Author's response to reviews

Title: The association of 9p21-3 locus with coronary atherosclerosis: A Systematic Review and Meta-Analysis

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Author's response to reviews: see over
We appreciate the reviewers’ comments and suggestions. Here are our point-by-point responses.

Editorial:

*Acknowledgements: We strongly encourage you to include an Acknowledgements section between the Authors’ contributions section and Reference list. Please acknowledge anyone who contributed towards the study by making substantial contributions to conception, design, acquisition of data, or analysis and interpretation of data, or who was involved in drafting the manuscript or revising it critically for important intellectual content, but who does not meet the criteria for authorship. Please also include their source(s) of funding. Please also acknowledge anyone who contributed materials essential for the study.

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Response: We added the Acknowledgments in the manuscript.

Reviewer 1:

In their manuscript, the authors describe a literature search and meta-analysis on associations of the 9p21 region with severity of coronary artery disease. While this is a potentially interesting topic, there are some issues pertaining mainly to the methods employed.

Major compulsory revisions

1. Background: Please elaborate on the rationale of the study. While the association of the 9p21 region with CAD has been established, it does not become clear why this region should have an effect on CAD severity. Related to that, more details should be provided on this specific region concerning the possible functionality, relationship with other phenotypes etc.

Response: Functional studies of this region have revealed that variation at this locus may lead to increased smooth muscle cell proliferation as well as pro-inflammatory effects (Visel 2010 Nature Genetics, Harismendy 2011 Nature Genetics). We therefore hypothesized that variation in this region may also influence severity of coronary artery disease. In addition, (Murabito et al Circ Cardiovasc Genetics) and colleagues have found that the 9p 21 locus is associated with inter-individual variation in the ankle-brachial index, a marker of atherosclerosis severity in the lower extremities.

2. Methods: The search itself needs to be described in much more detail. This includes answering the questions: which terms were searched for in which data bases at which date with which restrictuions and with which connections? I was not able to reproduce even part of the search based on the descriptions. Did the authors also try to identify further studies by going through the reference lists and through personal contacts? In addition, it is not clear how many hits were made in each of the data
bases before removing any duplicates, i.e., the upper part of the flow chart as recommended by PRISMA is missing.

**Response:** We agree with the reviewer and attached the exact search strategy as a supplemental file to this manuscript. We didn’t conduct reference mining as our search strategy was developed by an experienced librarian in our institution who has developed over 100 search protocols. At the very beginning of our project, we decided that reference mining would not bring many benefits. We updated our flowchart using PRISMA template and replaced the old flowchart. However, we can’t provide the number of duplications removed as our library search program automatically deleted these duplications and we did not track the number.

3. Methods: The authors state that „In studies reporting > 1 SNP-outcome association, we chose the SNP not elsewhere tested in other data set ...“. Why was this done?

**Response:** This was done to allow testing of as many markers as possible so that all known markers in the locus were captured to determine locus-outcome association.

4. Methods: A number of different outcomes were analysed using a number of different genetic models. How was multiple testing accounted for?

**Response:** In meta-analyses, currently there is no consensus about when the multiple testing problem should be taken into account, or which statistical method should be used (Cochrane Handbook for Systematic Review, Chapter 16; and Methods Guide for Effectiveness and Comparative Effectiveness Reviews. AHRQ Publication No. 10(14)-EHC063-EF). However, we agree that the multiple testing problem can increase type 1 error. We added this in the limitation. We also removed two genetic models and only reported additive genetic models in the manuscript.

5. Methods: In a related manner, how was power ensured for those outcomes with no detected association?

**Response:** In meta-analyses, optimal information size (OIS, a similar concept to sample size in clinical trials) is not routinely calculated. Here we calculated for each outcome and discussed them in the manuscript (Table listed below). We found in all of the outcomes, except all cause mortality, triple vessel disease, and Gensini Score, the total sample size reported in the studies were less than the OIS. We, thus, were unable to reach conclusive findings for these outcomes.

<table>
<thead>
<tr>
<th>Power=80%, a=5% (2-tailed)</th>
<th>Assumed effects of outcomes in comparisons</th>
<th>Optimal information size (OIS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-cause mortality</td>
<td>0.08 vs. 0.05</td>
<td>870</td>
</tr>
<tr>
<td>Recurrent MI</td>
<td>0.05 vs. 0.04</td>
<td>13490</td>
</tr>
<tr>
<td>Revascularization</td>
<td>0.15 vs. 0.13</td>
<td>9448</td>
</tr>
<tr>
<td>Triple vessel disease</td>
<td>0.50 vs. 0.40</td>
<td>776</td>
</tr>
<tr>
<td>Gensini Score</td>
<td>30 vs. 20 (SD: 20)</td>
<td>252</td>
</tr>
<tr>
<td>DPI</td>
<td>0.25 vs. 0.20 (SD: 0.8)</td>
<td>2010</td>
</tr>
</tbody>
</table>
6. Results: Whenever results are presented for the different outcomes, please state how many studies and how many individuals were included.

Response: We added the number of studies and the number of patients in Table 2.

7. Results: In presenting the studies, please also state which genetic variants were included for every study. Also, add further information on the genetic variants such as genotyping method, base position, allele frequencies and linkage disequilibrium.

Response: We added the information in Table 1.

8. Discussion: The authors are strongly encouraged to tone down the interpretation of their results considering the small effect sizes and lack of adjustment for multiple testing, as well as lack of power estimates for the negative outcomes.

Response: We added a limitation section and softened our conclusion.

Minor essential revisions:

1. Background: Risk variants associated with CAD have increased in number, and more recent publications should be given. For example, in PMID: 23202125 46 variants are given.

Response: We agree with the reviewer and added the reference in the background.

2. Background: The authors speak of „the 9p21 variant“. However, 9p21 is a region that contains a number of variants.

Response: we now refer to the 9p 21 locus rather than 9P 21 variant.

3. Methods: Concerning the selection of studies, the authors say that „Applicable study designs included GWAS and observational studies (case-control, cohort, and cross-sectional) ...“. This is misleading, because GWAS are also observational studies (mostly case-control). Instead, a possible distinction would be GWAS and candidate association studies.

Response: We agree with the reviewer and deleted it in the manuscript.

4. Methods: For the included studies, the ethnic background of the subjects is described. How was this accounted for in the analyses?

Response: Majority of the studies included white or mixed ethnicities. We decided that the studies did not present enough information to investigate the difference among ethnic groups.
5. Methods: In the meta-analysis, three different genetic models were applied. This increases the multiple testing burden (see below) and is not really required, since information on the adequate genetic model should be provided by the original articles.

Response: We thank the reviewer for this suggestion. We now only reported additive genetic models in this current manuscript.

6. Results: Concerning the result on the number of diseased vessels, the authors state that „Homozygotes (HR) for the risk allele had 33.7% greater risk in the additive genetic model ...“. What does that mean in the context of a semi-quantitative measure; is it a risk increase by one vessel?

Response: This is an overall risk increase of triple vessel disease. We clarified this in the manuscript.

7. In table 2, please provide the p values instead of “NS”, and provide exact values instead of “<0.001”. Also, please provide the number of studies included.

Response: We agree with the reviewer and changed these numbers in Table 2.

8. Concerning figure 3, why is the plot shown for mortality, for which there was no effect? It would be, in my eyes, be more informative to see the heterogeneity for those outcomes with associations.

Response: Although we didn’t find significant associations, mortality is the most important outcome measure to patients and clinicians. We believe a separate plot is necessary in this case.

9. Discussion: The authors state that „All analysis were repeated using the add. rec. and dom. models where possible, with similar results across all 3 models“. Similar results from different genetic models do not really strengthen the results, because this is not necessarily expected.

Response: In response to the reviewer suggestions, we now only report results from additive genetic models, as these are the most likely.

10. Discussion: As a limitation, the authors are advised to include their selection of studies, which is restricted to association studies. Different results might be obtained if linkage and function studies were included as well, if there are any.

Response: We added the following sentence in the limitation section:

Our meta analyses was restricted to Association studies as no linkage analyses have identified this locus to be associated with CAD.

Reviewer 2

The manuscript by Munir et al. examines the relationship between the 9p21.3 locus and various phenotypes associated with coronary atherosclerosis in a systematic meta-analysis. The questions addressed were whether the 9p21.3 risk variant associates with the severity of coronary artery disease
and whether the 9p21.3 variant predicts adverse clinical outcomes in those with documented coronary artery disease.

There have been many studies examining this question, and a recent report by Chan et al. in JACC in 2013 has provided a larger collaborative meta-analysis to address these questions. Munir et al. find essentially the same result as reported by Chan et al. except that they claim to report, for the first time, an association of the risk allele with elevated (modified) Gensini index. This is factually incorrect as stated. Dandona et al. (JACC, 2010) were the first to report an association between the 9p21 risk allele and Gensini index. The authors should state instead: “Our meta-analysis is the first to confirm an association between the 9p21.3 HR genotype and a higher Gensini score”. The authors should also be careful not to associate the 9p21.3 locus with risk of a given phenotype, but rather the 9p21.3 risk allele with risk of a given phenotype.

Response: We appreciate the reviewer’s comments and suggestions. Chan et al. 2010 focused on two outcomes, angiographic CAD and MI. In this study, we analyzed measures of severity of coronary atherosclerosis [number of diseased vessels, Gensini Score, Duke CAD Prognostic Index (DPI)], angiographic outcomes [change in minimum lumen diameter (∆MLD) and number of new lesions at follow-up], and key clinical outcomes (all-cause mortality, recurrent myocardial infarction and the need for coronary revascularization). We clarified this in the manuscript. We changed the words to state that our study is the first to confirm the association. We also changed our words to reflect 9p21.3 risk allele instead of 9p21.3 locus.