Author's response to reviews

Title: Endothelial Nitric Oxide Synthase Gene Polymorphism (Glu298Asp) and Development of Pre-eclampsia: a nested case-control study and a meta-analysis.

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Version: 4 Date: 26 January 2006

Author's response to reviews: see over
Dear Editor,

Thank you for the comments of the referees that have been used to prepare the enclosed revised manuscript. Since our submission of the manuscript two additional studies have been published, and the authors conscious of that decided to up-date the meta-analysis until November 2005. The conclusions of the manuscript remain unaltered, and the appropriate changes have been conducted. We have given specific responses to the reviewer’s recommendations:

Thank you

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Reviewer 1

1. Reference 28 should not be superscript.

Response: Thank you. This has been corrected.

2. Reference 30 was cited before reference 29 in the manuscript.

Response: Thank you. This has been corrected as suggested and amended in the reference.
Reviewer 2

1. It is well known that Q is a rather unreliable for addressing heterogeneity. Not significant heterogeneity is detected in figure 1, but this may be due to low paper. The appropriate measure is I2 [Higgins]. Moreover, it is not advisable to decide about the meta-analytic model (random effects or fixed effects) on the basis of a p-value. I suggest random effects models to be applied as default, since homogeneity is rather the exception in genetic association studies.

Response: Random effect models are now used throughout the manuscript. As suggested, we have introduced the I^2 measure to evaluate the degree of heterogeneity observed.

2. You need to address further sources of heterogeneity for the meta-analysis as the genotyping method and the compliance to HWE. The studies are not enough to explore them, but you need to consider them.

Response: We agree that other sources of heterogeneity, mainly study-level covariates, have to be considered. Unfortunately the number of studies is a substantial limitation at this stage in order to conduct such analysis in a robust manner. However, we now include data on use of blinding of the genotyping staff as another possible source of heterogeneity. Compliance to HWE could not explored since in all the control samples from the studies included were in HW equilibrium.

3. Please make a proper stratified analysis for the nested case-control study by ethnicity, and report the estimates.

Response: We thanks to the reviewer for her thoroughness with the revision of the manuscript. The authors want to clarify that although the original study from which the participants were selected was prospective study, for logistic reasons we were unable to select a random set of controls matched to the cases, as it should be in a nested case-control design. Therefore, the authors now consider that the study design is a case-control study and therefore logistic regression models are used for its analysis. Though, the word “nested” has been deleted from the revised version of the manuscript to be in agreement.

4. Here are some further comments to improve the presentation
   a. In table 1, SD or 95% CI should be reported along with the point estimates for continuous outcomes. The p-value for primiparous and smokers cannot be based on ANOVA, I guess you did a X2 analysis?

Response: The median and interquartile ranges (for non-normally distributed variables) have been added to Table 1. For the non-continuous outcomes such as primiparous and smokers, we did carry out a χ^2 analysis and the appropriate amendments have been done in Table 1.
b. Are the studies in the forest plots in random order? If yes, order them by date of publication or sample size. Next to the polled effect size, print the point estimate and the 95% CI.

Response: The study are now sorted by total sample size. We have added the point estimate and the 95% CI in each forest plot.

c. For the nested case-control study add a 3x2 table for the numbers per genotype in cases and controls.

Response: We have included that information in the text, (Page 9, seventh line from the bottom).

d. In table 2, add the ORs for the clinical factors included in the model.

Response: We thank the reviewer for her careful analysis on the manuscript. However, we consider that those clinical factors should not be added. None of those (Maternal age [OR:1.02 (95%CI: 0.9, 1.06)], Smoking [OR: 0.88 (0.3, 2.2)] and ethnic background[^1] [Caucasian: 0.81 (0.4, 1.3); other-group: 0.69 (0.2, 1.7)]) were statistically significant. In addition, we consider that by doing that, we may disperse the readers attention from the genetic factors, the main exposure to be evaluated. Estimate of the effect for those clinical factors can be obtained elsewhere.

e. Statistical analysis methods are mixed with the systematic review methods. The fact that the cases in the nested case-control study are in HWE corroborates the conclusion of no association between Asp and pre-eclampsia.

Response: We now separate the statistical methods from the case-control study and those from the systematic review.

[^1] For ethnic background the Afro-Caribbean group was set as the reference group; while for smoking the non-smoker group was set as a reference.