Reviewer’s report

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

Version: 2 Date: 28 Apr 2018

Reviewer: Jennifer Smith

Reviewer's report:

Building on previous demonstration of the relationship between variants in the mitochondrial genome and maternally-inherited essential hypertension (MIEH), this manuscript evaluates whether an 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.

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.

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?
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?

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?)

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?

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.

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.

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.

5. Please discuss additional study limitations, such as not including people treated with antihypertensives, etc.

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 …"

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

3. Page 5, line 98 - should be "… blood pressure and this increases…”
4. Page 5, line 108 - since this isn't a cohort study, please avoid use of the word "cohort"

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

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

7. Page 6, line 122 - should be "not receiving antihypertensive medication; (5)…”

8. Page 7, lines 151-153 have some repetitive information

9. Page 10, lines 200-207 could be a single paragraph

10. Page 10, line 210 - LEUCINE does not need to be in all caps

11. Page 12, line 264 - extra space between "that" and "mtDNA"

12. Page 14, line 288 - should be "ATP8 and ATP6"

13. Page 14, line 301-302 - the sentence that begins with "in terms" is awkward

14. Page 15, line 316 - should be "our findings may be generalizable"

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

16. Table 1 - should "smoking" 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.

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?

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

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes
Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

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