than having an effect on mRNA stability. Since the authors did not use a reference database to impute untyped variants (e.g. a re-sequencing sample) to figure out tagged SNPs associated with ALI, the whole paragraph should be re-focused to discuss other potential alternatives for the association: 1) that the association might be due to other common or rare variants of the TIRAP gene associated with ALI; 2) that the association might be due to LD with variants from nearby genes.

11) Discussion. The manuscript will benefit from a cautious discussion relating the findings of the study with other studies of the field strengthening the relevance of TLR pathways in sepsis and ALI development.

12) Discussion, last paragraph. The authors claim that one of the strengths of the study is that racial admixture was reduced since all samples were collected from Han Chinese. However, no empirical assessment for the presence of population stratification or a correction for it was considered. This is a limitation of the study that needs to be clearly identified and discussed. Another main limitation of the study that needs to be replicated in independent studies.
Minor Essential Revisions.

1) Abstract, third sentence of methods. Introduce “frequencies” after “haplotype”.
2) Abstract, first sentence of results. Please, provide 95%CI and alleles at-risk along with ORs and p-values.
3) Abstract, last sentence of results. Substitute “…and Bonferroni correction” by “…and for multiple comparisons”.
4) Background, first paragraph. Substitute ref 3 by the more recent: Flores et al. Crit Care 2008, 12:R130.
5) Methods, SNP selection and genotyping. The sentence “Six SNPs in the TIRAP gene region on chromosome 11q24.2 were used to identify tag SNPs” is confusing and can be safely removed.
6) Methods, SNP selection and genotyping. The program used for tagging SNP selection should be provided (I guess was Tagger within Haplovie). Additionally, it would be helpful for some readers to have an idea of the size of the tagged area.
7) Methods, Statistical analysis. Please, state that LD was calculated in terms of r2 values.
8) Results, Associations of the TIRAP gene SNPs with ALI risk. Please, check that MAFs are correct, as those from rs3802813 and rs8177375 seem to be switched with respect to HapMap.
9) Results, Associations of the TIRAP gene haplotypes with ALI risk. The haplotype analysis is not adding much more information to the manuscript than the analysis of SNPs alone (either in terms of ORs or in terms of p-values risk haplotype is essentially driven by rs595209). Given this, and the fact that haplotype blocks are strongly dependent on the SNPs being tested in the region, this part of the analysis should be removed.
10) Discussion, first paragraph. The authors state that no differences were found between healthy controls and sepsis patients but no result is shown. Although not significant, these results may be considered as supplementary results as they might be important for future studies (e.g. meta-analyses).
11) Discussion, second paragraph. The authors found no association for rs7932766 and rs8177374. The authors declared that the study had limited power given their MAF in East Asians. However, they should provide power calculations to support the statement.

Discretionary Revisions.

1) Abstract, second sentence of background. Please, introduce “gene” somewhere in the sentence.
2) Abstract, first sentence of methods. I suggest starting it with “DNA samples from 298 healthy subjects,…” The authors need to make clear here that the samples are from Han Chinese.
3) Abstract, second sentence of methods. What do the authors mean with
“spanning the entire TIRAP gene”? To make it simpler I suggest re-writing it as "Three tagging single nucleotide polymorphisms (SNPs) of TIRAP gene and two additional SNPs that have previously showed association with susceptibility to other inflammatory diseases...”

4) Methods, SNP selection and genotyping. The second paragraph can be safely removed from the manuscript since it is not adding any information. Relevant data from the supplementary table 1 can be added to table 3 so that this supplementary table can be removed from the manuscript.

5) Methods, SNP selection and genotyping, third paragraph. The authors refer to the supplementary table 2 for PCR methods. I suggest including this information (primer sequences and PCR conditions) as part of the main text in methods and not as a supplement. This will allow the authors to better explain their methods since, as it is now, the reader has to “guess” that the two first SNPs from the table were genotyped together within an assay. The next three were genotyped together as well but with a different assay.

Additionally, other minor issues are:
1) Substitute “variations” by “variants” throughout the text and title.
2) Please, make sure that TIRAP is in italics when referring to the gene.
3) Abstract, last sentence of methods. “Logistic” should be written all in lowercase.
4) Methods, SNP selection and genotyping, first paragraph, last sentence needs re-writing. A suggestion would be: “Additionally, two coding SNPs (rs8177374 predicting Ser180Leu, and rs7932766 predicting Ala186Ala), were also genotyped in this study as they have showed evidence of association with other inflammatory diseases [15-19]. These were not genotyped as part of the HapMap project”.

5) Methods, Statistical analysis, second sentence. Substitute “departure” by “departures”.

6) Methods, Statistical analysis, third sentence. Substitute “appropriated” by “appropriate”.

7) Results, Characteristics of the study population, third sentence. Substitute “were” by “are”.

8) Results, Associations of the TIRAP gene SNPs with ALI risk, second paragraph, second sentence. Substitute “The minor allele of…” by “The alleles...”.

9) Discussion, third paragraph, third sentence. Substitute “This is in accordance with the recent founding...” by “This is in agreement with recent findings...”.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being
published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests