Author's response to reviews

Title: The PPARGC1A Gly482Ser polymorphism is associated with left ventricular diastolic dysfunction in men

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Author's response to reviews: see over
September 25, 2008

Melissa Norton, MD
BMC Cardiovascular Disorders, Editor-in-Chief
BioMed Central Ltd
Middlesex House
34-42 Cleveland Street
London W1T 4LB, UK

Dear Dr. Norton,

Thank you for the valuable comments regarding our manuscript ‘The PPARGC1A Gly482Ser polymorphism is associated with left ventricular diastolic dysfunction in men’ (MS: 3679997892102204). We have responded to the reviewer comments and have revised the manuscript accordingly. Please find attached a point-by-point Author Response to each comment with specific notations of the manuscript changes.

We greatly appreciate the time and insights provided by the reviewers of our manuscript. We believe that the peer review process has improved the manuscript. We hope that the revised manuscript is now acceptable for publication in BMC Cardiovascular Disorders.

With best wishes,

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Comments of Chao-Qiang Lai

Comment 1. Page 11, Results: Please check if the genotypes of PGC-1 Gly482/Ser are in Hardy-Weinberg equilibrium in the men, women, both, respectively. This should be clarified in the results.

Author response: We thank the Reviewer for the insightful comments, and we have revised the manuscript accordingly. The frequency distribution of the PPARGC1A Gly482Ser polymorphism was in agreement with the Hardy-Weinberg expectations (P>0.97) in men and women, jointly and separately. This has been clarified at page 7:

The frequency distribution was in agreement with the Hardy-Weinberg expectations in men and women, jointly and separately (P>0.97 for all).

Comment 2. Page 9, 7th line from the bottom: Please list out the ABI assay ID and primer sequences if available, and rs# for the PGC-1 Gly482/Ser polymorphism.

Author response: We agree that this is important information, and have added it at page 4 and page 9, as can be seen below. There is no ABI assay ID, since this is an ‘assay on demand’ meaning that the sequence is sent to ABI for design. We have added also this information.

At page 3:

We hypothesized that the PPARGC1A Gly482Ser (rs8192673) polymorphism would be associated with diastolic dysfunction measured by Doppler-derived diastolic filling indexes.

And at page 7:

The PPARGC1A Gly482Ser polymorphism (rs8192673) was genotyped using an allelic discrimination assay (assay on demand) performed with an ABI 7900 system (Applied Biosystems Inc., USA) using the PCR primers; 5’-TGGAGAATTGTTCATTACTGAAATCACTGT-3’ (forward) and 5’-GGTCATCCCAGTCAAGCTGTTTT-3’ (reverse) and TaqMan MGB probes, Fam-5’-CTATTGACGCAGAAAG-3’ and Vic-5’-CTCCTATTGACCCAGAAAG-3’.

Comment 3. Page 11, line 3: Given that PGC-1a interacts with estrogen receptors, one should examine if interaction between Gly482/Ser genotype and menopause status in women influence on the risk of diastolic dysfunction.

Author response: We appreciate the suggestion and have examined this interaction using age 55 as a cutoff as a proxy for menopausal status, since information about exact menopausal age was not available. We have added the following text to the manuscript at page 9:

There was no significant interaction between PPARGC1A Gly482Ser and age (p=0.61) or between PPARGC1A Gly482Ser and menopausal status (using age 55 in women as a proxy; p=0.24).
Comment 4. Table 3, females: Based on Table 2 –females, the Ser allele seems to act recessively on the risk of DD in females, i.e., G/S subjects have an increased risk whereas S/S have a decreased risk. For this reason, authors should compare SS vs GG+GS, instead of GG vs GS+SS as listed in Table 3 –females.

Author response: We agree with the Reviewer that this would be an alternative way of presenting the data. As can be seen in Reviewer Table 1 below, the results are similar with significant associations in men (with the effect in the opposite direction as could be expected), whereas the results in women still are non-significant.

Reviewer Table 1. Associations between the *PPARGC1A* Gly482Ser polymorphism and diastolic dysfunction, in males and females. Data presented as odds ratios (OR) with 95% confidence interval (CI).

<table>
<thead>
<tr>
<th></th>
<th>Ser/Ser</th>
<th>Gly/Ser+Gly/Gly</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males</strong></td>
<td>N=48</td>
<td>N=451</td>
<td></td>
</tr>
<tr>
<td>DD, n (%)</td>
<td>3 (5.1)</td>
<td>76 (17.6)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Covariates</th>
<th>OR</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.0</td>
<td>4.42 (1.20 – 16.25)</td>
<td>0.025</td>
</tr>
<tr>
<td>Age, BMI</td>
<td>1.0</td>
<td>5.17 (1.34 – 19.86)</td>
<td>0.017</td>
</tr>
<tr>
<td>Age, LTPA</td>
<td>1.0</td>
<td>4.46 (1.21 – 16.44)</td>
<td>0.025</td>
</tr>
<tr>
<td>Age, BMI, LTPA</td>
<td>1.0</td>
<td>5.14 (1.33 – 19.86)</td>
<td>0.018</td>
</tr>
<tr>
<td>Age, hypertension</td>
<td>1.0</td>
<td>5.18 (1.34 – 20.03)</td>
<td>0.017</td>
</tr>
<tr>
<td>Age, diabetes</td>
<td>1.0</td>
<td>5.26 (1.35 – 20.53)</td>
<td>0.017</td>
</tr>
<tr>
<td>Age, hypertension, diabetes</td>
<td>1.0</td>
<td>5.66 (1.42 – 22.46)</td>
<td>0.014</td>
</tr>
<tr>
<td>Age, BMI, LTPA, hypertension, diabetes</td>
<td>1.0</td>
<td>5.92 (1.50 – 23.40)</td>
<td>0.011</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Ser/Ser</th>
<th>Gly/Ser+Gly/Gly</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Females</strong></td>
<td>N=61</td>
<td>N=472</td>
<td></td>
</tr>
<tr>
<td>DD (%)</td>
<td>2 (6.1)</td>
<td>56 (13.7)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Covariates</th>
<th>OR</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.0</td>
<td>3.79 (0.81 – 17.82)</td>
<td>0.091</td>
</tr>
<tr>
<td>Age, BMI</td>
<td>1.0</td>
<td>3.30 (0.71 – 15.42)</td>
<td>0.128</td>
</tr>
<tr>
<td>Age, LTPA</td>
<td>1.0</td>
<td>3.80 (0.81 – 17.90)</td>
<td>0.091</td>
</tr>
<tr>
<td>Age, BMI, LTPA</td>
<td>1.0</td>
<td>3.36 (0.72 – 15.72)</td>
<td>0.124</td>
</tr>
<tr>
<td>Age, hypertension</td>
<td>1.0</td>
<td>4.25 (0.87 – 20.66)</td>
<td>0.073</td>
</tr>
<tr>
<td>Age, diabetes</td>
<td>1.0</td>
<td>3.48 (0.76 – 16.02)</td>
<td>0.109</td>
</tr>
<tr>
<td>Age, hypertension, diabetes</td>
<td>1.0</td>
<td>3.94 (0.83 – 18.79)</td>
<td>0.085</td>
</tr>
<tr>
<td>Age, BMI, LTPA, hypertension, diabetes</td>
<td>1.0</td>
<td>3.56 (0.76 – 16.61)</td>
<td>0.106</td>
</tr>
</tbody>
</table>
As can be seen, only 48 men and 61 women were homozygous for the minor allele (Ser), and only 3 and 2 of those, respectively, had diastolic dysfunction. Therefore, we prefer to present the data using individuals being homozygous for the major allele (Gly) as reference group, as this gives more reliable results. If the Reviewer and Editor would prefer so, we would be glad to include this table as a supplementary table.

Comment 5. Page 12, line 7: PGC-1a variants have been shown to be associated with CVD before, so this may not be the first finding.

Author response: We thank the Reviewer for notifying us about this, and we have now updated the reference list with these more recent publications. We have rewritten the corresponding section at page 11-12:

Comparisons with Previous Studies

Recently, other PPARG1A polymorphisms were shown to be associated with presence of self-reported cardiovascular disease, and it was suggested that this association was mediated via DNA damage.[34] Also, the PPARG1A Gly482Ser polymorphism has been associated with hypertrophic cardiomyopathy.[35] Further, previous studies have demonstrated the Ser allele of the PPARG1A Gly482Ser to be associated with an increased risk of diabetes [11-13], but a recent meta-analysis have implied only a modest role for the polymorphism in the development of diabetes [14]. Moreover, the Ser allele has been associated with obesity in some studies [18, 19], whereas other have failed to find significant associations between the PPARG1A Gly482Ser polymorphism and various body fat measures and the metabolic syndrome in different study populations [36-38]. The PPARG1A Gly482Ser polymorphism has also been associated with hypertension in previous studies [15-17]. In two of these studies, the Ser allele conferred a lower risk of hypertension [15, 16], whereas one study demonstrated an increased risk of hypertension for carriers of the Ser allele [17]. However, the latter study consisted only of subjects with diabetes; hence this inconsistency could be a result of a gene-environment interaction. A recent large meta-analysis comprising of 13 949 individuals from 17 studies, of which 6 042 were from previously unpublished datasets, failed to find an association of the PPARG1A Gly482Ser polymorphism with hypertension overall. However, they reported higher systolic and diastolic blood pressure in younger individuals (<50 years of age) with the Ser allele, whereas there were no such associations in elderly.[39]

To the best of our knowledge, this is the first epidemiological study of associations between the PPARG1A Gly482Ser polymorphism and diastolic dysfunction. We observed a protective effect of the Ser allele, with lower risk of diastolic dysfunction for each copy of the allele in men. This contrasts with prior findings that the Ser allele is associated with higher risk of diabetes,[11-13], but agrees with findings that it is associated with lower risk of hypertension,[15, 16] both of which are major risk factors for diastolic dysfunction. In our investigation, the findings were independent of the presence of hypertension and diabetes. Further studies are needed to confirm our results and to further elucidate the role of PPARG1A in the development of diastolic dysfunction.

Comment 6. Page 15, Conclusions: Given that PGC-1A Ser allele is associated with increased risk of type 2 diabetes, and type 2 diabetes is an independent risk factor of cardiovascular
disease, one would expect that the Ser allele should associate with increased risk of diastolic dysfunction. But the finding from this study is totally opposite to what we expected. Please check the data analysis. Switching in phenotype or genotype coding could lead to an opposite result. If indeed this observation is biological, sex-specific regulation of PGC-1α expression in cardiac myocytes could be the key.

Author response: We agree with the Reviewer that this seems a bit odd. However, there are also studies showing lower prevalence of hypertension in individuals with the Ser allele. As hypertension also is a major risk factor for diastolic dysfunction, this would work in the opposite direction. We have checked the data, and it is correct, but we agree that this potential inconsistency should be highlighted in the manuscript. The possibility of a sex-specific regulation of PGC-1α expression in cardiac myocytes is discussed at page 13-14. We have added the following at page 12:

We observed a protective effect of the Ser allele, with lower risk of diastolic dysfunction for each copy of the allele in men. This contrasts with prior findings that the Ser allele is associated with higher risk of diabetes,[11-13], but agrees with findings that it is associated with lower risk of hypertension,[15, 16] both of which are major risk factors for diastolic dysfunction. In our investigation, the findings were independent of the presence of hypertension and diabetes. Further studies are needed to confirm our results and to further elucidate the role of PPARGC1A in the development of diastolic dysfunction.
Comments of Vimalesswaran Karani Santhanakrishnan

Comment 1. The authors need to explain why the stratification based on gender was done when there was no interaction between the SNP and gender. The p value for interaction between the SNP and gender should be mentioned in the results.

Author response: We thank the Reviewer for the insightful comments, and we have revised the manuscript accordingly. The plausible reason for this discrepancy is probably low statistical power to detect interaction in formal testing. The analyses were sex-specific according to our a priori analysis plan, and were based on the hypothesis that the relations would differ by sex. We have updated the manuscript with this information at page 8:

Analyses were stratified on sex according to our a priori analysis plan based on the hypothesis that the association between PPARGC1A Gly482Ser and diastolic dysfunction could differ by sex.

At the following at page 14:

The interaction between PPARGC1A Gly482Ser and sex was non-significant upon formal testing, which could be due to low statistical power to detect interactions. However, the analyses were stratified on sex according to our a priori analysis plan based on the hypothesis that the association between PPARGC1A Gly482Ser and diastolic dysfunction could differ by sex.

Comment 2. Did the authors look at the association of Gly482Ser with ventricular diastolic dysfunction in the entire cohort? Data on the entire cohort has to be given.

Author response: We are not certain what the Reviewer means by “the entire cohort”. The association was studied in the participants that had undergone an echocardiography (n=1058), a sub-sample of the full Vara cohort (n=1811). Only 26 participants were excluded due to failed genotyping, inadequate echocardiography, or prior heart failure, so the analyses were performed in 1032 individuals. If the Reviewer means that we should perform sex-pooled analyses, we would be glad to do so and to present them in a Supplementary Table. However, we are not confident that this would be a correct procedure, given the pronounced sex differences.

Comment 3. Power of the study to detect an association with the SNP studied should be mentioned?

Author response: We appreciate the comment, and have added the following power statement at page 9-10:

With a significance level of 0.05, we had 87% (men) and 90% (women) power to detect a quantitative trait locus that accounted for 1.5% of the residual variance.
Comment 4. Were any of the study subjects related? How was this determined? If they were related, how was this taken account into analyses?

Author response: No, none of the individuals were related, as least as far as we know. We have added the word “unrelated” in the following sentence at page 4:

Between 2001 and 2003, 1811 unrelated participants were accordingly included (participation rate 82%).

Comment 5. Did the authors observe any association between Gly482Ser and diastolic blood pressure?

Author response: There was no association between Gly482Ser and diastolic blood pressure, as can be seen below in Reviewer Table 2. These data are also included the new Table 2, in response to another Reviewer’s suggestion.

Reviewer Table 2. Association of the Gly482Ser polymorphism and diastolic blood pressure

<table>
<thead>
<tr>
<th></th>
<th>Gly/Gly</th>
<th>Gly/Ser</th>
<th>Ser/Ser</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>74 (9.6)</td>
<td>73 (9.6)</td>
<td>73 (9.6)</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>70 (9.4)</td>
<td>69 (9.4)</td>
<td>70 (9.3)</td>
</tr>
</tbody>
</table>
Data are means (standard deviations).

Comment 6. Did the authors undertake any quality control measures for genotyping? What was the call rate for genotyping?

Author response: We agree with the Reviewer that this should be included in the manuscript. We have added the following text to page 7:

Genotyping efficiency was 99.8%, and 100% genotyping accuracy was shown when 5% of the samples were re-run.

Comment 7. The p values for the Hardy-Weinberg equilibrium should be mentioned in the genotyping section.

Author response: We appreciate the comment, and have clarified this at page 7:

The frequency distribution was in agreement with the Hardy-Weinberg expectations in men and women, jointly and separately (P>0.97 for all).

Comment 8. The rs number for the polymorphism under study should be provided in the text.
Author response: We agree that this is important information, and have added it at page 3:

We hypothesized that the PPARGC1A Gly482Ser (rs8192673) polymorphism would be associated with diastolic dysfunction measured by Doppler-derived diastolic filling indexes.

And at page 7:

The PPARGC1A Gly482Ser polymorphism (rs8192673) was genotyped using an allelic discrimination assay performed with an ABI 7900 system (Applied Biosystems Inc., USA) using the PCR primers; 5’- TGGAGAATTGTTCATTACTGAAATCACTGT-3’ (forward) and 5’- GGTCATCCCAGTCAAGCTGTTTT -3’ (reverse) and TaqMan MGB probes, Fam-5’-CTATTGACGCAGAAAG -3’ and Vic-5’-CTCCTATTGACCCAGAAAG -3’.

Comment 9. The authors need to give a reference for the rural population in Vara being very homogenous.

Author response: We have added a reference supporting this statement at page 8:

The rural population in Vara is very homogenous [33] and there are thus no population stratification concerns to address.

Comment 10. The work ‘ratio’ in Table 1 needs to be corrected.

Author response: We have corrected this in Table 1.

Comment 11. The abbreviated terms such as BMI, LTPA in Table 2 and 3 needs to be expanded.

Author response: We have expanded all abbreviations in all tables.
Comments of Norbert Stefan

Comment 1. The relationships of the genotypes with age, BMI, LTPA, diabetes and blood pressure in men and women need to be shown.

Author response: We thank the Reviewer for the insightful comments, and we have revised the manuscript accordingly. We have added a new table showing these relations, Table 2 – and renamed the other tables accordingly.

Comment 2. The largest study (n>13000) addressing the relationship between the Gly482Ser SNP with hypertension (Vimaleswaran et al. J Appl. Physiol. 2008) showed a higher risk of hypertension in young (<50 years) Ser482 allele carriers. No relationship was found in older subjects. These data need to be discussed in the light of the present findings.

Author response: We thank the Reviewer for notifying us about this, and we have now updated the reference list with this more recent publication. We have rewritten the corresponding section at page 12:

The PPARGC1A Gly482Ser polymorphism has also been associated with hypertension in previous studies [15-17]. In two of these studies, the Ser allele conferred a lower risk of hypertension [15, 16], whereas one study demonstrated an increased risk of hypertension for carriers of the Ser allele [17]. However, the latter study consisted only of subjects with diabetes; hence this inconsistency could be a result of a gene-environment interaction. A recent large meta-analysis comprising of 13 949 individuals from 17 studies, of which 6 042 were from previously unpublished datasets, failed to find an association of the PPARGC1A Gly482Ser polymorphism with hypertension overall. However, they reported higher systolic and diastolic blood pressure in younger individuals (<50 years of age) with the Ser allele, whereas there were no such associations in elderly.[39]

Comment 3. The discussion is very well written. The authors should also address the important role of estrogen-related receptors in the pathogenesis of cardiomyopathy.

Author response: We appreciate the suggestion of the Reviewer, and have added a section about the potential role of estrogen-related receptors in the pathogenesis of cardiomyopathy at page 14:

Moreover, PPARGC1A interacts with estrogen receptors and enhances their transcriptional activity [44]. Estrogen receptors are expressed in vascular endothelial and smooth muscle cells, as well as in myocardial cells, and there are gender differences in expression [45]. Estrogen and estrogen receptors have repeatedly been demonstrated to be involved in cardiovascular disease and several recent animal studies have implied a role for estrogen receptors in the development of cardiac dysfunction [46-48]. Also, a recent study of patients with hypertrophic cardiomyopathy demonstrated polymorphisms in the estrogen receptor alpha (ESR1) gene, as well as in the androgen receptor (AR) gene to be associated with left ventricular wall thickness in men, but not in women.[49]
Similarly, another recent study reported a 1.8-fold increase in estrogen receptor alpha mRNA levels in patients with end-stage dilated cardiomyopathy.[50]

Comment 4. **PPARGC1A is the HUGO-approved gene symbol in humans.**

**Author response:** We agree with the Reviewer that it is preferable to use the HUGO-approved gene symbols, and have changed this throughout the manuscript.