Author's response to reviews

Title: Association of ADAM33 gene polymorphisms with COPD in a northeastern Chinese population

Authors:

Xinyan Wang (wxylyfly@163.com)
Lei Li (lilei75@hotmail.com)
Jinling Xiao (xiaojinlingxiao@yahoo.com.cn)
Chengzhen Jin (jcz912@hotmail.com)
Kun Huang (huangkun515@sina.com)
Xiaowen Kang (xiaowen801124@yahoo.com.cn)
Xiaomei Wu (wu.xiaomei@hotmail.com)
Fuzhen Lv (yyhww@yahoo.com.cn)

Version: 2 Date: 3 August 2009

Author's response to reviews: see over
RE: MS: 1597150544270192 “Association of ADAM33 gene polymorphisms with COPD in a northeastern Chinese population”

Thank you for providing us the opportunity to resubmit the manuscript. The manuscript has been revised based on the reviewers’ comments and the changes we have made in response to the comments were underlined for you to review. Below, we provide an itemized summary of the changes. Reviewers’ comments are shown in bold, followed by our responses.

Reviewer #1 (7402129852751858_comment):
The investigation of polymorphisms in a population is always valuable; however there are some Minor Essential Revisions that have to be made:
1. The language used. Corrections needed in "Background" 2nd paragraph: some verbs are not present or the subject of sentence is not in position.
Answer: Thank the Reviewer for careful check. We changed the sentence "The work by Ilaria Puxeddu et al shown that a truncated, soluble form of ADAM33 containing the catalytic domain causes rapid induction of endothelial cell differentiation in vitro, and angiogenesis both ex vivo and in vivo, and TGF-β2 enhances ADAM33 release" as following: "Puxeddu et al. showed that a truncated, soluble form of ADAM33 containing the catalytic domain caused rapid induction of endothelial cell differentiation in vitro and angiogenesis ex vivo and in vivo". Follow the Editor’s
suggestion, we invited a native English speaking colleague to improve the English used in the manuscript.

2. The selection of patients. It is stated that COPD patients had NO previous treatment. It is assumed that they are newly diagnosed?

Answer: Thank the Reviewer for the question to sampling method. We recruited patients who had not been previously treated with theophylline, β2-adrenergic receptor agonists, or glucocorticosteroids because these samples were not only used for this association study but also for other subsequent experiments. Although these patients are newly diagnosed, they are conclusively diagnosed.

Moreover the you say that the patient group had "no exacerbation within the preceding month". Why do you use this exclusion criterion?

Answer: The expression of “and (6) no exacerbation within the preceding month” used in the paper is simply to emphasize that the patients were in stable conditions. There was no intention to use this as an exclusion criterion. Since this has been mentioned in the early part of the paper already this sentence was removed to eliminate any confusion.

3. You have also investigated these polymorphisms in asthma and allergic rhinitis. Do you have a suggestion regarding the pathogenetic mechanism?

Answer: Thank the Reviewer for the question. On one hand, the pathogenetic mechanisms of asthma, allergic rhinitis and COPD are different from each other, and each disease has its specific diagnosis. On the other hand, there may be some overlapping aspects in their pathogenetic mechanism. In the last paragraph of Discussion, this point has been emphasized.

4. You should provide the p values in table 4.

Answer: Table 4 was revised according to reviewer’s suggestion.

5. Regression analysis could be valuable, since this analysis is more appropriate for matched data and will strengthen the presentation.

Answer: The allele frequencies for the COPD patients and control-group individuals were statistically compared using the χ² test with SPSS software. We didn’t choose the Regression analysis method because it was more suitable for the measurement
6. You have to be more specific on their difference from the Dutch results.
Answer: We have described the conflicting result of different studies and strengthened it in the second paragraph of Discussion.

Reviewer #2:
1. The authors firstly selected eight Tag SNPs for genotyping. The author mentioned that they selected SNPs from published ADAM33 SNPs associated with excess decline in FEV1 and/or presence of COPD. References should be added after the sentence.
Answer: Thank you. We simplified the sentence in the Methods section of Abstract "Eight polymorphic loci (V4, T+1, T2, T1, S2, S1, Q-1 and F+1) of ADAM33 were selected for genotyping, which were previously associated with excessive decline of lung function and/or presence of COPD" as following: "Eight polymorphic loci (V4, T+1, T2, T1, S2, S1, Q-1 and F+1) of ADAM33 were selected for genotyping". The same meaning sentence was presented and referenced in the last paragraph of Background as "Recent studies revealed that SNPs within ADAM33 confer susceptibility to COPD in the general population and are associated with airway inflammation in COPD [21, 22]".

2. Linkage disequilibrium among the selected eight SNPs should be explained with actual D’ and r2 values and allele frequencies. It would be better to explain about differences of linkage disequilibrium between northeastern Chinese and Caucasian populations.
Answer: Haplotype frequencies for multiple sites in phase-unknown samples were estimated using the expectation-maximization method by Haploview software. The LD plot of the eight SNPs is as followed. We didn’t get a LD block of relatively high LD scores when we carried this analysis. As the eight SNPs exists in a small chromosome region (<5kb), and such haplotype structure was not also analyzed in some published papers (Reference 17 for example), we didn’t present the haplotype structure in the manuscript. But we will delete the haplotype analysis section if you
think it’s reasonless.

Fig. The LD plot of the eight SNPs

3. How many SNPs with minor allele frequency >5% will be captured with the finally genotyped eight SNPs in human ADAM33 genes?

Answer: The MAF of all genotyped SNPs was >5%, and deviation from the Hardy-Weinberg equilibrium was not seen in the COPD or control group for each polymorphism.

4. In European children, M+1 SNP was associated with lower FEV1 [24]. Why the authors did not include the SNP in this study?

Answer: Thank you. Actually, to reduce genotyping cost, we developed a PCR-RFLP approach to determine the genotypes. We excluded M+1 SNP because we couldn’t find the restriction endonuclease for this locus.

5. The present study showed that the T1, T2, S2, and Q-1 were significantly associated with COPD. The authors should also discuss about the direction of allelic association in previous studies and this study.

Answer: We have described the conflicting result of different studies and strengthened it in the second paragraph of Discussion.

6. The authors reported association studies of ADAM33 polymorphisms in adult allergic asthma and rhinitis in a Chinese Han population [30]. The similarities
and differences of the results among the studies should be discussed in detail.

Answer: In the last part of “Discussion”, this point has been emphasized.

Again, I would like to thank you for your effort and help in the revision of this manuscript. Please feel free to contact me should you have any questions. The validated email address has been changed to fuzhenlv46@yahoo.cn.

We look forward to your reply.

Yours sincerely,

Fuzhen Lv, M.D., Ph.D.
Dean of Respiratory Department, the Second Affiliated Hospital
Harbin Medical University
Harbin 150081
Tel: 86-451-86605445
Email: fuzhenlv46@yahoo.cn