Author’s response to reviews

Title: Apolipoprotein E Gene Polymorphism and the Risk of Cardiovascular Disease and Type 2 Diabetes

Authors:

Sudong Liu (vanguard_1987@163.com)
JING LIU (13823886749@163.com)
RUIQING WENG (weng1988@126.com)
XIAODONG GU (guxiaodongmz@163.com)
ZHIXIONG ZHONG (zhongzx@aliyun.com)

Version: 1 Date: 08 Jul 2019

Author’s response to reviews:

Point by point response

Dear editors and reviewers,

We are very grateful to receive your comments on our paper and thank you very much for thinking positive of our paper. We have tried our best to revise our paper according to your advices. Furthermore, the language of manuscript has been improved by detailed grammar check and proofread. Every correction we made has been highlighted in RED in the manuscript. Here is a point-by-point response to the questions raised by reviewers.

Reviewer#1

The comments of Reviewer#1 are included in the reviewed manuscript. We have corrected our manuscript according to the comments in our revised version. Here we respond to the comments point-by-point.

1) Comment 1

Respond: According to Reviewer#1’s suggestion, “in Hakka Population” has been removed from the title in our revised manuscript.
Comment 2
Respond: The sentence has been removed in the revised manuscript.

Comment 3
Respond: The sentence has been reconstituted as “Clinical data such as gender, age, diabetes, obesity, hypertension and family history was collected prospectively.”

Comment 4
Respond: The word “incidence” has been replaced by “frequency” as Reviewer#1 suggested.

Comment 5
Respond: The word “CVD” has been replaced by “CAD” as Reviewer#1 suggested.

Comment 6
Respond: Thanks for your suggestion. We decided to remove this sentence in the revised manuscript. Also, we reorganized this part of results in our revised version. Please check these changes.

Comment 7
Respond: Thanks for your kind remind. P-value has been added as footnote under the table.

Reviewer#2
Statistical analysis

1) Authors should inform if data distribution were tested for normality (p values included). Suitable tests must be applied for parametric or non-parametric data.

Respond: Thanks for your suggestion. The normality of data distribution has been assessed before suitable tests were applied. We have rewritten the statistical analysis in the revised version as follow:
“Data were assessed for normality using Kolmogorov–Smirnov test. Continuous data were expressed as mean ± standard deviation (SD) or median ± interquartile range based on the normality of distribution. Groups were compared using Student’s t-test or Mann-Whitney test. Categorical variables were expressed as frequency and compared using Chi-square ($\chi^2$) test or Fisher’s exact test.”

Please check these changes in the revised version.

2) Hardy-Weinberg equilibrium of genotype distribution must be tested.

Respond: Thanks a lot. According to your suggestion, Hardy-Weinberg equilibrium was used to test the genotype distribution of APOE and the results were shown in Table 2 in the revised version.

3) Authors should include the use of blood pressure control medicaments as a correction factor in multivariate regression analysis.

Respond: Thank you for the question. In Table 5, multivariate regression analysis was applied to determine the correction factors of T2DM, CVD or both. We did not include the blood pressure control medicines, because the subjects of the control group were all bearing normal blood pressure and did not take medicines. In the revised version, we have stated our inclusion and exclusion criteria of control group in the Materials and Methods as this: “The control group included 211 subjects with fasting blood glucose < 6.11mmol/L. Exclusion criteria were hyperlipidemia, hypertension, cardiovascular disease or family history of cardiovascular disease, diabetes, malignant tumors, liver and kidney diseases, and autoimmune diseases.”

Thus, we think blood pressure control medicaments might not be a proper correction factor in our analysis. I hope you will agree with us.

4) Authors did not inform if comparisons of biochemical variables between groups had taken into account the use of blood pressure control, cholesterol or glycemic control medicaments.

Respond: Thanks for your suggestion. Either CVD or TM is a complicated and chronic diseases and generally with many other complications. Thus it is not easy to record all of the medicaments for these participants. In our study, we stated in our Method that fasting lipid profiles and glucose were examined the next morning after admission; the blood pressure was examined after they check in the hospital. So, though comparisons of biochemical variables had not taken in the account the use of control medicaments, we have tried our best to reduce the impact that was exerted by medicaments. We completely hope the Reviewer would understand this and agree with us.
Results

1) The term "Baseline characteristics of subjects" may be misleading by suggesting that this is a follow up study. The authors show only patients and control variables which were used in the association analysis.

Respond: We thank Reviewer #2 for reminding. We have modified the subtitle “the characteristics and biochemical variables of the study population”.

2) The indiscriminate use of terms CVD and CAD is confusing.

Respond: We’re sorry that we didn’t make it clear. The subjects in the present study are patients with CVD. We have corrected them in the revised manuscript.

3) Lines 153 to 156: "At the same time, a significant sex difference was found between controls and T2DM patients without CVD, CVD patients without T2DM, T2DM patients with CVD, respectively, which suggested that women are more likely to develop cardiovascular disease or T2DM." This information/suggestion is not supported by data shown in table 1. The only sex ratio difference is found in the control group, but not in any of the patient's group. It is not correct to compare patients sex ration with control sex ratio because control sample was selected by the authors and should had been designed to match a 50:50 male: female proportion.

Respond: Thanks for your valuable suggestion and we completely agree to it. According to your advice, we replaced a part of control samples to satisfy a 50:50 male: female proportion. We reanalyzed the sex ratio in Table 1 and no difference was observed between patients and controls. Correspondingly, we have modified the description in the revised version. Please check it.

4) Line 179 to 182: "In CVD+T2DM patients, the frequency of E3/E3 genotype was lower (p = 0.556), while the frequency of E3/E4 genotype and epsilon 4 allele were higher than that of CVD group (p = 0.070 and 0.124, respectively)." These differences are not significant and the authors should say so.

Respond: Thanks for your suggestion. To make our point clear and correct, we have rewritten this part of results as follow:

“The most frequent genotype is E3/E3 in our study population. In comparison with control group, the frequencies of E3/E3 and Allele ε3 significantly decreased (p < 0.01 and p < 0.01, respectively), while the frequencies of E3/E4 and Allele ε4 significantly increased in CVD patients and CVD+T2DM patients (p < 0.05 and p < 0.01, respectively) (Table 3). Otherwise, no significant difference was observed between DM(+) and DM(-) in the subgroup of CVD(-) and CVD(+).”
Please check these changes in the revised version.

5) Line 182 to 183: "In addition, compare with controls, ε2 allele was significantly lower in T2DM, CVD without patients, T2DM with CVD patients (p = 0.003)" This sentence is difficult to understand and must be rewritten.

Respond: Thanks for your kind advise. As we respond in the Q4, we have rewritten this part of results. Please check these changes in the revised version.

6) Lines 208 to 222: "Relationship between Lipid profiles and ε4 allele" The analysis presented in this topic should take into account the use of cholesterol control drugs.

Respond: Thanks for your suggestion. We have stated in the Method that the fasting lipid profiles were examined the next morning after admission, which meant that most of the subjects enrolled in our study didn’t take cholesterol control drugs. Meanwhile, all of the controls have healthy lipid profiles and didn’t take cholesterol control drugs.

Thus, we didn’t take into account the use of cholesterol control drugs in our analysis. We hope you would understand.

Discussion/Conclusion

1) Lines 225 to 249: Authors present a lot of information that does not really discuss their findings. Some of this information could be in the Introduction section of the article.

Respond: Thanks for your suggestion. According to the Reviewer’s suggestion, some information of AOPE in the Discussion has been moved to Introduction section. Please check these changes in the revised version.

2) I believe there is an error in control sample composition, because authors included age and sex bias by not matching properly controls and patients regarding these variables. Considering this problem, authors should not suggest age and sex as risk factors based on their association analysis.

Respond: Thanks a lot for pointing out this error. We are sorry for the mistake in choosing the controls. In the revised manuscript, we replaced some of the subjects in the control group to meet 50:50 male: female proportion and matched age. Correspondingly, we have reanalyzed the data and renewed the Tables based on the new controls. Please see these changes in revised version.