Author’s response to reviews

Title: rs1234313 and rs45454293 are risk factors of cerebral arterial thrombosis, large artery atherosclerosis, and carotid plaque in the Han Chinese population: a case-control study

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Author’s response to reviews:

Response letter:

Editor Comments:

1. The authors should explain the mechanism underlying the relationship between several SNPS and the risk of atherosclerosis. Why this study was of great significance?

Re: per your suggestions, the “Several SNPs have been reported to regulate cardiovascular diseases [15]. SNPs or rare mutation can directly mediate the expression of atherosclerosis-related genes, which further participate in the regulation of inflammatory responses.” was added in line 96-99.

The “, which can be used as potential targets in the screening of patients with ischemic stroke.” was added in line 108-109.

2. The authors should explain the strengths and limitations of this study

Re: per your suggestions, sentence “To date, there is no report on the SNPs of TNFSF4 in Chinese patients with ischemia stroke. Our study is the first that demonstrates the two TNFSF4 SNPs in specific genetic model might be associated with the risk of calcification and thick plaque of carotid plaque.” was added in line 328-331;
The limitations were stated in discussion section in line 347-355. From “However, there are some limitations of this study. ---” To “--- echolucent core and thin fibrous cap.”

3. The authors should explain the different findings of this study compared with prior studies, such as both in different race, different area, lifestyle.

Re: Yes, we already compared with prior studies. such as following sentences:

“These results were consistent with the study by Gardener et al. [17], which indicates that rs1234313 was associated with the carotid plaque phenotypes in stroke patients. We also investigate the association between the calcification of carotid plaque and atherosclerotic cerebral infarction, which is not included in the study by Huang et al [16].” in line 304-308.

sentence “Studies have shown that TNFSF4 rs2205960 and rs844648 SNPs are associated with the susceptibility to systemic sclerosis [24]. Meanwhile, a meta-analysis study demonstrates that TNFSF4 rs2205960 SNP may confer susceptibility to SLE (systemic lupus erythematosus) in different populations and that the TNFSF4 rs1234315 SNP is associated with the susceptibility to SLE in Asian [25] and Malaysian populations [26]. Lian et al., [27] have demonstrate that rs844648 and rs704840 SNPs of TNFFS4 are associated with an increased risk of NMOSD (Neuromyelitis optica spectrum disorders) in different genetic models” in line 320-327.

the differences in line 328-331 “To date, there is no report on the SNPs of TNFSF4 in Chinese patients with ischemia stroke. Our study is the first that demonstrates the two TNFSF4 SNPs in specific genetic model might be associated with the risk of calcification and thick plaque of carotid plaque.” was added.

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Reviewer reports:
Jun Young Chang (Reviewer 1): The authors intended to evaluate the association of TNFSF4 SNP (rs1kj234313 and rs45454293) and the risk of atherosclerosis by conducting prospective case control study. The surrogate markers for atherosclerosis in this study are calcification, thickness, and multiplicity of the plaque. Prior studies could not find any association between TNFSF4 polymorphism and ischemic stroke.

4. According to the study, the genotype and allele frequencies of TNFSF4 SNP (rs1234313 and rs45454293) seemed to be not significantly different between the case and control groups. The association of TNFSF4 polymorphisms (rs1234313 and rs45454293) and carotid plaque features were compared only within the case group. The association of TNFSF4 polymorphisms (rs1234313 and rs45454293) and carotid plaque features between the case and healthy control needs be mentioned first. Also a cut off level of statistical significance needs to be corrected by multiple comparisons.

Re: In this study we used genetic models to compare “the association of TNFSF4 polymorphisms (rs1234313 and rs45454293) and carotid plaque features” between the cases and the controls. We also calculated the OR values. Our focus is the relative risk of the SNPs, therefore, we did not perform multiple comparisons.

5. The definition of calcified plaque (signal with strong echo) is ambiguous and needs to be clarified. The presence of carotid plaque calcification only does not represent a plaque vulnerability. Rather than calcification, plaque features including irregular, ulcerative surface, echolucent core and thin fibrous cap, are associated with future ischemic stroke events. The association between TNFSF4 polymorphisms and vulnerable plaque features seems more clinically meaningful.

Re: We also analyzed the irregularity of the plaque and added the following information. In the line 136-138 “Atherosclerotic plaque was defined as the ratio of the thickness of blood vessel linings to the peripheral vascular wall greater than 50% in common carotid artery, internal carotid artery and bifurcation” was added. In line 140-141 “The irregularity of the sclerosis plaques was analyzed.” was added.

The sentence “If we had more samples, the allelic and dominant genetic models of rs1234313 might show statistical significance.” was added in line 353-355.
We didn’t collect other information, such as ulcerative surface, echolucent core and thin fibrous cap. In line 353-355 “other features of ischemic stroke were not analyzed and classified except of carotid plaque” was replaced with “the association between ischemic stroke and other features of carotid plaques were not analyzed. In our future study we will include other features, such as ulcerative surface, echolucent core and thin fibrous cap”. Thanks.

6. The definition of thick plaque (maximal carotid plaque thickness>1.9mm) is somewhat arbitrary. Sensitivity analysis using another index of atherosclerotic plaque burden including total plaque area calculated by the sum of areas of all plaques seen, or plaque thickness as a continuous scale needs to be performed to clarify the association of rs45454293 SNP and subsequent risk of ischemic stroke.

Re: The definition of thick plaque was published previously (Rundek T AH, Boden-Albala B, Elkind MS, Paik MC, Sacco RL: Carotid plaque, a subclinical precursor of vascular events: the Northern Manhattan Study. Neurology 2008, 70(14):1200-1207.), so we’ve used the same definition according to this article.

7. According to the methods, the authors matched the control using age and gender, but table 1 showed significant differences in age and gender between the case and control. The reason of the imbalance needs to be explained.

Re: per your suggestions, “The differences in age, gender and smoking status between the patients and the controls imply that these factors might be risk factors for ischemic stroke. Therefore, we included these factors as variables in logistic analysis.” was added in line 282-285.

8. Regarding the Inclusion criteria in this study, enrollment period, definition of acute ischemic stroke including acute period, confirmation of ischemic stroke by MR needs to be mentioned. The reason why patients with atrial fibrillation were excluded is not clear. If the authors intended to include the acute ischemic stroke with large artery atherosclerosis, the criteria also include that point. Dysfunction of heart/lung/kidney needs to be clarified more clearly and the reason for exclusion should also be explained.

Re: Yes, “No time limits on the duration between the onset and diagnosis;” was added in line 116.

Enrollment period was indicated on line 115 (between Oct. 2016 and Apr. 2017).
“atrial fibrillation” was deleted from the exclusion criteria 120.

We analyzed LAA sub-types and the results are statistically significant.

We deleted “Dysfunction of heart/lung/kidney” in line 120.

9. There was a substantial loss of case subjects due to absence of basic information. The characteristics of the patients who did not included in the analysis needs to be mentioned also.

Re: We could not retrieve the data on the characteristics of the patients who were not included in this analysis.

“We analyzed the risk factors of ischemic stroke in all genetic models with or without concomitant variables (age, gender and smoking status). Therefore, our analysis reflects the association between TNFSF4 polymorphism and stroke.” was added in the line 282-288.

Shuna Yang, M.D. (Reviewer 2): The authors aimed to investigate the association between TNFSF4 single nucleotide polymorphisms (SNPs) and the risk of cerebral arterial thrombosis, which is of interest and important. But I have several questions.

10. why patients underwent thrombolysis treatment were excluded? which will increase the chosen bias. The authors chose acute ischemic stroke patients as case group. As we all know, the genotype will not changed even if the ischemic stroke patients underwent thrombolysis treatment.

Re: The use of chemotherapy will affect the gene (TNFSF4) expression, hence, we collected patients’ samples before they received any treatments. We have added the statements in the discussion section.

in line 331-333 “In addition, we have excluded the patients who had received any type of treatments. Therefore, our findings can be used for predicting the risk of disease onset in patients without treatments.” Was added.
11. Did the authors classify patients according to TOAST classification? Maybe the correlation was more strong in LAA stroke patients.

Re: We have added the analysis of LAA.

“TOAST classification

According to the TOAST classification methods [21] [22], the LAA (large artery atherosclerosis) and SVD (small vessel disease) subtypes were analyzed. The diagnostic criteria were as follows: 1) LAA: carotid artery occlusion or stenosis was 50% greater than the arterial cross section by carotid artery ultrasound; the lesion of cerebellum or cerebral cortex, or cortical involvement/subcortical infarction was greater than 1.5 cm by MRI. 2) SVD: the maximal diameter of the stroke lesion was smaller than 1.5 cm by MRI; atypical lacunar infarction existed in clinic samples, but was not detected by MRI.” was added in line 145-153.

“LAA and SVD subtypes analysis

There were 138 patients with LAA and 97 patients with SVD. We found that rs1234313 was greatly associated with the risk of both LAA and SVD in all genetic models (Table 6), indicating that allele G, GG and GA might be protective compared to allele A and AA genotypes. We also found that rs45454293 was greatly associated with both LAA and SVD only in the allelic model (G vs A) (Table 6). When compare to allele A, the allele G was a risk factor in LAA and a protective factor in SVD.” was added in line 270-276.

And table 6 was added.

“We discovered that all the genetic models including allelic, dominant and genotypic models of rs1234313 were greatly associated with LAA and SVD, suggesting that allele G, GG or GA might reduce the risk of LAA and SVD, compared to A and AA genotypes. In addition, rs45454293 had a reverse effect on the risk in LAA and SVD in the allelic models (G vs A). These results indicate the different mechanisms between LAA and SVD. Meanwhile, the ischemic stroke might be regulated by distinct SNPs of the TNFSF4 gene. No study had reported the relationship between SNPs of TNFSF4 and LAA or SVD subtypes of ischemic stroke. Our study provides an important direction for a better understanding of the mechanisms of stroke.” Was added in line 290-298.
“However, we found that rs1234313 has a significant correlation with the LAA and SVD of stroke in all genotypic models; rs45454293 had a significant correlation with the LAA and SVD of stroke in allelic and genotypic models. In addition,” was added in line 363-365.

“large artery atherosclerosis and” was added in line 1-2.

“Neck ultrasonography and magnetic resonance imaging (MRI) were used in all patients to detect large artery atherosclerosis (LAA) and small vessel disease (SVD) of stroke, as well as the thickness and calcification of carotid artery.” Was added in the line 41-44.

In the line 49-54 “rs1234313 SNP has a significant correlation with the calcification of carotid plaque in dominant (GG/GA vs AA, p=0.022) and genotypic (GA vs AA, p=0.01) models. rs45454293 SNP has a significant correlation with the thick plaque of carotid plaque in allelic (G vs A, p=0.01) model analysis.” Was replaced with “rs1234313 SNP had a significant correlation with the LAA and SVD subtypes in allelic (G vs A), dominate (GG/GA vs AA) and genotypic (GA vs AA; GG vs AA) models, as well as with the calcification of carotid plaque in dominant (GG/GA vs AA, p=0.022) and genotypic (GA vs AA, p=0.01) models. rs45454293 SNP had a significant correlation with the LAA and SVD subtypes in allelic (G vs A) and genotypic models, as well as with the thick carotid plaque in allelic (G vs A, p=0.01) model.”.

12. The manuscript need some language editing.

Re: the manuscript was edited by a PHD in the USA. Thank you.