Author’s response to reviews

Title: Screening key genes for abdominal aortic aneurysm based on Gene Expression Omnibus dataset

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Version: 3 Date: 28 Dec 2017

Author’s response to reviews:

Dear Barry Palmer,

We are submitting the revised manuscript with the ID BCAR-D-17-00607R2, entitled "Screening key genes for abdominal aortic aneurysm based on Gene Expression Omnibus dataset" for publication in BMC Cardiovascular Disorders. According to the comments, we have already revised the manuscript. All revisions have been marked in red in the revised manuscript. The following are our reply to the comments, item by item.

Once again, thank you for your kind reconsideration.

Yours sincerely,

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Reviewer reports:

Emanuela Bostjancic (Reviewer 1): In an article by Wan et al authors described comprehensive meta-/bioinformatic analysis of GEO datasets from the abdominal aortic aneurysm in comparison to normal individuals. However, the article would benefit from inclusion of comment on discrepancy between bioinformatic and immunohistochemically evaluated expression of PDGFA.

Answer: We thank the Reviewer for his/her kind evaluation of our work. We are sorry for omitting the discussion of the discrepancy. In fact, we enrolled a patient with AAA and a normal individual for IHC experiment. To be frank, the sample was small. The sample size may account for the discrepancy between bioinformatic and immunohistochemically evaluated expression of PDGFA. We have to admit that this is a limitation of our study. We have added to the discussion of the discrepancy and the limitation of our study. All changes have been marked in red in the “Immunohistochemical (IHC) staining for CCR7 and PDGFA” section (line 116-117, page 6), “Discussion” section (line 224-226, page 11) and “Conclusions” section (line 273-275, page 13) of the revised manuscript. Thanks again!

Lei Tian (Reviewer 2): The authors detected key genes for abdominal aortic aneurysm (AAA) based on multiple public gene expression datasets with bioinformatic data analysis. They further validated their results with immunohistochemistry and ROC curve analysis. In conclusion, they found some potential diagnostic biomarkers and 3 potential drug targets for AAA. It is an interesting study, and this study provides insight of relationship between vascular smooth muscle contraction and AAA. However, I have the following comments and suggestion which might further improve the study:

1. Please compare the results with previous studies. E.g. the overlapping of DEGs, enriched pathways.

Answer: We thank the Reviewer for his/her kind evaluation of our work. We are appreciated for providing the wonderful suggestion. In this study, we found that ZBTB16 was down-regulated in AAA, which was consistent with a previous report. After searching related articles, we found that vascular smooth muscle cell was associated with AAA. Similarly, we also found that vascular smooth muscle contraction was involved in the development of AAA. Moreover, we found that three down-regulated genes including RAMP1, ROCK1 and ROCK2 were involved in the signal pathway. After searching related literature, RAMP1 was also decreased, which was in line with our result. However, ROCK1 and ROCK2 were increased in AAA lesion, which was
not consistent with our study. The study method or the sample type may interpret the differentially expression of ROCK1 and ROCK2. As suggested, we have compared above genes and signal pathway with the previous study. All changes have been marked in red in the “Discussion” section (line 212-213, page 10; line 231-233, 235, page 11; line 252-256, page 12) of the revised manuscript. Thanks again!

2. There are much more down-regulated DEGs than up-regulated DEGs, is this because of less normal individuals with a higher false positive rate for down-regulated DEGs detection? What if the authors sample equal numbers of individuals in both groups? Authors should at least mention this issue in the manuscript.

Answer: We thank the Reviewer for his/her kind evaluation of our work. Indeed, the sample size in the normal controls was small. In this study, we selected the useful dataset according to the standard strictly. We searched datasets from the Gene Expression Omnibus (GEO) database with the keywords abdominal aortic aneurysm [All Fields] AND ("gse"[Filter] AND "Homo sapiens"[Organism]). The study type was described as “expression profiling by array.” All selected datasets were genome-wide expression data of AAA group and/or normal group tissue samples. Those standardized or primary datasets were included in this study. Based on the standard, we finally obtained three datasets. To be frank, there were only 26 normal individuals, which may lead to the higher false positive rate for down-regulated DEGs detection. Whatever, it is a wonderful suggestion that we should sample equal numbers of individuals in both groups (normal and case groups) in our further studies. As suggested, we have added the limitation and marked in red in the “Discussion” section (line 277-280, page 13) of the revised manuscript. Thanks again!

3. What does "+" mean in Table 1 at Samples column? How many patients are included in GSE57691?

Answer: We thank the Reviewer for his/her kind evaluation of our work. We are sorry for omitting the detailed information. In fact, there is 49 patients (20 small AAA and 29 large AAA) are included in GSE57691. Small/large is the disease state of AAA. For convenience, we have corrected the number at 49 and marked in red in the revised Table 1. Thanks again!
4. The fold changes should be presented in Table 2.

Answer: We thank the Reviewer for his/her kind evaluation of our work. In fact, we didn’t calculate the fold change. Instead, we calculated the combined effect size. We calculated the combined effect size either from classical or moderated t-tests for each study and combined these effect sizes. Combined effect size was similar to the fold change. As suggested, we have added the combined effect size and marked in red in the revised Table 2. Thanks again!

5. Please state which enriched pathways are up/down-regulated. E.g. the proportion of up-regulated genes in all genes assigned to a pathway.

Answer: We thank the Reviewer for his/her kind evaluation of our work. In the KEGG enrichment signal pathways of DEGs (Table 4), all genes were down-regulated. Maybe, the small size of normal individuals in the selected datasets may account for the result. To be frank, there were only 26 normal individuals, which may lead to the higher false positive rate for down-regulated DEGs detection. Whatever, we should sample equal numbers of individuals in both groups (normal and case groups) in our further studies. As suggested, we have added the limitation and marked in red in the “Discussion” section (line 277-280, page 13) of the revised manuscript. Thanks again!

6. P.6 line 174: bioinformatics —> bioinformatic

Answer: We thank the Reviewer for his/her kind evaluation of our work. We are sorry for our poor English writing. As suggested, we have corrected and marked in red in the “Validation of CCR7 and PDGFA in AAA” section (line 175, page 8; line 177, page 9) of the revised manuscript. Thanks again!

7. P.13 line 266: diagnosis biomarker —> diagnostic biomarkers

Answer: We thank the Reviewer for his/her kind evaluation of our work. We are sorry for our poor English writing. As suggested, we have corrected and marked in red in the “Background” section (line 78, page 4; line 270, page 13) and “Conclusions” section (line, page) of the revised manuscript. Thanks again!
8. Overall, the language needs to be improved. Some phrases need to be modified.

Answer: We thank the Reviewer for his/her kind evaluation of our work. We are sorry for our poor English writing. As suggested, we have checked and corrected the spelling and grammar mistakes. All changes have been marked in red in the section (line 24, 40, page 2; line 54, 57, page 3; line 70, 85, page 4; line 101-102, 106, 109, page 5; line 112, 128, page 6; line 140, 149, 151, 154, page 7; line 156, 165, 176, page 8; line 187, 198, page 9; line 199, 214, 220, page 10; line 240, page 11; line 252, page 12) of the revised manuscript. Thanks again!