Author’s response to reviews

Title: Genetic Variants in CYP4F2 were Significantly Correlated with Susceptibility to Ischemic Stroke

Authors:

Yuan Wu (yiyi7229@163.com)
Junjie Zhao (zhaojunjie_2009@126.com)
Yonglin Zhao (zhaoyonglinlin@163.com)
Tingqin Huang (guge88@qq.com)
Xudong Ma (747001758@qq.com)
Honggang Pang (646613359@qq.com)
Ming Zhang (zhangming_361@163.com)

Version: 1 Date: 10 Jul 2019

Author’s response to reviews:

Dear Editor and Reviewers,

On behalf of my co-authors, we thank you very much for giving us an opportunity to revise our manuscript, we appreciate you very much for your positive and constructive comments on our manuscript entitled “Genetic Variants in CYP4F2 were Significantly Correlated with Susceptibility to Ischemic Stroke” (ID: MGTC-D-19-00166R1). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and made correction. We hope meet with approval.

We have carefully read the reviewers’ critical comments and thoughtful suggestions and revised the manuscript point-by-point. Revised portion are marked using the track changes mode in the marked manuscript.

The main corrections in the paper and the responds to the reviewer’s comments are as follows:

Responds to the reviewer’s comments:

1. Abstract
* Line 17, if SNPs has been defined, then can continue to say "SNPs".

Response: We thank the reviewer’s for scrutinize. The error was due to negligence. We have revised the error in the line 8 in page 3 according to the reviewer's suggestion.

2. Introduction

*Is there a reference for the first sentence? (Page 5, line 3)

Response: Thank the reviewer for scrutinize. We have cited the reference in line 26 in page 5.

*Typo on page 5, line 56 with "regulates"; and page 7, line 9 with "The all cases met".

Response: Thank the reviewer for pointing out such a mistake. I'm sorry for the miscalculation in our manuscript. And we have revised the error in line 50 in page 6 and line 79 in page 7 according to the reviewer's suggestion.

3. Methods

Was the any sample QC performed, for example to exclude related individuals, those with mixed ancestry etc. Any efforts to identify possible population stratification?

Response: We thank the reviewer’s for scrutinize. All studied individuals were unrelated and could be traced back at least three generations. We have added this information to the line 90-91 in page 7-8. Meanwhile, we also performed QC for DNA sample by NanoDrop 2000 (Thermo Scientific, Waltham, MA), which have been described in detail in line 102-104 in page 8.

And in the progress of statistics, we conducted the stratified analysis for age and gender to further explore the relationship between CYP4F2 SNPs and IS risk, which may contribute to the risk assessment, early diagnosis and prevention of IS.

4. Discussion

(1) The rs2108622 SNP that has been previously reported, is this in LD with any of the SNPs in this current study?

Response: Thanks for the reviewer’s scrutinize. The rs2108622 in CYP4F2 gene used in this article was only to demonstrate that CYP4F2 gene polymorphisms were associated with the risk of IS. Our aimed to explore other sites in this gene whether were associated with IS in the population or not, providing valuable insights into the pathogenesis of potential prevention and treatment strategies for IS.
(2) Is there any further work that could be done to support the claims made in this study? Any plans to replicate the results within an independent sample?

Response: Thanks for the reviewer’s scrutinize. We also planned to verify this result from the expression level. But our sample size is not very large, obtaining credible genotype-based mRNA expression results is difficult to a large extent. But, we have been continuing to collect samples to perform genotype-based mRNA expression analysis and further explore the potential role of the selected polymorphisms by methods of cell biology and molecular biology. Ultimately, we hope our subsequent study could lay a good foundation for the treatment and drug development of IS.

(3) The sample size is small, but could these variants be looked up within a larger independent Chinese Han population and a meta-analysis be formed?

Response: Thanks for the reviewer’s scrutinize. The sample size was relatively small compared with the Genome-wide association analysis (GWAS). However, our sample has met the requirements of statistics. In the process of data analysis, we used the analytical method often used in this kind of research, such as Pearson’s chi-square, so our results were credible.

In the early stage of our experiment, the relationship between CYP4F2 gene and IS risk was analyzed in the Han Chinese population. Moreover, the subjects were all Shaanxi Han population, so the results may not be applicable to other ethnic groups. Therefore, a larger and more diverse population is needed to verify our experiment. What’s more, more well-designed experiments are needed to further explore the pathogenesis of CYP4F2 in the occurrence and development of IS in future.

(4) Is there any functional evidence that could be found for the SNPs investigated to support the role they play in disease susceptibility?

Response: Thanks for the reviewer’s scrutinize. Genetic predisposition has been suggested to significantly participate in the pathogenesis of IS. Genes encoding proteins involved in lipid metabolism, thrombosis, atherosclerosis and others are thought to be potential genetic factors for stroke. Duanxiu Liao et al [1] found the 20-HETE level of IS patients was significantly higher compared with that of the controls, and genotype ‘G/G’ of rs9333025 and genotype ‘G/G’ of rs2108622 can increase capability to metabolize AA, resulting in increased 20-HETE production. 20-HETE constricts cerebral arteries and regulates cerebral vascular tone by activating intracellular signaling pathways, including protein kinase C (PKC), which are involved in apoptosis and cell death[2]. 20-HETE also depolarizes vascular smooth muscle cells through inhibition of the largeconductance Ca2+-sensitive K+ channel and Na+, K+-ATPase activity together with an increase in Ca2+ influx through Ltype Ca2+ channels [3]. These showed some functional evidence that could be found for the SNPs investigated to support the role they play in IS susceptibility.
Reference


(5) Any bioinformatic work that could be done to suggest the functional implications (if any) of these variants. I think some more detail could be given to provide evidence of this variants and gene region having a role in IS. How does this study compare with other stroke studies that have been performed in the same population? For example by Lee et al, 2017 (https://www.nature.com/articles/s41598-017-14355-3).

Response: Thanks for the reviewer’s scrutinize. We have added the bioinformatic work in line 128-129 in page 9 and line 195-201 in page 12-13. In addition, we compared our results with other stroke studies that have been performed in the same population. The detailed description could be found in line 250-259 in page 15.

Responds to the editor’s comments:

1. Rationale of selecting the SNPs of interest should be stated, instead of ‘randomly selected’ - functional variant? Strong LD? Previously reported?

Response: Thank the editor for raising this kind issue, and we have revised the description in line 64 in page 6. Many candidate SNPs in the CYP4F2 gene were selected with minor allele frequencies > 5% in 1,000 genome project (http://www.internationalgenome.org/). In addition, the genotype distributions of the SNPs in control group were in accordance with Hardy-Weinberg equilibrium (HWE) (p > 0.001). Then, the Haploview software package (version 4.2) was used to estimate pairwise linkage disequilibrium (LD) at CYP4F2 gene polymorphisms. When r2 (the measure value of LD) > 0.8, the SNP can represent all the polymorphisms in a block. According to the above selection criteria, we selected the five SNP (rs3093203, rs3093193, rs12459936, rs3093144, and rs3093110) in CYP4F2 gene as the gene variation to the study. And we have also revised the detailed description in line 93-98 in page 8.
2. The role of CYP4F2 in IS pathogenesis could have been elaborated in Introduction rather than in the Discussion.

Response: We thank the editor for raising this kind issue. We have carefully revised the introduction and discussion section. In the introduction, we used some reports to illustrate the importance of CYP4F2 in IS. In our discussion, we combined our results and more previous articles to elaborate on the role of this gene in IS.

3. The authors carried out the haplotype analysis based on all the 5 SNPs studied. Since they found 3 SNPs that were significantly associated with IS, why not try to take a look at the haplotype analysis based on the 3 SNPs?

Response: We thank the editor for raising this kind issue. According to your suggestions, we have added the haplotype analysis based on the 3 positive SNPs (rs3093193, rs12459936 and rs3093144) in Table 6B using the SNPStats (https://www.snpstats.net/start.htm?q=snpstats/start.htm). And we have described the results carefully in line 191-194 in page 12. Moreover, we have discussed this portion in line 242-249 in page 14-15.

4. Table 2: clinical examinations @ parameters used to diagnose IS should be shown.

Response: We thank the editor’s for raising this kind issue. We have added clinical examination used to diagnose IS in Table 2. And we have also described the information in line 137-140 in page 10.

5. Table 3 & 4 could be listed as supplementary material.

Response: We thank the editor for raising this kind issue. In this case-control study, we firstly conducted the overall association analysis between the selected SNPs and the susceptibility to IS in the allele and multiple genotype models, and then conducted another stratified analysis of age and gender. Finally, the linkage disequilibrium and haplotype analyses of the CYP4F2 polymorphisms in the cases and controls were further studied. Table 3 & 4 reflected that the association between the selected SNPs and the susceptibility to IS on the whole, which was the main result in this research. Therefore, we think it is better to put table 3 & 4 in the main tables instead of the supplementary material.

6. Grammatical and typos were spotted throughout the text. Suggest to send the manuscript for proofreading and language checking before resubmitting.

Response: We thank the editor for raising this kind require. We have revised the article according to your suggestion.
We appreciate for Editors/Reviewers’ warm work earnestly, the reviewer’s comments are quite helpful. We revised our paper point-by-point and grammatical problems. Finally, we hope that the correction will meet with approval. Looking forward to hearing from you.

Thank you and best regards!

Yours sincerely,

Ming Zhang,