Author's response to reviews

Title: Association between polymorphisms in the adiponectin gene and cardiovascular disease: a meta-analysis

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Author's response to reviews: see over
Dear Dr David Meyre,

I am submitting the revised manuscript entitled “Association between polymorphisms in the adiponectin gene and cardiovascular disease: a meta-analysis” (manuscript ID: 9308729683847085) for your review.

We have responded, point by point, to each of the suggestions and comments of the reviewers. We have highlighted the revised portions (tracked changes) in the revised manuscript.

We appreciate the thoughtful comments provided by BMC Medical Genetics referees and believe that our manuscript has been greatly strengthened after this revision. My co-authors and I would like to thank you for the opportunity to resubmit our work for potential publication in BMC Medical Genetics.

Sincerely yours,

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Response To Reviewers

Response To Reviewer 1: Frédéric Fumeron

In this meta-analysis, the authors have examined the associations of 3 ADIPOQ SNPs with CVD and CHD. Their conclusions support the associations, but they also report a significant heterogeneity between studies and the need for more high quality studies. The study has been well conducted and analyzed. The paper is well written.

Minor essential revision

1) The +45T>G (rs2241766) and +276G>T (rs1501299) SNPs are in high linkage disequilibrium. Therefore, it would be interesting to assess whether their effects are independent (or not) by haplotypic analyses or whatever. Could it be possible to examine this point in the studies retrieved for this meta-analysis, or to test this by the meta-analysis per se?

Response: Thanks for your nice suggestion. We are unable to perform haplotypic analysis or conditional haplotype tests to test whether SNPs have independent effects in our meta-analysis without individual genotypes. Nevertheless, several studies evaluated the association between rs2241766-rs1501299 haplotypes and CVD risk. (Alireza Esteghamati et al, Simonetta Bacci et al, Imen Boumaiza et al, Emanuela Filippi et al). The results suggested that SNP276G>T, rather than SNP45T>G, is more strongly associated with CVD risk. However, these studies didn’t test whether SNPs have independent effects, either.

2) The authors speculate on the mechanisms of these associations by mentioning the effects of these SNPs on adiponectin levels. Nevertheless, these particular SNPs are far from being the most influent on adiponectin levels, and their association is weak and/or controversial. This should be discussed.

Response: Thanks for this comment. We revised our manuscript and discussed this point in the discussion section accordingly (Page 9).

3) It is claimed that the associations are significant but weak. Nevertheless, the magnitude of these associations is in the range of all positive associations found with SNPs in multifactorial polygenic disorders, even with the “top ten” SNPs from GWAS. This should be indicated.

Response: This is a very good point. The magnitude of these associations is in the range of the GWAS-identified SNPs, but the results were nearly "crude" ones, other important risk factors for CVD or CHD might diminish the significance of the results, so we
mentioned that the associations are significant but weak. We indicated this in paragraph 1 of the discussion (Page 8).

4) In discussion, most of the numeric values from other published data should be omitted, because it makes the reading uneasy. Usually, in a discussion, when associations are mentioned, it is obvious that they are significant.

**Response:** We omitted most of the numeric values from other published data in the discussion section. Thanks for the suggestion.

5) Full reference 27 is lacking.

**Response:** Reference 27 has been rectified (reference 31 in the revised manuscript).

**Response To Reviewer 2:** Ya-Wei Xu

This study summarized the results of published studies on the association between adiponectin gene polymorphisms and cardiovascular disease. The result of this study is useful for the ongoing genetic epidemiology research in the field. However, a few issues remain.

1. *P*<0.05 was used in this meta-analysis to identify the presence of potential publication bias. However, in most of the papers, a significance level of 0.1 was used as an indication for the presence of potential publication bias.

**Response:** In previous meta-analyses of genetic association studies, both 0.05 and 0.1 were used as significant levels. A significance level of 0.1 seems more appropriate for detecting publication bias. We changed the significant level in our meta-analysis. If 0.1 is used as the significant level, the publication bias in the studies of rs1501299 was significant (p=0.077).

2. The authors need to discuss the other meta-analysis that was published on the topic (*Claudia Menzaghi et al.*, Genetic Influences of Adiponectin on Insulin Resistance, Type 2 Diabetes, and Cardiovascular Disease. *Diabetes*, 2007) which included more studies hence more individuals and 2 other variants of the Adiponectin gene and underscore the main differences between the two studies.

**Response:** The meta-analysis conducted by Claudia Menzaghi et al was an important study on this topic by mainly addressing the associations between ADIPOQ polymorphisms and circulating adiponectin levels, HOMA_{IR}, T2D, and BMI. For the
meta-analysis of coronary artery disease, the authors discussed several studies and analyzed data for rs1501299 from four populations (including 827 cases and 1887 controls). And this has already been reported by Qi et al (Qi L, Doria A, Manson JE, Meigs JB, Hunter D, Mantzoros CS, Hu FB: Adiponectin genetic variability, plasma adiponectin, and cardiovascular risk in patients with type 2 diabetes. Diabetes 55:1512–1516, 2006). We have cited both of the papers in our study. The result suggested a protective effect of the T allele, which was similar to that of our study.

Response To Reviewer 3: Christina Wassel

Reviewer’s report:
This paper meta-analyzes data from 34 different studies which examined the associations of 3 common polymorphisms of the adiponectin gene (ADIPOQ) with cardiovascular disease (CVD). These SNPs have been studied extensively, but there are conflicting results as to the strength and direction of the associations with CVD. Studies contributing to the meta-analysis represented different ethnic groups, both men and women, and a varying sample sizes. Studies were given quality scores based on the NCI-NHGRI Working Group on Replication in Association Studies guidelines, and the MOOSE guidelines were followed in the meta-analysis. The three SNPs, rs2241766, rs1501299, and rs266729 had significant but weak associations with CVD in the meta-analysis, and the authors conclude that further high quality studies are still needed, especially for rs2241766. Overall a well-written and well done paper, but there are some points to clarify or improve upon.

Major Comments
1. The authors are missing several papers from the literature on the association of adiponectin and CHD which found inverse associations (i.e. Laughlin et al, Kanaya et al, among others).

Response: Thanks for your comment. Several large cohort studies have reported the inverse association between adiponectin levels and CHD (as mentioned, Laughlin et al, Kanaya et al, references listed below). These papers have been discussed and listed in our revised manuscript.

2. Laughlin GA, Barrett-Connor E, May S, Langenberg C: Association of adiponectin with


2. The quality scores seem low for these studies. Is this typical with use of the NCI-NHGRI Working Group on Replication in Association Studies guidelines? What are average scores using this metric for assessing quality?

**Response:** NCI-NHGRI Working Group on Replication in Association Studies guidelines is a typical scoring system. Some studies used this to assessed quality scores in meta-analysis (i.e. Wang Q, et al. PLA2G7 gene polymorphisms and coronary heart disease risk: a meta-analysis. *Thromb Res.* 2010 Dec;126(6):498-503.). Some studies used a modified scoring system (Sun K, Li Y, Wei C, Tong Y, Zheng H, Guo Y: Recessive protective effect of ADIPOQ rs1501299 on cardiovascular diseases with type 2 diabetes: A meta-analysis. *Mol Cell Endocrinol* 2012, 349(2): 162-169.). The quality scores assessed by these two methods were similar, by comparing the results of meta-analysis conducted by Sun et al and ours. Studies with a quality score ≥8 was considered as high quality studies.

3. Figure 1 states that one of the reasons 65 papers were excluded was “detail data unavailable”. Can the authors better define what this means? How many papers were excluded for this reason? As it stands, this is a bit vague.

**Response:** In our meta-analysis we used the genotype data of cases and controls in the published studies to evaluate the associations between the studied SNPs and CVD. So the number of persons in different genotypes in cases and controls is necessary in the analysis. Four studies related to our meta-analysis should be included but the authors didn’t report the genotype distribution of SNPs in case and control groups, instead they only reported ORs or p-values. The results of these studies were basically consistent with ours. Figure 1 “detail data unavailable” was changed to “detailed genotype distribution data unavailable”.

Those studies were listed below:


4. What about population stratification? Did any of these studies adjust for population stratification within ethnic groups? This could be contributing to the between study heterogeneity!

Response: Studies included in our meta-analysis didn’t adjust for population stratification.

Most of the study populations were racially homogeneous as indicated in the studies.

Population stratification may influence the observed associations, but we could not control for population stratification because we did not have access to individual data.

5. Use of the word “risk” throughout the manuscript is too loose – these are case-control studies, and odds ratios, which do not equate to relative risk. More appropriate to say “greater odds” rather than “greater risk”.

Response: We revised our manuscript as your suggested, and used “odds” instead of “risk” when referring it.

6. Why do authors use random effects? As Lebrec et al (Dealing with heterogeneity between cohorts in genomewide SNP association studies. *Stat Appl Genet Mol Biol*. 2010;9(1):Article 8) point out, the random effects hypothesis is appropriate for clinical trials but results in relatively reduced power for genetic association/GWAS detection of SNPs which show association in at least one study. The random effects model has less power to detect effects than fixed effects in almost all situations (Han et al, Random-effects model aimed at discovering associations in meta-analysis of genome-wide association studies. *Am J Hum Genet*. 2011 May 13;88(5):586-98), thus does not improve the ability to detect additional associations. It would probably be more appropriate to just use fixed effects for all 3 SNPs in this situation based on the work of Lebrec et al and Han et al.
Response: Thank you very much for your advice. We used fixed effect method to calculate the pool ORs. The associations were more statistically significant while the ORs were similar to that calculated by random effect method (see table below). The results were also presented in our revised manuscript.

<table>
<thead>
<tr>
<th></th>
<th>Random effect</th>
<th></th>
<th>Fixed effect</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR(95%CI)</td>
<td>P-value</td>
<td>OR(95%CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>+45T&gt;G (rs2241766)</td>
<td>1.22 (1.07, 1.39)</td>
<td>0.004</td>
<td>1.12(1.05, 1.19)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>+276G&gt;T (rs1501299)</td>
<td>0.90 (0.83, 0.97)</td>
<td>0.007</td>
<td>0.93(0.89-0.97)</td>
<td>0.001</td>
</tr>
<tr>
<td>-11377C&gt;G (rs266729)</td>
<td>1.09(1.01, 1.17)</td>
<td>0.032</td>
<td>1.07(1.02-1.13)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

7. Also what meta-analysis method exactly is used to combine the odds ratios from each study, i.e. inverse variance weighted? Please give a little more detail of Ref 24 since many readers may not be familiar?

Response: The pooled OR was calculated by the inverse-variance weighted method, and the significance of the pooled OR was tested by Z statistic. We gave more detail about the meta-analysis method in our revised manuscript.

Minor Comments
1. In paragraph 2 of the introduction – would say “also found significant inverse associations” rather than negative associations.

Response: The word “negative” has been changed to “inverse”.

2. Page 5, first line of statistical analysis section, “compare contrasts” does not make sense – maybe the authors just mean “compare”?

Response: The words “contrasts of ” have been deleted.

3. Page 6, second line, “…consist of 36 case-control studies…” – should be “consisting”

Response: The word “consist” has been changed to “consisting”.

4. Page 6, third line, should be ‘polymorphisms” instead of “polymorphism”

Response: The word “polymorphism” has been changed to “polymorphisms”.

5. Table 2 – may consider labeling the column “studies” as “No. studies” to make it clear that this is the number of studies contributing to that particular analysis.

Response: The labels of table 2 have been changed. Thanks.