Reviewer’s report

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

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Reviewer: Tirunilai Padma

Reviewer's report:

Hypertension is a common complex condition posing a major risk to life due to associated complications like stroke, coronary artery disease, end stage renal disease etc. adding to the mortality rate. Therefore there is need to investigate the causes including genome variation that would help in preventing the origin and associated complications leading to mortality. The major attempt to reach this goal is to identify the genes and their variants causing high risk of susceptibility to the development of hypertension. Among the genes that are studied, NOS3 located on chromosome 7q36 position is worked by several investigators to assess the risk caused to the development of EHT. Variations in the associations reported with EHT differ between the populations and analysis comparing the results will help in drawing the conclusions that can be approved to improve the management of the clinical condition. The attempt made by the authors in studying the contribution of NOS3 gene polymorphisms to EHT patients in Sudanese population can be accepted since such studies in the Sudanese population are not carried out that regularly.

The manuscript should be rewritten as it has major objectionable points. It should be modified thoroughly based on the points mentioned below.

1. The sample size of the patients is 260 and the controls 144 and the frequency of the sexes in the two groups is not comparable. The frequency of males and females are respectively 43% and 57% among patients and 77% and 33% among controls. The low no. of females among controls is acceptable only to certain extent since usually aged females do not co-operate to participate in the studies and hence in many studies the frequency of female controls will be lesser than the males.

2. Were the control subjects verified in detail regarding the presence of EHT, family incidence and other associated conditions like diabetes etc.

3. Is the age of the subjects studied represents the age at onset/diagnosis of EHT or the age reported at the time of recording the cases?.
4. The mean age of patients among patients (59.68) is significantly higher than the mean age of the controls (36.12). This could have happened because of more no. of control subjects at the lower age group as compared to patients. A table of distribution of age groups with 3-5 yrs of class intervals among controls and patients should be worked out. It will show the frequencies of age of controls and patients at the lower age groups recorded by the authors.

5. The younger control subjects must be carrying susceptible genotypes of causative genes and may express the EHT at a later age. Therefore frequency of the age groups of controls should match with the frequency of similar age groups among EHT patients.

6. The age range in patients is 28 to 87 and in controls is 19 to 70 years. So the controls below the age of 28/30 years should be removed from the data & entire data should be analysed again.

7. The EHT and cardiac diseases are known to have early onset in certain populations like India (around 30yrs) as compared to Western populations. Similarly is the onset of EHT is at younger age among the Sudanese.

8. Are the EHT patients associated only with one or more than one condition like diabetes, MI, stroke, renal failure or hypercholesteremia.

9. The data collected should be grouped as 1) patients only with hypertension 2) patients with EHT and diabetes 3) patients with EHT and MI etc. and comparison of distribution of polymorphisms should be made between the groups (if the no. of cases in each group are sufficient). Is EHT expressed in patients first and later on followed by the occurrence of other associated conditions. Renal failure might have occurred due to prolonged and malignant hypertension. Comparison of the data of patients only with EHT and those with EHT and Diabetes (with 80 cases) can be made since the association of a gene variant may be with diabetes rather than EHT among patients with the two conditions.

10. Under results, only the mean levels of systolic pressure is mentioned and not the diastolic pressure. It should be added.

11. The title of table -1 can be: Primers, restriction enzymes, and fragment lengths of the major 614 and minor alleles of 615 rs1799983, rs2070744, and VNTR polymorphisms of NOS3 gene

12. The details given in tables 4,5 and 6 can be combined into one table since the information categorized are similar in the 3 tables.

13. Since the information in tables 7, 8 and 9 are statistically insignificant. So they can be removed and the details can be given in text under results.
14. The title of table -10 can be : Pairwise linkage disequilibrium between the markers rs1799983, VNTR, and rs2070744

15. Haplotype analysis can be done after modify the data as suggested.

16. A brief comparison of the detection of the contribution of NOS3 gene polymorphisms to EHT by investigations in different populations is preferred. A couple of references are :-


2. Sushma Patkar, et. al, Risk conferred by 786 T>C polymorphism of NOS3 gene to Essential Hypertension in synergy with smoking and elevated Body Mass Index International J of Current Res. Jan 2011,

Since it is an attempt to conduct the study of the Sudanese population the manuscripts can be considered for publication after modification

**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.

No

**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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