Author’s response to reviews

Title: The BRCA2 variant c.68-7T>A is associated with breast cancer

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Author’s response to reviews:

General comment:

Going though all details again, we found one typo: there are 17 independent families, not 18 as erroneously noted previously. This did not have any bearing on the conclusions.

Reviewer #1

We interpret this as this reviewer has accepted our paper.

Reviewer #2:

This reviewer now state that: ‘I note the responses to the questions of methodology but it does not seem to me that the relatively small numbers involved are themselves a reason not to employ the most robust and established statistical methods. As there have been no changes to the manuscript in this regard there is nothing further that I would add to the original review.’

Response

We have modified our response to this reviewer’s previous comments on methods which we not completely follow, and copy the reviewer’s initial comments and our modified response in below:

Reviewer #2:

Segregation analysis - why are only 3 of 18 pedigrees included in the analysis?
Response: Defining youngest case with breast or ovarian cancer determined to be carrier as proband in the family as suggested in the reference, no family besides the first separately described had more than one informative meiosis. Likelihood segregation analyses in families with less than 2 informative meioses was not found relevant.

Reviewer #2:

Acknowledging that it is the author's own described method, why use a significantly simplified segregation method that requires pruning of most of the pedigree data when other robust options such as the full likelihood method, or the co-segregation likelihood method are capable of managing this and are now openly available and widely supported by the literature?

Response:

We have now used co-segregation likelihood method as requested, see modified text. The reference states “Another important assumption is that "causal" UV's show the same penetrance as the known deleterious mutations in BRCA1 and BRCA2.” which is in conflict with our conclusion of a putative lower than average penetrance. The reference states: “One of the critical steps in our analysis is the selection of the proband. In most cases, this will be the youngest affected family member that was tested positive for the UV. The algorithm analyses the segregation of the UV from the proband and the results can differ according to which person is used as proband.” Redefining who were the probands this way, no family besides the first had more than one informative meiosis. Applying the advocated scoring method (LR threshold for causality of 1,000:1 (LR>1000) and for neutrality of 100:1(LR<0.01)), the results of analyzing the family in Fig 1 was inconclusive whatever way we estimated the unknown information needed by the algorithm. The .ped file we used to arrive at LR=0.36 was as follows:

```
1 15 2 58 2 58 0 0 1
2 16 4 0 38 2 38 0 0 1
3 15 2 0 61 2 0 0 0 0
4 0 0 0 82 2 80 0 0 2
5 16 4 0 81 2 0 0 0 2
6 2 1 5 0 61 2 0 0 1
7 2 1 5 0 47 2 47 0 0 1
8 2 1 5 0 46 2 0 0 0
9 1 7 7 0 25 1 0 0 1
```
Reviewer #2:

Prospective follow-up / annual incidence: again this is a very simplified analysis on small numbers. At least a standardized incidence ratio is needed to account for the age distribution of the carriers in follow-up. Even so the width of the confidence interval around the annual incidence figure means that the strictly correct interpretation of this figure is that it provides no significant additional support for pathogenicity at this stage.

Response: Agreed, we mention what we have observed but there is no conclusion to be made from this. If enough other centres have enough data, one may combine them all to arrive at conclusions. There was no events before 50 years of age or above 70 years of age. Annual incidence rates from 50-69 years of ages were:

<table>
<thead>
<tr>
<th>Age group</th>
<th>Observation years</th>
<th>Breast cancers observed</th>
<th>Annual incidence rate in age group</th>
</tr>
</thead>
<tbody>
<tr>
<td>50-54 yrs</td>
<td>14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>55-59 yrs</td>
<td>6</td>
<td>1</td>
<td>0.1667</td>
</tr>
</tbody>
</table>
Reviewers #2:

With only 18 index cases/families found to be carriers it would be useful to include a table summarising their clinical features, particularly in comparison to the cohort as a whole i.e. age of cancer dx, history of ovarian cancer, bilateral breast cancer etc.

Response: A new Table 1 is now included with details on all female first degree relatives. Our files did not contain the information needed to expand the table to include second degree relatives.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Cases</th>
<th>Ovarian</th>
<th>Breast</th>
</tr>
</thead>
<tbody>
<tr>
<td>60-64 yrs</td>
<td>14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>65-69 yrs</td>
<td>13</td>
<td>1</td>
<td>0.0769</td>
</tr>
</tbody>
</table>

We do not find it reasonable to include these details in the report.