Reviewer’s report

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

Version: 0 Date: 23 Feb 2017

Reviewer: Caitrin W. McDonough

Reviewer's report:

The paper entitled "NOS3 gene polymorphisms in Sudanese patients with Essential Hypertension" sought to investigate the association of SNPs within NOS3 and association with essential hypertension in a Sudanese EH case-control study. Overall, the study is novel, as this is a novel population, and little is known about the genetics of EH in Sudanese. However, there are several points in the methodology that need to be clarified and addressed, particularly in the statistical methods. Specifically:

1. How where the SNPs studied selected? Was there a prior literature review, or do these SNPs have particular interest in Sudanese based on reported allele frequencies or functions? The authors should include this in the introduction or the methods. There are parts of each of these sections that can be deleted - so there should be room (see comment #10). Additionally, why was the GWAS significant level SNP in NOS3 that has been reported in at least 3 HTN GWAS studies not included? (rs3918226). The authors need to justify their SNP selection, and why they included some SNPs in this gene, but not others that have been studied and highly associated.

2. It is unclear if the genotyping methods are accurate. The authors should include duplicate concordance rates, and genotyping rates for each SNP/variant. Additionally, for rs1799983, as it was digested with two different restriction enzymes - where the results from these two digestions consistent and concordant? These numbers should be reported. Also, how were the RFLP results determined: by just one person reading the gels, or by two people reading them, and making sure their results were concordant and employing a third person or additional method when the two people didn't agree? Finally, how do the other allele frequencies compare to 1000 Genomes, and do the authors feel comfortable with the deviation from 1000 Genomes they observe for rs1799983? Does it make sense given the individual African populations - as opposed to the overall African population? Or with other populations that could represent any admixture that is occurring within the Sudanese population?

3. Another issue is in the statistical methods. Currently all of the results are unadjusted and there is no correction for multiple comparison. The authors need to either adjust for multiple comparisons, or state why they are not adjusting for multiple comparisons. Given
the differences between the cases and controls in Table 2, adjusted analyses seem more appropriate (logistic regression), adjusting for at least age, and gender, and possibly smoking status.

4. The linkage disequilibrium analysis does not make sense. P-values are not usually presented with linkage disequilibrium results, just the D' and r-squared values. Additionally, it seems the authors are perhaps unclear on what the differences between D' and r-squared are, based on the discussion. All of the SNPs are within moderate-high D’ - which would be expected as they are all probably in the same LD block, or at least close to one another as they are in the same gene. The r-square values however, are low, indicating that the SNPs cannot be used as proxies for one another. Thus they likely did not evolve at the same time. Likely SNPs/variants were just created/occurred on one allele version of the SNPs/variants that already existed - giving higher D' values, and lower r-squared values, as the allele frequencies would be different and not predictive of one another. Using a program like haploview or PLINK may be useful for the LD analysis.

5. The difference in age between the cases and controls is a little alarming. Was family history of EH collected within the controls? It is very possible that with controls that are younger than the cases, they could develop EH later in life, especially if they have a family history of EH. This should be discussed as a limitation within the discussion section.

6. The authors to discuss the small sample size. A formal power calculation would be nice to determine if there is enough power to observe associations with the SNPs/variants they are testing.

7. What does "a positive home blood pressure monitoring" mean in line 123?

8. What is meant by "present as a single copy in the haploid human genome." On line 96? Human have a diploid genome - meaning we have two copies of every gene.

9. What is meant by "trans linkage" on line 354? If this is meaning that there is LD between two SNPs even though there is another variant between them - this is not a correct use of the word trans. In genetics 'trans' usually refers to very far effects from a gene - in relational to eQTLs and expression. And usually the trans relation describes loci on different chromosomes or VERY far apart on the same chromosome.

10. There are paragraphs in the introduction and methods that could be removed or shortened as they contain information that should be known by the audience or that could be summarized much more succinctly or referenced elsewhere:

a. Paragraph on the NOS3 gene lines 93-100

b. DNA sample preparation could be shortened: lines 143-151
c. Paragraph on PCR and Taqman could be shortened or referenced elsewhere if available: lines 164-177

d. Paragraphs on gel electrophoresis and cloning could be shortened: lines 178-198.

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

No

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.

I am able to assess the statistics

Quality of written English
Please indicate the quality of language in the manuscript:

Acceptable

Declaration of competing interests
Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?
5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

No COI

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal