Author's response to reviews

Title: PALB2 variants in hereditary and unselected Finnish Prostate cancer cases

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Author's response to reviews: see over
Dear Bjorn Olsen,

Thank you for your editorial letter of October 23, 2009 and for the accompanying thorough and sound criticism provided by the Reviewing Editor. We now enclose a revised manuscript, in which we have addressed the concerns of the Reviewer to our best understanding.

The comments of the Reviewer (in order of their appearance) have been dealt as follows:

1. **A more detailed description of what unselected cases and controls are typed for which variants in the Methods section would be helpful. Differences between different cohorts of control patients are not described at all (i.e. use of 470 controls vs. 760 controls).**
   
   **Answer:** A more detailed description of what sample sets have been used for which variants have been inserted into the Methods section (pages 5-6, lines 25 and 1-6). The text is now in following format: “Unselected cases for screening the whole gene were early onset cases with Gleason score over seven. Additional analyses were carried out on four variants that showed a trend for association (PALB2 c.1592delT, 1674A>G, 2993G>A and 3300T>G). Unselected cases for additional analysis included 95 early onsets, aggressive cases and 368 non-aggressive cases with average age at diagnosis 67 years (range 63-77). Control samples in both primary and additional analysis were anonymous male blood donors from the Finnish Red Cross.”

2. **The acronym HPC is not defined, although I can guess it means “high-risk prostate cancer” family.**
   
   **Answer:** The acronym HPC has been defined now as “hereditary prostate cancer” (page 8, line 5).
3. **Loss of heterozygosity analysis on tumor samples is described in the Methods section, but no results are given in the Results/Discussion section.**

   Answer: Results of loss of heterozygosity analysis on the tumor samples have been added into the Results/Discussion section (page 9, last section).

4. **A haplotype analysis of at least two of the most common detected variants (i.e., 1674A>G and 2993G>A) might add further insight into the role of PLAB2 and prostate cancer; I strongly suggest doing it. These two variants appear to have a frequency in prostate cancer cases that is higher than the Finish population control frequency. The third variant 3300T>G has a similar frequency between cases and controls, although it could be included too. A haplotype analysis might show a stronger association with prostate cancer than the individual variants examined.**

   Answer: A haplotype analysis of the three detected variants (i.e., 1674A>G, 2993G>A and 3300T>G) has been carried out. The used method has been described in Methods section (page 7, lines 6-9), under “Statistical analyses”. The results are discussed in Results/Discussion section (page 8, lines18-25). Disappointingly the haplotype analysis did not add further evidence on the role of PALB2 in prostate cancer risk. Consequently no table or figure was included into the manuscript. However, the detailed results are presented below.

**Other changes**

In addition to changes described above, we have made a few minor changes: On page 3, lines 11-12, corrected the acronym “PRCA”; on page 8, lines 6-7 added “the entire coding region of ”and “and controls” on line 12; on page 9 added “61” on line 5 and corrected “368” on line 11.

We believe that we have thoroughly addressed the comments made by the reviewer, and that the manuscript is now improved. We hope that you will find the revised manuscript suitable for publication in *Journal of Negative Results in BioMedicine*.

Sincerely,

Johanna Schleutker and co-authors
This table depicts the results of the possible haploblocks formed by the three $PALB2$ variants.

<table>
<thead>
<tr>
<th>Block</th>
<th>Haplo Frequency</th>
<th>Case, Control Ratio Counts</th>
<th>Case, Control Frequencies</th>
<th>Chi Square</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA</td>
<td>0.906</td>
<td>1711.0 : 173.0, 1378.2 : 145.8</td>
<td>0.908, 0.904</td>
<td>0.149</td>
<td>0.6997</td>
</tr>
<tr>
<td>GG</td>
<td>0.079</td>
<td>144.0 : 1740.0, 123.8 : 1400.2</td>
<td>0.076, 0.081</td>
<td>0.271</td>
<td>0.6026</td>
</tr>
<tr>
<td>AG</td>
<td>0.015</td>
<td>29.0 : 1855.0, 22.0 : 1502.0</td>
<td>0.015, 0.014</td>
<td>0.052</td>
<td>0.819</td>
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<tr>
<td>Block 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGA</td>
<td>0.905</td>
<td>1709.9 : 174.1, 1374.4 : 149.6</td>
<td>0.908, 0.902</td>
<td>0.327</td>
<td>0.5672</td>
</tr>
<tr>
<td>TGG</td>
<td>0.077</td>
<td>143.0 : 1741.0, 119.6 : 1404.4</td>
<td>0.076, 0.078</td>
<td>0.077</td>
<td>0.7813</td>
</tr>
<tr>
<td>Block 3</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>0.982</td>
<td>1854.0 : 32.0, 1494.0 : 30.0</td>
<td>0.983, 0.980</td>
<td>0.349</td>
<td>0.5549</td>
</tr>
<tr>
<td>GA</td>
<td>0.010</td>
<td>19.0 : 1867.0, 16.0 : 1508.0</td>
<td>0.010, 0.010</td>
<td>0.015</td>
<td>0.9028</td>
</tr>
</tbody>
</table>

This figure shows the linkage disequilibrium (LD) of three $PALB2$ variants. LD chart shows D’ measures of pairwise LD.