Author's response to reviews

Title: A de novo complete BRCA1 gene deletion identified in a Spanish woman with early bilateral breast cancer.

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Version: 2 Date: 23 September 2011

Author's response to reviews: see over
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Valencia, 24 May 2011

Dear Editors,

Please find enclosed the original manuscript of the article prepared by our group entitled “A de novo complete BRCA1 gene deletion identified in a Spanish woman with early bilateral breast cancer” and referenced as 7689819775421449 by your editorial office.

In this new version of the manuscript we have met the requirements requested by the assistant editor of the journal which are:

- A written consent of the patient reported in the manuscript giving her authorization for the publication of personal data. This document has been sent by email (to editorial@breast-cancer-research.com) on Tuesday May 24.
- Furthermore, I sent the author’s competing interest form also by email to the editorial office on Tuesday 17.
- Finally, we have included in the body of the manuscript a sentence stating that “written consent to publish was obtained from the patient”.

As previously stated, we consider this manuscript of interest for publication in BCR journal because we describe the first case of a patient with no strong family history of hereditary breast cancer who developed early-onset bilateral breast cancer with a de novo complete BRCA1 gene deletion in the germinal line characterized by MLPA and array CGH.

I hope that this new version of the manuscript achieves now the scopes of the journal to be considered for publication.

Sincerely yours,

Dr. José Antonio López-Guerrero  
Corresponding author

Breast Cancer Research  
Editorial Office

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Dr. José Antonio López-Guerrero  
Corresponding author
Editorial comments:

- Was the testing of relatives part of standard care? If not please can you confirm that appropriate ethical approval was obtained for this and include details in the manuscript.
As a part of the Universal Genetic Counselling in Cancer Program of the Comunidad Valenciana (Spain), the Unit of Genetic Counselling of hereditary cancer of our institution offers genetic testing exclusively to relatives of probands who harbour any genetic alteration. As stated in the manuscript, all the closed relatives were invited to participate in the segregation study. Relatives who accepted the study signed an informed consent.

- Please also confirm that the subject and the family members described in the study gave permission for the publication of their clinical details. A statement to this effect should be included in the methods section.
We have included the sentence “Written consent to publish was also obtained from the patient”. You can find attached a copy of this consent from the patient.
I, D. CARAVEN COTANO VILLACLOOS, accept that the anonymous data on my clinical case can be published in a peer review scientific journal.

Fecha/Date: 25/05/2021.

Nombre mayúsculas/Name Capital letters:

Firma/Signature: D. CARAVEN COTANO VILLACLOOS
- Can you also add in the letter in the paragraph about formatting: We also feel that your manuscript should be considered as a case report and not a research article (http://www.biomedcentral.com/bmcmedgenet/ifora/?txt_jou_id=2014&txt_mst_id =2006).

We also have considered this option and finally we decided to submit our manuscript as Research Article. This decision is supported by two reasons. First, methodologically we invested a lot of personal efforts and material resources in order to characterize well this new LGR; and second, we also provide an extended revision of the literature in order to highlight that the alteration we report is being described for the first time (a de novo complete BRCA1 deletion). Adapting our manuscript into a Case Report format would lose emphasis in the novelty of the finding.

Associate Editor's comment:

This is an interesting manuscript that needs some revisions according to the reviewers' suggestions. In particular, mutation designation must adhere to HGVS standards, as outlined by reviewer 1. The English style needs very careful revised throughout the manuscript, as pointed out by reviewer 2.

As Editor and reviewers suggest mutation designation using HGVS nomenclature was included for alterations detected in this study. However, all other genetic alterations appear in the text as they have been previously cited in the original papers. An explicative note was added on the bottom of table 1 to emphasize this point.

Additional comments:

- On p. 4, the lengthy description of the regional genetic counselling program and selection criteria for BRCA testing should be deleted, as these are not scientifically relevant.

We have incorporated the suggested changes.

- On p. 5, 2nd paragraph: correct "medular" > "medullary".

We have incorporated the suggested changes.

- On p. 9: as pointed out by reviewer 1, it would be appropriate to use additional software to obtain independent prediction of splicing effects. In addition, since such programs are only predictive, and usually not very reliable except for splice consensus sequences, the word "confirmed" should be replaced with "predicted".

We have also tested the base change detected in BRCA2 sequence using the Human Splicing Finder as suggested by reviewer 1 and replaced “confirmed” with “predicted”.

- In the Results section the authors state that ZFPM2 is likely amplified (likely present in more than two copies). Further on, in the Discussion, they discuss the amplification as a verified event (mosaic amplification), hypothesizing a mechanism related to BRCA1 dysfunction. This discrepancy should be solved.
particular, the authors should clearly state if and why the quantitative RT PCR values obtained are highly indicative of a mosaic amplification, referring to appropriate controls. Alternatively, if the mosaic amplification is only a suggestion, the discussion on the underlying mechanisms is not relevant and should be deleted.

Perhaps we have not been able to transmit what we wanted to say and probably we have generated some confusion. We found two alterations: first, a complete loss of the \textit{BRCA1} locus produced during the gametogenesis of our patient’s mother; and second, an amplification of the region corresponding to the \textit{ZFPM2 locus} (8q23) detected by Array CGH and confirmed by specific CNV assays using health controls and the proband’s relatives. In addition, in this region there is no any CNV reported according to Genomic Workbench Standard Edition 5.0. As the level of amplification is less than 3, it would suggest that this alteration is not present in all cells (which would mean an extra copy of the gene), but that has been produced during development in a subpopulation of cells (mosaicism). Hence, we hypothesize that in our patient, \textit{BRCA1} deficiency could have propitiated (surely together other factors) \textit{ZFPM2 locus} amplification in a subpopulation of cells in the early phase of the development.

-References for the reports on the previously published complete \textit{BRCA1} deletions should be provided in the second sentence of the discussion on p. 11.

We have provided the references as suggested.

-Finally, it would be interesting to know whether the deletion occurred on the paternal or maternal chromosome. Perhaps analysis of intragenic polymorphisms could be helpful to this purpose.

In order to assign the parental origin of \textit{BRCA1} deletion we have analysed the genotype of 7 intragenic polymorphisms for the patient’s mother and father. The results of this analysis were fully informative because the patient’s father has the same homozygous haplotype than the patient; on the contrary the mother presented the complementary homozygous haplotype for the seven analysed polymorphisms (Table).

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Patient</th>
<th>Patient’s mother</th>
<th>Patient’s father</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs8176144</td>
<td>DelT</td>
<td>T/T</td>
<td>DelT/DelT</td>
</tr>
<tr>
<td>rs1799949</td>
<td>T</td>
<td>C/C</td>
<td>T/T</td>
</tr>
<tr>
<td>rs16940</td>
<td>C</td>
<td>T/T</td>
<td>C/C</td>
</tr>
<tr>
<td>rs1799966</td>
<td>G</td>
<td>A/A</td>
<td>G/G</td>
</tr>
<tr>
<td>rs3092987</td>
<td>G</td>
<td>A/A</td>
<td>G/G</td>
</tr>
<tr>
<td>rs8176235</td>
<td>A</td>
<td>G/G</td>
<td>A/A</td>
</tr>
<tr>
<td>rs11654396</td>
<td>A</td>
<td>C/C</td>
<td>A/A</td>
</tr>
</tbody>
</table>
Reviewer: Laura Papi

Minor Essential Revisions
1) Abstract, Result section: replace “…. VAT1 locus to the beginning of gene….,” by “…. VAT1 locus to the beginning of NBR1 gene…..

We have incorporated the suggested change.

2) Methods, Patients section: specify the age of onset of breast cancers also in the second paragraph.

We have specified the age of breast cancers as suggested.

3) Methods, Mutation analysis of BRCA1 and BRCA2 section: The mutation nomenclature must follow also the HGVS format (http://www.hgvs.org/mutnomen/); moreover, the appropriate GenBank reference sequence and version number for both genes studied should be given.

As Editor and reviewers suggest mutation designation using HGVS nomenclature was included for alterations detected in this study. However, all other genetic alterations appear in the text as they have been previously cited in the original papers.

4) Results, Mutation analysis of BRCA1 and BRCA2: replace “….change was confirmed not to affect splicing,” by “….change was predicted not to affect splicing,”. Maybe, Authors would like to add results of another bioinformatics tool to predict splicing signals (i.e. Human Splicing Finder, URL: http://www.umd.be/HSF/)

We have also tested the base change detected in BRCA2 sequence using the Human Splicing Finder as suggested and replaced “confirmed” with “predicted”.

5) Discussion: MIM numbers of NBR2 and ZFPM2 are missing.

We have provided the MIM number of ZFPM2 gene but there is not a MIM number for NBR2.

6) Discussion, ZFMP2 amplification paragraph: replace “…and hence it would be prone to accumulate genetic...” by “…..and we may speculate that it would be prone to accumulate genetic…..”.

We have incorporated the suggested change.

I do not agree that the paper of Tirkkonen et al (1997) support the finding of a mosaic amplification of ZFPM2 in the Spanish patient. Tirkkonen et al (1997) found 8q gains in BRCA1-associated tumors as well as in BRCA2-associated and sporadic breast cancers supporting the view that 8q gains are common in breast cancers independently by the presence of a BRCA1/2 germinal mutation.

We have considered the reviewer’s comments on this topic reaching to the same conclusion. Hence, we have decided to eliminate this hypothesis from the manuscript.

• Discretionary Revisions
1) Abstract, Conclusion section: replace “….in hereditary breast and ovarian cancer families…” by “….in young breast cancer patients without family history, as well as in hereditary breast and ovarian cancer families.

We have incorporated the suggested change.

Reviewer: Thomas Hansen

Minor Essential Revisions
1) The manuscript needs careful editing throughout, e.g. abstract, line 5, “analized” should be changed to “analysed”; CGH array should be changed to array CGH; page 5, line 9 “presented a no previous” should be changed to “presented no previous”; page 11, line 12 “which seem result” should be changed to “which seem to result” amongst others.

We have incorporated the suggested changes.

2) The nomenclature should be changed throughout the manuscript, e.g. c.744+14C>T should be changed to nucleotide 744+14C>T, since “c.” refers to the coding DNA sequence, where nucleotide +1 is the A of the ATG translation initiation codon. Moreover it should be mentioned that this mutation is seen in the homozygote state. Finally this mutation is not undescribed (described in UMD-BRCA2 mutation database), but uncharacterized.

As Editor and reviewers suggest mutation designation using HGVS nomenclature was included for alterations detected in this study. However, all other genetic alterations appear in the text as they have been previously cited in the original papers.

Concerning the base change detected in BRCA2 sequence, we have mentioned that it is seen in heterozygote state and we have changed “undescribed” with “uncharacterized” in the text.

3) Page 13, line 24, reference 43 should be included.
The reference was included as suggested.

4) I do not understand why the authors use the term “mosaicism” on page 10, line 11 and page 11, line 16.
We have used the term “mosaicism” in order to indicate that the detected amplification of ZFPM2 do not correspond to an extra copy of the gene in all the cells (2.68 and 2.37 copies of the target sequence by CNV assays), hence different population of cells with different genotypes are present.

5) Does the symptoms caused by defects in the ZFPM2 gene mentioned on page 11, line 19-21 correlates to a duplication of this gene?

The symptoms described do not correlate with a duplication of ZFPM2, we comment them just in order to describe that this gene is involved in some human disorders. In particular, Pizzuti et al. (2003) suggested that ZFMP2 mutations (ser657 to gly (S657G)
or glu30 to gly (E30G) may contribute to some sporadic cases of tetralogy of Fallot (TOF). On the other hand, Bleyl et al. (2007) identified alterations in exon 7 in some patients with congenital diaphragmatic hernia.
