Author’s response to reviews

Title: Association of NOS3 gene polymorphisms with Essential Hypertension in Sudanese patients: a case control study

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Response letter

Tirunilai Padma (Reviewer 1):

There was some mistake in my suggestion made about the title of table -1. Now it can be stated as: Table-1 Primers, restriction enzymes, and fragment lengths of the major and minor alleles of rs1799983, rs2070744, and VNTR polymorphisms of NOS3 gene

Response: the title of the table was done as suggested

Caitrin W. McDonough (Reviewer 2):

While the authors have addressed most of my comments, questions, and concerns, and the paper is much stronger and reads much better; there are still a few things that need to be addressed:

1) During the discussion of the SNP selection in the Methods section, the authors should include that the literature review to select the SNPs was conducted in 2011, and thus more recent SNPs associated with EH and NOS3 were not included. Currently the authors just justify the SNPs they selected, and make no comment as to why others were left out.

Response: a paragraph was added mentioning the date of the literature review (lines 133-134).

2) The results from the logistic regression analyses that include age, gender, and smoking as covariates should be shown, so that the reader can see that these factors are not influencing
the results. Additionally, as BMI is different between the case and control groups, BMI should also be included as a covariate to adjust for.

Response: BMI was added as a covariate and the results of the regression were mentioned in the results section (lines 218-227 and lines 244-252).

3) In regards to the discussion of the linkage disequilibrium results in the results section, the authors need to also remove the statement "All comparisons showed statistical significance", or they need to explain further how they calculated this in the methods section. As once again, the statistical significance part of the LD analysis does not make sense to me.

Response: the statistical significance was calculated by chi square test looking at the difference between the observed and expected frequencies

\[ \chi^2 = \sum (\text{obs} - \text{exp})^2 / \text{exp} \]

obs: haplotype frequency

exp: product of allele frequencies

But the statement was removed to get out of the confusion

4) While many of the limitations of the study have been addressed in other parts, there should still be a limitations paragraph included in the discussion. And this should include reference to the small sample size, potential lack of power, and then the differences between the case and control group (age, gender, BMI, smoking).

Response: a paragraph was added in the discussion mentioning the limitations of the study (lines 327-330).