Author's response to reviews

Title: Systematic analysis of the clinical and biochemical characteristics of maternally inherited hypertension in Chinese Han families associated with mitochondrial genome mutations

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Author's response to reviews: see over
Revised
Review 1:

Major Compulsory Revisions
1. Page 2 Line 16: T7492C is the old and now non-standard notation, the correct m.7492T>C notation style must be used for all mutations reported in the paper.
Revised.

2. Page 7 Line 7: I would recommend the use of Phylotree (http://www.phylotree.org/) in addition to MitoMap to make an initial search for population variants, and explained below mtDB is hopelessly outdated, having not been updated since March 2007.
Revised.

3. I have looked at the m.7492T>C variant, and it is not listed as a marker on phylotree, but will not look up all 24.
The m.7492T>C was not reported before, which was a novel variant reported here.

4. Please see the following: 2. ?Exaggerated status of "novel" and "pathogenic" mtDNA sequence variants due to inadequate database searches? Bandelt HJ, Salas A, Taylor RW, Yao YG. Hum Mutat. 2009 Feb;30(2):
Revised.

5. FIGURE 2: This has been taken from MitoMap: See Figure 1b: Mitochondrial DNA Map (color), do the authors have permission to reproduce this figure in the paper submitted here?
https://www.mitomap.org/bin/view.pl/MITOMAP/MitomapFigures
Figure legends, these require clarification before the paper can be published.
Figure 2 was deleted.

Minor Essential Revisions
1. Page 3 Line 16: Would word limits permit more details as to how the controls were characterized? As an example how long, were they tracked clinically?
Revised. The control underwent physical examination, the family history, laboratory assessment for at least twice in one year.
Page 3 Line 3: You might want to consider citing the following paper, as it is an excellent study supportive of mtDNA variation in this category of common disease. Mitochondrial DNA haplogroups and risk of transient ischaemic attack and ischaemic stroke: a genetic association study? Chinnery et al 2010, Lancet Neurol

Replace the reference with your suggestion.

Page 3 Line 7: Estimates of genetic variance range from 20% to 50%. [5-7]? you might comment mtDNA variation has been excluded from many GWAS, as such might be a candidate for some of the missing heritability known to exists in association with this group of common complex disorders.

Yes, these articles suggest that genetic variance contribute to high blood pressure, including mtDNA variation. The early or more recent investigations showed that significant maternal familial aggregation of high blood pressure was presented, which suggested the contribution of mitochondrial genome to hypertension.

Page 7 Line 7: CAUTION mtDB (http://www.genpat.uu.se/mtDB) is really out of date, the cover page of the database will tell you that it was last updated March 2007. It has a large number of sequences from patients with mitochondrial disease and is and sequences have a European bias.

Revised.

Page 7 Line 8: Comparison with the Cambridge? reference sequence (CRS)? should read Comparison with the revised Cambridge? reference sequence (rCRS)?

Revised.

Page 7 Line 13: It is not sufficient to call a variant novel based on the database cited in reference [24] as it has not been updated since March 2007, and has only 10% of the current mtDNA sequences available on the public database. As mentioned, MitoMap and Phylotree should be consulted, as a minimum. The authors might also look at the following paper, Bandelt HJ, 2009, Human Mutation, which talks about how to use database to test is a mutation is novel.
Summary

These novel variants, including 2448G>A, 2534G>A, 2673G>A, 2695G>A, 2706A>G in 16SrRNA and 14686G>A mutations in tRNA\textsuperscript{Glu}, were detected by Google engineering without same variants reported before [Bandelt et al].

Page 9 Line 27: "In these 172 variants, there’re 30 novel 28 variants not indentified in the control or the 2704 mtDNAs."

I have not commented on the discussion, as this is likely to change after the database searches have been conducted using up-to-date databases

These 30 variants were not identified in the control or the 2704 mtDNAs, not means “novel” vatiants, which have been listed in the supple 1.

Reviewer 2:
The coauthors performed sequence analysis of mitochondrial genome in patients with maternally inherited hypertension and identified several novel loci that may be related to the development of hypertension. In general, this was an interesting and important investigation trying to identify genetic causes of hypertension. There are two major concerns. First, although they identified 24 novel variants in hypertension patients that were not present in the controls, they did not provide sufficient evidence that these novel variants were related to hypertension. The second concern was about readability of the manuscript. There were numerous unclear sentences, grammar errors and typos throughout the manuscript.

Major Compulsory Revisions:
Although they discovered novel variants in the patients of maternal lineages, they did not relate these variants directly to hypertension. I feel that they only worked half way for their aims.

In Clinical and inheritance evaluation of 9 families (page 5, line 19), the authors referred to figure 1 and figure 1 for three male probands, HT-1, HT-8 and HT-9. I looked the figure carefully, and was not clear which individuals in the figure were these male probands they referred to. Therefore, the inheritance pattern was not clear by reading the figures.

The arrow showed the probands of the families, HT-1, HT-8 and HT-9, were male, without offspring presented with hypertension. Maternal transmission of mitochondrial means only the offspring of the affected mother may affect. So they were consistent with maternal inheritance. More detail see the article.

On page 3, line 22, the coauthors stated that they performed segregation analysis. What segregation analysis did they perform? Only by looking at the disease status in family members (described on page 5 line 19 through page 6)?
Segregation analysis was performed to exclude the other patterns of inheritance, including autosomal recessive, autosomal dominant, X-linked, or Y-linked inheritance as a method prescribed in reference 17.

Hypertension is one of the risk factors for cardiovascular diseases. Is hypertension considered as a cardiovascular disease?

Hypertension is also considered as a cardiovascular disease.

Numerous typos and grammar errors hampered the importance of the manuscript. Below are several examples. The whole manuscript should be proof-read by expert in the field and by English speaking individuals to improve the readability.

1. On page 6, line 17 ?We collect the clinical date of 9 probands? maternal members ?. see table 2). I guessed that ?the clinical date? would be ?clinical data?. Table 2 could not be found following the main context. Instead, Table 2 was found in the supplementary materials.

   Table 2 showed the comparison between the 64 maternal members (from 9 maternal inherited families) and 216 controls.

2. On page 7, line 12, ?In these 151 variants, there?re 24 novel variants not indentified in the control or the 2704 control mtDNAs, and 22 nonsynonymous variants.? This sentence has grammar error and typos, therefore the meaning of this sentence was not clear.

   Revised.

3. They used ?they?re? or ?there?re? in many places, should be ?they are? or ?there are? revised.

4. On page 7 line 8-9 ?All these 172 variants were compared with the 216 control?, grammar error. They can?t compare variants with controls.

   Revised.

5. On page 2, line 14-15, ?we identified 7 amino acid changes presented in the 9 maternal inherited hypertension families?. First, number less than 20 should be written as English word. Second, should be ?maternally inherited?.

   On the same page, line 16, should be ?more interestingly ??

   Revised.

6. On page 4, line 1. ?There?re 99 probands were excluded?. ?so there were 9 probands proved with maternal inherited hypertension?, grammar errors in both sentences.

   Revised.

7. On page 4, line 4, what is ?the past history?, did they mean by ?health history? or ?disease history??

   Means the past health history.
8. They should provide units for height and weight in calculating BMI.
   BMI (Kg/m$^2$)

9. On age 9 line 14, ?essential HTN is polygenic?, should be polygenic disease
   Revised.

10. On page 10. Line 23 ? line 25, the sentences are run-on sentences without clear meaning.
    Revised.

11. Sometimes they used ?HT?, other times, they used ?HTN? to refer to hypertension. It was confusing. Abbreviations should be consistent in the manuscript.
    Revised.