Author's response to reviews

Title: Two polymorphisms (rs699947, rs2010963) in the VEGFA gene and diabetic retinopathy: An updated meta-analysis

Authors:

Yan Lu (luyan366@126.com)
Yirui Ge (zgjr@hotmail.com)
Yuhua Shi (oculistsyh@sina.com)
Jie Yin (yinjie73@163.com)
Zhenping Huang (huangzhenping1963@163.com)

Version: 2 Date: 30 July 2013

Author's response to reviews: see over
**Reviewer's report**

**Title:** Two polymorphisms (rs699947, rs2010963) in the VEGF-A gene and diabetic retinopathy: An updated meta-analysis

**Version:** 1  **Date:** 9 June 2013

**Reviewer:** Fuu-Jen Tsai

This is a study about VEGF polymorphisms and DMR, using meta-analysis. The relation of VEGF and DMR is important and well known recently. Meta-analysis collects published data and reanalysis them. This paper used updated analysis methods, but there still limited information we can obtain from this new and gathered materials article. Moreover, this paper divide the patients into different ethnic groups; this make the patients of each groups are not enough for support the results for readers.

Response: Thanks for your comments.

First, compared to previous meta-analyses on this topic, our updated meta-analysis included more studies and more subjects. The results of our meta-analysis indicated that there was a significantly association between VEGF rs699947 polymorphism and risk of DR after exclusion of outliers, whereas rs2010963 polymorphism might be not associated with risk of DR. Our findings were somewhat different from those from previous three meta-analyses.

Second, we agreed with you that the subgroup analyses by ethnicity may lower the statistical power for each subgroup. However, as the minor allele frequency of each polymorphism is different across various ethnic groups, the effect of each polymorphism on diabetic retinopathy may be different. Indeed, we found rs699947 polymorphism was marginally associated with the risk of DR among European populations but not among East Asian populations. In addition, we added the limitation of small sample size among each ethnic group in the Discussion section (please see page 8).

Again, we thank for your valuable suggestions. We hope the revisions will satisfy your expectation.
Referee 2
Reviewer's report
Title: Two polymorphisms (rs699947, rs2010963) in the VEGFA gene and diabetic retinopathy: An updated meta-analysis
Version: 1 Date: 10 June 2013
Reviewer: Dongfeng Zhang
Reviewer's report:
Comments to the Author
Lu Y et al. investigated association of two polymorphisms (rs699947, rs2010963) in the VEGFA gene and diabetic retinopathy by conducting an update meta-analysis. 8 studies with 1204 cases and 1198 controls for rs699947 polymorphism and 10 studies with 1666 cases and 1782 controls for rs2010963 polymorphism were included in this meta-analysis. They concluded that rs699947 polymorphism might be associated with the risk of DR among Europeans but not among East Asians; rs2010963 polymorphism was not associated with DR. However, I have several important issues in this meta-analysis.

Major Compulsory Revisions
1. Exploring the potential sources of heterogeneity is the essential part of meta-analysis. In the present analysis, significance of heterogeneity was found across all studies and subgroups. So how did the authors explain the source of heterogeneity across studies? Besides the authors should conduct the further analysis after excluding the articles that caused the
Response: Thanks for your suggestions. We performed meta-regression analysis to explore source of heterogeneity. We introduced variables including publication year, ethnicity, sample size in cases and controls. However, these variables can not explain the source of heterogeneity. Then, we drew Galbraith figure to further explore the outliers. The pooled results after exclusion of these outliers are listed in Table S1. The between-study heterogeneity for two polymorphisms disappeared, and non-significant association for rs2010963 polymorphism with DR remained (Table S1). However, the association between rs699947 polymorphism and DR changed to be significant under homogeneous co-dominant model (OR=1.64, 95%CI=1.18-2.28, $p=0.003$, Figure 4), even after multiple comparison correction was performed.

2. The authors performed multiple comparisons, i.e. homogeneous co-dominant model and heterogeneous co-dominant model. But this multiple comparisons could result in the risk of an inflated Type I error rate. How do the authors deal with this problem in this manuscript?

Response: Thanks for your suggestions. In this meta-analysis, we used four genetic models for each of two polymorphisms, and Bonferroni method was used to correct for multiple comparisons ($p=0.05/8=0.00625$) (please see page 5).

3. The authors referred the homogeneous co-dominant model, heterogeneous co-dominant model, dominant model and recessive model. The authors should explain the models in the article. Besides, co-dominant model should be added.

Response: Thanks for your suggestions. We have explained the four genetic models in the modified manuscript and Table 3 and 4. In addition, co-dominant model included homogeneous and heterogeneous co-dominant model.

4. The authors did not include all qualified studies. (for example: Association of vascular endothelial growth factor 2 634C/G polymorphism and diabetic retinopathy in type 2 diabetic
Han Chinese. Experimental Biology and Medicine 2010; 235: 1204 – 1211). The authors should identify all possible studies fully.

Response: Thanks for your suggestions. The study (Association of vascular endothelial growth factor 2 634C/G polymorphism and diabetic retinopathy in type 2 diabetic Han Chinese. Experimental Biology and Medicine 2010; 235: 1204 – 1211) should not be included in the present meta-analysis. In that study, the genotype frequency of 634C/G polymorphism was not in Hardy-Weinberg Equilibrium in controls.

5. We do not think literature and search strategy is suitable with only PubMed and EMBASE literature databases. Please supplement the qualified articles from other databases.

Response: Thanks for your suggestions. Web of Science and Google Scholar were further searched. However, no further potentially relevant studies met the inclusion criteria.

6. The authors say that this was an update meta-analysis. Two meta-analyses by Abhary et al. [17] and Zhao et al. [18] have investigated association of VEGFA gene and DR. However, another meta-analysis by Qiu et al. have assessed VEGF# 634G>C (rs2010963) polymorphism and DR risk in 2013, and this meta-analysis had shown significant association between rs2010963 polymorphism and DR. The current meta-analysis by Lu Y et al added only two new studies and the result changed to no significant association between rs2010963 polymorphism and DR under all genetic models. Please explain the results.

Response: Thanks for your suggestions. The recent meta-analysis by Qiu et al. focused on only 634G>C (rs2010963) polymorphism, but our study included two polymorphisms. Although Qiu et al. found the positive association between rs2010963 polymorphism and DR, it should be noted that the association was only marginally significant as p value under allelic model and recessive model was 0.03. Most importantly, the recent meta-analysis by Qiu et al. omitted two studies (Buraczynska, 2007 and Feghhi,2011) and included one study (Yang, 2010) where the genotype frequency of 634C/G polymorphism was not in Hardy-Weinberg Equilibrium in controls. Thus, the findings from the meta-analysis by Qiu et al. were
Minor Essential Revisions

1. You state that two researchers extracted data with the inclusion and exclusion criteria independently and reached a consensus. Please clarify if two investigators searched articles as well.

Response: Thanks for your suggestion. Yes, two investigators searched articles as well. We have added this point in the modified manuscript. (please see page 4)

2. The flow chart was not clear.

Response: Thanks for your suggestion. The flow chart was clarified.

3. About table 3 and 4, please indicate that which pooled ORs and 95% CIs were for REM and which were for FEM.

Response: Thanks for your suggestion. We have noted the pooled estimates with REM or FEM in table 3 and 4.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**
I declare that I have no competing interests

**Reviewer's report**

**Title:** Two polymorphisms (rs699947, rs2010963) in the VEGFA gene and diabetic retinopathy: An updated meta-analysis

**Version:** 1  **Date:** 10 June 2013
Reviewer: Seydi Okumus

Reviewer’s report:

Reviewer Comments to Authors:

Manuscript title 'Two polymorphisms (rs699947, rs2010963) in the VEGFA gene and diabetic retinopathy: An updated meta-analysis'

Limited interest meta-analysis study. But, there are a few suggestions.

1-Check your text for spelling, grammatical errors and correct.

Response: Thanks for your comments. We have corrected spelling and grammatical errors through the whole manuscript.

2-Results and conclusion section in the abstract should be rewritten.

Response: Thanks for your comments. Results and conclusion sections in the abstract have been rewritten.

3-Discussion section in the text should be rewritten.

Response: Thanks for your comments. Discussion section in the text has been rewritten.

Level of interest: An article of limited interest

Quality of written English: Not suitable for publication unless extensively edited

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests.

Editorial Comments:

As you will see from the referees’ reports, several major concerns have been raised that we would like you to address in a revised manuscript. Please note that these revisions are an opportunity for you to thoroughly respond to these specific concerns and I would like to
highlight the fact further consideration of your manuscript for publication strongly depends on the quality of your point-by-point response and your revised manuscript. We therefore urge you to make every effort to fully address the criticisms during this revision.

Response: Thank you. We have provided point-by-point responses and highlighted the changes in red in the modified manuscript. We thank you again for giving us an opportunity to revise this manuscript.

**We would be grateful if a cover letter accompanied your revised manuscript submission. This should provide a point-by-point response to each of the referees' concerns, detailing exactly how you responded to each point and where you can find the amendment in your revised manuscript (e.g. document line and/or page numbers). Please include as much detail as possible in your point-by-point response, so that the editorial team and referees can assess your corrections efficiently.**

Response: Thank you. We have tried our best to provide point-by-point responses and highlighted the changes in red in the modified manuscript.

-- In addition to the Referees' comments, could you please also address the following editorial points?

Response: Thank you. Yes, we have addressed the following editorial points.

1. Please include an Acknowledgements section in your manuscript:
   - Acknowledgements
   Please acknowledge anyone who contributed towards the article by making substantial contributions to conception, design, acquisition of data, or analysis and interpretation of data, or who was involved in drafting the manuscript or revising it critically for important intellectual content, but who does not meet the criteria for authorship. Please also include the source(s) of funding for each author, and for the manuscript preparation. Authors must describe the role of the funding body, if any, in design, in the collection, analysis, and
interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication. Please also acknowledge anyone who contributed materials essential for the study. If a language editor has made significant revision of the manuscript, we recommend that you acknowledge the editor by name, where possible.

Response: Thank you. We have added Acknowledgements.

2. Please include a formal Conclusions section in your manuscript.

Response: Thank you. We have included a formal Conclusions section in the modified manuscript.

3. Please copyedit your manuscript:

Further consideration of your manuscript is conditional on improvement of the English used - please bear in mind that as we are a free-access publisher, we cannot bear the costs of copyediting English ourselves. Please ensure particular attention is paid to the abstract. You should have a native English speaking colleague help you with this, if possible, or you may need to use a professional language editing service. For authors who wish to have the language in their manuscript edited by a native-English speaker with scientific expertise, BioMed Central recommends Edanz (www.edanzediting.com/bmc1). BioMed Central has negotiated a 10% discount to the fee charged to BioMed Central authors by Edanz. Use of an editing service is neither a requirement nor a guarantee of acceptance for publication. For more information, see our FAQ on language editing services at http://www.biomedcentral.com/authors/authorfaq/editing.

Response: Thank you. We have copyedited our manuscript.

Please also ensure that your revised manuscript conforms to the journal style (http://www.biomedcentral.com/info/ifora/medicine_journals ). It is important that your files are correctly formatted.

Response: Thank you. Our files have been correctly formatted.