Reviewer's report

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

**Version:** 1  **Date:** 1 October 2013

**Reviewer:** marquitta white

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Minor issues not for Publication

1. Introduction, 1rst Paragraph

   Line 2: “unacceptable” should be “unacceptably”

   Line 10: The sentence beginning with “Recently, there is…” needs to be rephrased. One suggestion is as follows “Recently, there has been increasing evidence supporting the involvement of genetic factors, particularly single nucleotide polymorphisms (SNPs), inter-individual variation in sepsis susceptibility and severity [2].”

2. Introduction, 2nd Paragraph

   Line 6: Delete “Genes containing”

3. Introduction, 3rd Paragraph

   Line 4: “attributable” should be “attributed”

4. Methods, (Statistical Analysis Subsection, Line 2)

   Perhaps change “dominant (BB+AB vs. AA)” to “dominant (AA vs. BB+AB)” to match the format of how you described the recessive model.

5. Methods, (Statistical Analysis Subsection, Line 12)

   “excluded” should be “excluding”

6. Results, Line 4

   “supplied” should be “supply”

7. References

   There appears to be a typo in reference 30 (Wells 2011) “wwwohrica” should be www.ohri.ca

**Discretionary Revisions**

1. Abstract (Methods subsection)

   Instead of mentioning the software that was used here (since this information is presented in the methods section of the manuscript), perhaps highlight the fact that analysis was performed in the total dataset and in ethnicity and sepsis severity defined subgroups?

2. Abstract (Results subsection)
Remove “and in non-Asian populations, 2 allele (OR=1.30, 95% CI=0.98-1.73, P=0.07) also conveyed an increased sepsis risk as well as 2/2 and L/2 genotype (OR=1.34, 95% CI=0.97-1.85, P=0.08).” The author’s have set a statistical significance cut-off of p < 0.05. As both of these results have p > 0.05 their associated odd’s ratios are not significantly different than 0, indicating that there is no statistically significant association with sepsis risk in non-Asian populations for this polymorphism.

3. Abstract (Results subsection)
Presentation of the results in this section seems a bit awkward, perhaps this section can be reworded to convey the pertinent results more succinctly?

4. Materials and Methods (Qualitative assessment subsection)
Perhaps include a supplementary table showing the tabulation of the quality scores for all included studies.

Minor Essential Reviews
1. Figure 1

2. Figure 1
The last box in Figure 1 is misleading. There were not 31 studies included in the meta-analysis, there appear to be 20 (18 were from full text articles, and the Johnson 2012 study was divided into two separate studies by population (Caucasian/African-American). This box needs to be corrected either to indicate that the “31” here does not describe unique studies included in the meta-analysis, and that individual studies genotyped multiple polymorphisms so that certain studies will appear in more than one group when they are divided by polymorphism.

3. Figures 2,3,4
These are not forest plots as the figure legends indicate but are instead funnel plots representing publication bias. The figure legends are incorrect and need to be edited so that they describe the actual figures being presented.

4. Figures 2,3,4
The funnel plots depicted in these figures are all missing the accompanying dotted lines on either side of the middle dotted line to create the “funnel”, please add these to the figure.

5. Table 1
Perhaps add “A=Major Allele, B=Minor Allele” to the table legend.
6. Table 1
The superscript b (b) needs to be defined in the figure legend. It appears that b= (AB+BB).

7. Table 2
If the bold font used in the table was meant to emphasize statistically significant results then the only results that should be in bold print are those with significant odds ratios as defined by the authors in the methods sections (P < 0.05). For example, the dominant and allelic models for IL1RN VNTR L/2 under the non-asian subgroup are bolded, but these results are not significant with pvalues of 0.08 and 0.07, respectively.

8. Table 2
In the Total row of IL1B-31C/T, under the recessive model, na = 6, when it should =5 (as the Asian study could not be evaluated under this model). This may be a typo, but still needs to be addressed

9. Introduction, Paragraph 3
The word “deferences” should be “differences”

10. Statistical analysis section, Paragraph 1
The word “exited” should be “existed”

Major Compulsory Revisions
1. The authors perform both total (including all studies) and subgroup (separated by ethnicity and sepsis severity) analyses. The ethnicity subgrouping is defined as “Asian” and “Non-Asian”. Upon review of all included studies, I found that of the 20 unique individual studies (counted the Johnson study as two separate studies due to ethnicity differences in the sample), the ethnicity of the participants broke down as follows: Asian: n=6, Caucasian: n=10, African American = n=1, Hispanic: n=2, Jewish/Arabic: n=1. The ethnicity with the largest number of samples in Caucasian, followed by Asian. It would seem like the ethnicity subgrouping definition that would reduce the most heterogeneity caused by differences in ethnicity would be to separate the studies into three categories: “Asian”(Gu 2010, Wan 2012, Shimada 2011, Watanabe 2005, Zhang 2005, Ma 2002), “Caucasian”(Johnson-1, Emonts 2010, Shalhub 2009, Barber 2004, Balding 2003, Treszl 2003, Fang 1999, Sole-Violan 2010, Garcia-Segarra 2007, Amalich 2002), and “Other” (Davis 2010, Johnson-2, Zapata-Tarres 2013, Bessler 2004). By separating the studies into Asian and Non-Asian, this lumps together Caucasian, African-American, Jewish/Arabic, and Hispanic populations; populations that have been known to show differences in allele frequencies as well as immune response. Assumedly, the authors separated the studies this way to try and increase power in subgroup analysis by keeping the non-asian group large (n=14), but it appears that increasing sample size in this case would only lead to an increase in power if the samples were ethnically homogenous. The way that the authors defined their ethnicity subgroups needs to be addressed. Additional analysis according to the subgrouping described above
should be performed in order to determine if new associations are revealed and to ascertain whether this subgrouping scheme leads to a decrease in between study heterogeneity estimates. If interesting results/decreased heterogeneity are shown to be the result, then this could be presented in supplement perhaps?

2. Materials and Methods Section, Data Extraction and Methodological Approach subsection

Here the authors state that “For studies including subjects of different populations, data were extracted separately”, but numerous places elsewhere in the manuscript they mention that there are 19 studies in their meta-analysis. This is misleading. Since the Johnson study contained both Caucasian and Black subjects, they were extracted separately (Johnson-1, Johnson-2) in accordance with the author’s listed methods and were treated as two independent studies, effectively bringing the number of studies included in the meta-analysis to 20 and not 19, which may be a bit confusing to readers. An extension to the last sentence that states that after the data were extracted separately, they were treated as independent studies (as in the case of Johnson-1 and Johnson-2) would make things more clear. Also this should be addressed elsewhere in the manuscript where the author’s mention “19” studies.

3. Results Section

The author’s do not show any information about Hardy-Weinberg equilibrium tests being performed in the control samples of each of the individual studies. Where possible, these tests should be performed as deviation from equilibrium could indicate a source of bias in there analyses. Please perform these calculations and present them (perhaps as a part of table 1 after the last column).

4. Materials and Methods section, Qualitative Assessment subsection

The authors state that a score of 7 or greater indicates high quality, but then they state in the results section (paragraph 1) that quality scores ranging from 5 to 9 indicate high quality. These two statements are not in agreement. If 7 stars indicate high quality, than do scores below 7 indicate low quality, and if so then why were studies with quality scores below 7 included in analysis? The authors do not state explicitly what their quality score “cut-off” value is, if this value is 5, which is below the value of 7 which indicates good quality, what is the justification for this?

5. Material and Methods section, Statistical Analysis

The author’s cite Higgins 2003, when describing the tests that they performed to assess heterogeneity. However, the author’s mention using a Q test (assumedly the standard Cochran Q test), which is not the method developed by Higgins et al in the paper they cite. Higgins et al, present the calculation of inconsistency (I2) as a superior method to calculate the amount of heterogeneity as it measures the amount of variation across studies that is truly due to heterogeneity rather than chance. This would be a superior measure of heterogeneity in this case as the standard Cochran Q test is known to perform poorly in meta-analyses where the total number of studies is low. This fact is somewhat minimized by using a cut-off of p<0.10 (which seems to be the standard practice, which the authors have employed). But since the authors reference Higgins here a statement about I2
should be included, also the calculated I2 values and associated p-values should be presented with the Q values where the authors discuss heterogeneity. If this reference was included in error and the authors did not in fact use the Higgins et al method then this needs to be corrected in this paragraph as well as in the reference section.

6. In Table 1, in the IL-1B-511A/G section – Watanabe 2005 study

The authors present that for the Watanabe 2005 study there are 39 (19 case, 20 controls) individuals with at least one copy of the B allele, and 74 individuals (49 case, 25 controls) individuals who are homozygous for the major allele. Upon review of the Watanabe et al study, the authors (Watanabe et al) present in Table 3 (line 3) and in Table 4 (column 4, lines 1 and 2) data that states that there are 39 individuals who are homozygous for the major allele (*2-) and 74 individuals who are heterozygous or homozygous for the minor allele (*2+). Watanabe et al define the less frequent (minor allele) as the *2 allele on page 1183 of their published paper. This information indicates that the authors of this manuscript (Zhang et al) have flipped the genotype counts in Table 1 for the Watanabe study, and the correct genotype distribution should be: Cases: AA=19, AB+BB=49, and Controls: AA=20, AB+BB=25 (B allele=*2 allele, so *2- would be individuals without the B allele so AA individuals, *2+ would be individuals with the B allele so AB and BB individuals).

Because of the difference in allele coding between Watanabe 2005 and this manuscript, interpretation between the two articles is challenging, and it is possible that I have misinterpreted something when moving between the two papers. The authors of this manuscript should check the Watanabe 2005 paper and their analyses and confirm that the genotype distributions are correct. If it is determined that there is a discrepancy, then the source of the error must be determined. There is the possibility that the error is merely a typo when formulating the table and the analyses was in fact correct. There is also the possibility that the analysis was performed using the wrong genotype counts, in which case reanalysis would be necessary.

7. Table 1, IL-1B-31C/T – Shalhub 2005

The authors list 147 sepsis cases and 451 (SIRS and trauma) controls. Upon inspection of the Shalhub 2005 dataset, Shalhub et al state that their primary case definition was complicated sepsis defined as septic shock and/or sepsis with organ failure. The authors of this manuscript classify the cases in this study as “sepsis” when it appears that this should perhaps be classified as “SS and SSH”.

Of further note, and perhaps more importantly, Shalhub et al state that their study contained 147 cases (complicated sepsis) and 451 controls. However, included in their control population were 248 individuals with uncomplicated sepsis (Watanabe et al 2005 Figure 2). As the current manuscript is exploring the impact of IL-1 gene polymorphisms with sepsis risk, it does not seem appropriate to include a study that contains individuals with sepsis (any form) in both the case and control populations; because this study would not be evaluating the effect of polymorphism genotype on sepsis risk, but the effect of that genotype on
sepsis severity only. A few suggestions for how this study could perhaps be reanalyzed to evaluate sepsis risk would be:

a. 278 sepsis cases vs 320 controls with no sepsis
b. 43 septic shock cases vs 320 controls with no sepsis
c. 104 severe sepsis cases (sepsis complicated by organ failure) vs 320 controls with no sepsis

Presently, the inclusion of this study seems inappropriate for the assessment of sepsis risk. The authors should provide justification for inclusion of the study the way that it was analyzed or reanalyze the study to address sepsis risk and recalculate any associated pooled odds ratios. The results section should be revised after reanalysis.

8. Table 1, IL-1RN VNTR L/2, Sole-Violan 2010.

The authors list 324 cases of SS and SH and 828 controls with CAP for the Sole-Violan 2010 study. These samples appear to come from the 1152 CAP cases mentioned in the Sole-Violan 2005 study. Upon further investigation, Sole-Violan et al states that all 1152 CAP cases had some form of sepsis (Sole-Violan 2005, Table 1). This would appear to mean that the 828 “controls” listed in the current manuscript all have sepsis (non-severe). So the odds ratio obtained from comparing cases to controls in this case would actually be addressing susceptibility to severe sepsis and SSH vs sepsis. The authors state that they are interested in sepsis risk which would mean comparing some form of sepsis (cases) vs. individuals who do not have sepsis (controls).

Although it would be interesting to investigate susceptibility to sepsis on a CAP background, in order to do so appropriately there would need to be individuals with CAP who contracted some form of sepsis (Cases) and individuals with CAP who did not get sepsis (Controls). In the case of Sole-Violan there are no individuals with CAP who did not also develop sepsis of some form.

Presently, the inclusion of this study seems inappropriate for the assessment of sepsis risk since all individuals in this cohort in fact have some type of sepsis. The authors should remove this study and recalculate the pooled odds ratios for any polymorphism analysis that it was included in or provide justification for its inclusion addressing the concerns above.

9. Note about Sole-Violan and Watanabe datasets

As noted above, the Sole-Violan and Watabnabe datasets do not appropriately assess sepsis risk as presented in this manuscript. However they do appropriately assess sepsis severity (individuals who develop SS and/or SSH vs. those who develop less severe sepsis). If the authors wanted to pursue this they could perhaps include in their main aims that they wish to assess sepsis risk and severity and include these two studies in a separate analysis of sepsis severity? This is just a suggestion for an additional exploratory analysis if the authors are in fact interested in evaluating sepsis severity as well as sepsis risk, but if sepsis risk is to remain the main focus of this manuscript then this suggestion need not be addressed.
10. Results Section, Heterogeneity analysis subsection, Paragraph 2

I applaud the authors for further examining heterogeneity in this study through the use of meta-regression. I would however, like to have been given more information on how the meta-regression was conducted. For instance, was a random or fixed effects model used for the meta-regression? Was each covariate analyzed independently or were they all included in one regression model? Also, source of control subjects is a possible cause of heterogeneity in meta-analyses. Since the authors have access to this information, source of controls should be included as a covariate/possible heterogeneity source in the meta-regression analysis. Also there is no mention of the meta-regression that was performed in the materials and methods section of the manuscript and perhaps there should be?

11. Figures

The authors do not present any forest plots to visualize the individual odds ratios (or pooled odds ratios) that they present in table 2. Forest plots allow readers to see the directionality and effect size of the individual studies which is helpful in the intuitive interpretation of the pooled odds ratios. These plots should be created and included in the manuscript. As the plots are mentioned in the incorrect Figure legends (for figures 2, 3, 4) it seems like the authors have indeed created these plots but may have accidentally omitted them from this submission. Please address this.

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