Author’s response to reviews

Title: ARLTS1 polymorphism is associated with an increased risk of familial cancer: evidence from a meta-analysis

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Author’s response to reviews:

Dear Editor:

Thank you for your letter 18 Jan 2017 regarding our manuscript entitled “ARLTS1 polymorphism is associated with risk of familial cancer: evidence from a meta-analysis” (HCCP-D-16-00036).

Sorry for replying your letter so late. We carefully considered the reviewer’s comments and suggestions and amended the manuscript accordingly. In addition, we have put serious efforts into revising the manuscript and made numerous editorial changes, including re-wording some sentences and correcting various spelling and grammar errors. We hope that the revised version is suitable for publication in your journal.

Thanks again for your reconsideration.

Point-by-point replies to the reviewers’ comments are listed below.

With kind regards,

Hui-yi Lv
Point-by-point responses to the reviewer’s comments

We thank the reviewers for their positive and constructive comments. We amended the manuscript to accommodate the comments and revised the manuscript extensively. We hope that the revised version is now acceptable for publication.

Reviewer #1:

This is a meta-analysis of 5 SNPs in the ARLTS1 gene (official gene name is ARL11 - should this be used or at least referred to?), which has found a significant association of one SNP (Cys148Arg) with both sporadic and familial cancer risk. And therefore claim that this gene should be considered as an important potential target for personalized medicine in cancer treatment.

We fully agree with your suggestions. According to your suggestions, we have referred to ARL11 in our Manuscript. (Introduction: paragraph 1, lines 1-2)

It is a well conducted study that has followed the "checklist for meta-analysis" published by M.W.Russo (Gastroenterol. Hepatol, 2007). An OR of 1.36 for sporadic and 1.26 for familial cancer risk was shown for Cys148Arg SNP and I am wondering how they interpret the risk to be high enough for it to be a potential target for personalized medicine?

We thank the reviewer for these comments. According meta-analysis theory, our result showed Cys148Arg variant has a significant association with sporadic cancer (CC vs. TT: OR=1.36, 95% CI=1.18-1.55) and familial cancer (CC vs. TT: OR=1.26, 95% CI=1.12-1.43). Consequently Cys148Arg SNP was associated with increased sporadic cancer risk as well as familial cancer risk statistically. We believed Cys148Arg SNP play a important role in cancer risk. Cys148Arg SNP will be a potential target for personalized medicine.
I am not a statistician and therefore I am not commenting on statistical methods used. But I do have one "statistical" question; SNPs are subject to the same multiple testing issues in meta-analysis as in single sample studies, but this has not been adjusted for or discussed (i.e. Bonferroni correction).

We sincerely appreciate that comment. We did think use Bonferroni correction to adjust p-values. But the genetic association between Meta analysis of the model of genes is not very independent, there are also some kind of relationship, in addition, this kind of method is too conservative, and the possibility of correction of excessive (increasing the incidence of false negatives).Therefore, this method is suitable for the genetic association between Meta analysis results of correction

Major essential revisions

* In addition to refer to SNPs as Cys148Arg, also include C148R and rs numbers which is mostly used for SNPs today. Maybe make a table showing all the different names of the 5 SNPs (polymorphisms)

According to your suggestions, we replaced the all the different names of the 5 SNPs in Table 1 (Tab 1).

* In result section; can be summarised by writing the following at the end of the 1st paragraph in results:

  o Not all studies genotyped all SNPs included in this meta-analysis, therefore number of included studies will differ between the SNPs. All results will be presented like this: OR (95% CI), p-value.

  o By doing this you can write results as this in next paragraph; …pooled into the meta-analysis (CC vs. TT: 1.27 (1.15-1.41), p=0.000 Fig2; CC + TC vs. TT: 1.13 (1.02-1.27), p=0.026, etc.) = much easier to follow.

  o P-values in a lot of the result in section Cys148Arg is missing in the paragraph.

We are grateful for your careful review of our manuscript and have rewritten the result section according to your suggestion.( Results: paragraph 1, lines 9-11; paragraph 2, lines 4-6, 10-11, 13-17; paragraph 3)
In section Trp149Stop, Por131Leu, etc. it says: Overall, significant main effects on cancer risk…. But the results are not significant (p=0.700 and p=0.635). In Table 4 though the results in that section says p=0.001 for both. Which is correct? In the same section all results show the same, so can be shortened to say: No significant results are observed for and of these SNPs and refer to table 4. No need to repeat the same thing for each SNP and all the numbers are in the table.

We thank the reviewer for these comments. Firstly, the p values in Table 4 is for heterogeneity for Q-test but not for correlation between SNP and cancer. It indicated which effect model we should use. If between-study heterogeneity was significant (p < 0.05 for the Q-test), we used a random-effects model (DerSimonian–Laird method), otherwise the fixed-effects model (Mantel–Haenszel’s method) was used. Besides, we have rewritten Trp149Stop, Por131Leu, Ser99Ser and Leu132Leu section according to your suggestion. (Results: paragraph 3)

In Discussion (line 30-35) you write; "Yet, it did not mean that other variant have no association with cancer. These variants may be with minor effects individually, but they are likely to contribute adequately in combination to lead the failure of immune response". Why have you come to this conclusion as there is no indication at all that they contribute towards cancer development? I can understand this reasoning if you saw a trend towards an association but not without.

We thank the reviewer for these comments. We come to this conclusion according to a deduction. There are many gene-gene interactions to increase risk of cancer. For example, the study1 have demonstrated that a significant association was found only between the CDH13 SNP rs3865188 with cancer and none of the four APN SNPs (rs2241767, rs3821799, rs3774261 and rs6773957) showed evidence of any association and cancer. But when the four APN SNPs interacted with rs3865188, the results showed the combined genotypes were significantly associated with cancer risk. In addition, a meta-analysis2 indicated that GSTM1 and GSTT1 genes mutation was not associated with an increased gastric cancer risk separately. Whereas the results showed that the mutation genotype of GSTM1 might increase gastric cancer risk associated with the GSTT1 mutation genotype. So a similar situation may be described for SNPs of ARLTS1. No significant results are observed for and of these SNPs (Trp149Stop, Por131Leu, Ser99Ser and Leu132Leu). However, through gene-gene interactions the combination increased the cancer risk possibly.

In Discussion (line 53-59);"… it is outstanding that this study is the first time to manifest the association between these five SNPs and cancer risk. Also our research provides powerful evidence for future large scale population-based cohort study and case-control study". First of all you did not find an association between 5 SNPs and cancer risk, just one of them and
secondly, I am not sure you can say the study provides powerful evidence for future large scale population-based cohort study and case-control study from the findings you present.

We thank the reviewer for these comments. For the first question, it should be ambiguity of our expression. So we have rewritten this section according to your suggestion. (Discussion: paragraph 3, lines 9-12) For the second question, the main reason why our study provides powerful evidence was based on the following. Firstly our study included up to now all researches discussed the association between ARLTS1 and cancer risk. Secondly the studies included in our meta-analysis had high quality generally. Moreover the most important point is among all studies didn’t show any statistical evidence of publication bias and sensitivity analysis also met the criterion. Consequently our results were stable and robust and our study provided powerful evidence.

* Conclusion - one of your major findings is that you have excluded the association of any of the other 4 SNPs with cancer risk. This should be included in the conclusion.

We are grateful for your careful review of our manuscript and have rewritten the conclusion section according to your suggestion. (Conclusion: paragraph 1, lines 2-3)

* English grammar needs attention.

We sincerely appreciate these comments and completely agree with your suggestions. We have rewritten the manuscript according to your suggestion.

* Discussion p.2 (line 3-5); "…..reduction or absence of ARLTS1 expression contributes to DNA mutation with LOH in breast cancer (23) - this article does not talk about ARLTS1 expression, DNA mutations with LOH in breast cancer. Make sure you reference correctly. Same goes for next sentence and reference 7 (does not say methylation in thyroid cancer). Go through all references and make sure you are saying what you are referencing!

We are grateful for your careful review of our manuscript and have rewritten the discussion section according to your suggestion. (Discussion: paragraph 1, lines 13-15)
Minor Essential Revisions

* Introduction p.2 (line 1-2); "..... which was published in the New England journal (2)" - no need to state the journal when you are referencing and if you do use the correct name New England Journal of Medicine.

We are grateful for your careful review of our manuscript and have rewritten the introduction section according to your suggestion. (Introduction: paragraph 2, lines 2-3)

Introduction p.2 (line 8-9); "However, to up date the results of these studies have remained inconsistent". What do you mean here? Sentence does not make sense.

We thank the reviewer for that comment. We mean that some studies believe ARLTS1 polymorphism is associated with cancer risk but some are not. So we said "However, to up date the results of these studies have remained inconsistent". We have rewritten this section according to your suggestion. (Introduction: paragraph 2, lines 5-8)

M & M p.1 (line 21-22); suggest changing sentence to "Database searches of Medline, PubMed, Embase, Web of Science and China National Knowledge Infrastructure (CNKI) were performed. If you need to pub in timeline - you might want to justify why you have chosen to start in 2005.

We fully agree with your suggestions, which helped us improve the quality of our manuscript. The sentence was adjusted and the timeline was retained. Because we found that articles before 2005 did not report any relationship between ARLTS1 and cancer. So the timeline was retained and the starting point was set for January 2005. Details could be seen in the M & M p.1 (line 21-23)

It is normal to refer to SNPs with rs numbers today, maybe a table with Cys148Arg, C148R, genotype and the SNPs rs number would be a good idea?

According to your suggestions, we replaced the all the different names of the 5 SNPs in Table 1 (Tab 1).

M&M p.1 (line61); "… were extracted from each study; ..... ethnicity, study design. Might be a good idea to say "ethnicity of study population".

We are grateful for your careful review of our manuscript and have rewritten according to your suggestion. Details could be seen in the M&M p.1 (line61).
M&M p.2 (line 2); "Different cancer types were was classified…" Can't use were was - choose the correct one.

We chose were as the correct one and the Details could be seen in the M&M p.2 (line 2).

M&M p.2 (first line in Quality assessment) is hard to read - suggest changing to "The quality of the included publications was assessed according to a quality assessment scale (Table 1), which was modified from previously published meta-analysis (8-10). In the next sentence, delete word: severally.

We are grateful for your careful review of our manuscript and have rewritten according to your suggestion. Details could be seen in the M&M p.2.

M&M p.2 (line48); "ARLTS1 polymorphism and cancer risk" should read "ARLTS1 Polymorphisms and cancer risk".

We are grateful for your careful review of our manuscript and have rewritten according to your suggestion. Details could be seen in the M&M p.2 (line48).

M&M p.2 (line51); "…. and studies quality." should read "… and study quality".

We are grateful for your careful review of our manuscript and have rewritten according to your suggestion. Details could be seen in the M&M p.2 (line51).

M&M p.3 (line5); change the work "…. Besides Egger's test" to "…. in addition Egger's test".

We are grateful for your careful review of our manuscript and have rewritten according to your suggestion. Details could be seen in the M&M p.3 (line5)

Add references in Table 2 (behind Author names)

The references were added behind author names. Details could be seen in Table 2.
Results p.1 (line 11): You are referring to 10 articles meeting the inclusion criteria, but up until now you have talked about included studies. Change articles to studies in next two sentences. Same paragraph; line 15 - add "ss" to polymorphism as you are referring to more than one.

The word articles was replaced with the word studies. We also add ‘s’ to the word polymorphism. Details could be seen in Results p.1 (line11-17).

Results p.1 (line 26-27) - suggest changing to; "..... 11 studies were included in the meta-analysis for SNP C148R with 7,152 cases and 6,698 controls."

We are grateful for your careful review of our manuscript and have rewritten according to your suggestion. Details could be seen in the Results p.1 (line26-27).

Results p.1 (line 35): change "…was listed in Table 3" to "….are listed in Table 3".

We are grateful for your careful review of our manuscript and have rewritten according to your suggestion. Details could be seen in the Results p.1 (line35).

Results p.1 (line 37-38) - English grammar: "controls source" should be "control source" x 2, "studies quality" should be "study quality". "CC polymorphism" should be "CC genotype" + add studies behind population-based and hospital based in the next sentence.

We are grateful for your careful review of our manuscript and have rewritten according to your suggestion. Studies were added in the next sentence. Details could be seen in the Results p.1 (line37-39).

Results p.1 (line 43) - suggest changing sentence to; "For study quality, the significant results only existed in high quality studies….". Also in line 48 you refer to significant results as positive results.

We are grateful for your careful review of our manuscript and have rewritten according to your suggestion. Details could be seen in the Results p.1 (line43, 48).

Discussion p.1 (line 48); change "incompletely clear" to "unclear". Also not possible to follow thought when saying …' unclear such as pathways, …". What do you mean here? That we don't
know what pathways the gene work in? Need to make this clearer. Next sentence starts with "The possible mechanism is that…" Mechanism of what?

The pathways mean the access of ARLTS1 gene mechanism. The possible mechanism is our speculation that how the ARLTS1 gene activated to suppress tumor formation. We have rewritten to make clearer according to your suggestion.

Discussion p. 1 (line 53-56) - English grammar; delete the work "on" before acceptors, change "become" to "becoming" and write "the ARLTS1 gene".

We are grateful for your careful review of our manuscript and have rewritten according to your suggestion. Details could be seen in the Discussion p.1 (line53-56).

Discussion p.2 (line 7-14) - English grammar; say "immune responses resulting in…", add "s" to polymorphism x 2 (when you talk about more than 1 SNP).

We are grateful for your careful review of our manuscript and have rewritten according to your suggestion. Details could be seen in the Discussion p.2 (line7-14).

Discussion p.2 (line 53-54): "outstanding that this study is the first time to manifest" - you are not the first study to associate the 5 SNPs and cancer risk (and you only found association in 1 SNP!), you can say first meta-analysis.

We are grateful for your careful review of our manuscript and have rewritten according to your suggestion. Details could be seen in the Discussion p.2 (line53-54).

* Declarations: You have 2 Authors' contribution - combine it to 1.

We thank the reviewer for that comment. But Jiang and Zhao made the same contribution on the study. We hope they shared first author.

Reviewer #2:

Introduction: The nomenclature for the variants/polymorphisms should be HGVS - standard if you are discussing sequence variants or rs- positions if you are discussing SNPs. The reason for using the chosen nomenclature should nevertheless be stated. (Earlier studies have used it etc.)
Definitions of genetic normal variation, SNPs vs genetic susceptibility alleles and disease-causing variants should be mentioned.

We are grateful for your careful review of our manuscript and have rewritten the highlights according to your suggestion. And we listed the different names of the 5 SNPs in Tab 1.

Method and material:

The methods for the meta-analysis and the statistical tests are described thoroughly and nice. I can't however fully review the statistics for this type of study.

Results: "Characteristics of included studies", should be placed in Methods- chapter? A forest plot to show previous results from the chosen studies would be nice.

We thank the reviewer for these comments. We believe "Characteristics of included studies" was the result of including the studies. Consequently, it was placed in Result. A forest plot has been uploaded separately and not been attached to the manuscript.

Discussion: Some discussion on the difference in significance between nonsense mutation Trp149 Stop, and the other four variants which are missense could be included. (shown in table 4) The fact that polymorphisms in ARLTS1 leads to different types of cancer, but with altogether low risk estimates, 1.27 (1.15-1.41) for Cys148Arg CC, should be elaborated on. On its own this polymorphism is only a weak cancer risk factor, but may contribute in carcinogenesis in a way that is common for different cancers.

We fully agree with your suggestions, which helped us improve the quality of our manuscript.
