Reviewer's report

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

Version: 2 Date: 12 November 2013

Reviewer: marquitta white

Reviewer's report:

Minor Issues not for Publication
Abstract, Methods
1. Line 1: change “literatures” to “literature”,
Line 1: change “were searched” to “was retrieved”

Abstract, Results
1. Line 4: “no associations” should be “no significant associations”
2. Line 5: “and” should be “or”

Abstract, Conclusions
1. Line 3: “large” should be “larger”
2. Line 4: “samples” should be “sample sizes”
3. Line 4: “and homogenous population” should be “from homogenous populations”
4. Line 4: Change “should be necessary” to “ are needed”

Introduction, Paragraph 1
1. Line 2: Change “are” to “have been”
2. Line 4: Change “mortality” to “mortality rates”
3. Line 5: Change “response,,” to “response, and”
4. Line 7: Delete “studies have” replace with “research”
5. Line 11: Add a comma after “genes”
6. Line 13: Delete “will” replace with “may”
7. Line 13: Delete “the patients with sepsis” replace with “sepsis patients”

Introduction, Paragraph 2
1. Line 16: Delete “of the” replace with “in”
2. Line 17: Delete “of” replace with “in”

Introduction, Paragraph 3
1. Line 2: Add “the” after “However,”
2. Line 2: Add “reported from these studies” after “results”
3. Line 2: Add “are” before “inconsistent”

Materials and Methods, Paragraph 1
1. Line 4: Delete “the”
2. Line 8: Add “and” after “controls),”
3. Line 9: Delete “For” replace with “In cases of”
4. Line 9: Delete “one” replace with “study”

Materials and Methods, Paragraph 2
1. Line 4: “frequency” should be “frequencies”

Materials and Methods, Paragraph 4
1. Line 5: Delete “the”
2. Line 9: “Multivariable” should be “Multivariate”
3. Line 16: Add “individual” after “excluding”
4. Line 16: Delete “study” replace with “studies”

Results, Paragraph 2
1. Line 2: Delete “studies”
“Caucasians” should be “Caucasian”
“Asians” should be “Asian”
2. Line 3: Add a comma after “African-American”
Insert “populations” before “, respectively”
3. Line 5: “study” should be “studies”
4. Line 7: Delete “Different” replace with “Several different”
5. Line 9: The last sentence is awkward. Consider rewording.

Results, Paragraph 3
1. Line 2: Add “the” before IL-1A-889
Add “, rs1800587,” after “polymorphism”
2. Line 3: Add “a” before “significant”
3. Line 4: Delete “was” replace with “were”
“statistical” should be “statistically”
“significance” should be “significant”
Insert “associations observed” before “between”
4. Line 5: Change “and” to “or”

Results, Paragraph 4
1. Line 3: “Removing” should be “Removal of”
2. Line 4: “altered from the pooled” should be “alter these”
Delete “there was also no association in each”
3. Line 5: Insert “analyses” before “based”
Insert “did not detect any significant association between rs16994 and sepsis risk” before “under any genetic model”

Results, Paragraph 5
1. Line 1: Insert “the” after “For”

Results, Paragraph 6
1. Line 1: Add “were identified” after “28]”
2. Line 2: Delete “with”
3. Line 4: Delete “only for” replace with “in the”
4. Line 6: Delete “remained to be” replace with “persisted in the”
5. Replace “group” with “subgroup”

Results, Paragraph 7
1. Line 1: Insert “the” after “For”
2. Line 2: Insert “of” after “compromised”
   “result” should be “results”
3. Line 7: Insert “a” after “ethnicity,”
4. Line 12: “and “ should be “or”
5. Line 13: Delete “We also performed stratified analysis according to the quality score of studies”
6. Line 14: Delete “of”
7. Line 16: Perhaps change “Moreover” to “Unsurprisingly”

Results, Paragraph 8
1. Line 3: Insert “and” after “models,”
Delete “genetic”

Results, Paragraph 9
1. Line 1: Delete “in meta-analysis assessing” replace with “in our assessment of the”
2. Line 4: Insert “and” after “controls,”
Delete “showed” replace with “did not indicate”
3. Line 5: Insert “any of” before “these factors”
Delete “all probably not the”
4. Line 7: “attribute” should be “be attributable”
   Insert “that” before “regrettably”
Delete “, due to insufficient data, it was difficult to identify their sources only using meta-regression” replace with “remain undefined due to insufficient data”
5. Line 8: Insert “the” after “For”
Results, Paragraph 10
1. Line 1: Perhaps change “deleted” to “removed”?
   Delete “single”
   Change “check” to “evaluate”
2. Line 2: “to” should be “on”

Discretionary Revisions
1. Abstract (Background Subsection)
   Consider rewording the first sentence. Suggestion: “Previous epidemiological studies have presented conflicting evidence regarding associations between interleukin-1 (IL-1) polymorphisms and sepsis susceptibility. We have performed a meta-analysis to evaluate possible associations between IL-1 polymorphisms and sepsis risk.

2. Abstract (Results Subsection)
   I would suggest removing the sentence “For IL-1RN VNTR polymorphism, although only borderline statistical significance was observed in overall comparison and Asians”. Again, when a significance threshold is set at a certain level, as the authors have done in this manuscript (p<0.05) results with p-values greater than this are simply not significant. And in this particular instance, the Asian subgroup only contained 2 studies, 1 of which was of low quality (quality score < 7), making the results from this subgroup analysis highly questionable. I do not think this result should be emphasized in the abstract as it detracts from the presentation of results that are actually significant for the VNTR polymorphism, such as all the models in the sepsis subtype defined subset analysis for example.

3. Results, Paragraph 4
   Line 1: The first sentence is a little awkward; perhaps consider rewriting it to improve sentence flow. A possible suggestion: “Seven studies [13-16, 18, and 33] totaling 745 cases and 750 controls, were identified in order to investigate the association between sepsis risk and rs16944”

4. Results, Paragraph 5
   Line 2: The sentence containing “the overall, removing one low score study ([17]) with score=6) and subgroup result based on ethnicity and sepsis severity suggested no significant association with sepsis risk” would be greatly improved by restructuring. One suggestion would be something along the lines of: “there were no significant associations detected between rs1143627 and sepsis risk overall or in any subgroup analysis for any genetic model even after removal of one study with a low data quality score [17].”

Minor Essential Reviews
1. Introduction, Paragraph 2
The sentence describing the mouse model was unclear to me as it did not say whether the mice showed increased or decreased tolerance to bacterial infection. Upon review of the cited reference, I discovered that while Riedemann et al mention several animal models the only ones given for IL-1 were for IL-RA and IL-6, but I did not see a mouse model of IL-1 specifically (there was mention of an HGMB1 model (HGMB1 causes downstream up regulation of IL-1#)). Could this citation be misplaced possibly? This sentence should be revised for clarity, removed, or updated to include citation to an appropriate reference.

2. Table 1
Though the authors have added a column to the table to describe whether individual studies deviate from Hardy-Weinberg Equilibrium, they have not defined HWE in the figure legend. Also, the authors should present the actual HWE p-values that were calculated and not simply “yes” or “no” in this column. A description of the HWE p-value significance criteria is also missing from the Methods section and should be added so that readers will know what the authors classified as a significant deviation from equilibrium (was it p<0.05? p<0.001?). Please address this.

3. Table 2
A note should be added to the table legend that statistically significant results are highlighted in bold (if this is in fact what the authors are doing here). If instead the bold font merely highlights results which the author’s believe to be noteworthy then that needs to be stated in the table legend.
If the bolded results are supposed to indicate statistical significance then bold font should be removed from: The TOTAL row for IL-1RN VNTR (allelic model), the Asian subgroup allelic model for IL-1RN VNTR, and the >7 Quality score (allelic model) for IL-1rn VNTR

4. Figure 2
Perhaps add a superscript 1 to “Events” and then a definition in the figure legend: Events1: Number of individuals with TT genotype
Perhaps change “Favours [Experimental]” to “TT decreases risk” and “Favours [control] to “TT increases risk”
Editing the forest plots from the output that is generated directly from STATA to customize hem to your particular dataset makes them more easily interpretable for readers.

5. Figures 4, 5, 6
It might be nice if the size of the circles representing the individual studies were proportional to the weight of that study (in terms of contribution to the pooled OR). This is just a suggestion, but it might be nice for readers to be able to visualize this when assessing the figures.

6. Statistical analysis section, Paragraph 1
Please add the p-value cut-off that was considered statistically significant in the evaluation of HWE to this section.
7. Statistical analysis section, Paragraph 1
The word “Multivariable” should be “Multivariate”

8. Results Section, Paragraph 2
The word “were” should be “are” in the first sentence.
The word “Expect” should be “Except” in the third sentence.
A citation for Table 2 needs to be added after the description of HWE.

9. Results, IL-1b+3594 Section
Please add the HWE pvalue for the Fang et al study, it is stated that this study deviated from HWE but we don’t know the significance of this.

10. Results Section, Sensitivity analysis subsection
The word “considered” should be “identified”

11. Results, Publication Bias Subsection
The word “testing” should be inserted after “bias”

12. Discussion, Paragraph 1
The word “and” in the last sentence should be “or”

13. Discussion, Paragraph 8
Please consider rewriting this paragraph. The first sentence is not clear. The authors do not state under what conditions Salanti implies spurious results might be magnified just that they might be magnified somehow, making the first sentence a bit confusing. And the second sentence does not seem to be connected to the first.

14. Discussion, Paragraph 9
I believe the word “were” should be “was” and the word “point” should be “pointed”

Major Compulsory Revisions
I would first like to commend the authors for the additional information that they have added to the text, tables, and figures in response to initial reviews as this has greatly improved the quality and interpretability of this manuscript.

1. Figure Legends
The addition of forest (Figures 2-3) and funnel (Figures 4-6) plots by the authors greatly improve the interpretability of the results that they present in the text, and the figures themselves are of adequate quality. Although forest and funnel plots are fairly standard for meta-analysis studies, not all readers will be familiar with these types of graphic, therefore 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. An example of pertinent information that should be included in the funnel plot figure legends would look something like this: Each circle represents an independent study; for
each study the OR was plotted against the standard error of the log of the odds ratio. Center dotted line represents the pooled OR and sloping dotted lines represent the 95% confidence interval of the pooled OR. OR, odds ratio; SE, standard error. An example of necessary information that should be included in the forest plot figure legends would be: Squares represent individual study odds ratios, with 95% confidence intervals represented by corresponding horizontal lines. Area of squares is proportional to the weight of the individual study to the overall pooled odds ratio. Diamond at the bottom of the graph represents the pooled odds ratio and 95% confidence interval. It is important to note that because there was no information in the included figure legends I am assuming that the above statements concerning what the symbols in the figures indicate are accurate based on previous experience with these types of graphics. 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.

2. Results Section (IL-1RN VNTR) Issue: inclusion of Bessler in Allelic Model for IL-1RN VNTR

As previously requested, the authors include an assessment of HWE in controls for all analyzed studies. As mentioned before, departure from HWE in control samples introduces statistical bias at the individual study level as well as at the meta-analysis level. Allelic models are particularly vulnerable to the inclusion of studies with deviations from HWE in controls (select references: Trikalinos 2006, American Journal of Epidemiology; Sasieni 1997, Biometrics). Trikalinos notes that while inclusion of studies deviating from HWE seldom changes the effect size of pooled ORs in meta-analyses, it has been shown to impact the significance of results. This fact was evidenced in the association tests performed for IL-1RN VNTR in the allelic model – the inclusion of the Bessler study resulted in a non-significant result (p=0.05), while its removal resulted in a significant association (p=0.04) between VNTR and sepsis risk under the allelic model in the overall analysis. Although the authors mention this in text, they present the results for the association including this study in Table 2, Figure 3, and throughout the text of the manuscript. Knowingly including a study that shows deviation from HWE in controls is questionable at best, but including it in allelic models, which have demonstrated sensitivity to the bias created by their inclusion, is inappropriate. Studies deviating from HWE in controls should not be included in the allelic models for any tested association. 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.

3. Figure 3

The analysis presented in the forest plot should not include Bessler 2004 for the
reasons outlined in the previous comment. Please remove this study and redo the analysis and forest plot or provide justification for presenting the figure as is.

4. Results Section (IL-1RN VNTR) Issue: Presentation of results for allelic model of Asian subset

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.

5. Discussion, Paragraph 2

The authors state that the IL1b+3594 polymorphism has been reported to influence the production of IL-1 beta protein and site Pociot et al, as a reference. Upon further review, Pociot et al clearly state the 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.

6. Discussion, Paragraph 3

Please revise this paragraph in response to the issues mentioned to Point 4 above (presentation of Asian subset results for VNTR), or provide justification for the current presentation of results discussed here.
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: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I declare that I have no competing interests.