Author’s response to reviews

Title: The Interaction Effects between TLR4 and MMP9 Gene Polymorphisms Contribute to Aortic Aneurysm Risk in a Chinese Han Population

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The Interaction Effects between TLR4 and MMP9 Gene Polymorphisms Contribute to Aortic Aneurysm Risk in a Chinese Han Population

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BMC Cardiovascular Disorders

Dear editor,

Thank you for giving us a chance to revise our article. The editor and reviewers gave constructive suggestions which are all valuable and very helpful for revising and improving our paper. According to suggestion of reviewers, we have made correction which we hope meet with approval. Revised portion have been marked in red in the revised manuscript. The main corrections in the paper and the responds to the reviewers’ comments are attached below. Thank you very much for your work and kind consideration.

Yours sincerely,
Response to reviewers:

Reviewer 1:

1. How were the healthy controls recruited? Did they undergo a computed tomography angiography to rule out the disease of the aorta?

Answer: Thanks sincerely for your valuable question. All the patients and healthy controls were recruited from the First Hospital of China Medical University between September 2016 and November 2017. The controls were recruited from the physical examination center of our hospital with both age and gender matched to the patients. We ruled out the disease of the aorta on the basis of CT or ultrasonic examination. We have added an explanation about how the healthy controls were recruited, as shown in “Method-Study population” part of our revised manuscript.

2. The origin of the patients and controls is unclear. Were these clinic outpatients, community individuals, normal volunteers? Were these patients with hypertensive or had any other disorder requiring access to hospital?

Answer: Thank you for your professional reminding. All the patients and healthy controls were recruited from the First Hospital of China Medical University between September 2016 and November 2017. The patients were enrolled from the cardiac or vascular surgery department of our hospital in which hospitalized patients received the treatment of aortic aneurysm. The control subjects were recruited from the physical examination center of our hospital without aortic aneurysm. All the participants with coronary heart disease, congenital heart disease, severe vascular stenosis, autoimmune disease, severe organ failure, infectious disease, hematological system disease or malignant tumor were excluded. We have added an explanation, as shown in “Method-Study population” part of our revised manuscript.

3. In Page No6, line number 21, - it is mentioned that "randomly selected samples had repeat genotypes carried out and the results were 100% consistent." How to randomly?
Answer: Thank you for your question. To ensure the accuracy of SNP genotyping results, we randomly selected 10% samples for verification. In the system random sampling, the population samples were first numbered from 1-970 successively, then a random number was extracted from 1-10 as the first unit of the sample, and then one was extracted from every ten holes. For example, we first randomly selected number 3 sample, and then we extracted number 3, 13, 23, 33, 43……963 samples to have repeat genotypes and the results were 100% consistent.

4. In Page No6, line number 41, - it is mentioned that "Associations were calculated by odds ratios (ORs) and 95% confidence intervals (95%CIs) with adjustments for age, gender, hypertension, diabetes and dyslipidemia." What is the diagnosis of hypertension, diabetes and dyslipidemia in this study? The number of hypertension, diabetes and dyslipidemia subjects according to the diagnosis should be provided and listed in Table 1 (with chi-square test used for comparative purposes)

Answer: Thank you for your valuable suggestion. We have added the diagnosis of hypertension, diabetes and dyslipidemia in this study, as shown in “Method-Study population” part of our revised manuscript. In addition, the number of hypertension, diabetes and dyslipidemia subjects according to the diagnosis have been provided and listed with chi-square test used for comparison, as shown in revised Table 1.

5. What is the rational for including "diabetes and dyslipidemia" as conventional confounding factors in this study?

Answer: Thank you for your precious question. Aortic aneurysm is a multifactorial disease affected by complex genetic and potential cardiovascular risk factors. This study mainly explored genetic interaction effects between TLR4 and MMP9 polymorphisms in the risk of aortic aneurysm as well as its subtypes. Therefore, in order to avoid the influence of possible confounding factors, we performed multivariate logistic regression with adjustments for potential cardiovascular risk factors. Hypertension is a well-known risk factor associated with aortic aneurysm; besides, diabetes and dyslipidemia are also recognized as cardiovascular risk factors associated with most cardiovascular pathologies including aortic aneurysm. More importantly, there were statistical differences in the distribution of hypertension, diabetes and dyslipidemia between patients and controls in this study. Therefore, we adjusted them as confounding factors.

6. In Page No7, line number 3, - it is mentioned that " In our unpublished data, there were no significant associations of TLR4 rs11536889 and rs1927914 polymorphisms with the risk of AA and its subtypes (P > 0.05) in the general and subgroup analysis stratified by AA location and size. MMP9rs17576 AA genotype was related to a higher risk of overall AA (OR=1.897, P=0.015), AAA (OR=2.291, P=0.007) or small AA (OR=1.977, P=0.024) compared with GG genotype. However, no significant link between MMP9 rs17576 polymorphisms and the risk of TAA and large AA were observed (P > 0.05)." The data should be provided in the manuscript.
7. This study was performed among a preselected population. This might considerably bias the sensitivity and specificity of the test. Hence the issue of selection bias must be described in the discussion section.

Answer: Thanks sincerely for your reminding. Although the genotype distribution of subjects was compatible with HWE, this study was performed among a preselected population and thus the results may be affected by selection bias. Within your guidance, we have described it as one of limitations in the discussion section of our revised manuscript.

8. Thoracic aortic aneurysm and abdominal aortic aneurysm are different diseases, which have different genetic background. It should be discussed in this manuscript.

Answer: Thank you for you precious advice. Indeed, abdominal aortic aneurysm and thoracic aortic aneurysm are different diseases and have heterogeneity in their inheritance, incidence and distribution along aorta, but they also share some similar pathological states and histological phenotypes, including inflammatory cell infiltration and extracellular matrix (ECM) degradation. However, different types of aortic aneurysm may have different genetic background and show varying sensitivity to the effects of genetic polymorphisms. Therefore, we evaluated the interactions between TLR4 and MMP9 polymorphisms not only in the risk of overall aortic aneurysm but also in the risk of its subtypes, including thoracic aortic aneurysm and abdominal aortic aneurysm. Within your guidance, we have discussed it in the “Introduction and Discussion” parts of our revised manuscript.

Reviewer 2:

GENERAL COMMENTS: The paper is clear and well written, and the methods are carefully explained; my major concern is the lack of a multiple testing correction. Statistical analysis was performed with a lot of comparisons, so it may happen that a comparison appears to be statistically significant by chance and not because it is true. Furthermore, in my opinion, a candidate gene approach is not appropriate in order to investigate the objective of this study.

REQUESTED REVISIONS:

Please perform a multiple test correction and test additional SNPs.

Answer: Thank you for your professional comments and suggestion. A cross-talk between TLR4 and MMP9 plays a vital role in aortic pathophysiology. The objective of this study was to evaluate the interactions between TLR4 and MMP9 polymorphisms in the risk of aortic
aneurysm and its subtypes. Just as you concerned, in order to ensure the reliability of our results and avoid the interference of potential factors, we have tried to perform all the comparisons with multivariate logistic regression analysis after adjusting possible confounding factors. However, multiple comparisons for significant P values should be performed indeed. Under your guidance, we used the Bonferroni correction to adjust P values for multiple tests as needed, which was described in the statistical analysis section. P values after correction have been presented in corresponding tables and supplementary tables in our revised manuscript.

In addition, we only involved two TLR4 SNPs and one MMP9 SNP in this study, which was one of our limitations and we have described that in the discussion part. Further comprehensive studies should be conducted with more functional tag-SNPs, which participate in SNP-SNP interactions between inflammation-related gene and ECM degradation gene pathways.