Author’s response to reviews

Title: The TXA2R rs1131882, P2Y1 rs1371097 and GPⅢa rs2317676 three-loci interactions may increase the risk of carotid stenosis in patients with ischemic stroke

Authors:
Jing Lin (22350277@qq.com)
Qiang Zhou (zhouqiang5613@163.com)
Xingyang Yi (1842942576@qq.com)
Ruyue Huang (zbyhry@163.com)
Zhenxiao Chai (yixingyang64@163.com)

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Author’s response to reviews:

Dear Dr. Dr. Samuel Harris and Dafne Solera,

Thank you very much for your decision letter and advice on our manuscript (MS ID: NURL-D-18-00486R2) entitled “The TXA2R rs1131882, P2Y1 rs1371097 and GPⅢa rs2317676 three-loci interactions may increase the risk of carotid stenosis in Chinese population”. We also thank the reviewers for their constructive and positive comments and suggestions. Accordingly, we have revised the manuscript. All amendments are highlighted in red in the revised manuscript. In addition, our point-by-point responses to the comments are listed below this letter.

We hope that the revision is acceptable for publication in your journal, and we look forward to hearing from you soon.

Sincerely yours,

Dr. Xingyang Yi and Jing Lin

Department of Neurology
Replies to Reviewers’ Comments

First of all, we would like to express our sincere gratitude to the reviewers for their constructive and positive comments.

Replies to Reviewer #1

1. The report by Lin et al. of gene-gene interactions among platelet aggregation and aggregates of platelet-leukocyte loci as it pertains to carotid stenosis is of interest. The methodology is sound and well described. I have only a minor suggestion, which would be to include lack of an independent sample for replication as a limitation in the discussion section.

Response: Thank Dr. Alessandro Biffi for your positive comments and suggestions. We added “Furthermore, lack of an independent sample for replication was also a limitation in this study” in the limitation section (page 16, Line 22, and page 17 Line 1).

Replies to Reviewer #2

1. In the Ethics statement section, the authors declared that "This study protocol was reviewed and approved by the Ethics Committee of the Ethics Committee of the Third Affiliated Hospital of Wenzhou Medical College and the People's Hospital of Deyang City", they should provide the approval certificate and ID number.

Response: Thank you for your suggestion. We uploaded the approval certificate as Supplementary file.

2. In the Study population section, the authors declared that "The diagnosis of IS is based on brain magnetic resonance imaging", this diagnostic criteria may not be suitable, according to the guidelines. What were the detailed diagnostic criteria of IS in this study?
Response: Thank you for your insightful suggestion. We very agreed the description “The diagnosis of IS is based on brain magnetic resonance imaging” was inaccurate. Thus, we added the detailed diagnostic criteria of acute IS in the Study population section of the revised manuscript (page 6, Lines 15-19).

3. In the inclusion criteria, the authors said that "IS in carotid artery territory", it was not clear in this way of saying. How about other arteries? Did they include IS only with carotid artery territory, or both with carotid artery and other arteries territory? There was the same problem in their exclusion criteria, "IS in vertebrobasilar territory".

Response: Thank you for your insightful suggestion. We very agreed the description "IS in carotid artery territory" and “IS in vertebrobasilar territory" were not clear. We revised these in revised manuscript (page 7, Lines 1-4).

4. In the Carotid ultrasonography section, "The patients with 15%-49% carotid stenosis or intracranial arterial stenosis were also excluded in this study", the authors should provide the (reference) link or tell us the reasons. Besides, "The patients with asymptomatic carotid stenosis were excluded in this study", but their exclusion criteria did not include these criteria.

Response: Thank you for your insightful suggestion. According to your suggestions, we added "(5) patients with 15%-49% carotid stenosis or intracranial arterial stenosis or asymptomatic carotid stenosis” in the exclusion criteria section of the revised manuscript (page 7, Lines 4-6). Our current study mainly investigate the association between variants in platelet activation-relevant genes and symptomatic carotid stenosis in IS patients. Also we referred the study of Worrall et al [Ref. #22]. For excluding the effect of 15%-49% carotid stenosis or intracranial arterial stenosis on the results, thus, the patients with 15%-49% carotid stenosis or intracranial arterial stenosis were also excluded in this study. We added these in revised manuscript (page 8, Lines 10-14).

5. What is the basis that choosing 28 randomly selected patients?

Response: Thank you for your insightful suggestion. The 28 patients were randomly selected from enrolled patients with carotid stenosis through the randomization office in our hospital by the Internet. The sentence was added to the Carotid ultrasonography section (page 7, Lines 19-21) in the revised manuscript.
6. The sample size of this study is limited, so how about the power of the SNPs in the BDNF gene which included in this study?

Response: Thank you for your insightful suggestion. According to a suggested sample size requirement for gene-gene interactions [Ref. #23], we calculated that our sample size of 230 patients with symptomatic carotid stenosis and 370 patients without carotid stenosis would provide 80% power at the 5% significance level calculated using three genetic models: the additive model, the dominant model, and the recessive model. We added several sentences to the Statistical analysis section (page 10, Lines 11-15) and Ref. #23 to the references section (page 23) in the revised manuscript.

7. In the methods section, the authors should provide their methods of DNA extraction.

Response: Thank you for your insightful suggestion. Genomic DNA was extracted from peripheral blood using a modified phenol/chloroform method and purified using the UNIQ-10 kit (Sangon Biotech Co., Ltd. Shanghai, China) in this study. The sentence was added to the Genotyping and selection of SNPs section (page 9, Lines 16-18) in the revised manuscript.

8. Please give a brief description of the diagnostic criteria for each high-risk factor. If not described in this article, please comment in table 1.

Response: Thank you for your insightful suggestion. We added the description of the diagnostic criteria for each high-risk factor to the Evaluation of risk factors section (page 9, Lines 1-9) in the revised manuscript.

9. In the result section, "While compared with patients carrying low-risk interactive genotypes of rs1131882, rs1371097 and rs2317676", there is no specific description in this article, Please describe in this article or comment in table 4, table 5.

Response: Thank you for your insightful suggestion. We described what are the high-risk interactive genotypes and low-risk interactive genotypes of rs1131882, rs1371097 and rs2317676 in the result section (page 12, Lines 19-20 and page 13, Lines 1-3), and Table 4 and Table 5 (page 30 and page 31) in the revised manuscript.

10. Table 4 lacks the heading row.
Response: Thank you for your carefulness. According to other studies, it usually use one variant genotypes as the heading row (such as Rs1131882 CC, TT, TT, CT….. in Table 4 in this study) to analyze the associations between different genotype combinations and disease.

11. It is suggested to streamline the results section, some of which can be described in detail in the discussion section.

Response: Thank you for your insightful suggestion. According to your suggestion, we streamlined the results section, some of which were described in the discussion section in the revised manuscript. We added several sentences to the discussion section (page 15, Lines 10-22, and page 16, Lines 1-2), and Ref. #30, Ref. #35 and Ref. #36 to the references section (page 23 and page 24) in the revised manuscript.

12. In the result part, the author's narration is inaccurate, and the detailed description is suggested according to the locus. It should be described in detail the correlation of different SNP genotypes and combination of different SNP. There should be corresponding annotations for specific SNP genotypes under tables and supplementary notes for which no specific description in this paper.

Response: Thank you for your insightful suggestion. According to your suggestion, we revised Table 3, Table 4 and Table 4 (page 29, page 30 and page 30) in the revised manuscript.

13. There were not described whether to use statins, antiplatelet therapy, and their effect on arterial stenosis.

Response: Thank you for your insightful suggestion. There were no significance differences in conventional risk factors and previous drugs treatments (including statins, antiplatelet therapy, antihypertensive drugs hypoglycemic drugs) by univariate analysis (Table 1). One sentence was added to the results selection (page 11, Lines 12-14) in the revised manuscript. Furthermore, multivariate logistic regression analysis was reanalysed to assess risk of carotid stenosis when we added previous statins and antiplatelet therapy as variables. The results showed that previous statins and antiplatelet therapy were not independently effect on risk of carotid stenosis. Accordingly, we revised the Table 6 [page 32] in the revised manuscript.

14. In this study, subjects and controls both are IS patients, so these words, "in Chinese population" in their title, may not be appropriate.
Response: Thank you for your insightful suggestion. The title “The TXA2R rs1131882, P2Y1 rs1371097 and GPⅢa rs2317676 three-loci interactions may increase the risk of carotid stenosis in Chinese population” was changed to “The TXA2R rs1131882, P2Y1 rs1371097 and GPⅢa rs2317676 three-loci interactions may increase the risk of carotid stenosis in patients with ischemic stroke” in the revised manuscript (page 1, Lines 2-3).

However, we do not knew if our the revised manuscript is enough to satisfy the reviewer. If our the revised manuscript have some problems, please let us knew, and give us another chance to re-revise our manuscript.