Author's response to reviews

Title: Fetal ERAP2 variation is associated with preeclampsia in African Americans in a case-control study

Authors:

Lori D Hill (hillld4@vcu.edu)
DaShaunda D Hilliard (ddh9@hermes.hood.edu)
Timothy P York (tpyork@vcu.edu)
Sindhu Srinivas (ssrinivas@obgyn.upenn.edu)
Juan P Kusanovic (jkusanov@med.wayne.edu)
Ricardo Gomez (rg.cedip@gmail.com)
Michal A Elovitz (melovitz@obgyn.upenn.edu)
Roberto Romero (prbchiefstaff@med.wayne.edu)
Jerome F Strauss III (jfstrauss@vcu.edu)

Version: 2 Date: 24 March 2011

Author's response to reviews: see over
March 24, 2011

Dear Editors,

Thank you for your consideration of our recent submission: MS:1274824451503480, “Fetal ERAP2 variation is associated with preeclampsia in African Americans in a case-control study.” Please find below our detailed responses to the reviewers’ comments.

Sincerely,

Jerome F. Strauss III, M.D., Ph.D.

I. Please note the addition of DaShaunda D Hilliard as a coauthor.

II. Section Editor Concerns:

In the abstract, it is said "We found no associations between rs17408150 and risk for preeclampsia in either population." This is incorrect as this SNP was not tested in the American African population.

Response: This point has been clarified in the abstract.

b. Table 2 should report genotype counts so that readers can address the validity of Hardy Weinberg distribution and data could be later used for meta-analysis if needed

Response: Genotype counts have been added to Table 2.

c. In the Chilean population, the pairwise r^2 between rs17408150 and rs2549782 is ~0.08. What about D'? Haplotype analysis of the two SNPs must be provided.

Response: We chose to report r^2 for this situation since, in association studies, when the minor allele frequency of one SNP is very small compared to the other SNP, it has been shown r^2 represents a better measure of LD than D' (Wray 2009). This is because distribution of D' is overrepresented at 1 in situations where the frequency of one allele is low and r^2 is better suited to measure LD. In our study D'=1.0 and r^2=0.08. This marked difference can be attributed to the low minor allele frequency of the rs17408150 SNP. This combination of D' and r^2 “implies a tendency to the presence of only 3 haplotypes” when one would expect four (2^2 = 2 SNPs ^ 2 alleles). In our study rs2549782 has a MAF ~ 0.33 whereas rs17408150 has a MAF ~ 0.04, thus the r^2 measure is a more appropriate measure of LD vs D’. Since there two SNPs were not observed to be in LD by r^2, haplotype analysis was not justified in this study.


d. The Michigan vs Pennsylvania analysis is obscure and must be clarified. In particular, it is not clear in which samples (cases, controls, both?) allele frequencies reported in table 3 are derived from? The rs2549782 showed difference in MAF between Pennsylvania and Michigan (0.3980 0.4284) of nearly similar amplitude than
those reported in table 2 for fetal DNA. The authors should demonstrate that the significant difference in table 2 is not due to population mixture and is observed in both fetal African American samples.

Response: Table 3 has been revised to show allele frequencies from controls only at each location. This has been included in the table legend. We tested for differences in proportions (allele and haplotype frequencies) between each SNP/Haplotype and found no significant differences. This has also been added to the legend. Finally, the results of two additional tests that specifically addressed the issue of population stratification were included at the end of the results section. The results of these analysis show that the observed association between the fetal rs2549782 ERAP2 and preeclampsia was not the result of population stratification. Additionally, there is no literature to support African Americans being different between the two sites, and more importantly, there is no literature to support differences in preeclampsia between African American groups.

please also check for grammar and typos:

eg page 6:

"and the previously described altered expression of ERAP2 in placentas before maternal symptoms develop[29]; suggests that the fetal ERAP2 gene contributes to the development of preeclampsia."

Response: grammar corrected as requested

"In the present study, we investigated whether the previously described associations between ERAP2 and risk for preeclampsia(ref) replicated in other ethnic"

Response: reference has been added

III. Reviewer: Matthew Peter Johnson

a. ABSTRACT: Conclusions

"...this gene in a New Zealand population..." needs to be changed to read "...this gene in an Australian/New Zealand population..."

Response: correction has been made as requested

b. RESULTS: African American Population

Was the genetic similarity assessment between the Pennsylvania and Michigan African American cohorts formally tested or were the minor allele frequencies “eyeballed” prior to the decision to merge the two cohorts? Between the Pennsylvania and Michigan cohorts, do the ERAP2 minor allele frequency differences of ~3% and ~3.8% in the maternal and fetal groups, respectively, constitute enough of a difference to potentially stratify the association results? If formal statistical tests were performed to assess these differences, then they should be included in the manuscript.

Response: please refer to the response to comment d. in the section editor’s concerns.

c. DISCUSSION: 6th paragraph
“While the Johnson et al. reports a maternal association, they did not include fetal samples in their study, and thus cannot rule out that the observed association was driven by fetal genes that were shared with the mother.” This reviewer acknowledges the plausibility about shared genetic components between the mother and fetus and their contribution to preeclampsia susceptibility. However, in stating the highlighted sentence (above) the authors need to be aware of, and make the reader aware of, ERAP2 residing within a region of known genetic linkage for maternal preeclampsia susceptibility (Johnson et al. Molecular Human Reproduction 2007). The study design in the Johnson 2007 article interrogated maternal preeclampsia susceptibility in a sub-set of families that were used in the Johnson 2009 article. Therefore, there is good evidence that ERAP2 may contribute to both the maternal and fetal genetic components of preeclampsia susceptibility. Given the heterogenic nature of preeclampsia, ERAP2 may very well have independent effects primarily towards the maternal and fetal components in the Australian/New Zealand and African American cohorts, respectively.

Response: We acknowledge the above point made by the reviewer and have adjusted the discussion (paragraph 7) to reflect the possibility of genetic heterogeneity explaining different genetic associations between maternal and fetal genotypes in ethnically different populations.

CONCLUSIONS
The rs2549782 association with maternal preeclampsia risk was specific to the Australian/New Zealand population and not the Norwegian population. An association of maternal preeclampsia risk in the Norwegian population was specific to the rs17408150 SNP. Therefore, the third sentence needs to be changed accordingly.

Response: the change has been made as requested.

IV. Reviewer: Sara Sedano-Balbas

a. Why did the authors used those specific three genes to establish the genetic similarity between samples from both hospitals? Could the author include in the text a reason due to they have chosen those genes?

Response: The results section of the manuscript has been revised to more clearly show why these genes were chosen. They were chosen based on the following: 1. MTHFR and COMT show variation not only between ethnicities, but also within large ethnic groups. 2. 5 SNPs in these genes were available for comparison and 3. The 4 SNPs in the COMT gene form ethnic specific haplotype structures that allowed for the comparison of a more complex genetic structure.

The results of this study support a relationship between fetal rs2549782 G allele in the ERAP2 gene and preeclampsia. No maternal rs2549782 G allele was however associated in neither of the populations investigated in this study. The results can be more completed if an example of other study where fetal genes are accounting for the development of pregnancy outcomes was included in the discussion?
Response: A fetal genetic contribution has been established in the literature and has been reviewed in the manuscript. We feel that no further precedent is needed to support fetal genes contributing to the development of preeclampsia. The contribution of fetal genes to preeclampsia is discussed at the following places in the manuscript:

Background: “A genetic susceptibility to preeclampsia has been established with both maternal and fetal genes contributing to disease[2, 10-17].

Discussion: “Preeclampsia is usually diagnosed after 20 weeks of gestation, but it is thought that problems arising early in pregnancy, especially during placentation, are the origin of this disorder. \textit{ERAP2} is expressed in the syncytiotrophoblast and it has been reported that expression of this gene was down-regulated in first trimester placentas of women who subsequently developed preeclampsia[23, 29].”

Discussion: “Additionally, maternal-paternal ethnic discordance has been associated with an increased incidence[2].” Paternal genes act through the fetus.

c. In Abstract Conclusions: What do the author mean by maternal associations? Do the author mean preeclampsia association? or any other women adverse condition during pregnancy?

Response: the end of this sentence “\textit{ERAP2} has now been associated with preeclampsia in three populations.” Explains that these associations are all with preeclampsia.

In results; what BMI stands for has to be included in the text.

Response: this change has been made as requested

In results; Clinical Characteristics of the study population; Last sentence Significant differences en fetal sex have been reported in the literature, but results are “mixed”. What does the author mean by mixed? Contradictory?

Response: “mixed” refers to the fact that some studies have found more female fetuses, some studies have found more male fetuses, and still others have found no difference in fetal sex in association with preeclampsia.

In discussion; In the sentence Preeclampsia is associated with…..”What Th1 and HLA stands for should be included on the text”.

Response: these have been included in the text as requested

In discussion; can it be clarified what ethnic group is referred to by white women?

Response: per the new NIH guidelines, “white” is the preferred ethnic name previously designated as Caucasian.

In discussion; what MHC stands for in “ Finally two haplotypes of ERAR2…”
Response: MHC stands for Major Histocompatibility Complex and this has been added to the text as requested

In Conclusions; Include reference ; ...of a previous study “by Johnson et al.” that found an association .... Norwegian population.

Response: The reference has been included as requested

What next?
Examination of maternal and fetal samples for variations in the ERAP2 gene from subjects with preeclampsia including a wider range of samples and from different ethnic backgrounds.

Response: the first step to extend this study is stated at the end of the Discussion section “Future studies, increasing the number of markers to saturate the maternal and fetal ERAP2 genes, are needed to distinguish between maternal and fetal effects of this gene and to characterize the haplotype structures of each group.” Before extending to different ethnic groups, better characterization of the maternal and fetal ERAP2 genes within the populations already established to have an association with preeclampsia is needed.