Author's response to reviews

Title: Association of genetic variants in chromosome 17q21 with adult onset asthma in a Chinese Han population

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

Thanks for your hard work for processing the manuscript. We are pleased to know that our findings are of interest for the readers of this field. We have carefully evaluated the reviewers’ comments and thoughtful suggestions, responded to these suggestions point-by-point listed below (reviewer’s suggestion in black, the reply in blue), and revised the manuscript accordingly.

We are looking forward to receiving your decision on the revised manuscript.

Sincerely yours,

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Major Compulsory Revisions

1. Statistical analysis

The authors reported in Table 3 single-marker associations between asthma and five SNPs. Such analyses were not adequate to clarify the genetic associations, and the authors need to confirm the results by adjusting for important covariates such as age and sex using multivariate regression. It would also be interesting to know whether 17q21 locus is linked to spirometric parameters. Besides, the results should be adjusted for multiple statistical comparisons. In addition, the authors need to analyze the association between asthma and haplotypes based on the five studied SNPs.

R: We calculated the p value for each SNP adjusted for age and sex by using logistic regression with PLINK software, and the adjusted P are added in Table3. For we selected 5 SNPs for analysis, multiple statistical correction should be performed. However, due to our sample size, we can only get a marginal P after correction, although correction for multiple test would reduce type I error, it will increase the type II error. Also, for these 5 SNPs are in completed linkage disequilibrium situation, multiple test correction can be ignored. Haplotype analysis has been added in the result section.

2. Clinical phenotypes

Most published studies reported 17q21 locus to be important for the susceptibility of childhood-onset asthma. A substantial number of children with asthma continue to suffer from this disease as adults, but some adults may report uncertain or even inaccurate recall as to the onset of their disease (especially the milder cases). The authors need to acknowledge this issue as a limitation. The other major issue relates to the source from which controls were recruited. Were they healthy volunteers from the community, or attendants of the hospital who did not have asthma history? I am concerned about their lung function (Table 1), with low FEV1 and/or FVC in a subgroup of these controls. Lastly, how did the authors select the 61 cases for studying gene expressions? Were they consecutive patients seen in their clinics or only a biased group of subjects who consented for this additional testing?

R: We added relevant description in discussion: “But, we have to acknowledge the limitation that recall bias of self-reported onset age may have a subtle influence on the results.” The controls were recruited from the Health check-up center of Qilu hospital, they are from many communities organized by government for physical examination, only those without any symptoms or history of asthma, or other pulmonary diseases or atopy were included in our study.

For the 61 cases for gene expression analysis, we added in method: We selected 61 asthma patients who were met to the following criteria for transcripts level analysis: 1) First episode patients, and 2) no any medication used within one month.

Minor Essential Revisions
1. SNP selection
The authors chose five SNPs that were reported to be significant in published studies. Were these SNPs also tagging for 17q21 locus in the Chinese Han population? If not, why did the authors not include the more important tagging SNPs in their population? Were the five SNPs studied in linkage disequilibrium with other SNPs on this locus.
**R:** For 17q21 locus is in a high linkage disequilibrium region, these 5 SNPs can be the proxy SNP for other SNPs.

2. References
The citations for several references are incomplete (e.g. #2, #5, #18 and #20). The authors also need to cite the GWAS meta-analysis recently published by the GABRIEL Consortium (Moffatt MF et al. N Engl J Med 2010;363:1211-21), and discuss the findings of this manuscript in the context of that landmark GWAS.
**R:** We have made changes in first paragraph of discussion.

Reviewer: Manuel Ferreira
Minor issues not for publication

*Page 4, please rephrase “61 asthma patients who were first onset and no any medication used within one month were recruited [...]”*
**R:** Has been revised.

*Page 5, add PLINK’s reference instead of website.*
**R:** It has been added in the reference.

*Page 6, transcript level analysis, please add % variance in expression explained by each SNP.*
**R:** Thanks for reviewer’s suggestion, for the 3 SNPs analyzed in the expression are in completed LD status with other SNPs, we think they are just markers and may not be the causative variants. So the results showed in figure 2 should be appropriate.

*Page 7, lines 6 to 10, the association between asthma and 17q21 variants reported by Ferreira et al [18] could be driven by the childhood-onset cases included in that study. As it was not possible to test for age-of-onset effects, it is a bit of a stretch to state that results from that study partly support your findings. I suggest that these two sentences should be deleted.*
**R:** Has been deleted.

*Page 7, paragraph 2, rephrase “it can't address gene function [...]”.*
**R:** Has been changed.