Author's response to reviews

Title: Association of PALB2 sequence variants with the risk of familial and early-onset breast cancer in a South-American population.

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Reviewer Lauren G Aoude

Minor essential revisions:

1. The term “OC” was defined in Methods, paragraph 2.
2. Transcript IDs were included in Methods. Amino acid changes were annotated at the first mention of the three variants in Results, paragraph 1.
3. We included the discussion requested.
4. We checked the number of decimal points used when quoting population frequencies in the Tables, and in the text we used one decimal for percentages. For allele frequencies, the literature typically provides two decimal places, and therefore we used this conventional form.
5. We deleted the sentence “variant is probably pathogenic”.
6. We corrected the error in Table 3.

Discretionary revisions:

1. To date, no high-penetration gene similar to BRCA1, BRCA2 or TP53 has been identified. Therefore, no authors mention this possibility. We think that is
theoretically possible, but we feel that it is not necessary to state this explicitly.

2. We checked whether families carrying the variants c.1676A>C and c.2993C>A showed cases of pancreatic cancer. Only four families showed pancreatic cancer (one case in each of these families). Therefore, an analysis considering pancreatic cancer was not performed.

Reviewer George Priya Doss C

With respect to points 1 and 2: The results of this paper include patients from 436 families (one case per family). Therefore, Table 1 summarizes the characteristics of the families. We have the pedigrees of all families, which we can attach if required.

3. We performed additional bioinformatic analysis for the c.2993C>T variant using SIFT and PROVEAN tools, and both programs indicated that this variant is probably pathogenic. We also applied these tools for the c.1676A>C and c.1861C>A variants, and both programs indicated that these variants are not pathogenic. To evaluate the protein stability, we used the i-Mutant program, and the results obtained are included.

4. We performed a modeling analysis to determine whether the amino acid change produces protein structure alterations. We included the 3D model obtained.

5. We do not have experience with the molecular dynamics simulation analysis. To date, we have been unable to connect with an expert to perform this analysis.

Reviewer Dirce Carraro

Mayor compulsory revisions

1) We added a brief description of the methodology used for the mutation identification in the PALB2 complete sequence.

2) Transcript ID was included in Methods section.

3) The primers used correspond to previously described by Tischkowitz et al. In fact the reference was wrong and we change it. For he exón 9 we designed new primers because we needed to have an amplicon more longer.

4) With respect to mayor information for the primer designing, we think that it is enough to indicate the software that we used.

5) We now read the paper of Antoniou et al., N Engl J Med 2014 Aug 7: 371(6): 497-506, that probably was published when we finished the manuscript written. The paper of Antoniou et al. Establish that “the pathogenic mutation in PALB2 has an absolute breast cancer risk of 58% for those women with two or more first-degree relatives with breast cancer at 50 years of age. This aspect was added in the discussion of our manuscript. Nonetheless, the variants studied in our manuscript are not described in the Antoniou et al (2014) article.

6) In the discussion we included a paragraph that explain the origin of the
contemporary Chilean population. This is a mix 60% Caucasian (Spanish) and 40% Amerindian and had not another admixture. The samples of cases and controls are of median and low socioeconomical strata from Santiago city (>90%), therefore the geographic origin was well controled and the age was an easy controlled variant. We added in Methods section that the samples live in Santiago (>90%)

7) With respect to bioinformatic analysis of PALB2 variants:

7.a.) We accept the suggestion and change the sub-title “Bioinformatic analysis of PALB2 variants” by “Prediction of functional effects of PALB2 variants” in line 259.

7.b.) We change “PolyPhen software indicated” by “predicted”, and changed the word “indicate(d)” by “predict(ed)”

7.c.) We classified the c.1861C>A as a variant of unknown significance and added “or as a rare variant”

7.d.) We added that the c.2993C>T could be classified as a variant of unknown significance.

Minor essential revisions

1) We changed the term “polymorphism” by “variant” or other.

2) We change “database of PALB2 polymorphism and mutation” by “variation database of PALB2 gene”.

3) We accept the suggestion for Table 4.

Thank you very much for your deep revision.