Author's response to reviews

Title: Genetic association study of selected candidate genes (APOB, LPL, LEPTIN) and telomere attrition in obese and hypertensive individuals.

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Author's response to reviews:

To

Editor-in-Chief,

BMC Medical Genetics,

Dear Sir/Madam,

I am submitting the revised manuscript (MS: 9657831702395905) entitled, “GENETIC ASSOCIATION STUDY OF SELECTED CANDIDATE GENES (APOB, LPL, LEPTIN) AND TELOMERE ATTRITION IN OBESE AND HYPERTENSIVE INDIVIDUALS.” authored by Birajalaxmi Das, Nilambari Pawar, Divyalakshmi Saini and M. Seshadri to your esteemed journal.

We have tried our best to respond all the comments and suggestions made by both the reviewers point by point and incorporated those in the revised manuscript.

We have revised all the sections in the manuscript. We have incorporated a total number of 5 tables and three figures in the manuscript. Table 3 contains allele frequency data for better clarification in results section. We have also incorporated figure 3A and figure B.
I am also providing two separate reply for the reviewer 1 and 2. below.

Awaiting for a positive reply.

Thanking you,

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Reply to the comments of Reviewer 1.

We profusely thank the reviewer for the valuable suggestions and comments for the manuscript entitled “Genetic association study of selected candidate genes (APOB, LPL, LEPTIN) and telomere attrition in obese and hypertensive individuals.”

We have revised our manuscript as per your suggestions and comments and incorporated the necessary suggestion. We also have tried our best to answer all your queries raised by you point by point. We have revised all the sections in the manuscript. We have added an extra table on allele frequency data for better clarification in results section. We have also incorporated figure 3A and figure B

1. We have performed Hardy-Weinberg equilibrium tests to assess the appropriateness of the selected samples for the association studies. (The P values are mentioned in table no. 5 for all the three loci)
2. In the “Materials and Methods section”, we have described in detail the statistical analysis method applied for the association studies of the three repetitive loci (ApoB VNTR, Leptin and LPL microsatellites).

3. In the present investigation the telomere length was measured from the DNA of peripheral blood mononuclear cells (PBMC)/leucocytes. As per your suggestions we have also incorporated this statement in the “Materials and Method section”.

4. We fully agree to your suggestions that the samples details with the confounding factors should be mentioned in the manuscript. In the “Materials and Method section, we have incorporated the sample details.

Since telomere length gets shortened with aging, we have determined the telomere length with respect to age and the scatter plot is provided in the figure 3A. Due to the limitation of sample size we did not provide the analysis for normal, obese na dhypertensive individuals separately. However we did the analysis of Male and female individuals with respect to age and determined the telomere length data in the text as well as a scatter plot in figure 3B. We also have tried to minimize the confounding factors. In the present study we have included normal individuals without obesity, hypertension and diabetes. Similarly the hypertensive individuals included in the study were not obese and independent of diabetes. The obese individuals included in the study were without hypertension and diabetes.

We also did not include smokers or individuals drinking alcohol in our study as it could influence the telomere attrition.

5. As rightly pointed out by the reviewer, the association between telomere length and hypertension is not clear yet although one can not exclude the effects if any. As suggested by you we have deleted the word “prediction” from the text by deleting that sentence. We have modified the discussion section and discussed about telomere in detail.

MINOR COMMENTS:

1. Abbreviations like Cp, T/S ratio have been explained in the text of the revised manuscript. “HVR” term was not appropriate in the text. Therefore we have
deleted it from the text. As per the suggestion, we have deleted “RTL” from the “Methods”.

2. As per your suggestions we have cited references in the fifth paragraph. “telomere attrition is known to be associated with several diseases like diabetes, coronary heart disease.”

3. In the second paragraph of Material and Methods section under the heading of sample collection we have changed “the systolic/diastolic 120/80” to “equal to under”.

4. As suggested we have deleted the sentence “Obesity is a condition in which the natural energy reserve, stored in the fatty tissue of humans and mammals, is increased to a point where it is associated with certain health conditions or increased mortality” from the second paragraph of “sample collection “ in Methods.

5. We have written in the figure to as “normal” instead of “normotensive” as suggested by you. The bars represent standard deviation and the numeral description is also provided in the revised manuscript.

6. As per the suggestions we have numbered the pages and we have taken care of the spacing between the words throughout the paper.

7. As suggested by you we have tried our best to give a thoughtful discussion in the revised manuscript. We have also compared the related papers suggested by you.

The comments and suggestions were extremely useful for revising the manuscript. Hope we have addressed all the comments point by point upto your satisfaction.

Awaiting for a positive response.

Reply to the comments of Reviewer 2.

We have revised all the sections in the manuscript as per the valuable suggestions and comments. We have added an extra table on allele frequency
data for better clarification in results section. We have also incorporated figure 3A and figure B

Major compulsory revisions:

1. As rightly pointed out this is one of the preliminary genetic association study in Indian population. We agree to your suggestions regarding the lack of comparisons of our data with other published reports. Hence, we have tried to discuss the comparison of other studies in the revised manuscript (Discussion Section”).

2. We fully agree that we have missed the important references about telomere and not discussed sufficiently about telomere length with reference to obese and hypertensive individuals as reported by others. In the revised manuscript we have viduals. We have incorporated those references “discussion section” and discussed about telomere length in hypertensive and obese individuals.

3. We also have tried to do the analysis of telomere length in relation to gender and age. Due to the limitation of sample size, we have not stratified our data according to normal, obese and hypertensive groups. However we have done the overall analysis of telomere length with respect to age and the analysis in Male and Female individuals separately.

4. Firstly, we are extremely sorry that our description was confusing regarding the telomere length calculation. We have modified the write up with our own words and hope it is understandable now. Secondly, the telomere length measurement is based on relative quantitation method using LC480 light cycler. It basically follows ddCT calculation. However, we do a standard curve for each plate/PCR reaction with serially diluted genomic DNA in order to show the efficiency of PCR. It gives a better picture that plate to plate variation is minimized. Even we do the PCR of each sample in triplicates and standard deviation is minimal (0.02-0.05). Anyways, we have modified the Materials and Methods section. Hope it is clearer now.

Minor Essential revisions:

1. As per your suggestion we have included table 5, where the allele frequency data for all the three loci (ApoB, LPL and Leptin ) has been described. Hope, it will convey the description of the loci better. We also have tried our best to avoid repetitions in the text.
2. We have taken care of the referencing, where we have mentioned about shorter telomere length and its association with cancer. We have tried our best to put the references in place and avoid confusing statements. We are extremely thankful to the reviewer for this important comment.

3. In figure 2 we have mentioned that the error bars are standard errors. It is also mentioned in the legends.

4. As per the suggestion, in the discussion section we have tried to write clearly which locus we are describing. We profusely thank the reviewer for this comment/suggestion.

5. As rightly pointed out, we have corrected the reference section by incorporating Frossard et al’s reference correctly.

Discretionary revisions:

1. We thank the reviewers for giving valuable comments on Materials and Methods. We have tried to address your query regarding the replicates. We have clarified these points in the “Materials and Methods section”. We have repeated the samples readings for quality control purpose and observed the standard deviation ranging from 0.02 to 0.05 as compared to the original values.

We hope we have answered all the queries point by point raised by the reviewers. We are very much thankful for the valuable suggestions and comments.

Awaiting for a positive response.