Author's response to reviews

Title: Polymorphisms of the endothelial nitric oxide synthase (NOS3) gene in preeclampsia: a candidate-gene association study

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Author's response to reviews:

TO:
Prof Carl Hubel
Editor, BMC Pregnancy and Childbirth
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Dear Prof Hubel,

We were pleased to hear that BMC Pregnancy and Childbirth is interested in a new revised version of our manuscript. We are grateful to the reviewers for their valuable input. We have addressed all these comments in this new revision. In more detail:

Reviewer #1

Minor Essential Revisions

Comment 1: The Abstract indicates a “case-control sample of 102 patients with preeclampsia and 176 healthy females”. It would be better to indicate that the controls were women with a history of uncomplicated pregnancy (the genotype distribution of these controls could differ from the general population of healthy females).

Response 1:

In Abstract, paragraph 2, the text previously reading: “Methods: We examined the association of three common variants of the NOS3 gene (4b/a, T-786C and G894T) and their haplotypes in a case-control sample of 102 patients with preeclampsia and 176 healthy females.”

was changed to:
“Methods: We examined the association of three common variants of the NOS3 gene (4b/a, T-786C and G894T) and their haplotypes in a case-control sample of 102 patients with preeclampsia and 176 women with a history of uncomplicated pregnancies.”

Comment 2: Are all of the inclusion/exclusion criteria for cases and controls included in Methods? Please indicate whether or not all of the controls were delivered at term and whether patients with previous renal disease, diabetes, or history of metabolic disorders were excluded from the patient sample.

Response 2:
In Methods section, paragraph 1, the sentence previously reading: “The controls were unrelated by blood to cases.”
was changed to:
“The controls were unrelated by blood to cases, and all apart from 3 (1.7%) were delivered at term.”
Moreover, in Methods section, paragraph 1, the following sentence was added: “All study participants were of Caucasian origin whereas women with previous renal disease, diabetes, or history of metabolic disorders were excluded from the patient sample.”

Comment 3: Table 1: Please indicate significant differences. Please also include maternal pre-pregnancy body mass index (BMI) if available.

Response 3:
In Table 1, significant differences were indicated. Unfortunately, pre-pregnancy body mass index (BMI) of the participants is not available.

Comment 4: It may be assumed that all patients were Caucasian but race status should be indicated somewhere.

Response 4:
In Methods section, paragraph 1, the following sentence was added: “All study participants were of Caucasian origin whereas women with previous renal disease, diabetes, or history of metabolic disorders were excluded from the patient sample.”

Discretionary Revisions

Comment 1: It is recommended that a few sentences and reference be added to explain WHY the “endothelial nitric oxide synthase gene (NOS3), ..... , has emerged as a logical candidate gene in the development of preeclampsia” (beyond just the association with hypertension).

Response 1:
In Background section, paragraph 2, the following text was added: "The leading hypotheses, concerning the pathogenesis of preeclampsia, are based on disturbed placental function and impaired remodelling of the spiral arteries [5]. Endothelial nitric oxide synthase (NOS3) is an important regulator of vascular tone and contributes to the reduction of the uteroplacental resistance seen in normal pregnancy [6-8]."

Moreover, the sentence previously reading: "The endothelial nitric oxide synthase gene (NOS3), located at the 7q35-q36 region, has emerged as a logical candidate gene in the development of preeclampsia."

was changed to:
"Therefore, the endothelial nitric oxide synthase gene (NOS3), located at the 7q35-q36 region, has emerged as a logical candidate gene in the development of preeclampsia."

Comment 2: It might be useful to mention/discuss that the linkage recently reported for eNOS possibly reflects its relationship with (essential) hypertension rather than preeclampsia, and that preeclampsia is more than just gestational hypertension.

Response 2:
In Background section, paragraph 2, the sentence previously reading: "A whole genome-scan meta-analysis for preeclampsia has already identified the locus of NOS3 gene as a promising candidate for preeclampsia susceptibility [2]."

was changed to:
"A whole genome-scan meta-analysis for preeclampsia has already identified the locus of NOS3 gene as a promising candidate for preeclampsia susceptibility [2], although linkage studies seem to support a relationship between NOS3 and hypertension rather than preeclampsia [12, 13]."

Comment 3: Regarding the statement, "Interestingly, 13 studies [5, 18-29] have reported association for the variants NOS3 gene..." please clarify whether these represent a mixture of positive and negative findings. Please also consider adding Hillermann R e al J Hum Genet (2005) (Glu298Asp study) to this mix of references.

Response 3:
In Discussion section, paragraph 4, the text previously reading: "Interestingly, 13 studies [5, 18-29] have reported association for the variants NOS3 gene, and the two published meta-analyses did not replicate the findings [8, 9]."

was changed to:
"Interestingly, 13 studies [9, 24-35] have reported positive association for the variants NOS3 gene, whereas the two published meta-analyses and other individual studies did not replicate the findings [14, 15, 36, 37]."
Reviewer #2
Comment 1: the authors have described the frequencies of polymorphisms in NOS gene in their cohort of patients with preeclampsia and compared them with women with normal pregnancies. They did not find any difference in the frequencies studied.
They have not tried to do any subgroup analysis, such as according to severity of PE or SGA deliveries.
Response 1:
We did not perform subgroup analysis for three reasons: 1) the sample is small to detect the minor contribute role of the NOS variants in the development of PE, 2) the overall analysis showed no association and 3) any possible significant result in a subgroup analysis will be due to false-positive results and definitely it is going to data-driven. In addition, the study was not designed to investigate specific PE populations.
Comment 2: The authors themselves acknowledge that the sample size is too small to detect differences, if any. Moreover, it is clear on the basis of current knowledge that genotypic differences in a single gene are very unlikely to explain a disorder like PE. The authors had the opportunity to study other genes that code for vasoactive compounds, which would have given greater information.
Response 2:
Indeed, the simultaneous study of other genes that code for vasoactive compounds would have given greater information regarding the pathogenesis of preeclampsia. Nevertheless, our resources (especially the financial) were limited and therefore, we did not study other genes.
Reviewer #3
Comment 1: However, by employing the above strategy of a single gene polymorphism (even if it includes three previously studied intergenic polymorphisms with potential functional significance), the putative significance or insights for a genetic association of the NOS3 gene with a multifactorial disease such as preeclampsia are very limited. This weakness is well recognized by the authors and should be clearly phrased in the manuscript (Abstract, Results and Discussion).
Response 1:

In Discussion section, paragraph 4, the following text was added: “Since predicting the functional credentials of most genetic variants remains problematic, obtaining robust replication of positive association findings proves difficult [38].”

Moreover, in Abstract section, paragraph 4, the sentence previously reading: “The evidence of our study does not support the major contributory role of these common NOS3 variants in preeclampsia.”

was changed to: “Given the limitations of the candidate-gene approach in investigating complex traits, the evidence of our study does not support the major contributory role of these common NOS3 variants in preeclampsia.”

Comment 2: The authors could consider expanding the NOS3 haplotype in the Greek population by evaluating additional SNPs flanking the NOS3 gene. The data generated by such an approach, could provide additional insights on the role and the contribution of the region on the development of preeclampsia.

Response 2:

In this study we aimed to replicate previous findings on the association between three commonly investigated, and potentially functional, NOS3 polymorphisms and preeclampsia. We may consider evaluating additional SNPs flanking the NOS3 gene in a future study.