Author's response to reviews

Title: A genetic variant of the atrial natriuretic peptide gene is associated with left ventricular hypertrophy in a non-diabetic population - The Malmo Preventive Project Study

Authors:

Amra Jujic (amra.jujic@mah.se)
Margrét Leósdóttir (margret.leosdottir@med.lu.se)
Gerd Östling (gerd.ostling@med.lu.se)
Petri Gudmundsson (petri.gudmundsson@mah.se)
Peter Nilsson (peter.nilsson@med.lu.se)
Olle Melander (olle.melander@med.lu.se)
Martin Magnusson (martin.magnusson@med.lu.se)

Version: 4 Date: 26 February 2013

Author's response to reviews: see over
Dear Editor,

We are writing with regards to our manuscript “A Genetic Variant of the Atrial Natriuretic Peptide Gene is Associated with Left Ventricular Hypertrophy in a Non-Diabetic Population – The MPP Study” to gauge your potential interest for publication in BMC Genetics. Since this is a submission of a revised manuscript, please see below for a point-by-point description of the changes made. This work describes the association of a common genetic variant, rs5068, and left ventricular hypertrophy. We suggest that rs5068 or genetic variant in linkage disequilibrium might affect susceptibility for left ventricular hypertrophy and support the possible protective role of natriuretic peptides, mediated by the same polymorphism. The major contribution of the current work is that the association between rs5068 and left ventricular hypertrophy was investigated in non-diabetic patients, since we believe diabetic cardiomyopathy could affect the results.

We therefore believe that our work, as described in this manuscript, would be very much of interest to the scientific community, but especially to scientists in the field of genetics, endocrinology and cardiology. Therefore, we devise further development and application of this work to accelerate discoveries concerning polymorphisms which mediate circulating levels of natriuretic peptides.

Drs Martin Magnusson, Olle Melander, Margret Leosdottir and Amra Jujić, have all participated in the conception and design of the study and all authors (Drs Martin Magnusson, Olle Melander, Margret Leosdottir, Peter Nilsson, Gerd Östling, Petri Gudmundsson and Amra Jujić) have contributed in the analysis and interpretation of data and also in the drafting and revising of the manuscript. Before submission each author have read and thereafter given his final approval of the manuscript. The manuscript has not been published and is not being considered for publication in whole or part in any language. None of the authors have stated any conflicts of interest.

Thanks for considering whether our work fits into your own editorial plans for BMC Genetics.

In case of any questions please do not hesitate to contact us anytime.

Corresponding author: 
Amra Jujić, Department of Clinical Sciences, Skåne University Hospital, Inga Marie Nilssons Gata 42, floor 2, SE 205 02 Malmö, Sweden. Telephone +46 40 33 85 62 Fax: +46 40 33 62 09. E-mail: amra.jujic@mah.se.

Sincerely

Amra Jujić MS. and Martin Magnusson MD., PhD.
Response letter

The authors would like to thank the reviewers for their time and their valuable comments, which will help us improve the manuscript. We revised our manuscript, and quite a lot of changes have taken place. So, hereby we send the revised version containing all the changes to be visible.

REVIEWER 1

Reviewer's report
Title: A Genetic Variant of the Atrial Natriuretic Peptide Gene is Associated with Left Ventricular Hypertrophy in a Non-Diabetic Population - The Malmö Preventive Project Study
Version: 3 Date: 27 November 2012
Reviewer: Maria Hurle

Reviewer's report:
The manuscript “A Genetic Variant of the Atrial Natriuretic Peptide Gene is Associated with Left Ventricular Hypertrophy in a Non-Diabetic Population – The Malmö Preventive Project Study”, authored by A Jujic, M Leosdottir; G Östling, P Gudmundsson, P Nilsson, O Melander and M Magnusson deals with the influence of the SNP rs5068 on the lack of left ventricular hypertrophy in non diabetic patients. The authors belong to a group that should be commended for a large long term prospective initiative that is providing much relevant population-based information.

Response: We thank the referee for these comments.

Reviewer’s comments:
Comment 1. Left ventricular mass index for the complete group of patients and the normal and hypertrophic LV cohorts is not shown.
Response: Data showing left ventricular mass index for the complete group of patients, both normal and hypertrophic LV cohorts, added (Table 3, page 19) in accordance with referees wishes.

Comment 2. When summarizing the results of the logistic regression analysis (Table 2, model 2) the authors do not include the coefficient B and Wald test values of all independent variables and this conceals to the reader the probably low relative statistical weight of the SNP rs5068 for the prediction of LV hypertrophy as compared with some of the other independent variables studied. The only marginally significant predictive power of the SNP, together with the paucity of other relevant data raises in the reader the suspicion that the effect of this polymorphism may be of low relevance as it is relatively “ejected” of the model by the rest of the heavier variables.
Response: Tables containing additional data on the coefficient B and Wald test values of all independent variables in both model 1 and model 2 added (Table 4 model 1, page 20, Table 5 model 2, page 21) in accordance with referees wishes.

Comment 3. Also in model 2, the sensitivity, specificity, percent of overall correct and results of the Hosmer-Lemeshow test (#2 and significance) are not given.
Response: Results for Hosmer-Lemeshow test as well as sensitivity, specificity and percent of overall correct for model 2 are now added (page 22, Table 6).
**Comment 4.** A similar reasoning can be applied to the summary of results of the multiple linear regression analysis (Table 3). Neither the full equation of the model (with F, adjusted R2 and significance values), nor coefficient B and beta values of every single significant independent predictor variable are given and, again, when all important variables are included in the model the SNP under study becomes statistically non-significant.

**Response:** Tables containing additional data on full equation model with F, adjusted R2 and significance values, coefficient B and beta values of all independent variables in both model 1 and model 2 added (Table 7 model 1, page 23, and Table 8 model 2, page 24). Regarding the general remark that indexed left ventricular mass becomes non-significant in fully adjusted model, we believe that left ventricular hypertrophy as a dichotomous value is a truer variable, which we also addressed in the discussion (page 8 line 14 – page 9 line 8). We attend to objectively discuss the results and we do acknowledge that the association is weak.

**Comment 5.** With these important limitations of the key finding the rest of the discussion is speculative.

**Response:** A comparison of our results with those of others, as well as reflections on adequate correction of left ventricular mass calculations is added to the discussion section (page 8 line 14 – page 9 line 8), which we believe makes the manuscript clearer.

**Level of interest:** An article of limited interest

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:** I declare that I have no competing interests

**REVIEWER 2**

**Reviewer's report**

**Title:** A Genetic Variant of the Atrial Natriuretic Peptide Gene is Associated with Left Ventricular Hypertrophy in a Non-Diabetic Population - The Malmö Preventive Project Study

**Version:** 3  **Date:** 1 December 2012

**Reviewer:** Jose-Luis Perez-Castrillon

**Reviewer's report:**

This interesting paper titled "A genetic variant of the atrial natriuretic peptide gene is associated with left ventricular hypertrophy in non-diabetic population (The Malmö Preventive Project Study)" have as objective to test the hypothesis that rs5068 allele suppresively regulates the development of LVH in a non-diabetic population. The authors showed this association although the effect was attenuated in the multivariate logistic analysis. The Malmö Preventive Project is a large cohort with consecutive re-examinations. There was 968 eligible subjects in the final sample. The methodology is correct although the size of population is small.

**Response:** We thank the referee for this these comments.

**Comment 1.** The genotype frequencies of rs5068 must be described

**Response:** A table containing genotype frequencies of rs5068 for the complete cohort, subjects without diabetes and subjects with diabetes added (Table 2, page 18) in accordance with referees wishes.
Comment 2. The circulating plasma levels of NT-proANP could be measurement. This data could be interesting to establish the causality of association

Response: We agree that data on ANP-levels would be interesting and could help establishing a causal association, however, those measurements were not undertaken at the time of the study being conducted, and are therefore not available. Also, we have previously shown, in another cohort from Malmö, that the minor allele of rs5068 is strongly associated with ANP levels. (Newton-Cheh C, Larson MG, Vasan RS, Levy D, Bloch KD, Surti A, Guiducci C, Kathiresan S, Benjamin EJ, Struck J, Morgenthaler NG, Bergmann A, Blankenberg S, Kee F, Nilsson P, Yin X, Peltonen L, Vartiainen E, Salomaa V, Hirschhorn JN, Melander O, Wang TJ: Association of common variants in nppa and nppb with circulating natriuretic peptides and blood pressure. Nat Genet, 2009;41:348-353)

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests: I declare that I have no competing interests

REVIEWER 3
Reviewer's report
Title: A Genetic Variant of the Atrial Natriuretic Peptide Gene is Associated with Left Ventricular Hypertrophy in a Non-Diabetic Population - The Malmo Preventive Project Study
Version: 3 Date: 13 December 2012
Reviewer: Carolina Medina-Gomez
Reviewer's report:
In this paper Jujic et al. shown an association of the polymorphism rs5068 with left ventricular hypertrophy after adjustment for sex and age which became borderline not significant after further adjustment systolic blood pressure, antihypertensive and/or cardioprotective treatment, body mass index and fasting plasma glucose in 968 non-diabetic individuals form the Malmö Preventive Project. The authors concluded that rs5068 might affect susceptibility to left ventricular hypertrophy and support the possible protective role of natriuretic peptides.
This clearly written manuscript reports a well-designed effort seeking the understanding of the effect of a previously described variant associated with blood pressure in cardiomyopathy. Nevertheless, the statistical methods, results and discussion require further clarification.
Response: We thank the referee for this these comments.

Major Compulsory Revisions:
Comment 1. Genotype frequencies of rs5068 (for control and cases) as well as Hardy Weinberg equilibrium significance should be reported. Quality control steps for the genotyping should be specified in one sentence in the methods.
Response: A table containing genotype frequencies of rs5068 for the complete cohort, subjects without diabetes and subjects with diabetes added (page 18, Table 2) in accordance with referees wishes. Data on Hardy Weinberg equilibrium significance has been added to the results section (page 8, line 4-5). Regarding quality control, twenty five percent of the genotypes were run in duplicates without discrepancies, and this information has been added to the methods section (page 6, line 7-8).
Comment 2. The authors show in tables 2 and 3 the results for the different models only for the genetic variant rs5068, which is the main predictor under study. Nonetheless, results from all variables included in the model would enrich the reported results as well as give stronger bases to some of the hypothesis stated on the discussion. I believe the inclusion and further discussion of OR/effect sizes as well as P values for the different variables included in model 1 and 2 for the two outcomes under study are of large importance and might bring opportunities to approach mediators/confounders. The phenotypic variance explained by each model in general and perhaps emphasizing on rs5086 ought to also be reported.

Response: We agree. Data on all variables included in analysis for left ventricular hypertrophy is now presented in tables for both model 1 and model 2 (Table 4 model 1 and Table 5 model 2, page 20-21), as well as additional data for left ventricular mass analysis (Table 7 model 1, and Table 8 model 2, page 23-24) in accordance with referees wishes.

Comment 3. In the discussion there is little related to the results for LVM, although you fully describe possible bias introduced for the measurement.

Response: We agree. We addressed the issue of left ventricular mass in the discussion section, since we believe that left ventricular hypertrophy as a dichotomous value is a truer variable (page 8 line 14 – page 9 line 8).

Minor Essential Revisions:

Comment 1. In the discussion when the authors contrast their work with Ellis and co-workers, did they mean Cannone et al. (reference 14) or both? In the article from Cannone it is stated “…The analysis of left atrial volume, LV structure and function as determined by echocardiography (LV ejection fraction, LV dimensions, LV mass, and LV volume index) did not reveal any significant associations with the rs5068 genotype…”.

Response: We mean both Cannone and Ellis, and this error is now corrected. In both introduction (page 3, line 19) and in the discussion (page 8 line 14 – page 9 line 8) the statement is corrected and refers now to both Cannone (reference 14) and Ellis (reference 15).

Comment 2. In the abstract and conclusion: “These findings suggest that rs5068, or genetic loci in linkage disequilibrium, might affect susceptibility to left ventricular hypertrophy and support the possible protective role of natriuretic peptides”. Could be better read by changing the word loci to variants.

Response: We agree. In accordance with referees wishes we have now changed the word “loci” to “variants” in both abstract and conclusion sections.

Discretionary Revisions:

Comment 1. In order to prove if congestive heart failure impacts your results, authors could simply do a sensitivity analysis by excluding those four patients and comparing those results with the ones reported in the manuscript.

Response: We agree, and have done so; the results of analysis with the four patients with heart failure excluded did not differ from the results reported in the manuscript. This issue is now addressed in the discussion section (page 10, line 6).

Comment 2. Based on MAF (which I suppose based on literature for the present study will be less than 0.1) and on the previous studies as those mentioned in references 14 and 15 would not the authors increase power using a dominant model?
Response: The referee is right to point out that at dominant model might increase power. During the study design we made a decision to use an additive model, but we are fully aware that the dominant model could have increased power and will definitely use a dominant model in future calculations.

Comment 3. In the statistical models tested, particularly for the logistic regression I would be cautious with multicollinearity between the variables included in the fully adjusted model and recommend you check for this type of issue.
Response: We agree. We performed an multicollinearity analysis, and our calculations do not indicate a multicollinearity problem (please see supplementary table in this letter).

Comment 4. Authors could report kappa statistic for inter-observer agreement to emphasize the low levels of subjectivity in the echocardiography reports.
Response: We agree, however, we do not have access to kappa statistics for intra-observer agreement and are therefore not able to report the data.

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: Yes, and I have assessed the statistics in my report.
Declaration of competing interests:
I declare that I have no competing interests

Supplementary table for multicollinearity

<table>
<thead>
<tr>
<th>Model</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>t</th>
<th>Sig.</th>
<th>Collinearity Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Constant)</td>
<td>-25,573</td>
<td>6,939</td>
<td>-3,685</td>
<td>.000</td>
<td></td>
</tr>
<tr>
<td>BP</td>
<td>.053</td>
<td>.017</td>
<td>.090</td>
<td>3,099</td>
<td>.002</td>
</tr>
<tr>
<td>FPG</td>
<td>-1,323</td>
<td>2,937</td>
<td>-.014</td>
<td>-.450</td>
<td>.653</td>
</tr>
<tr>
<td>gender</td>
<td>-3,274</td>
<td>.747</td>
<td>-.134</td>
<td>-4,381</td>
<td>.000</td>
</tr>
<tr>
<td>rs5068</td>
<td>-1,282</td>
<td>.922</td>
<td>-.039</td>
<td>-1,390</td>
<td>.165</td>
</tr>
<tr>
<td>age</td>
<td>.537</td>
<td>.060</td>
<td>.272</td>
<td>8,925</td>
<td>.000</td>
</tr>
<tr>
<td>BMI</td>
<td>1,033</td>
<td>.089</td>
<td>.347</td>
<td>11,552</td>
<td>.000</td>
</tr>
<tr>
<td>AHT</td>
<td>2,140</td>
<td>.678</td>
<td>.093</td>
<td>3,154</td>
<td>.002</td>
</tr>
</tbody>
</table>