Author's response to reviews

Title: Chromosome 1p13 genetic variants antagonize the risk of myocardial infarction associated with high ApoB serum levels.

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Author's response to reviews:

Stockholm, September 13th 2012

Dear Editor,

attached please find the revised version of our manuscript entitled "Chromosome 1p13 genetic variants antagonize the risk of myocardial infarction associated with high ApoB serum levels".

We have addressed all the points raised by the Reviewers and we hope that our manuscript is now suitable for publication on BMC Cardiovascular Disorders.

We have added novel data on the effect of the two SNPs under investigation on serum lipid levels and the results are reported in Table 2; the results of the interaction between the genetic variants and total- and LDL- cholesterol are now reported in the new Table 4; the limitation of our study are more clearly outlined in the Discussion. A new table describing the lack of association between the two genetic variants and the tryglicerides, HDL and ApoA1 serum levels has been included as Supplemental Table II

In addition several part of the manuscript have been largely re-written to fully discuss Reviewers comments. In particular the abstract and the Results section, and new references have been included.

All the changes have been highlighted in yellow throughout the manuscript.

We would like to thank the Editor and the Reviewers for the careful revision of our work that has certainly improved the quality of our manuscript.

Sincerely,
Bruna Gigante

Bruna Gigante
Associate professor
Division of Cardiovascular Epidemiology
Answer to Reviewer 1 (Dr Orestis Panagiotou)

The Authors would like to thank the Reviewer for his comments and suggestions that have certainly improved the quality of our work.

Major Compulsory Revision

1. As a reader, I feel that the authors should explain in more detail why the 3 specific SNPs were selected for genotyping and examination for modifying MI risk. E.g. rs660240 as well as other SNPs in the same gene region and/or loci are also associated with lipid levels. Same question applies to SNPs in other regions. In the current manuscript it is not clear enough what the selection criteria were: e.g. whether the strongest SNPs in each locus were selected, etc. The possibility of selective reporting cannot currently be ruled out: i.e., the authors have tested many SNPs but the report here only those with positive results. Also, it would be useful to report the OR for MI that pertains to the 2 SNPs as these were found in the cited papers (in the Introduction section), especially in the largest studies or in meta-analysis of GWA studies. Hence, one could compare the risk in the current population against the GWAS and see whether the effects are replicated in the SLEEP.

The Authors agree with the Reviewer that the rationale behind the selection of the SNPs investigated in the present study should be more clearly outlined. The three SNPs analyzed in the present study are the most commonly investigated with regard to the association with LDL-cholesterol levels as well as with risk of CAD.

This is now clearly stated in the Methods, Single nucleotide polymorphism genotyping section, page 7, first paragraph and outlined as a limitation of our study in the Discussion, page 15, last paragraph.

As suggested by the Reviewer, the odds ratio as well as the relative 95% CI OR in former GWA studies is now reported in the Introduction, page 4, 2nd paragraph, last 6 lines. In addition, the effect of these genetic variants on total and LDL cholesterol levels as compared to the data obtained in our study is reported in the Results, page 10, 4th paragraph, lines 3-9.

The part of the discussion where the differences between our study and the studies previously performed has also been extended, page 13, 2nd paragraph, line 5 until page 14.

2. I am not sure why an SI=0.62 (0.34-1.12) provides evidence of antagonism.
The 95% CIs are on both sides. Is the interpretation somewhat different?

In the former version of our paper we have defined this as a borderline antagonistic effect. We recognize that this phrasing may confound the reader and we have reformulated the sentence pointing out the larger confidence interval and the lack of statistical significance.

The changes are reported in the Results section, page 12, second paragraph.

3. It would be useful if the authors could report the allele frequencies of the SNPs examined, as these were found in their population.

The allele frequencies at the two investigated SNPs is now reported for cases and controls also in the main text in the Result, page 11, 2nd paragraph, lines 5-7.

4. Does the result about HWE refer to cases, controls or both? Also, they should report which threshold was used for HWE (P=0.05 or 0.1) and which the P-value for each SNP was.

Concordance to HWE was calculated in cases and controls with p threshold value of 0.05. This information is now provided in the Methods, Statistical Analysis section, page 7, 1st paragraph, last 2 lines. The actual p values for cases and controls are reported in the Results, page 10, 3rd paragraph.

5. A few more descriptive information regarding the baseline characteristics from Table 1 could be reported in the main text.

According to the Reviewer suggestion, more data are now reported in the main text, Results page 11, 1st paragraph.

6. I think that the authors should present the results of interaction analyses for LDL and Total-Chol. After all, they are more clinically relevant than ApoB.

Large part of the Results section describing the interaction analysis has been re-written and a new table, Table 4 where the interaction analysis between genetic variants and serum lipid levels, has been added to the manuscript. The results of the interaction analysis for total- and LDL-cholesterol are also reported in the main text, Results page 12, first paragraph line 3.

7. Having said the above (point 6), I would suggest moving the results for the overall population from Supp Table 2 to the main text; especially ORs for ApoB should be presented. Sex-specific results could remain in the suppl.

According to the Reviewer’s suggestion OR and 95%CI relative to the interaction analysis are now reported in the main text in the new Table 3.

8. It is not clearly enough described why the authors use median (IQR) and non-parametric tests to compare lipid levels in cases and vs controls? Are these
traits non-normally distributed in their population? Or is there some other reason? Similarly, I would expect to see the effect of the selected SNPs on lipid levels through a linear regression. Is there a reason why they have not performed such an analysis? This could give a more clear picture about the change in lipid levels according to the number of the reference allele

Serum lipid levels were not normally distributed in the SHEEP. Normality of trait distribution was assessed by Kolmogorov Smirnov test. This is now stated in the Methods, Statistical Analysis section, page 7, first paragraph, line 3-4.

To analyze the effect of the two SNPs on serum lipid levels we have therefore used the weighted least square regression analysis that does not assume constant variance for the regression residuals. The analysis is described in Methods, Statistical Analysis section, page 8, first paragraph, lines 1-6. The results of this analysis, that show a progressive reduction in the lipid serum levels according to the number of G and C alleles, are now reported in the main text Results, page 10, last line, and page 11, first two lines.

9. Is the additive model used in the analysis the same as a per-allele model?
In the logistic regression analysis we have tested three different models the additive, the dominant and the recessive. The genotype comparisons in these three models are now clearly stated in the Methods, page 8, lines 8-9.

The interaction analysis is called additive or biological. To avoid potential confusion in the reader the term biological is now used throughout the manuscript when referring to the interaction analysis.

10. The limitations of the current work should be more clearly acknowledged.
A paragraph on the limitation of our work has been added to the Discussion page 15-16. In addition we added to the discussion (pages 13-14) a paragraph to include the differences, the strengths and the weaknesses of our work as compared to other published papers.

Minor Essential Revision
1. Gene names should be written in italics.
Gene names are now reported in italics
2. There should be some footnote explaining what the parentheses in table 1 refer to: is it SDs, percentages etc?

Table 2 has been corrected according to the Reviewer’s suggestions.

Discretionary Revisions
1. Maybe the authors would find it useful to comment on the cumulative evidence that is likely to be produced in the future from GxE analyses and underline the importance of evaluating this evidence, as potential implications for future
research.

This is now discussed in the Discussion, page 16, 2nd paragraph.

Answer to Reviewer 2 (Dr Ioanna Tzoulaki)

The Authors would like to thanks the Reviewer for her comments and suggestions that have certainly improved the quality of our work.

Major Compulsory Revision

1. The authors should explain why they selected the examined SNPs among others in the 1p13 locus. Similarly, they should explain why the apoB biomarkers was selected and not other lipid fractions.

The Authors agree with the Reviewer that the rationale behind the selection of the SNPs investigated in the present study should be more clearly outlined. The three SNPs we have analyzed in the present study are the most commonly investigated with regard to the association with the LDL-cholesterol levels as well as the risk of CAD.

This is now clearly stated in the Methods, Single nucleotide polymorphism genotyping section, page 8, first paragraph and outlined as a limitation of our study in the Discussion, page 15, last paragraph.

In the former version of our manuscript only data related to ApoB were reported because the interaction between ApoB and the genetic variants was shown to be the strongest. In the revised version of our paper we have included the results of the analysis of the interactions between the two SNPs and total and LDL-cholesterol levels (new table 4).

In addition we have also included the distribution of tryglicerides, HDL and ApoA1 across the genotype strata (new Supplemental table II).

2. There is no information on the population, number of cases, age etc. in the abstract. Please add relevant information.

The abstract has been largely re-written and more information regarding the SHEEP study, the methods and the results have been included.

3. Interactions as hard to measure. Please discuss the owed of your study to identify interactions, please add more on your study limitations.

We have discussed the rationale behind the choice of the biological method to estimate the presence of interaction between serum lipid levels and genetic variants at 1p13 in the Discussion page 15, 2nd paragraph, lines 3-6.

The limitations of the interaction analysis and of our study have been more clearly written in the Discussion, page 15-16.

4. You give the median and IQR values however the phenotypes studies
(cholesterol) are usually normal distributed. In addition, you perform logistic regression which is a parametric test. Why did you do initially non-parametric tests?

Serum lipid levels were not normally distributed when cases and controls were analyzed together. Normality of trait distribution was assessed by Kolmogorov Smirnov test.

To analyze the effect of the two SNPs on serum lipid levels, as suggested by dr Panagiotou, we have therefore used the weighted least square regression that does not assume equal variance for the regression residuals. The results of this analysis, that show a progressive reduction in the lipid serum levels according to the number of G and C alleles, are now reported in the main text Results, page 10, 3rd paragraph, last three lines.

The logistic regression was used to assess the association between genetic variants and the risk of MI under three different models additive, dominant and recessive.

This is now stated in the Methods, Statistical Analysis section, page 8, first paragraph, lines 6-10 and the three models are more clearly described and in the Results, page 10, 2nd paragraph, where also the OR and 95%CI for the three models are reported..

Minor Essential Revision
Cases and controls are matched for age, why do you further adjust for this?

Cases and controls were matched by age, sex and residential area, however more controls than cases were included in the analysis because 5 controls were matched for each case. When the first control replied late and another control had already replied both were kept into the study and into the analysis. This has resulted in the inclusion of more controls. This is now explained in the Methods, Study Population section, page 6, first paragraph, last 6 lines.

Please include the OR results in main text.

The OR are now included in the Results section, page 11, second paragraph, last 6 lines.