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.

Authors:

Christina KH Yu (chrissieyu@aol.com)
Juan P Casas (rmhajpc@ucl.ac.uk)
Makrina D Savvidou (msavvidou2005@yahoo.co.uk)
Manpreet K Sahemey (manpreetsahemey@yahoo.com)
Kypros H Nicolaides (kypros@technocom.com)
Aroon D Hingorani (a.hingorani@ucl.ac.uk)

Version: 2 Date: 20 December 2005

Author's response to reviews: see over
Re: 'Endothelial Nitric Oxide Synthase Gene Polymorphism (Glu298Asp) and Development of Pre-eclampsia: a nested case-control study and a meta-analysis.'

Manuscript: 1452598236642891
20th December 2005

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

Christina Yu
Harris Birthright Research Centre for Fetal Medicine,
King’s College Hospital,
Denmark Hill,
London SE5 9RS, U.K.
Tel: 0044 207 346 3070
Email chrissiyu@aol.com

Juan P. Casas
Department of Epidemiology and Population Health
London School of Hygiene & Tropical Medicine
Email: Juan.pablo-Casas@lshtm.ac.uk
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 in 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 have now included data on use of blinding of the genotyping staff as another possible source of heterogeneity. Compliance to HWE could be explored since in all the control samples from 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 thank the reviewer for her thoroughness with the review of the manuscript. The authors want to clarify that, the design we adopt for this genetic study was a case-control study (ratio 1:4) with frequency matching by maternal age and ethnicity. Therefore, the logistic regression models including the matching variables in the multivariate model are used for the analysis. The word “nested” has been deleted from the current version of the manuscript.

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 χ^2 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 study 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\textsuperscript{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 have separated the statistical methods from the case-control study and those from the systematic review.

\textsuperscript{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.