Author's response to reviews

Title: Li-Fraumeni-like syndrome associated with a large BRCA1 intragenic deletion

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Author's response to reviews: see over
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Dr Christna Chap
Executive Editor of BMC Cancer

Ref.: MS 7967875316700537
Li-Fraumeni-like syndrome associated with a large BRCA1 intragenic deletion

Dear Dr Chap

Thank you for consideration of our manuscript for publication in your journal. We have reviewed the above manuscript according to the reviewer’s comments. In addition, the entire text of the manuscript was reformatted and shortened to address only the relevant findings. Below, please find our responses to the comments.

Yours sincerely,

Ana Krepischi, PhD
Researcher AC Camargo Hospital
Reviewer's 1 report
Reviewer: Dr Dafydd Evans

The authors report testing for large rearrangements in BRCA1/2, PTEN, CHEK2 and TP53 in 27 breast cancer cases from LFL/LFS families that have tested negative for point mutations in TP53. One novel BRCA1 deletion was found in a woman with breast cancer at 41 and endometrial cancer at 44. The authors report this as an association between BRCA1 and LFL. The authors have used quite loose criteria for LFL including the Eeles criteria which is the criteria for the BRCA1 family. The report would be more convincing if the deletion had been found in a more typical family with sarcoma.

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Major revisions
1. In the abstract and text the authors use ‘hardly’ detected by most commonly used molecular methods. Large rearrangements are not detected by sequencing or other methods for detecting point mutations, but MLPA is now widely used especially for BRCA1 where it may account for >20% of mutations. The authors should rephrase the abstract and text.
   R. The text was modified accordingly the indication of the Reviewer.

2. The authors state that 85% of HBOS is due to BRCA1/2 mutations, but this is fairly strictly defined HBOS and the authors should allude to this.
   R. This mistake was corrected throughout the text as the reviewer indicates.

3. At the end of the introduction they state that large rearrangements occur in as much as 30% of cancer genes. Don’t they mean can occur as the cause of up to 30% of the genetic aberration in cancer predisposing genes. Otherwise they are saying it doesn’t occur in 70% of genes.
   R. We have clarified this in the introduction section; the correct statement is that large rearrangements were already reported associated to cancer susceptibility in at least 30% of known cancer genes.

4. Last sentence of intro: ‘germinative’ should be changed to ‘germline’ … with (a) clinical.
   R. The word ‘germinative’ was changed to ‘germline’.

5. The size of the BRCA1 deletion changes from exons 9-19 in the results to 9-17 in the discussion. Which is it? I am not sure why the deletion needs a separate paper to write it up fully.
   R. We agree with the Reviewer; data regarding the breakpoint sequencing of this BRCA1 microdeletion was added to the manuscript. Therefore, the text was changed to make clear the exact size of the deletion according to the sequencing analysis of the breakpoints.
6. Can the authors say why they have not used MLPA for BRCA1/2? How many single exon deletions would their dosage test have missed?  
R. Considering that the array-CGH analysis have been conducted in a 180K platform, a single exon copy number change could be missed as the Reviewer pointed. Accordingly, we performed a BRCA1/BRCA2 screening in the patients using MLPA; no further deletion/duplication was found in those genes.

7. Can the authors provide any details on the childhood brain tumor and hepatoblastoma. These are critical to this being considered an LFL family.  
R. Pedigree was reviewed but unfortunately no additional information could be found on the child with SNC.

8. The authors should provide details of the LFL/LFS cancers in FDRs in table 1.  
R. Details of the LFL/LFS cancer were added to the Table 1.

9. Discussion: the reason large rearrangements are not common on the IARC database is partly because most families have not had MLPA. How many TP53 point mutations have the authors found in LFL/LFS families?  
R. Indeed, the TP53 gene is not commonly screened for copy number changes. We have screened the TP53 gene by sequencing in a large cohort of non-related LFS/LFL cancer patients, and we have found a frequency of 28.9% of point mutation (Achatz, et al., 2007). Further, we screened by MLPA 73 LFS/LFL cancer patients who tested negative for TP53 point mutation; TP53-exon copy number deletions or duplications were not detected in this group, suggesting that TP53 large rearrangements are not a common cause of Li-Fraumeni (unpublished data).
Reviewer's 2 report
Version: 2 Date: 12 May 2011
Reviewer: Dr Ed Hollox

This paper describes an analysis of 27 LFL/LFS patients which were negative for TP53 mutations. In one of the patients a hemizygous deletion of part of the BRCA1 gene was identified.

This is a well-written clear paper, of high scientific quality. Yet only a small amount of data is reported. The one deletion found by aCGH is reported but only aCGH data shown: the MLPA analysis and breakpoints are described but the data is not shown, and this data appears to be forming part of another paper in preparation.

To be worthy of publication, the paper should show the aCGH data, the MLPA data and verification of the breakpoint, and the breakpoint sequence so the exact sequence breakpoints can be shown.

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R. The paper was re-written in order to describe additional data. We have performed BRCA1/BRCA2 MLPA analysis in all patients, and have analyzed by sequencing the breakpoints of the detected BRCA1 rearrangement. The exact breakpoint sequence is now provided.
Reviewer's 3 report
Version: 2 Date: 19 May 2011
Reviewer: Dr Jenny Leary

The study is a potentially important contribution to the understanding of cancer susceptibility in Li-Fraumeni (LFS) and Li-Fraumeni-like (LFL) families with respect to large genomic rearrangements in high risk and moderate risk breast cancer susceptibility genes however there are some critical points that should be addressed with the study as described below.

• Discretionary Revisions (which are recommendations for improvement but which the author can choose to ignore)
  1. An indication as to whether the families that appear to have a mixed hereditary breast cancer (HBC)/ hereditary breast-ovarian cancer (HBOC) phenotype and the LFS/LFL phenotype have had any other testing of BRCA1/2 would be an informative addition to the clinical picture of these families.

R. The selection and clinical description of the patients was improved by adding all the information that was available. We selected 23 breast cancer patients with an indication for TP53 mutation testing due to a Li-Fraumeni or Li-Fraumeni-like phenotype according to the classical criteria (Li and Fraumeni, 1969) or at least one of the LFL definitions: Chompret, Birch or Eeles. In all families, TP53 mutation testing was negative. Additionally, some of these families also fulfilled mutation testing criteria for other hereditary breast cancer syndromes, as described in the NCCN Practice Guidelines in Oncology – v.1.2010 (see Table 1). We have further performed a BRCA1/BRCA2 MLPA screening in all patients.

• Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
  1. Table 1: the classification system should be defined or at the very least appropriately referenced and the formatting for individual Y0029 more clearly presented.
R. Changes were made as indicated by the reviewer.

  2. The method section could be more detailed and include more detail about the DNA extraction procedures, DNA quality, the resolution of the array and information about what reference DNA was used as the normal comparator.
R. We have clarified this in the method section by adding details of the DNA quality and the reference DNAs (positive and negative controls) that were used in the MLPA experiments.

  3. If a premise of the study is that the breast cancers in LFS/LFL families are influenced by more moderate risk breast cancer susceptibility genes such as PTEN and CHEK2 it is reasonable to not undertake any further analysis beyond large genomic rearrangements. However if the family history of breast cancer is such that the involvement of the high risk genes BRCA1 and BRCA2 is suspected (several families are described as
HBC/HBOC and LFS/LFL (Table 1) then a more extensive analysis of these genes is warranted. This point should be discussed.

R. These breast cancer patients were first selected because of the indication for TP53 mutation testing due to a Li-Fraumeni or Li-Fraumeni-like phenotype; all of them tested negative for TP53 mutations. Some of these patients also fulfilled criteria for genetic testing other high risk genes (BRCA1 and BRCA2); however, unfortunately we were not able to perform a point mutation analysis of these genes.

We agree that due to the patient’s family history of high risk, the investigation of moderate risk breast cancer susceptibility genes is not justifiable at this time, and data and discussion regarding those genes were excluded.

The aim of this study was to address the role of large rearrangements affecting the 3 major cancer genes in the breast cancer susceptibility of this group of LFS/LFL patients. Therefore, we have performed a MLPA screening for TP53, BRCA1, and BRCA2, and the manuscript was re-formatted accordingly.

In the discussion and conclusion sections we addressed the need of a comprehensive investigation in patients with a cancer family history consistent with genetic testing criteria for multiple breast cancer syndromes.

- Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached)

1. The title of the manuscript requires re-thinking. It is interesting and important to determine whether large genomic rearrangements are a feature of breast cancers in the LFS/LFL syndrome. However before it can be asserted that the LFL syndrome in the family in whom the BRCA1 intragenic deletion was described has actually resulted from this deletion, as the title suggests, segregation analysis would need to be conducted to determine whether the deletion observed in the breast cancer patient Y00054 has been inherited from the maternal history that that satisfies criteria for a hereditary breast cancer phenotype or from the paternal history representing the LFL phenotype. If in fact the deletion segregates with the other breast cancers in this family it is likely that the breast cancer in Y00054 is part of a HBC syndrome not the LFL syndrome in this family. If this analysis cannot be done, the discussion should cover this point and the assertion in the title of the study cannot be as strong as it currently is.

R. We agree with the reviewer that a segregation analysis in paternal and maternal families of the patient carrier of the BRCA1 deletion would clarify the inheritance pattern of the disease. Unfortunately, affected relatives of the patient Y54 could not be investigated for the presence of the BRCA1 deletion either because they were deceased or were not available. We managed to test two non-affected relatives (III.13; III.16) and found one carrier (III.16). We included this remark in the discussion. We have decided to maintain the title of the article since the patient carrying the BRCA1 deletion was first selected for fulfilling Li-Fraumeni-like criteria, and at this time this possibility could not be rule out.

2. The authors have indicated in the introduction (paragraph 2) that the frequency of germline BRCA1/BRCA2 mutations is approximately 85%. Whilst it if often asserted
that “BRCA1 and BRCA2 account for the approximately 85% of all cases of hereditary breast and epithelial ovarian cancer” (Shulman 2010), it is not strictly true to say that the frequency of germline mutations is 85%. In reality the frequency of mutation detection is little more than 25%.

R. We agree that the frequency of germline mutation detection is lower than we previously stated; the text of the manuscript was changed accordingly.
