Author’s response to reviews

Title: Association study of two inflammation related polymorphisms with susceptibility to hepatocellular carcinoma: a meta-analysis

Authors:

Jiajing Liu (11211020006@fudan.edu.cn)
Bo Xie (bbyn0208@163.com)
Shuilian Chen (catherinezp06@gmail.com)
Feng Jiang (jf20000@sina.com)
Wei Meng (wmeng@shmu.edu.cn)

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Author’s response to reviews:

Dear Editors and Reviewers:

Thank you for your favorite consideration and the reviewers’ insightful comments concerning our manuscript entitled “Association study of two inflammation related polymorphisms with susceptibility to hepatocellular carcinoma: a meta-analysis” (MS: 3656396948498446). Those comments are highly valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied these comments carefully and have made corrections which we hope meet with approval. The main corrections in the paper and the responds to the reviewer’s comments are as following:

Part A (Reviewer 1#Dr. Wenlei Zhuo).

1# The reviewer’s comment: Why didn’t the authors use two recently published meta-analyses and have a discussion?(articles list as follows)


The authors’ Answer:

It is really true as the reviewer suggested that we should consider all meta-analyses that have been focused on this same issue and have a discussion. But when we search the internet for all eligible studies, we set the publication date up to February, 2013. Also, we submitted our paper at 19 March, 2013. So it is beyond our capability to have found these two studies that have been published later than our submission date.
Considering the Reviewer’s suggestion, we have searched the internet and read these two meta-analyses very carefully. Then we make our discussion part more perfect by adding a comparison between our findings and the results of these two articles.(we underlined the changes in revised paper)

2# The reviewer’s comment: Can the authors combine Chinese with Korean as Asians for analysis? Does significant genetic difference exist between Chinese and Korean?

The authors’ Answer:
This is a very useful advise for us. It obviously that the reviewer must have carefully read our article in order to put forward such an insightful comment. Firstly, by carefully search the Hap-Map, we find the G allele frequency of MiRNA-146a is 0.535 for Chinese population and 0.354 for Korean population. We worries that the significant genetic difference existed between Chinese and Korean may damage the credibility of the analysis that combining Chinese and Korean as Asians. Secondly, by dividing the population into Chinese and others, sub-group study can reduce the heterogeneity brought by different populations. For example, under the CC versus TT model, the overall group shows great heterogeneity (I²=63.8%, P value=0.040), while subgroup largely reduced the heterogeneity in Chinese (I²=73.4%, P value=0.053) and Other(I²=29.0%, P value=0.235). The significant difference in this comparison support the point that population is a major source of heterogeneity.

Considering the Reviewer’s suggestion, we have tried to combine Chinese and Korean as Asian for analysis, but the association came back negative and huge heterogeneity can be detected in most of the models. This may due to fact that the sample size of other population is pretty limited. So we advised to interpret the result with caution. Well-designed studies with more ethnic groups are required to further validate the results. Thus, we thinks this subgroup analysis is more suitable for our study, and hope our answers meet your approval. If the reviewer still thinks corrections needs to be made, we would be willing to do it.

3# The reviewer’s comment: One study by should be deleted from the overall analysis; otherwise, the overall data might be incredible.

The authors' Answer:
In our sensitive analysis for rs3746444, we found one studies (Zhao et al. list as follows) changed the between-study heterogeneities materially in every model comparisons.

We highly agree with the reviewer that this study contributes to the significant between-study heterogeneity and should be delete in our overall analysis. But meta-analysis required the criteria for study selection been set before the computer-based search to avoid the author tampering the result. This study do meet the criteria. Also, due to the limit studies existing, it didn’t feel right to arbitrarily deleted any information that might be useful. However, we also share the reviewer’s concern related to the credibility of the result. That is why we excluded this study in sensitive analysis in order to asses its affect (Table 7).
After excluded this study mentioned, the heterogeneity vanished, while the association remain insignificant.


Genetic model Test of association Test of heterogeneity Publication bias
(P value)

<table>
<thead>
<tr>
<th></th>
<th>OR 95% CI</th>
<th>P value</th>
<th>Model I2 P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C v T</td>
<td>0.968 0.804-1.165</td>
<td>0.733 F</td>
<td>41.8% 0.179 0.414</td>
</tr>
<tr>
<td>CT v TT</td>
<td>0.907 0.699-1.176</td>
<td>0.462 F</td>
<td>2.9% 0.357 0.868</td>
</tr>
<tr>
<td>CC v TT</td>
<td>0.998 0.647-1.541</td>
<td>0.994 F</td>
<td>0.0% 0.479 0.266</td>
</tr>
<tr>
<td>CC/CT v TT</td>
<td>0.916 0.716-1.174</td>
<td>0.489 F</td>
<td>24.8% 0.264 0.836</td>
</tr>
<tr>
<td>CC v CT/TT</td>
<td>1.070 0.756-1.514</td>
<td>0.704 F</td>
<td>0.0% 0.107 0.216</td>
</tr>
</tbody>
</table>

Table 7 – Sensitive-analysis of the miR-499 rs3746444 polymorphisms and HCC risk

4# The reviewer’s comment: In the discussion section, lengthy analysis about other diseases such as SLE is unnecessary.

The authors’ Answer:
It is really true as Reviewer suggested that lengthy analysis about other diseases such as SLE is unnecessary. We are very sorry for our negligence of this mistake. Because most of the authors are majored in studying autoimmune disease such as SLE. We kind of hope our study of those two inflammatory cytokines stimulation miRNAs can also enlighten our further study. We have made correction according to the reviewer’s comments and only left a small part as a proposal to follow-up on the paper. (we underlined the changes in revised paper)

5# The reviewer’s comment: in table 4, the results between the subgroups about genotyping methods are different from each other. Can the authors have a discussion on this subject?

The authors’ Answer:
Thank you very much for your insightful comment on this issue. We are very sorry for our negligence of such important information. Considering the Reviewer’s suggestion, we have added some discussion concerning this issue.

Massarry is a relatively new genotyping method compare to PCR-RFLP. It has many advantages such as complete multiple sites in large sample size. The traditional method PCR-RFLP is a less money consuming golden standard for genotyping, but cannot afford large sample size. Massarry has a relatively high false positive rate, which could reflect on this significant association. The studies of massarry tend to have a larger samples size, which may increase the detection power to find a possible small effect of the gene polymorphism on
We take the reviewer’s advice completely and made some changes in our revised papers. In this way, our discussion section got strengthened. (we underlined the changes in revised paper)

Part B (Reviewer2: Dr. Hua Zhao).

1# The reviewer’s comment: In the abstract, it is confusing to declare that "miR-146 and miR-499 are involved in inflammation" as the reason to link these two with HCC.

The authors’ Answer:

It is really true as reviewer suggested that we arbitrarily declare that "miR-146 and miR-499 are involved in inflammation". We are very sorry for our negligence of this problem and more than grateful that you point it out. We focused mainly on linking HCC to inflammation and forgot to list evidences how these two miRNA function in inflammation.

It is well known that MicroRNAs (miRNAs) are small evolutionarily conserved, endogenous, single-stranded, non-coding RNA molecules with typical length of 20~22 nucleotides. These noncoding small RNAs, such as miR-146 and miR-499, have been shown to participate in tumor genesis, such as inflammation, cell cycle regulation, differentiation, apoptosis, and invasion. The C allele gene displayed decreased production of mature miR-146a compared with G allele. The mature miR-146a failed to inhibit the target genes including IL-1 receptor-associated kinase 1 (IRAK1), TNF receptor-associated factor 6 (TRAF6) and papillary thyroid carcinoma 1 gene (PTC1). By this way, the Toll-like and cytokine receptors kept activating result in enhance the inflammatory responses. The optimal free energy was decreased from -62.30 kcal/mol for T to -61.90 kcal/mol for C alleles, that change the conformation of the secondary structure and thereby directly affect both the binding to target mRNAs and the miRNA maturation process. miR-499 had the potential to regulate all 4 of the cellular senescence induction exist including telomere shortening, oxidative stress, oncogene expression and DNA damage signaling types.

We have searched more articles related to "miR-146 and miR-499 involved in inflammation" and made corrections according to the Reviewer’s comments. (we underlined the changes in revised paper)

2# The reviewer’s comment: The discussion section needs to be strengthened.

The authors’ Answer:

Special thanks to you for your good comments. We may be inexperienced in strengthen our discussion. Sincerely considered the reviewer’s suggestion, we have read a lot of quality research papers and ask many skilled co-workers for advices. We have re-written discussion section. Hopefully these corrections will meet with approval. If the reviewer still thinks corrections needs to be made, we would be willing to do it. We are highly grateful for your advise.

Part C (Executive Editor)
We did our best to make the revised manuscript conforms to the journal style and also make sure the files are correctly formatted.

We have re-written some part according to suggestions to minimize typographical, grammatical, and bibliographical errors.

We tried our best to improve the manuscript and made many 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 underlined most of them in revised paper.

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

Once again, thank you very much for your comments and suggestions. We learned a lot during this process. Each author of this manuscript has reviewed this version of the manuscript and approved it for publication. We will be highly honored if our paper get accepted for publication.

Best wishes!

Jiajing Liu (first author)
Email: 11211020006@fudan.edu.cn
Department of Epidemiology, School of Public Health, Fudan University; Key Laboratory of Public Health Security, Ministry of Education, Shanghai 200032, China

Wei Meng* (Corresponding author)
Email: wmeng@shmu.edu.cn
MD, Ph.D, Professor
Department of Epidemiology, School of Public Health, Fudan University, 138 Yi Xue Yuan Road, Shanghai, 200032, P. R. China.