Author’s response to reviews

Title: A functional polymorphism in the promoter of miR-17-92 cluster is associated with decreased risk of ischemic stroke

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Author’s response to reviews:

Dear editor,

Thank you very much for your letter on 23 May 2019 enclosing the reviewer’s comments on our manuscript entitled “A functional polymorphism in the promoter of miR-17-92 cluster is associated with decreased risk of ischemic stroke” (MGNM-D-18-00369). We have revised the manuscript according to comments of editor and reviewer, and the amendments we made have been highlighted in red and were commented in the revised manuscript. Point by point responses to editor and reviewers’s comments are listed below. We hope that the revised version of the manuscript is now acceptable for publication in BMC Medical Genomics. We are looking forward to hearing from you soon.

Best wishes,
Yours sincerely,

Huatuo Huang; Guijiang Wei; Chunfang Wang; Yulan Lu; Chunhong Liu; Rong Wang; Xiang Shi; Jun Yang; Yesheng Wei

Answer to Editor’s Comments:

Major comments

1. There have been several large scale GWAS conducted for ischemic stroke in diverse populations (https://www.ebi.ac.uk/gwas/home). GWAS summary statistics may be available for some studies. I would suggest the authors to compare the findings from this study with other large-scale studies with GWAS summary statistics available in the same or different ancestry.

Response to the comment: Dear editor, thanks for your kind suggestion. We though it is a good suggestion which may help to improve our manuscript. We tried to visit the website you provided many times, but it was inaccessible when we tried to get some information from the search engine. So, we are sorry we can’t make the comparison at this time.

2. While miR-19a-3p has lower expression in IS patients than controls (i.e. higher expression is protective), rs9301654 G allele is protective for IS and yet G allele carriers have lower expression of miR-19a-3p in IS patients. These results seem contradictory. Also it is unclear the relationship between genotypes and gene expression is causal or consequence in patients. How is the association of rs9301654 genotypes and gene expression of miR-19a-3p in controls?

Response to the comment: Dear editor, thanks for your suggestion. The association of rs9301654 genotypes and gene expression of miR-19a-3p in controls has been analyzed and added in the manuscript. We found that controls with rs9301654GA or GG genotype also have lower level of miR-19a-3p compared with the AA genotype, however, the different was not significant (P=0.079). We thought there may be some explanations for this phenomenon. As it is known to all, the expression of a certain gene can be regulated by polymorphism in promoter of the gene. At the same time, polymorphism in the promoter of a gene is not the only factor that could affect gene expression. A large number of previous studies have provided us evidences. Protein encoding gene such as placental growth factor, transcription factor E2F1 and endostatin[33-35], and long noncoding RNA (IncRNA) such as IncRNA MEG3 and LncRNA H19[36, 37], and short hairpin RNA (shRNA) such as adenine nucleotide translocase 2 shRNA[38], have been reported to associated with abnormal expression of miR-19a in disease progression. Under normal circumstances, the normal expression of these genes does not affect the expression of miR-19a. However, during some pathologic process, abnormal expression of these genes may further down-regulate the expression of miR-19a based on the down regulatory effect of the
rs9301654 polymorphism. Hence, a gene-gene interaction analysis will better reveal the roles of miR-19a and its regulatory genes in the etiology of IS in the near future.

3. Discussion “This finding indicated that rs9301654 polymorphism in promoter of the miR-17-92 cluster may exert part of its protective role by decreasing the transcriptional activity of miR-19a-3p.” This sentence is in contradiction to the finding as mentioned above. A higher miR-19a-3p expression should be protective instead according to Fig 2. In general, it is incorrect to state whether a polymorphism is at risk or protective, the genotype that confer increase or decrease risk should be specified instead.

Response to the comment: Dear editor, thanks for your suggestion. Data from several previous publications have showed that miR-19a is a risk factor for ischemic stroke, and the expression of miR-19a was decreased according to their report. Our finding was in agreement with these previous reports. We thought there may be an explanation for the “contradiction” in Fig 2. As we know that ischemic stroke is a multifactor epidemic disease, and a large number of risk and protect factors for the disease have been found, such as inflammatory cytokines and ischemic stroke related microRNAs. However, a single risk or protect factor may not enough for causing or protecting ischemic stroke. As to the mistake on the conclusion which we state about the rs9301654 polymorphism, corresponding modification has been made and mark in red in the manuscript.

Minor comments

1. Table 4. There should be typo in the HapMap population. CEU & MXL are missing but HCB & MEX are added instead.

Response to the comment: Dear editor, thanks for your reminding. We have checked the manuscript once again carefully, and corresponding changes have been made and marked in red in the manuscript.

2. Table 5. The order of 3 SNPs contributing to the haplotypes should be specified. Usually the most common haplotype should be used as reference to calculate odds ratio, or one haplotype is compared to all other haplotypes combined. The method need to be clarified.

Response to the comment:

Dear editor, haplotype calculation in the present study was carried out by online SHEsis software (http://analysis.bio-x.cn/myAnalysis.php), this is a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci,
and the paper they published has been cited more than one thousand times (Shi YY, He L: SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell research 2005, 15(2):97-98).

However, we have made corresponding changes according to your suggestion, and the changes we made has been marked in red in the manuscript. Thanks for your suggestion.

3. Fig 1. Rs1491034 & rs982873 has r2=0.17. This is considered as low LD for association study.

Response to the comment: Dear editor, thanks for your kindly reminding. Corresponding changes has been made and marked.

Answer to Reviewer’s Comments:

Major concerns:

1. The study is not based on any power calculation to assess whether sufficient power could be expected for the different SNPs with 398 cases and 397 controls for such a common disease. A power calculation should be attempted to discuss what minimal sample size would have been required for each of the candidate SNPs.

Response to the comment: Dear reviewer, thanks for your kindly suggestion. Power calculation of the three polymorphisms have been performed and added to the manuscript. The changes we made have been marked in red.

2. There is no measure of quality control (e.g. DNA samples quality, genotyping). The authors should provide more information about the quality of genotyping and phenotype.

Response to the comment: Dear reviewer, genotyping method has been supplemented and marked in red. Thank you for your kind suggestion.

3. The statistical analyses are rather simplistic, yet some important information is missing:

a) How haplotypes were inferred.

Response to the comment: Dear reviewer, haplotype calculation in the present study was carried out by online SHEsis software (http://analysis.bio-x.cn/myAnalysis.php), this is a powerful
software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci, and the paper they published has been cited more than one thousand times (Shi YY, He L: SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell research 2005, 15(2):97-98).

b) The study focuses on dominant and recessive analyses, but the other genetic models should be better presented.

Response to the comment: Dear reviewer, thanks for your kindly suggestion. To our knowledge, in most of the genetic association studies usually will focuses on codominant, dominant, recessive, allele and haplotype analyses, and these analyses have been presented in our manuscript.

4. The cases and controls do not seem to differ in gender and age. Yet they differ significantly in diabetes, hypertension, TG, LDL-C, HDL-C, TCH. While some analyses seem to be adjusted for above items, this merits to be discussed further and how such ascertainment bias influence the results.

Response to the comment: Dear reviewer, thanks for your kindly suggestion. To our knowledge, it quite a common phenomenon to adjust the genetic association analyze by risk factors such as diabetes, hypertension, TG, which is of great important to reduce the influence of these risk factors on the analyze. However, it seems that they have not discussed how the adjustment on results.

5. Any information about life style status (e.g. smoking, alcohol consumption) remains unknown. It would need to be documented and discussed further.

Response to the comment: Dear reviewer, thanks for your kindly suggestion. We are sorry that when life style status were collected from medical record review of our hospital, information such as smoking and alcohol of several participant were missing. Therefore, we have no included these data no this statistical analysis.

6. The authors should address a multiple comparison problem.
Response to the comment: Dear reviewer, thanks for your kind suggestion. Multiple comparison has been performed in table 2, and the method we chose is Benjamin-Hochberg (B-H) method. If there are still any problem in this part, please feel free to contact us.

7. Manuscript requires grammatical review.

Response to the comment: Dear reviewer, grammatical review has been performed and the manuscript has been carefully revised by the help of someone who is good at English. If there are still any problem on grammatical, please feel free to contact us.

Minor concerns

1. When using abbreviations, write the full name of the abbreviated item followed by the abbreviation in parentheses at the point of first mention within the body of the manuscript. However in subsequent statement, authors should use abbreviations and unify the notation.

Response to the comment: Dear reviewer, we have checked the manuscript once again carefully, and abbreviations have been carefully revised and unified in the revised manuscript. Thank you very much for your reminding.

2. Please provide complete and appropriate footnotes below all tables (Table 3).

Response to the comment: Dear reviewer, footnotes have been completed and appropriated in this revise version. Thank you very much for your reminding.

3. Please insert page number.

Response to the comment: Dear reviewer, page number has now been inserted. Thank you very much for your reminding.

4. Recessive model should be "GG vs. GA+AA" et al in the Table 2. Table 5 Haplotype analysis of the four polymorphisms with risk of ischemic stroke should be "three". Figure 1 r2 = 17 should be r2 =0.17.

Response to the comment: Dear reviewer, the problem mentioned above have been carefully revised. Thank you very much for your reminding.
5. How many samples were used for the expression analysis in Figure 2 and 3?

Response to the comment: Dear reviewer, samples number have been added to the foot note of Figure 2 and 3, there are 60 and 60 case in cases and controls respectively.