Author’s response to reviews

Title: Mitochondrial DNA 7908-8816 region mutations in maternally inherited essential hypertensive subjects in China

Authors:
Ye Zhu (307971331@qq.com)
Xiang Gu (guxiang@yzu.edu.cn)
Chao Xu (cxu2@tulane.edu.cn)

Version: 3 Date: 01 Jun 2018

Author’s response to reviews:

Response to Reviewers’ comments

Dear Editor,

Thank you for carefully reviewing our manuscript previously titled “Mitochondrial DNA 7908-8816 region mutations in maternally inherited essential hypertensive subjects in China” for possible publication in the BMC Med Genomics. We are grateful to you and your reviewers for their constructive critique. We have revised the manuscript, highlighting our revisions in red, and have attached point-by-point responses detailing how we have revised the manuscript in response to the reviewers' comments below.

Thank you for your consideration and further review of our manuscript. Please do not hesitate to contact us with any further questions or recommendations.

Best regards,

Xiang Gu

Clinical Medical College, Yangzhou University, Yangzhou, Jiangsu 225001, China; Department of Cardiology, Subei People’s Hospital, Yangzhou, Jiangsu 225001, China

Tel: +86-18952781166

E-mail: guxiang@yzu.edu.cn
1. Editor Comments:

Thank you for including the reasoning for why verbal consent was taken in your last response letter. Please include this in the manuscript, in the Ethics approval and consent to participate section. Please clarify whether this method of consent was approved by the ethics committee.

Response: We agree that there should include the following sentence "Verbal informed consent was obtained from the subjects involved in the study" in the manuscript. This method of consent was approved by the ethics committee which was included in the revised article.

2. The Availability of data and materials section refers to the raw data used in your study. We strongly encourage all authors to share their raw data, either by providing it in a supplementary file or depositing it in a public repository and providing the details on how to access it in this section. If you do not wish to share your data, please clearly state this in this section along with a justification.

Response: We thank the editor for the comment. All the raw data of baseline clinical data of patients are available. For the patient's privacy, our hospital has the regulation that they do not fully support the disclosure of the patient's data in public. However, we can share the abnormal mtDNA mutations from part of the patients.

Reviewer reports:

Sivarajan Kumarasamy (Reviewer 1):

MY comments :

The article by Zhu et al screened for mtDNA mutations in maternally inherited essential hypertensive subjects in China. In particular, the authors screened for mutation in mtDNA regions from 7908 to 8816bp in MIEH vs controls subjects. Along with this mutation analysis report, the authors also studied reported BP changes, BMI, and biochemical changes between control and MIEH subjects. In total 40 SNP were identified between control and MIEH subject out of which 29 of them fell under non-synonymous variants and 11 under synonymous variation. The current study added 12 new SNPs to that list with less statistical significance. Overall, the study provided an association of this mtDNA SNP and biochemical changes in MIEH subjects compared to control subjects is interesting.
Comments

1. In general throughout the manuscript the author never said/discussed about the rationale for choosing region between mtDNA 7908 to 8816bp. This information will be useful for the reader and to the scientific community.

Response: For choosing region between mtDNA 7908 to 8816bp was the novelty of this article. The hottest spots of cardiovascular diseases were screened using oligodeoxynucleotides 7908–8816bp. Other regions of mtDNA were not screened in this article.

2. It's also important to discuss either similar mutation also exist/reported in genetic model for hypertension research? Again this information is important to the scientific community and may provide path to plan for future studies using novel genetic models for hypertension research.

Response: We are very honored to get your better improvement on our draft! It's also important to discuss either similar mutation also exist/reported in genetic model for hypertension research. We have discussed either similar mutation in the revised article.

3. Also It would be worth to discuss the importance of mitochondrial dysfunction in hypertensive genetic models and discuss in align with the SNP identified in MIEH subjects.

Response: We agree with the Reviewer. We have revised the article and discussed the importance of mitochondrial dysfunction in hypertensive genetic models and discuss in align with the SNP identified in MIEH subjects.

My comments

1. Please do spell check in page no 8 line 158

Response: We have changed FPG to FBG in page no 8 line 158.

2. Please check sentence page no 8 line no 167 to 170: Instead of stating "the mitochondrial tRNA….. Change to "Polymerase chain reaction was carried out to amplify mitochondrial tRNALYS gene using the following primers……."

Response: We have changed sentence page no 8 line no 167 to 170: Polymerase chain reaction (PCR) was carried out to amplify mitochondrial tRNALys gene using the following primers:
forward: 5′-ACGAGTACACCGACTACGGC-3′ and reverse: 5′-TGGGTGGTTGGTGTAATGA-3′.

3. Please do spell check in page no 9 line 197
   Response: We have checked the spell in page no 9.

4. The reference cited in page 13 line 273 is not relevant to the statement please check and provide appropriate ref

5. Please provide ref for page no 13 line 273 to 276

6. Statement from Page no 13 line 286 to 287: its generalized statement and should be discussed with rationale/ with lime light of literature evidence to support this argument.
   Response: We have not found abundant evidence to support the argument, so we deleted this statement.

7. Table: 1 change FPG to FBG both in table and legend
   Response: We have changed FPG to FBG both in table and legend.

8. Table: 3 it would be clear to split the table into synonymous and non- synonymous variants.
   Response: We have splited the table into synonymous and non- synonymous variants.
9. For all figures listed in this manuscript, figure quality could be better and providing the position of base pair for each chromatogram and highlighting the SNP reported from the current study with special feature like BOX or dotted line between will enhance the visibility for the reader.

Response: We are very honored to get your better improvement on our manuscript! We have improved figure quality and provide the position of base pair for each chromatogram which enhance the visibility for the reader.

Jennifer A. Smith, PhD, MPH (Reviewer 2): Building on previous demonstration of the relationship between variants in the mitochondrial genome and maternally-inherited essential hypertension (MIEH), this manuscript evaluates whether a DNA sequence variants are present at a higher degree in a sample of 300 MIEH cases and 300 controls. The authors find that the mitochondrial genomes in MIEH cases are significantly enriched for sequence variants in the 7908-8816 region, and that three amino acid changes are represented at a higher frequency in MIEH cases in the ATP8 and ATP6 genes. This timely study provides new insight into the role of the mitochondrial genome in hypertension etiology. The manuscript was well-written, and only minor substantive and typographical revisions are suggested.

Methods

1. It is not clear why the authors focused on region 7908-8816 specifically. It seems that all of the variants that have been previously found to influence MIEH were in other regions of the mitochondrial genome (for example in reference 7). In the Mitochondrial DNA analysis section, it states that "locations considered the main areas for CVD as described previously [12]" were used, but the finding from reference 12 (4329) was upstream of the evaluated region in this manuscript. The authors are encouraged to elaborate and justify their focus, and state why other previously-identified regions were not evaluated here.

Response: We thank the reviewer for the helpful comments. The hottest spots of cardiovascular diseases were screened using oligodeoxynucleotides 7908-8816bp. (29) The authors focusing on region 7908-8816 specifically was the novelty of this article.

2. Why were people who were receiving antihypertensive medication excluded? It seems that this would have eliminated quite a large number of people from the analysis, unless only
individuals with a new diagnosis of essential hypertension were allowed into the study. Please elaborate on this criterion and the possible implications for sample selection and bias.

Response: We agree with the Reviewer. At the time, we were thinking about avoiding the possible impact of antihypertensive drugs on the results of gene sequencing, but it lost a lot of gene analysis of patients with hypertension.

3. On page 8, line 156, it is stated "hypertension was defined as SBP>=140 or DBP>=90 on at least three different occasions". Does this refer to the study visit itself (measuring blood pressure 3x) or to having hypertension on at least 3 previous visits to the clinic?

Response: Good question! On page 8, line 156, it is stated "Hypertension was defined SBP>=140 mmHg or DBP>=90 mmHg measured three times on different days".

4. Please provide a more detailed description on how it was determined that "hypertension was transmitted by the mother or her relatives and not by the father". Functionally, how was this determination made, and what were the criteria specifically?

Response: The key identifying feature of a patient with hypertension transmitted by the mother or her relatives and not by the father, as the name suggested, are the presence of hypertension, and a family history of the conditions in maternal relatives.

5. What is meant by "no personal or family history of hypertension or any other disorder" in the control group? Which disorders were screened? Again, how was this operationalized? (were only parents considered, or grandparents, aunts/uncles/cousins?)

Response: We agree with the Reviewer. We have deleted this sentence “no personal or family history of hypertension or any other disorder" in the control group. Hypertension were screened. It was operationalized that there was no current hypertension.

Discussion

1. Although haplogroups of the three different variants are mentioned, it is not clear whether these variants are completely independent of one another. That is, is there linkage disequilibrium between any of the three amino acid changing variants that are related to MIEH? This is critical to understand, since if they are correlated with one another, it may
signal that there are only one or two causal mutations. In this same vein, what is the LD between these variants and other previously-discovered MIEH variants?

Response: The 3 different variants are all in weak LD. We calculated the LD using the reference data from 1000 Genomes Project Phase 3 release about 3 Chinese populations. The LD (r²-squared correlation) of the 3 variants were listed below. It is reasonable to assume they are mutually independent.

<table>
<thead>
<tr>
<th></th>
<th>MT:8414</th>
<th>MT:8584</th>
<th>MT:8701</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT:8414</td>
<td>1</td>
<td>0.021</td>
<td>0.149</td>
</tr>
<tr>
<td>MT:8584</td>
<td>0.021</td>
<td>1</td>
<td>0.005</td>
</tr>
<tr>
<td>MT:8701</td>
<td>0.149</td>
<td>0.005</td>
<td>1</td>
</tr>
</tbody>
</table>

2. The authors were not able to control for genetic principal components, which is typically essential in any genomic analysis. This should be mentioned as a limitation.

Response: We agree with the Reviewer. We were not able to control for genetic principal components, which is typically essential in any genomic analysis. We have mentioned it as a limitation in the revised article.

3. It is clear that the mechanistic link from mitochondrial variants and hypertension is not known. However, some speculation as to the potential underlying mechanisms would be nice in the discussion.

Response: We thank the reviewer for the helpful comments. We have revised the discussion with some speculation as to the potential underlying mechanisms.

4. The paragraph on page 14, starting at line 293 could be better supported by the literature. For example, what data/results are presented that support the claim in line 293-4? The end of this paragraph is also a little repetitive to other parts of the discussion.

Response: We revised the article and added the literature at line 293-4. We agree with the Reviewer. The end of this paragraph is also a little repetitive to other parts of the discussion, so we deleted the repetitive part.

5. Please discuss additional study limitations, such as not including people treated with antihypertensives, etc.
Response: We thank the reviewer for the helpful comments. We discussed additional study limitations, such as not including people treated with antihypertensives in the revised article. It lost a lot of gene analysis of patients with hypertension.

Minor edits:

1. Page 4, line 73 - suggest to change the sentence to read: "EH results from the interaction between environmental and inherited risk factors, which can be …"

Response: We agree with the reviewer. The sentence should be changed to: “EH results from the interaction between environmental and inherited risk factors, which can be caused by single-gene or multifactorial conditions”.

2. Page 5, line 96 - the semicolon needs to be a comma here

Response: We agree with the reviewer. It has been revised.

3. Page 5, line 98 - should be "… blood pressure and this increases…”

Response: The development of blood pressure and this increases with many factors that include the mtDNA mutation/background, nuclear genes and environmental factors.

4. Page 5, line 108 - since this isn't a cohort study, please avoid use of the word "cohort"

Response: We agree with the reviewer. So we deleted the word "cohort".

5. Page 6, line 112 - should be "of study in this"

Response: We agree with the reviewer. We changed "of study in this".

6. Page 6, line 120 and 131- should be "people's"

Response: We agree with the reviewer. We changed to people's.
7. Page 6, line 122 - should be "not receiving antihypertensive medication; (5)"
Response: We changed "not receiving antihypertensive medication; (5)"

8. Page 7, lines 151-153 have some repetitive information
Response: We agree with the reviewer. We have deleted some repetitive information.

9. Page 10, lines 200-207 could be a single paragraph
Response: We agree with the reviewer. We have changed lines 200-207 be a single paragraph.

10. Page 10, line 210 - LEUCINE does not need to be in all caps
Response: We thank the reviewer for the helpful comment. LEUCINE has been changed to Leucine.

11. Page 12, line 264 - extra space between "that" and "mtDNA"
Response: We thank the reviewer for the helpful comment. We have deleted extra space between "that" and "mtDNA".

12. Page 14, line 288 - should be "ATP8 and ATP6"
Response: We thank the reviewer for the helpful comment. We have changed it to be "ATP8 and ATP6".

13. Page 14, line 301-302 - the sentence that begins with "in terms" is awkward
Response: We thank the reviewer for the helpful comment. Regarding amino-acid changes and RNAs variants the MIEH subjects harbored more variants than the controls.

14. Page 15, line 316 - should be "our findings may be generalizable"
Response: We thank the reviewer for the helpful comment. We have changed the sentence to "our findings may be generalizable".

15. Table 1 - spacing between the variable and the units of measurement is not consistent

Response: We thank the reviewer for the helpful comment.

16. Table 1 - should "smoking" be "current smoking"?

Response: We thank the reviewer for the helpful comment. Yes, "smoking" should be "current smoking".

17. In Table 2, are these both homoplastic and heteroplastic mutations? Also, please include the name of the consensus sequence in the Table legend.

Response: We thank the reviewer for the helpful comment. In Table 2, these are both homoplastic and heteroplastic mutations. The name of the consensus sequence was listed in Table 3.

18. For Table 3, please elaborate in the figure legend what "previously reported" refers to. Do you mean that the variant was ever reported in a database, or that it was every reported to be associated with a disease?

Response: We thank the reviewer for the helpful comment. In the figure legend "previously reported" refers to the variant was ever reported in a database.

19. In Figure 1, "K" should probably be called tRNALys, consistent with the rest of the manuscript

Response: We agree with the reviewer, "K" should probably be called tRNALys. We have revised Figure 1 which consistent with the rest of the manuscript.