Author's response to reviews

**Title:** TP53 p.R337H prevalence in a series of Brazilian hereditary breast cancer families

**Authors:**

Nathalia M Cury (nathaliamcury@gmail.com)
Victor E F Ferraz (vferraz@usp.br)
Wilson A Silva-Jr (wilsonjr@usp.br)

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**Author's response to reviews:** see over
Changes made:

Referee 1: Edenir Palmero

The English language was corrected by a native speaker.

The manuscript title was changed.

Substitutions were made in the first and last paragraphs of the Introduction, in lines 5 and 2 respectively.

The characterization of tumor spectrum related to Li-Fraumeni syndrome was corrected.

We perform BRCA1 and BRCA2 mutation screening by sequencing in the two TP53 R337H positive females, with negative results for pathogenic mutation. The status of BRCA1/BRCA2 screening in the two TP53 R337H positive females was included in the methods and results section.

The phrase: “To investigate the association between this specific mutation and breast cancer in the southern region” was deleted because we agree that our results just sufficient to make conclusions about the prevalence of the R337H mutation in women from Ribeirão Preto, São Paulo, located in the Southeast region.

Genetic testing criteria according to NCCN Clinical Practice Guidelines in Oncology v.4.2013 for LFS were included in the paper. The inclusion of these criteria lead to some modifications not only in the results, elucidated in the 5th paragraph of the Results, but also in the discussion, elucidated in the 8th paragraph of the Discussion.

The last paragraph of the Results section was carefully rewritten to clarify the meaning. However, we maintained the comparison of the p value obtained in our study with the percentages reported by Custódio et al. 2013. This comparison has been made by various researchers, including Assumpção et al., who used the same overall frequency of R337H in southern Brazil to compare with their p values.

In the first paragraph of the discussion section, where we compared our results with previous work published by Achatz et al. and Assumpcao et al., we added information on differences in inclusion criteria.

We added a new paragraph, emphasizing the uncertainty concerning the role of the R337H mutation in breast carcinogenesis, mentioning the LOH results published by Achatz et al. and Assumpcao et al.

We added a statement concerning the weaknesses of our study.
Referee 2: Patrícia Ashton-Prolla

In the Introduction section, the first paragraph was rewritten to clarify the relation between HBOC, LFS, and the hereditary breast cancer phenotype. In addition, the high prevalence of the R337H mutation in Brazil was mentioned to better explain the focus of this study.

In the second paragraph, the meaning of early onset breast cancer was clarified; we also clarified the Chompret criteria, including LFL, in the text.

In the third paragraph, we specified that the work cited concerning R337H prevalence in the general population was conducted in a state in southern Brazil (Custodio et al. 2013).

We replaced throughout all text, including the title, the term R337H for the most current mutation denomination, TP53 p.R337H.

In the methods section, we added Table 3, showing that controls were age-matched to cases. We agreed with the considerations about vacutainer and deleted the detailed explanation about HRM reaction, since it has already been described in the literature by Bastien et al., 2008.

In the Results section, we agree that patient 1 fulfills Eeles criteria for LFL. We also agree that patient 2 could potentially fulfill Chompret criteria for LFL if the lung tumor of her uncle diagnosed at 52 years of age was bronchioalveolar. However, we can not use this criterion because we do not have information about his lung cancer type. In addition, we need to take into account that this uncle smoked, increasing his chances of lung cancer development, even with no genetic predisposition. However, NCCN Guidelines v.4.2013 for LFS and HBOC were included in the study. The HBOC criteria for all 28 patients in NCCN Guidelines v1.2010 remained the same in v4.2013. But considering genetic test criteria for LFS in NCCN v4.2013, we agree that patient 2 fulfills the criterion of early-onset breast cancer (< age 35) with a BRCA negative test result. Consequently, our results and discussion sections were rewritten to take this into account.

In the discussion section, the entire paragraph about the rationale for pathogenicity of the germline p.R337H sequence variant was rewritten. We emphasized the questionable pathogenicity of R337H due to the lack of a significant number of functional studies. We also deleted “the variety of tumor types found in families with the p.R337H mutation can be explained through the hypothesis of a specific tissue effect” because of a lack of support from the literature.

The last paragraph of the discussion was rewritten. Now it is clear that we are proposing that R337H testing be done simultaneously with BRCA testing in breast cancer patients who have HBOC genetic test criteria and family history of one or more tumors of the LFS/LFL spectrum, independent of age.

In the conclusions section, we added information about the lack of evidence of the role of the R337H mutation in breast carcinogenesis. With the proposal of simultaneous
genetic testing for BRCA and TP53, the statement that a negative R337H test does not preclude BRCA genetic testing in HBOC families is not necessary.

The questions about the use of NCCN Guidelines v4.2013 for LFS, the status of BRCA mutation test results for the subjects, and the review of the written English were already answered in our comments for referee 1.

Replies to the reviewer comments:

Referee 1: Edenir Palmero

In the abstract, we did not state that the TP53 R337H mutation is more common in Brazil than in other places. However, we adjusted the last two paragraphs of the introduction section to justify study of this particular mutation.

We did not test individuals with tumors other than breast cancer for the R337H mutation. Consequently, we did not conclude that other tumors are related to the R337H mutation. Our suggestion was that, in order to justify R337H genetic screening, a personal history of breast cancer and HBOC criteria is insufficient; there should also be a family history of individuals affected with other tumors of the LFS/LFL spectrum. We based this conclusion on our results that showed that women positive for the R337H mutation had a family history of individuals affected by other types of cancer, now specified as participating in the LFS/LFL tumor spectrum. Therefore, the inclusion of other cancer types at the end of the discussion and in the conclusion is justified.

Referee 2: Patrícia Ashton-Prolla

Combined BRCA and TP53 mutation testing were referenced in the 8th paragraph of the discussion.

In the discussion section, we do not agree that Assumpção et al. came to the same conclusion, since their subjects also include a subgroup of sporadic breast cancer cases (n = 78). We also do not agree that Gomes et al., indicated a significant association of p.R337H with breast cancer in Rio de Janeiro state, because they did not conduct statistical analyses. In this report, only two of 390 unselected breast cancer cases and none of the 324 controls carried the R337H mutation. The comparison is not significant by Fischer’s exact test analysis. However, we agree that our conclusions are not an important novelty and changed the discussion accordingly.