Author's response to reviews

Title: Genetic variants in ADAM33 are associated with airway inflammation and lung function in COPD

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Version: 2
Date: 8 August 2014

Author's response to reviews: see over
Reply to Reviewer #1

Summary: The authors aim to identify potential disease-related proteins using bioinformatics approach. Authors used "guilt by association" analysis and prioritized candidate proteins of human cardiomyopathies by building weighted human cardiomyopathy-specific protein-protein interaction networks for three subtypes of cardiomyopathies using the known disease proteins from OMIM as seeds. The authors observed that majority of candidate proteins with high scores shared disease-associated pathways with disease seed proteins. After validated their findings using the 2nd bioinformatics method, the authors concluded that their developed approach could be used for the identification of potentially novel disease proteins.

1. It is will be beneficial if the author can mention why they did not include S1 SNPs in their study. This SNPs has been linked with COPD some where else.

REPLY: Thank the reviewer very much for the suggestion. S1 has been linked with COPD in some studies. However, in our previous study [1], we tested the association between S1 and COPD in a northeastern Chinese population, and no association was found. Moreover, in a study which investigated the association between SNPs and COPD in Han population of northern China [2], no statistically significant difference in the relative risk of COPD in S1 was revealed. As a result, we did not include S1 in this study. We have added the part of discussion about S1 as follows:

In the Section of "Discussion":

"Since no association was found between S1 and COPD in our previous study in a northeastern Chinese population [29], we did not include S1 in this study."

In the Section of "Reference":


Reference:

2. Limitations of the work are not clearly stated.

REPLY: Thank the reviewer for identifying the insufficiency. We have added the limitations of our work as follows:
In the Section of "Discussion":

"We focused on the association of four polymorphisms (T1, T2, S2 and Q-1) of ADAM33 as well as their haplotypes with pulmonary function and airway inflammation of patients with COPD, thus, no sputum cells were obtained from control samples. More comprehensive data should be obtained to reveal the mechanism of COPD in further study."
Reply to Reviewer #2

*Major revision:*

1) *Should reveal Gold stage of all COPD patients, stable or unstable.*

**REPLY:** Thank the reviewer very much for the suggestion. 312 patients with stable COPD were recruited for this study. Patients with acute exacerbations two months preceding study assessment were also excluded. Disease severity was classified according to the criteria of Global Initiative for Chronic Obstructive Lung Disease (GOLD) and are shown in Table 1. We have revised the corresponding parts as follows:

In the Section of "Methods":

"Patients with acute exacerbations two months preceding study assessment were also excluded. Disease severity was classified according to the criteria of Global Initiative for Chronic Obstructive Lung Disease (GOLD) [1]."

In the Section of "Reference":

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In the Section of "Table 1":

"Table 1 - The clinical information of patients recruited.

<table>
<thead>
<tr>
<th></th>
<th>CASE *</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>312</td>
<td>319</td>
</tr>
<tr>
<td>Age (years)</td>
<td>60.5 (7.8)</td>
<td>61.5 (8.1)</td>
</tr>
<tr>
<td>Male: Female</td>
<td>186:126</td>
<td>192:127</td>
</tr>
<tr>
<td>Pack years of smoking†</td>
<td>35.46 (15.9)</td>
<td>32.54 (12.7)</td>
</tr>
<tr>
<td>FEV1 (% predicted) ‡</td>
<td>52.5 (8.6)</td>
<td>91.5 (9.6)</td>
</tr>
<tr>
<td>FEV1/FVC (%) §</td>
<td>47.5 (7.6)</td>
<td>90.5 (11.6)</td>
</tr>
<tr>
<td>Postbd FEV1 (% predicted)</td>
<td></td>
<td>58.5 (9.6)</td>
</tr>
<tr>
<td>Postbd FEV1/FVC (%)</td>
<td>49.2 (8.5)</td>
<td>91.5 (11.8)</td>
</tr>
<tr>
<td>GOLD status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I (mild)</td>
<td>52</td>
<td>-</td>
</tr>
<tr>
<td>Stage II (moderate)</td>
<td>140</td>
<td>-</td>
</tr>
<tr>
<td>Stage III (severe)</td>
<td>98</td>
<td>-</td>
</tr>
<tr>
<td>Stage IV (very severe)</td>
<td>22</td>
<td>-</td>
</tr>
</tbody>
</table>

* Data are means and standard deviations (in parentheses)
† Pack year: (packs per day) × (years smoked)
‡ FEV1: forced expiratory volume in first second
§ FVC: forced vital capacity
|| Postbd: post bronchodilator
** TLCO: transfer factor of the lung for carbon monoxide"

2) *What was the sputum cell differentials including total cells, macrophage, neutrophils, lymphocyte, and eosinophils between COPD and controls? Should be*
showed in the table.

**REPLY:** Thank the reviewer for the suggestion. We focused on the association between four polymorphisms (T1, T2, S2 and Q-1) of ADAM33 as well as their haplotypes and pulmonary function and airway inflammation of patients with COPD, thus, no sputum cells were obtained from control samples. We have revised the corresponding part as follows:

In the Section of "Discussion":

"We focused on the association of four polymorphisms (T1, T2, S2 and Q-1) of ADAM33 as well as their haplotypes with pulmonary function and airway inflammation of patients with COPD, thus, no sputum cells were obtained from control samples."

3) When the authors obtain peripheral blood and sputum cells, and pulmonary function stable or unstable status? How many patients do obtain sputum and blood? Should reveal number and quality.

**REPLY:** Thank the reviewer very much for the suggestion. Peripheral blood, pulmonary function and sputum cells were obtained in the stable clinical state, at least two months after any exacerbation. Peripheral blood and pulmonary function were obtained from all 312 COPD patients and 319 control samples, while only sputum cells were obtained from 312 COPD patients after pulmonary function measurement (Table 1).

Table 1 The number of COPD patients or control samples from which peripheral blood, pulmonary function and sputum cells were obtained.

<table>
<thead>
<tr>
<th></th>
<th>Blood</th>
<th>Pulmonary function</th>
<th>Sputum cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>COPD</td>
<td>312</td>
<td>312</td>
<td>312</td>
</tr>
<tr>
<td>control</td>
<td>319</td>
<td>319</td>
<td>-</td>
</tr>
</tbody>
</table>

We have added the descriptions as follows:

In the Section of "Methods":

"DNA was extracted from peripheral blood of both COPD patients and control samples, and genotyping was performed as described previously [1] using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis.

... Sputum samples were obtained in the stable clinical state of COPD patients, at least two months after any exacerbation.

... Pulmonary function testing of both COPD patients and control samples was evaluated on a Jaeger Transfer screen II (Erich Jaeger GmbH, Würzburg, Germany) and included: total lung volume; FEV1; vital capacity (VC); forced vital capacity (FVC); peak expiratory flow (PEF); and transfer factor of the lung for carbon monoxide (TLCO)."

4) What is the level of cytokines IL-8, TNF-A and VEGF between COPD and controls? Should be showed in the table.
REPLY: Thank the reviewer for the suggestion. We focused on the association between four polymorphisms (T1, T2, S2 and Q-1) of ADAM33 as well as their haplotypes and pulmonary function and airway inflammation of patients with COPD, thus, no sputum cells were obtained from control samples. That is, no levels of IL-8, TNF-A and VEGF were obtained.

In the Section of "Discussion":
"We focused on the association of four polymorphisms (T1, T2, S2 and Q-1) of ADAM33 as well as their haplotypes with pulmonary function and airway inflammation of patients with COPD, thus, no sputum cells were obtained from control samples."

Minor comments:
1) Conclusion you include in Asian people in conclusion.

REPLY: Thank the reviewer for the suggestion. We have added the corresponding part as follows:

In the Section of "Conclusion":
"In this paper, we confirmed for the first time that ADAM33 was involved in the pathogenesis of COPD in an East Asian population by affecting airway inflammation and immune response."

2) In Discussion You mentioned "Our study showed that the SNP T1 is significantly associated with macrophage and T2 is significantly associated with total cells in sputum of patients with COPD, Q-1 was significantly associated with IL-8, TNF-A and VEGF in sputum of COPD. Why do just macrophage and lymphocytes be associated with COPD except neutrophils and eosinophils? Should explain about that.

REPLY: Thank the reviewer very much for the suggestion. In this current study, our results showed that T1 and Q-1 affected lymphocyte and macrophage, implying that ADAM33 might affect the immune response of the airways and further promote the formation of COPD. Some studies have also found the association between macrophage and lymphocytes and immune responses of COPD. For example, Winkler et al. found that the products of activated macrophages have also been implicated in inflammation and tissue destruction, including in COPD [1]. Domagala-Kulawik et al. showed in their study that macrophages were involved in the inflammatory process caused by smoking in COPD and were associated with severe airflow limitation [2]. We have provided the explanation as follows:

In the Section of "Discussion":
"Some studies have also found the association between macrophage and lymphocytes and immune responses of COPD. For example, Winkler et al. found that the products of activated macrophages have also been implicated in inflammation and tissue destruction, including in COPD [35]. Domagala-Kulawik et al. showed in their study that macrophages were involved in the inflammatory process caused by smoking in COPD and were associated with severe airflow limitation [36]."
In the Section of "Reference":


Reference:


3) What is the clinical relevance that Haplotypes of ADMA33 related to airway inflammation and pulmonary function? How to apply this data in clinical filed?

REPLY: Thank the reviewer very much for the suggestion. Haplotypes of ADAM33 were related to airway inflammation and pulmonary function, which demonstrated that multiple SNPs could involve in COPD by working together. COPD high-risk groups could be screened out using these haplotypes of ADAM33 in the future, though much effort should be put into this clinical field. We have added the corresponding part as follows:

In the Section of "Discussion":

"COPD high-risk groups could be screened out using these haplotypes of ADAM33 in the future, though much effort should be put into this clinical field."