Author's response to reviews

Title: Hereditary breast and ovarian cancer: Assessment of point mutations and copy number variations in Brazilian patients

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Author's response to reviews: see over
Dear Editor

Editor-in-Chief – BMC Medical Genetics

Ref: MS: 4367729601163499

Thank you for your message and for sending us the concerns of the referees about our manuscript “Hereditary Breast and Ovarian Cancer: Assessment of point mutations and genomic rearrangements in Brazilian Patients”. The new version of the manuscript was fully revised addressing all points raised by the referees.

In addition, it was observed by one of the reviewers that the manuscript needed some language corrections. Prior to submitting our manuscript to BMC Medical genetics, we submitted it to a professional editing service (American journal of experts). After the corrections requested by the reviewers we sent for a second English editing (Nature publishing group language editing), and we expect that now the manuscript meets the required quality of written English.

In this letter you will find a point-by-point reply to all reviewers’ questions, as well as the paragraphs that have been modified, which appear underlined in the new manuscript version. We hope that the current version is now suitable for publication in the BMC Medical genetics.

We appreciate your attention and time.

Sincerely yours,

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REVIEWER 1:

Reviewer: Gregory J. Tsongalis

Reviewer's report:
Silva et al present a very thorough study of Brazilian hereditary breast and ovarian cancer syndrome patients. The design, methods and scope of the project are very well done. The manuscript is novel in that this is the first such study in Brazilian patients that encompass such a genomic profile including sequencing and microarray analysis. In addition the authors describe for the first time, detection of germline variants in the ATM and PTEN genes in this patient cohort. The figures and tables complement the manuscript very well. A minor issue is that here are numerous grammatical errors that will need to be changed.

Response: We thank Reviewer 1 for his positive analysis of our manuscript. Regarding the language corrections, we have sent our manuscript for a second English revision in a professional editing service and hope that this time the manuscript meets the required English written quality.

REVIEWER 2:

Reviewer: Pierre Hainaut

Reviewer's report:
This manuscript reports on the prevalence and types of mutations in breast cancer susceptibility genes in a single-center clinical series of 120 Brazilian women fulfilling the criteria for HBOC. Capillary sequencing and MLPA has been used to investigate mutations and rearrangements in BRCA1 and BRCA2. Additionally, the CHEK2del100 variant has been investigated. A further group of 14 genes has been investigated for LOH using array-CHG.

The main interest of this work is to provide information on the prevalence and distribution of BRCA1 and BRCA2 mutations in a Brazilian HBOC series. In this respect, the study is well executed and informative. The study also reports on the presence of CHEKdel100 in 1 case, suggesting that this form of predisposition is present in the population analyzed, although at low prevalence.

Response: We thank Reviewer 2 for the analysis of our manuscript and for his important suggestions. Below we address the major and minor revisions suggested by him.

Major compulsory revisions:

1) The selection criteria for the series of 120 patients are not given. It seems to be a consecutive series of patients recruited in one major center, but the recruitment
period is not given and it is unclear whether the series includes all patients matching HBOC criteria during a given period – or whether the series is an ad-hoc subgroup. One concern is that, based on previous publications from that center, there is in Southern Brazil a high prevalence of subjects with a rare germline TP53 mutations who develop a variant form of Li-Fraumeni Syndrome, a familial syndrome characterized by several cancers including Breast cancer. At least some of these patients and families are expected to meet HBOC criteria. If the group of 120 patients is unselected, it is recommended to test them for the rare germline TP53 mutation. If the series of 120 patients excludes patients with this rare TP53 mutation, this should be explained, together with details on how the series has been assembled.

Response: Regarding the inclusion criteria, we agree with the reviewer that it is not clear in the manuscript. So we included the period of patient’s selection and made clear that the inclusion criteria was based on the NCCN guideline for mutation screening of patients with HBOC (Page 7, 1st paragraph). A table of all selection criteria is in the manuscript (Table 1). In relation to the incidence of the Brazilian TP53 R337H mutation, we added the evaluation of this variant in all BRCA1/2 negative patients of our series and included the results in the manuscript. Interestingly, we detected three cases with this mutation, and we included a paragraph in the introduction to explain the reason of testing our cohort for this rare variant (Page 5, 3rd Paragraph), and discussed the importance to test this variant in all female with history of breast cancer (Conclusion section).

(2) Another concern regarding the selection of patients is that recruitment in one center in Sao Paulo does not capture the very broad ethnic and socio-economic diversity of the Brazilian population. Although defining ethnicity in this context is very complex and beyond the scope of the present study, it is expected that this group of patients may be biased in being mostly Caucasians of European descent, and of higher-than-average socio-economic status. These considerations should be taken into account when discussing whether the prevalence and mutation types reported here are representative of “Brazilian” population.

Response: We agree with the reviewer that our evaluation is based on an institutional registry and does not reflect the entire Brazilian population. This consideration was included in the discussion section (Page 13, 1st paragraph) and also reinforced it in the conclusion section paragraph.

Minor essential revision:
(1) The screening of LOH in 14 genes does not capture the whole range of molecular alterations that may predispose to breast cancer. Again, it is expected that a proportion of patients who are negative for BRCA1/BRCA2/CHEK2del100 may carry germline TP53 mutations. In series from other parts of the world, the prevalence of TP53 mutations in HBOC subjects varies between 2 and 7%, making it the third most frequently mutated gene in this pathology. It should be made
clear that scoring LOH does not amount to a comprehensive screening of the 14
genes.

Response: We agree with the reviewer that the search for CNVs in 14 genes previously
involved in breast cancer does not represent a comprehensive analysis and for that
reason we included a note saying that we cannot rule out the involvement of pathogenic
point mutations in these genes (page 16, 1st paragraph).

Final considerations:

In order to uniformly adequate our manuscript, we changed all “genomic
rearrangements” to “copy number variations”. In this regard, we had to change the
title of our manuscript to “Hereditary breast and ovarian cancer: Assessment of point
mutations and copy number variations in Brazilian patients”.

We corrected the title page, abstract and references to conform the journal style.

The most important alterations made throughout the manuscript are underlined.