Author's response to reviews

Title: Association of rs6982567 near GDF6 with neovascular age-related macular degeneration and polypoidal choroidal vasculopathy in a Han Chinese cohort

Authors:

Yuying Ji (jillea@163.com)
Xiongze Zhang (xiongze.zhang@gmail.com)
Kunfang Wu (kf.1012.k@163.com)
Yu Su (sy_daisy1206@qq.com)
Meng Li (drlimeng@163.com)
Chengguo Zuo (chengguozuo@163.com)
Feng Wen (wenfeng208@foxmail.com)

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Author's response to reviews: see over
Dear Erica Cruz:

Thank you for your letter on September 20, 2014, regarding the manuscript we submitted for publication, entitled “Association of rs6982567 near GDF6 with neovascular age-related macular degeneration and polypoidal choroidal vasculopathy in a Han Chinese cohort.” We are truly grateful for your and the other reviewers’ critical comments on and the thoughtful suggestions for our manuscript, and we have found them very helpful. Based on those comments and suggestions, we have made careful modifications to the original manuscript in content, format, grammar and spelling. The revised parts are marked in the article. Below, you will find our point-by-point responses to the reviewers' comments/questions.

We hope that these revisions are satisfactory and that the revised version will be acceptable for publication in BMC Ophthalmology.

Thank you very much for your work on our paper.

Sincerely,

Feng Wen, M.D., Ph.D.,
State Key Laboratory of Ophthalmology,
Zhongshan Ophthalmic Center,
Sun Yat-sen University
54 South Xianlie Road, Guangzhou, 510060, China
Phone: +86 20 87330292
E-mail: wenfeng208@foxmail.com
Responses to reviewers (MS ID:1208152472136917)

For reviewer 1:
Reviewer's report:
To the Editor and Authors,
Age related macular degeneration (AMD) is complex genetic disorder and is the third leading cause of blindness among worldwide. Genetic replication studies are gold standard for understanding the genetic etiology of the disease. In this paper Ji Y et al., provided an independent validation of the association of the rs6982567 near \textit{GDF6} with neovascular age related macular degeneration (nAMD) and polypoidal vasculopathy (PCV). Previous study by Zhang et al., (2012) has shown association of rs6982567 near \textit{GDF6} in AMD subjects. In this manuscript, Ji Y et al categorized subjects into nAMD and PCV. Since, AMD is a complex genetic disease; it is not surprising that this SNP was not associated with the disease.

1. Major Compulsory Revisions:
I do not see any major compulsory revisions

2. Minor Essential Revisions:
There are many spelling and spacing problems were present in entire manuscript and these needs to be corrected (line 65, 99, 109, 110, 119, 128 and 180)

Answer: Many thanks for the reviewer’s suggestion. These problems have been corrected. Furthermore, we have sent our paper to a professional English editing service, American Journal Experts.

3. Discretionary Revisions:
We cannot conclude lack of association of \textit{GDF6} by screening single polymorphism. If we want understand its role one should screen entire gene.

Answer: Many thanks for the reviewer’s suggestion. This was a limitation of our study. We had mentioned it in the Discussion and Conclusions section. A complete survey of the whole target SNPs in or near the \textit{GDF6} gene in a larger sample size would be needed to explore further the potential association between \textit{GDF6} and PCV.

Abstract (lines 44-45):” A complete survey of the GDF6 locus with a larger sample size is needed in future studies.”

Discussion (lines 207-212):” There were also some limitations of our study. First, we conducted it in a relatively small sample, so we could not fully identify the association between rs6982567 and PCV. Second, we only investigated a single SNP near the GDF6 gene in this study. So far, other SNPs in GDF6 locus and linkage disequilibrium (LD) analysis with this gene have yet not been completely investigated. Therefore, a complete survey of the whole target SNP in LD with the GDF6 gene with a larger sample size would be needed.”

Conclusions (lines 216-217):” Expanding the sample size and exploring the whole target SNPs in or near GDF6 gene are needed in future studies.”
For reviewer 2:
Reviewer's report:
1. The manuscript needs to be reviewed and edited by an English (as a first language) technical writer to resolve several grammatical and wording errors throughout the manuscript.
Answer: Many thanks for the reviewer's suggestion. We have already sent our paper to a professional English editing service, American Journal Experts. http://www.journalexperts.com.

2. Please discuss quality control of SNP genotyping - how many samples were genotyped and how many failed? What was the genotyping efficiency rate of the SNP?
Answer: Many thanks for the reviewer's comments. For the quality assurance of our samples, the genotyping call rates were 100% for rs6958967 in both the patient and control groups. To confirm the accuracy of the SNaPshot method, randomly selected subjects (10% of all samples) were analysed by direct sequencing. The consensus rate in the replicated samples (n=61) was 100%. We have added these data to the Results section of the revised paper.

Results (lines 132-136): "For the quality assurance of our samples, the genotyping call rates were 100% for rs6958967 in both the patient and control groups. The genotypes were determined using the SNaPshot method successfully in all of the subjects. To confirm the accuracy of the SNaPshot method, randomly selected subjects (10% of all samples) were analysed by direct sequencing. The consensus rate in the replicate samples (n=61) was 100%.”

3. What program was used to calculate power for the study?
Answer: G*Power software version 3.1.10 [Faul 2007], was used to evaluated the post-hoc power. It is mentioned in the Statistics part of Methods section (lines 124-125). This program has also been used in other SNP studies [Korolija 2010, Wu 2012, Su 2013].

Reference:

4. Please report the frequency of the SNP in HapMap, Exome Variant Server, and/or 1000 Genomes data - this will help the argument about whether the SNP allele frequencies are different across ethnicities.
Answer: Many thanks for the reviewer’s suggestion. In HapMap, the allele T frequencies of rs6982567 are 0.168 in Utah residents with Northern and Western European ancestry, 0.279 in Han Chinese people and 0.218 in Japanese people, exhibiting differences across ethnicities. No data on rs6982567 were found in the database of the Exome Variant Server or in the 1000 Genomes data. These frequencies help to explain the different results between our study and Zhang’s study in 2012. We have reported these data in the Discussion section. Thanks again.

Discussion (lines 202-206):" In the HapMap project, the allele frequencies of rs6982567 were 0.168 in Utah residents with Northern and Western European ancestry, 0.279 in Han Chinese people and 0.218 in Japanese people, exhibiting differences across ethnicities. These differences could be another explanation for the different outcomes."

5. What covariates were used in statistical analyses?
Answer: Many thanks for the reviewer’s comment. The characteristic factors in our study were not shown to be associated with the outcome, so we did not use any covariates in the statistical analyses.

6. Are there any additional SNPs in LD with this gene that could be genotyped to further explore the potential association in this sample?
Answer: Many thanks for the reviewer’s comment. Zhang et al (J Biol Chem, 2012) investigated the single SNP rs6982567 and found that it was significantly associated with AMD in Caucasians. They also demonstrated that rs6982567 was associated with decreased expression of GDF6 and increased expression of HTRA1. In this study, we performed this single SNP genotyping in an independent cohort of Chinese nAMD and PCV patients, and we found a possible weak association between this SNP and PCV. The difference in results might be due to the studied populations and sample sizes. So far, other SNPs in the GDF6 locus and linkage disequilibrium analysis with this gene have not yet been completely investigated. Therefore, a complete survey of the whole target SNPs in LD with the GDF6 gene in a larger sample size would be needed. We will investigate additional tagger SNPs in the GDF6 locus in a future study. Thanks again.

Abstract (lines 44-45):" A complete survey of the GDF6 locus with a larger sample size is needed in future studies."

Discussion (lines 207-212):" There were also some limitations of our study. First, we conducted it in a relatively small sample, so we could not fully identify the association between rs6982567 and PCV. Second, we only investigated a single SNP near the GDF6 gene in this study. So far, other SNPs in GDF6 locus and linkage disequilibrium (LD) analysis with this gene have yet not been completely investigated. Therefore, a complete survey of the whole target SNP in LD with the GDF6 gene with a larger sample size would be needed."

Conclusions (lines 216-217):" Expanding the sample size and exploring the whole target SNPs in or near GDF6 gene are needed in future studies."

Minor Essential Revisions
1. It would be beneficial to know the P-values for association when comparing controls to the nAMD and PCV combined (which would more accurately reflect the original analysis
Many thanks for the reviewer’s suggestion. We have revised the association analysis accordingly. The results showed a marginal association under a recessive model between this SNP and the combined group (p=0.0487), but could not withstand the Bonferroni correction. This marginal association between this SNP and the combined group might be attributed to that between this SNP and the PCV group. We have added these data to the Abstract, Result, Discussion section. Table 2 and Table 3 were revised accordingly. Also, we have modified the p value of the Bonferroni correction.

Abstract (lines 35-36): “The allele frequencies of rs6982567 were not significantly associated with nAMD, PCV or PCV and nAMD combined.”

Methods-Statistics (lines 122-124):” Bonferroni’s method was performed to correct the p values in multiple comparisons, with a p value <0.0056 (0.05÷3÷3) considered to be statistically significant.”

Results (lines 138-143):” The allele frequencies of rs6982567 were not significantly associated with nAMD, PCV or PCV and nAMD combined. The frequencies of the T allele were 29.12% for PCV, 25.48% for nAMD, and 27.94% for PCV and nAMD combined. The OR for the risk allele T of rs6982567 was 1.29 for PCV (95% CI, 0.97-1.73, p=0.0832), 0.91 for nAMD (95% CI, 0.65-1.28, p=0.6029), and 1.14 for PCV and nAMD combined (95% CI, 0.87-1.49, p=0.3444).”

Results (lines 150-154):” Combining PCV with nAMD as a group, the genotype distributions and additive and dominant models also showed no significant differences (all p values >0.05). Although there was a marginal association under a recessive model in the PCV and nAMD combined group (p=0.0487), it could not withstand the Bonferroni correction.”

Discussion (lines 167-168):” The marginal association under a recessive model in the PCV and nAMD combined group might be attributed to that between this SNP and the PCV group.”

Table 2

<table>
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<th>Status</th>
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<th>OR(95% CI)</th>
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<td>0.91(0.65-1.28)</td>
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<td>PCV+nAMD</td>
<td>0.6167</td>
<td>0.2794</td>
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Table 3

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<td>TC</td>
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<td>TT</td>
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<table>
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<td>Additive</td>
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