Author's response to reviews

Title: The Renalase Asp37Glu polymorphism is not associated with hypertension and cardiovascular events in an urban-based prospective cohort: the Malmo Diet and cancer study.

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

please find enclosed the revised version of the manuscript entitled “The Renalase Asp37Glu polymorphism is not associated with hypertension and cardiovascular events in an urban-based prospective cohort: the Malmö Diet and cancer study.” (Fava et al.) which we hope can be considered for publication in the “BMC Medical Genetics” journal. Please find enclosed also itemized responses to all of the points raised by the referee together with the manuscript with all changes highlighted.

We feel that the manuscript has improved after revision and it is our hope that you will find it acceptable for publication in your journal in its present form.

All authors have read and approved the submission of the manuscript; the manuscript has not been published and is not being considered for publication elsewhere, in whole or in part, in any language. One author, Gunnar Engström is employed as senior epidemiological scientist at AstraZeneca R&D. No other relevant conflict of interest are present for the other coauthors of the manuscript.

Yours Sincerely

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Itemized responses to referee
We thank prof. Gu for its comments and suggestions. Please find enclosed itemized responses to all of the points raised together with the revised manuscript with all changes highlighted.

Fava et al. genotyped rs2576178 A>G and rs2296545 C>G on RNLS gene in a population-based cohort including 5696 participants (MDC_CC), and interrogated that whether these two SNPs could affect blood pressure levels, hypertension prevalence, and risk of incident CVD events in middle-aged Swedes. Generally, the topic is interesting to the EH and CVD genetic research community, especially testing the hypothesis in a population-based cohort. However, there are several concerns to be considerable:

Major Comments
1. Blood pressure measurements were not obtained according to a standard protocol recommended by the American Heart Association. BP was measured once with the participant in the supine position rather than sitting position. What kind impact on subject’s blood pressures was yielded by this methodological issue compared with other relevant study?

Lots of epidemiological studies have stated that BP is relevant for cardiovascular events and some of them, including the MDC used supine rather than sitting BP (1,2,3). Also some clinical trial in Northern Europe used supine BP and this fact does not prevent them to find interesting findings about the effect of new and old antihypertensive medications. To our knowledge no study has clearly stated the superiority of one kind of measure of BP over another but we agree that international guidelines now clearly prefer sitting position even if it is only a convention. Thus we do not think that the use of supine BP should have affect markedly our results on future CV events. We have added a sentence in the discussion and two references to clarify this point (page 11 lines 19-23).


2. The rationale without excluding subjects with a clinical history cardiovascular diseases, diabetes, and kidney disease from the study, especially in the cohort at baseline for analysis needs explanation, since subject BPs could be changed when with those diseases and you have defined the first CVD event incidence during the follow-up period as the end point of observation.

We partially agree with the referee and think that both analyses could be of importance. This is an epidemiological urban-based longitudinal study and in our opinion it is important to assess the association of the 2 Renalase SNPs in the entire cohort, without excluding any subject, not to incur in a selection bias. On the other hand, as suggested by the referee, clinical history cardiovascular diseases, diabetes, and kidney disease could have changed BP in the affected subjects. Thus we made a duplicate analysis on BP after deleting these subjects and added the results to the “results” section (please see page 9 lines 20-23) and in the table S3 in supplementary material.

3. For BP analysis by logistic regression, was adjustment conducted for covariates including glucose (Glu), lipids such as triglycerides, total cholesterol, high density lipoprotein cholesterol and serum creatinine besides age, gender, BMI, smoking and drinking status?

No, we chose to adjust for “traditional covariates” such as age, BMI and age also because we have these covariates measured in almost all the subjects. We agree that also adjusting for other covariates could affect the results. Thus we added the results also of this analysis (please see page 9 lines 20-23 of the main manuscript and table S2 in the supplementary material).

4. What are the results for BP analysis when excluding a clinical history cardiovascular diseases, diabetes, and kidney disease?

Please see response to the 2nd question.

5. Is there any difference for clinical characteristics between subjects used and the majority not included in this analysis?

We are sorry but are not sure to have understood which subjects the referee refers. If the referee mean the subjects with previous CV disease, diabetes and chronic kidney disease (n=570) with respect to healthy subjects (n=5126) the answer is yes. Please find enclosed a table with their characteristics: if the referee and the editor think this is more representative of table 1 it can be used instead.
New table 1. Anthropometric and metabolic features of the whole sample and divided by people with either previous cardiovascular event, diabetes mellitus and chronic kidney disease (CKD).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All (n=5696)</th>
<th>With previous CV event or diabetes or CKD (n=570)</th>
<th>No previous CV event or diabetes or CKD (n=5126)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, male (%)</td>
<td>41.7</td>
<td>57.9</td>
<td>40.0</td>
</tr>
<tr>
<td>Age, years</td>
<td>57.47±5.94</td>
<td>57.21±5.93</td>
<td>59.75±5.51</td>
</tr>
<tr>
<td>Body mass index, kg/m² †</td>
<td>25.84±3.98</td>
<td>25.56±3.81</td>
<td>28.37±4.56</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>141.18±18.95</td>
<td>140.40±18.75</td>
<td>148.21±19.34</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>86.94±9.45</td>
<td>86.63±9.38</td>
<td>89.78±9.60</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>63.3</td>
<td>81.9</td>
<td>61.2</td>
</tr>
<tr>
<td>Antihypertensive therapy, %</td>
<td>16.2</td>
<td>40.4</td>
<td>13.6</td>
</tr>
<tr>
<td>Obesity, % †</td>
<td>13.6</td>
<td>31.3</td>
<td>11.6</td>
</tr>
<tr>
<td>Diabetes, % ‡</td>
<td>8.6</td>
<td>80.5</td>
<td>0</td>
</tr>
<tr>
<td>Current smoking, % §</td>
<td>27.6</td>
<td>25.6</td>
<td>27.8</td>
</tr>
<tr>
<td>Hypercholesterolemia, % ††</td>
<td>49.7</td>
<td>51.5</td>
<td>49.5</td>
</tr>
<tr>
<td>eGFR &lt;90 ml/min/1.73 m², % ‡‡</td>
<td>24.0</td>
<td>31.9</td>
<td>18.9</td>
</tr>
<tr>
<td>eGFR &lt;60 ml/min/1.73 m², % ‡‡</td>
<td>0.7</td>
<td>7.7</td>
<td>0</td>
</tr>
<tr>
<td>Alcohol consumption, gr/day §§</td>
<td>10.19±12.11</td>
<td>11.69±15.71</td>
<td>10.03±11.62</td>
</tr>
<tr>
<td>History of CV events</td>
<td>2.1</td>
<td>20.5</td>
<td>0</td>
</tr>
</tbody>
</table>

CKD, chronic kidney disease evaluated as a eGFR <60 ml/min/1.73 m²
Information missing in † 7 subjects, ‡ 549 subjects, § 322 subjects, †† 668 subjects, ‡‡ 873 subjects, §§ 726 subjects.

Minor comments
1. Does the genotype distribution of Sweden differ with that of Chinese? In addition to the different definition of hypertension, such genetic background information should be referred to.

   Thank you for this suggestion. Information about genotype distribution in Han Chinese has been added as a key to understand difference between the studies in the discussion section (please see page 11 lines 14-17)

2. The perspective part is almost duplicated with the last two sentences of discussion part.

   Thank you for this observation. We now deleted these redundant sentences (please see the final part of the discussion).