Author's response to reviews

Title: A novel germline PALB2 deletion in Polish breast and ovarian cancer patients

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Author's response to reviews: see over

Dear Dr Norton,

We greatly appreciate the reviewer’s comments and we corrected the manuscript accordingly. Enclosed we are sending the final version of the manuscript, as well as a response to the reviewer.

The last comment was related to the revision 1 version, not to the initially submitted manuscript. According to the suggestions we have analyzed the haplotype again, and in the light of findings from the literature with regard to various genes. We have also added a table with results of haplotyping and marker positions. We have responded to the comment as in the attached letter.

We think that our genotyping data are not easy to interpret. Since we had the last chance for revision, and to avoid another round of discussion through the editorial office, we contacted prof. Foulkes directly, and we believe that the version submitted now is already acceptable.

Sincerely yours
Jolanta Kupryjańczyk MD, PhD

REFEEEREE COMMMENTS and responses

Referee: William Foulkes

Before it is finally accepted, I would like the authors to comment on the local genomic structure around the deletion GA they report. Is there a run of GAGAGA that would explain this being a recurrent mutation? If not, I would question the haplotype data and would like them to show the data in the paper, maybe in a table instead of partially in the text (as it is now).
We greatly appreciate the comments. We have analyzed the haplotype again, and in the light of findings from literature with regard to various genes. We have also added a table with haplotyping data and marker positions. We think that our genotyping data are not easy to interpret. In our opinion they do not simply confirm a founder effect, but they do not exclude it either (they could indicate an ancient founder mutation since the highest variability was at the marker with the longest distance from the mutation).

The deletion is not placed in repeated sequences. We agree that we did not have a basis for calling this mutation a hotspot one, however, some deletion mutations are suggested to be hotspot ones even though they are not placed in repeated sequences e.g. Barkardottir et al. Haplotype analysis in Icelandic and Finnish BRCA2 999del5 breast cancer families. Eur J Hum Genet. 2001, 9.

The term “recurrent” is used in the literature quite often for description of a mutation of unknown origin, even if haplotypes are not studied, e.g. Cao et al. The prevalence of PALB2 germline mutations in BRCA1/BRC2 negative Chinese women with early onset breast cancer or affected relatives. Breast Cancer Res Treat 2009, 114; Garcia et al. Analysis of FANCB and FANCN/PALB2 fanconi anemia genes in BRCA1/2-negative Spanish breast cancer families. Breast Cancer Res Treat 2009, 113; Erkko et al. et al: A recurrent mutation in PALB2 in Finnish cancer families. Nature 2007, 446. Therefore, we would like to retain the term "recurrent" in the abstract and conclusions sections.

The main goal of our manuscript is to show a novel germline mutation of the PALB2 gene in Polish population, its association with familial breast cancer and its inclusion into screening programs. The origin of this mutation is of great interest and will be studied further; nevertheless, it has a lower a practical value.

Our current interpretation is reflected by the changes depicted below:

**Methods, PALB2 Haplotyping, page 8:**

We have added the following text:

Marker and PALB2 gene positions were reckoned from NCBI [21] Homo sapiens chromosome 16 genomic contig (reference sequence: NT_010393.16).

**Results section, page 11:**

Before:

Haplotype analysis
Genotyping of the seven c.509_510delGA deletion carriers was performed with three microsatellite markers: D16S417 which is distal to PALB2, and D16S481 and D16S403 that are proximal to this gene. PALB2 mutation-positive individuals had four variants of a haplotype. In particular, patients 293, 893 and the woman from the control group shared 4-2-3 alleles, while patients 375 and 802 shared 4-2-6 alleles. Each of the other two patients had a unique haplotype.

Now:

Haplotype analysis
Genotyping of the seven c.509_510delGA deletion carriers was performed with three microsatellite markers: D16S417 which is distal to PALB2, and D16S481 and D16S403 that are proximal to this gene. Genotypes are presented in Table 4. There was no common haplotype for all PALB2 mutations carriers with regard to the three markers analysed.
Discussion section, page 14, 2nd paragraph:

Before:
Some of PALB2 gene alterations in breast cancer were suggested to be founder mutations for some ethnic groups [8, 10]. The presence of the same deletion in seven unrelated women in our study might suggest that this was a founder mutation for the examined population from central Poland. However, the genotype analysis of the mutation carriers did not reveal a common haplotype. This indicates that the detected deletion might have arisen two or more times independently as a recurrent hot-spot mutation, and not by founding effect. More detailed analysis is necessary to determine the origin of this alteration.

Now:
Some of PALB2 gene alterations in breast cancer were suggested to be founder mutations for other ethnic groups [8, 10]. The presence of the same deletion in seven unrelated women in our study might suggest that this was a founder mutation for the examined population from central Poland. Although the genotype analysis of the mutation carriers showed differences in the haplotypes, one cannot exclude an ancient founder mutation. More detailed analysis is necessary to determine the origin of this alteration.

We have added Table 4:

Table 4 - Results of genotyping of PALB2 mutation carriers

<table>
<thead>
<tr>
<th>Proband no.</th>
<th>Marker</th>
<th>Position bp</th>
<th>D16S403</th>
<th>D16S481</th>
<th>PALB2</th>
<th>D16S417</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>22,977,651</td>
<td>23,188,290</td>
<td>23,554,483*</td>
<td>23,717034</td>
</tr>
<tr>
<td>293</td>
<td>Ov. Ca.</td>
<td>2, 3</td>
<td>1, 2</td>
<td>c.509-510delGA</td>
<td>2, 4</td>
<td></td>
</tr>
<tr>
<td>375</td>
<td>Ov. Ca.</td>
<td>2, 6</td>
<td>1, 2</td>
<td>c.509-510delGA</td>
<td>2, 4</td>
<td></td>
</tr>
<tr>
<td>ZB649</td>
<td>Control</td>
<td>4, 3</td>
<td>1, 2</td>
<td>c.509-510delGA</td>
<td>3, 4</td>
<td></td>
</tr>
<tr>
<td>802</td>
<td>Br. Ca.</td>
<td>1, 6</td>
<td>3, 2</td>
<td>c.509-510delGA</td>
<td>2, 4</td>
<td></td>
</tr>
<tr>
<td>2076</td>
<td>Br. Ca.</td>
<td>4, 5</td>
<td>1, 1</td>
<td>c.509-510delGA</td>
<td>2, 1</td>
<td></td>
</tr>
<tr>
<td>893</td>
<td>Br. Ca.</td>
<td>4, 3</td>
<td>1, 2</td>
<td>c.509-510delGA</td>
<td>3, 4</td>
<td></td>
</tr>
<tr>
<td>1540</td>
<td>Br. Ca.</td>
<td>4, 3</td>
<td>1, 1</td>
<td>c.509-510delGA</td>
<td>2, 4</td>
<td></td>
</tr>
</tbody>
</table>

Alleles for each microsatellite marker were numbered according to the size. Ov. Ca. – ovarian cancer patients; Control – a PALB2 mutation carrier from the control group; Br. Ca. – breast cancer patients.

* the position of PALB2 gene on chromosome 16 (NCBI reference sequence: NT_010393.16); the position of the deletion is 23,587,357 bp