Author's response to reviews

Title: A functional polymorphism in the SPINK5 gene is associated with asthma in a Chinese Han Population

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Author's response to reviews: see over
Dear editor,

Thank you for arranging a timely review for our manuscript. We are pleased to know that our findings is of important interest for the readers of this field. We have carefully evaluated the reviewers’ critical comments and thoughtful suggestions, responded to these suggestions point-by-point, and revised the manuscript accordingly. All changes made to the text are in red so that they may be easily identified.

Sincerely yours,

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Major Compulsory Revisions

It is stated that -206A/G polymorphism is significantly associated with asthma, but the correction for the multiple test was not performed. The method for the multiple test correction should be described and discussed further in the discussion section. After the correction of multiple testing, the results would be no more significant.

R: The reason that we got a marginal $P$ value may be due to the sample size. To be honest, after correction, there was no significant difference indeed. Although correction for multiple testing would reduce type I error, it is an extremely conservative measure and may increase type II error with a stringent criterion. Our results demonstrated that -206A/G is really a functional SNP and may have contribution to the etiology of asthma. Considering the functional evidence, together with the association result, we feel there is a strong argument for the involvement of this genetic variant in Asthma.

The authors showed that non synonymous SNPs were not associated with asthma. They should describe the power of the study to detect the differences and discuss the limitation of the study. It would be interesting to perform the meta-analysis to combine the results of several studies.

R: We have already calculated the power of our study according to the suggestion and we had a power of more than 80% at the alpha level of 0.05 with this sample size for a multiplicative model. The aim of this study is just to test the relation of SPINK5 gene and asthma in Chinese Han population and we think meta-analysis is not necessary in this paper but we think it is helpful for demonstrating that relationship of SPINK5 gene and asthma. Anyway, thanks for reviewer’s suggestion.

The authors performed the reporter assay to see the difference of the transcriptional activity. However, transcriptional activity in the 5’ flanking region of SPINK5 was not examined in details (i.e., transfection using constructs with various length of 5’-flanking region, transcription initiation analysis etc). How did the authors determine the region of promoter of SPINK5? If the authors refer the results of previous study, they should cite the appropriate references.

R: In the luciferase gene report assay experiment, we just wanted to show that the variants of -206A/G have different transcriptional activity. And the results confirmed that the two alleles have different transcriptional activity. We determined the region of promoter of SPINK5 according the ensemble website ([www.ensembl.org](http://www.ensembl.org)) based on transcription initiation site of the gene. According to the reviewer’s advice, we have made relevant revision in our manuscript.
In EMSA, why did the author show only the results of -206A allele? The binding activity of -206G allele should also be shown in the Figure 2. In order to determine whether or not GATA3 binds to the -206A/G polymorphic site, supershift assay or the competition assay using GATA specific oligonucleotide can be performed.

R: In the result of EMSA, we can see that cold -206A can inhibit the probe binding to nuclear extract, but -206G can not. The result demonstrated that -206G can not bind to the protein. In EMSA, supershift is necessary when trying to confirm that a specific protein binding to an oligonucleotide. The main aim of our EMSA experiment is just to show that -206A and -206G have different binding activity, not to determine whether the binding protein of -206A/G is GATA3 or not.

2nd review:
Major comment:
A) Genetics
- The association needs to be replicate in an independent cohort.
  R: We agree with the reviewer’s suggestion. The replication study does need to be confirmed in different cohort. To be honest, we don’t have other cohort’s sample. This will be our future endeavor to collaborate with other researchers in the field but it is beyond the scope of the current study.

- According to the number of SNPs, the standard is to apply a correction factor (ex. Bonferroni correction).
R: the point is well taken. however, due to the sample size, we got a marginal p value. After correction, there is no significant difference. But according to the function study, this variation is a true susceptibility locus. The aim of the correction is to exclude false positive result, but in our study, under the particular result, it may bring a false negative result.

B) Functional
- The potential implication of the GATA binding factor in the regulation of the SPINK5 gene expression should be confirmed by further experiment and literature review. A super shift assay with an antibody directed against GATA binding factor would clearly demonstrate it’s implication and allow the author to further extrapolate on this regulation pathways. Also, current knowledge on the potential role(s) of GATA binding factor and other transcription factors in asthma known molecular symptoms (Th2 activation, elevated serum IgE, recruitment of mast cell, etc…) should be addressed.
R: Thanks for the reviewer’s suggestion. In this paper, we just want to
demonstrate that -206A/G is a functional variant, and the result of luciferase report gene assay and EMSA have confirmed it. But, in order to study the detailed mechanism of -206A/G variant involved in the asthma supershift assay may be needed. In our EMSA experiment, we don’t think it is necessary. We have added some discussion on GATA binding factor and asthma in our revised manuscript.

Minor:
It would have been interesting to directly observe the different expression levels of the SPINK5 by real-time PCR experiment on control vs. asthmatic tissues or cell lines.
R: We agree on the point. Unfortunately, we don’t have the tissues or cell lines available for gene expression analysis. It will be our future effort in this regard.

- The first sentence of the second paragraph of the discussion beginning with: « Relative to the most obvious effects... » should be reformulated as it is not possible to include all mutations in coding sequence as having obvious effects in common diseases especially in complex traits as asthma an as it is way too inclusive and not exactly to say that « ...common diseases are more likely to be caused by subtle mechanisms affecting gene function, ».
R: We changed this sentence as “The variations in the regulatory sequences of genes may cause common diseases by regulating both the timing and cellular or tissue expression of the genes.”