Reviewer's report

Title: The intron 4c allele of the NOS3 gene is associated with ischemic stroke in African Americans

Version: 2 Date: 10 August 2007

Reviewer: Craig Lee

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General Comments:
• Grewal et al. conducted a case-control study investigating the association between two common genetic polymorphisms in NOS3 and risk of ischemic stroke. They observed a statistically significant association between presence of the intron 4c allele and ischemic stroke risk in African-Americans, which was predominantly driven by an etiology of large artery atherosclerosis. Overall, this study represents an important contribution to the literature validating previous observations.

Major Compulsory Revisions:
• Methods – The authors should provide a more detailed description of their statistical methods, including which covariates were considered and how the final covariates were selected for inclusion in their adjusted model. In particular, please clarify why cigarette smoking status and gender were not included, particularly since cigarette smoking status has been shown to modify the relationship between genetic polymorphisms in NOS3 and various cardiovascular phenotypes (endothelial function, ischemic stroke risk, CHD risk, etc.).
• The authors need to clarify why genotyping data for the Glu298Asp polymorphism was only obtained in a fraction of the population (missing in 32% of controls and 13% of cases), particularly if there were no genotyping failures. This needs to be addressed as a limitation in their analysis, which could have contributed to the lack of an association observed with ischemic stroke risk.
• The authors should add a paragraph to the Discussion commenting on the limitations in their analysis. This should include issues related to the small sample size and limitations in statistical power, particularly with the rare intron 4c allele. These limitations in power are apparent when looking at the confidence intervals in Table 3.

Minor Essential Revisions:
• The authors should use the term ‘race’ not ‘ethnicity’ throughout the manuscript to reference analyses across White/Caucasian and African-American individuals. Also, please clarify how race was defined (i.e., was this by patient self-report?).
• Results, paragraph 5 – The authors should state that the intron 4c allele is rare in both African-Americans and Caucasians, and the results should be interpreted with caution in both racial groups (the allele counts in the control group are only 2
for each racial group).

• Discussion, final paragraph – The authors attempt to pool the data from their analysis and reference #14 to obtain a “pooled” odds ratio. There are numerous limitations to such an approach, and this should be removed from the Discussion. The authors can simply state the OR from each individual study, and comment on the consistency of the observed relationships across population.

• Table 2 – Was the difference in the Glu298Asp T allele frequency in African-American cases (26.2%) and controls (15.4%) not statistically different? It may be helpful to add the regression analysis for the Glu298Asp polymorphism to Table 3, to more comprehensively present the data for the reader.

Discretionary Revisions:

• Introduction and Discussion – When discussing prior studies evaluating the relationship between genetic polymorphisms in NOS3 and ischemic stroke, the authors should clarify whether presence of the described variant alleles was associated with higher or lower risk of stroke. Moreover, the authors should include the well-characterized -786T>C (rs2070744) polymorphism in this discussion, particularly since it is functionally relevant and has been associated with risk of ischemic stroke in other populations. The authors should also justify why this polymorphism was not evaluated in their analysis.

• Discussion, final paragraph – The authors reference #14 as the only report evaluating the NOS3 gene in a biracial population; however, a recent publication evaluating the association between the Glu298Asp and -786T>C polymorphisms and risk of incident ischemic stroke in the ARIC population has been published (Lee et al. Pharmacogenet Genomics. 2006;16:891-99). This analysis should be cited and considered in data interpretation.

• Table 1 – Please include the racial distribution, independent of genotype, across the case/control groups. Please also include information on cigarette smoking status. Lastly, please provide a footnote describing what the allele counts refer to. I presume ‘2n’ are allele counts, but this needs to be clear for the reader.

What next?: Accept after minor essential revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests.