Author's response to reviews

Title: Gene Polymorphisms in Association with Emerging Cardiovascular Risk Markers in Adult Women

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Author’s response to reviews: see over
Response to Reviewers Comments

Title: Gene Polymorphisms in Association with Emerging Cardiovascular Risk Markers in Adult Women

Dear Editor-in-Chief,

We addressed reviewers’ comments as below.

General request for revision of abstract:
We would be grateful if you could address the comments in a revised manuscript and provide a cover letter giving a point-by-point response to the concerns.

Please also ensure that your revised manuscript conforms to the journal style (http://www.biomedcentral.com/info/ifora/medicine_journals). It is important that your files are correctly formatted.

We would also recommend you include some additional background information in the abstract on top of the aims. Guidelines for writing abstracts are provided at this page: http://www.biomedcentral.com/info/ifora/abstracts

Response: We addressed the above concerns. Additional background information was added to the abstract on top of the aims as requested.

Reviewer: Melanie Waldenberger
Major Compulsory Revisions
1. As the authors mention, the investigated markers are correlated with each other and with other phenotypes such as high blood pressure. The authors should present the degree of correlation between the analysed markers. It might well happen that the weak association between CRP concentrations and rs1801133 is based on the correlation of CRP and homocysteine concentrations. It might be of interest to further investigate these relationships (e.g. by conditional analysis).
Response: The degree of correlation between markers was shown in supplemental Table 1. The correlations between these markers ranged from 0.04 to 0.39. The correlation between CRP and homocysteine is very small (r=0.038). Thus it is not very likely that the weak association between CRP concentrations and rs1801133 is based on the correlation of CRP and homocysteine concentrations.

Minor Essential Revisions
1. The selection of covariates for the conducted analysis is hard to follow. The authors refer to a long list of potential a priori selected covariates and state that only significant associated covariates for a specific marker were retained in the adjusted models. It would be of interest to see the final adjusted models for each of the four markers.
Response: Information was added to the Methods section under Statistical Analysis, second paragraph to indicate the covariates in each model.

2. It would be of interest to the reader to discuss the present results with regard to the results of the recently published meta-analysis of GWA data for some of the investigated markers.
Response: Information and references were added to the last paragraph of the Discussion to address recent genome-wide association studies.

3. In the background section, the authors state that the selected candidate genes confer excess risk of cardiovascular disease. Properly by mistake, they reference a recent manuscript by Cambien and Tiret. In this manuscript other genes than the ones investigated here are discussed in relation to cardiovascular disease.
Response: Thank you for pointing this out. The objective of the review article was not to provide an exhaustive account of the numerous studies conducted on the genetics of CVD. Instead, a limited number of examples were discussed to provide insights into the causes and mechanisms of CVD. The reference was used to validate the statement that results are inconsistent.

4. The wording emerging biomarkers seems out of place for the well known and extensively studied markers CRP, fibrinogen, uric acid and homocysteine.
Response: As explained in the Cambien and TIRET reference, these markers are still controversial and are not used extensively in clinical practice.

5. The authors should carefully read the manuscript with respect to typos (e.g. it should read genes on page 2, paragraph 2 (methods), line 3)
Response: Agree. Several minor typos were corrected.

Reviewer: Jose M Soria
General Comments

The investigators conducted a classical population-based association study between several genetic variants and some traits in adult women. Although, the strategy used in this study is potentially interesting, their results are not new. They are mainly confirmatory regarding the relationship between some SNPs and some traits (i.e. MHFR and Homocysteine levels). In addition, some major concerns should be addressed to clarify the results.

Major Compulsory Revisions
1.- It is not clear how and why these candidate genes have been selected and not others, and how the SNPs has been selected. I assume that the SNPs have been selected based on their potential functional or regulatory effect. However, other SNPs in these genes with low LD among them should be included to capture the genetic variability of those loci. In fact, in this genomic era with the improvement of genotyping technologies, limiting the genetic analyses to only
few DNA variants within candidate genes is a poor strategy.
Response: Information and references were added to the Background section, third paragraph regarding selection of candidate genes and availability of SNPs.

2.- I concern about the selection of candidate gene, since it is not clear the implication of several of these genes in cardiovascular disease risk. The authors should provide with more information about that.

Response: Information and references were added to the Background section, third paragraph regarding selection of candidate genes.

3.- Recently, it has been reported a genome-wide association and replication study to identify genetic loci associated with plasma CRP concentrations. The lack of concordance between the effect on coronary heart disease risk of CRP genotypes and CRP levels argues against a causal association of CRP with coronary heart disease in this study (Elliot P et al JAMA 3002:37-48.2009). In addition, there are also information regarding several genome-wide with fibrinogen levels. It would be convenient that the authors incorporate this information into the manuscript.

Response: The second paragraph of the Discussion section addresses the lack of concordance with coronary heart disease. The suggested references were added.

4.- Although, I agree with the authors that the investigated traits are influenced by environmental factors which were not adequately captured by the current study (an important limitation), they would incorporate some data about the effect of the environmental factors that they have information with the trait levels.

Response: Environmental factors are likely a source of interaction rather than confounding. The Discussion section addresses the need for further interaction studies that are beyond the scope of this study.

Minor Essential Revisions
1.- The authors should incorporate in the Material and Methods how these phenotypes (i.e., Plasma c-Reactive, fibrinogen and Homocysteine) were measured.

Response: Since this information is readily available on the referenced website in the Methods section under Biochemical Analysis, we chose to omit details as is customary in order to conserve space.

Thank you for your consideration of publication.

Sincerely,

Amy Fan