Author's response to reviews

Title: Mutational analysis of BRCA1 and BRCA2 in hereditary breast and ovarian cancer families from Asturias (Northern Spain).

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Author's response to reviews:

Oviedo, March 28, 2013

Dear Editor,

We are most grateful for your kind consideration of our manuscript entitled “Mutational analysis of BRCA1 and BRCA2 in hereditary breast and ovarian cancer families from Asturias (Northern Spain). Identification and geographical distribution of eight novel pathogenic mutations” by Blay et al (Manuscript number 1186416216923749). We are re-submitting a revised version in which we have addressed all criticisms raised by the experts who have reviewed our paper. Their comments and criticisms have been dealt with as follows:

Reviewer: Ramunas Janavicius
We thank this reviewer for the thorough revision of our work and his positive comments about its importance. His comments have been addressed as follows:

Major Compulsory Revisions

1. The title is too long. It would be appropriate to leave the first sentence.
We have shortened the title, leaving only the first sentence.

2. The newest HGVS nomenclature version (v2.0) should be used for mutations (e.g. “*” instead of “X” for stop codons)
We have modified the text and the tables according to the new nomenclature, and have mentioned version 2.0 in the text (page 6).

3. The proportion (in percentages) of recurrent mutations should be presented
more clearly. Please also mention, that future haplotype analysis of identified recurrent frequent mutations for the more specific delineation of founder effect is necessary.

We have presented this information where missing in the Results section and discussed it in more detail in the Conclusions section.

We have also mentioned the necessity of future haplotype analysis to provide formal proof of founder effect, as correctly indicated by the reviewer.

4. Table 3. BRCA1 mutation was found in mucinous ovarian cancer type – this is very unusual, because it is generally regarded that mucinous OC is not associated with BRCA+ status. The decoding/pathology data should be critically reviewed.

The pathology of the particular case mentioned by the reviewer has been carefully reviewed again by an experienced pathologist. This was a very undifferentiated tumor, with mucinous features. We have modified the table to stress the undifferentiated characteristic of this tumor.

5. The UVS class according international classification system should be assigned for the reported variants.

Regarding assignment of the international classification system as proposed by Plon et al (Hum Mut 2008) for the variants of unknown significance, we have mentioned in the text the controversies persisting in the literature regarding the pathogenicity of most of these variants. It is also important to point out that even the Breast Cancer Information Core (BIC) Steering Committee is currently taking under revision the clinical significance of the described variants in BIC database. Tavtigian et al (Hum Mut 2008) also propose that an international classification of variants should be performed by panels of experts, and classification of variants should not be done by independent clinical testing laboratories. For these reasons we consider that it would be too risky to assign a value for most of the variants that we have found in our population and that have already been described in other populations. In any case, in order to improve the information provided in this regard, we have completed Table 6 with the reference to Leiden Open Variation Database (LOVD) for those variants that have also been published in this database, which includes bibliographic reference regarding functional or in silico studies for each variant. In several cases, functional predictions show conflicting results for a single variant. Also, in agreement with the reviewer’s request, we have included in the revised manuscript an extended discussion of the variants that, according to multiple studies, are suggested to be pathogenic and of those that are probably neutral.

Minor Essential Revisions
1. there are typos prevalent throughout the text (e.g. “en”, “pb” etc.), the words should be consistent (e.g. “Exons” to “exons”)

We have reviewed carefully our manuscript and we have corrected the typos mentioned by the reviewer.
Reviewer: Rupninder Sandhu

We thank this reviewer for the thorough revision of our work. His comments have been addressed as follows:

Minor Essential Revisions

1) Abstract: Title Conclusions is spelled wrong,
We have corrected this mistake.

2) In Conclusions of the Abstract, the last sentence needs revision...something like.. 'Our findings may help design a first step.....'
We have modified the text in accordance to the reviewer’s suggestion.

3) First paragraph in Background needs references at the end of second sentence that ends in 'certain genes'.
We have included now appropriate references to this sentence.

4) References 2 and 3 do not substantiate the claims made in the sentence in terms of proportion, the authors need to provide numerical proportion supported by references instead of using the term large proportion
We have modified this part in accordance to the reviewer’s indication.

5) Last sentence in first paragraph needs editing, maybe authors can modify to something like, Testing.....genes can make it feasible to identify...
We have modified the text in accordance to the reviewer’s suggestion.

6) Patient and Methods section-First paragraph contains the abbreviation HUCA that is not explained before (except in author affiliations). Authors need ti add the expanded form in the text at least once before using the abbreviated form
We have included the full designation of HUCA

7) Second paragraph in study population sub-section of Patient and Methods section-D. One .....and one OR more, the R in OR is missing, the authors need to add that.
We have corrected this mistake.

8) Second paragraph in study population sub-section of Patient and Methods section-H. This criteria is a little vague to understand what authors mean by term 'families close to fulfill any of the above criteria. It will be helpful if authors can be more specific.
We now provide a more detailed definition of the inclusion criteria for group H.

9) Last paragraph of study population sub-section of Patient and Methods section-third line starting with 'Samples were....member, it will be helpful to specify here what samples were taken whether tissue, blood....etc.
We have specified that blood samples were used to obtain DNA for the present
study.

10) Large genomic.... BRCA2-sub-section of Patient and Methods - The authors talk about normalizing the data to two control samples. However, there is no mention of details regarding control samples in terms of what they consisted of, how they were collected etc. The authors should include the details for control samples in parallel to the details of test samples.

We have specified the details about control samples.

11) Results and Discussion-First section (first paragraph)-the authors claim that highest mutation rate in BRCA1 was observed in certain groups but do not talk about Group Ga that had 50% according to their observations in Table 1. The authors need to integrate that finding in their results.

Following the reviewer’s indications we have now included this data in the text.

12) Results and Discussion-First section (first paragraph)--the authors do not discuss the results of BRCA2 at all (except for in last sentence in association with one patient). The authors should make observations/analyze parallel to BRCA1 here to make the manuscript stronger.

Following the reviewer’s indications, we have expanded the discussion of BRCA2 results in this section.

13) Results and Discussion-(second paragraph)-the link to BIC does not work. The authors need to provide a correct link.

We have replaced the link to BIC with the updated, functional one.

14) Results and Discussion-(seventh paragraph in the sub-section BRCA1 large genomic deletion) The authors are missing important information in third sentence 'The deletion was....with and with...' without which it is not possible to understand the sentence. The authors should provide that information to make it understandable.

We have deleted the extra “and”.

15) Results and Discussion-(tenth paragraph in the sub-section Deleterious BRCA2 mutations) The data is a little hard to understand the way it is presented, it is highly suggested that the authors revise it to make it more clear. Same paragraph has a grammatical error after reference 34 i is inserted, the sentence should read 'was identified' instead of 'was identify'. The authors should correct that.

We have revised this section and corrected the error pointed by the reviewer.

16) Results and Discussion-(twelfth paragraph in the sub-section Unclassified variants)-Third sentence reads 'We could......was death.....', death word needs to be replaced by 'dead or deceased.'

We have modified this sentence as indicated by the reviewer.

17) Conclusions section-first paragraph -second sentence-should end as 8 of
them being novel, the authors need to revise this.
We have modified this sentence as indicated by the reviewer.

18) Table 6 is not referenced at all in the text. The authors should either remove it or reference it appropriately.
We have referenced Table 6 at the end of the first sentence in the Unclassified variants section.

Discretionary Revisions
1) In the acknowledgement section, it is usually not customary to add 'to' after 'thank' but it is the authors' discretion
We have modified this section as suggested by the reviewer.

In the hope that this revised work can be now accepted for publication in BMC Cancer.

Sincerely yours

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