Reviewer's report

Title: Mutation analysis of PALB2 in BRCA1 and BRCA2-negative breast/ovarian cancer families from Eastern Ontario, Canada

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Reviewer: Katherine Nathanson

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In this manuscript Hartley et al used HRM/Sanger and MLPA del/dup analysis to identify variants in the PALB2 gene in families with breast and/or ovarian cancer. They identified two pathogenic mutations and three missense variants which they have classified as probably pathogenic. As noted in the Introduction, defining the patient populations for whom to offer PALB2 genetic testing is of utmost importance in the new era of clinical testing for moderate risk breast cancer genes. However, while Supplementary Table 1 proves some information, the definition of the patient population studied is quite confusing throughout the manuscript. The title states “...breast/ovarian cancer families...”; the Abstract-Methods “family histories of breast and/or ovarian”; the Abstract-Conclusions “breast/ovarian”; and the Introduction last paragraph “breast and ovarian cancer pedigrees”. In the discussion, the statement is made that “It should be noted that the majority (139/175) of the families we screened did not contain any reported cases of ovarian cancer”. As this manuscript is purporting to help genetic testing decisions, the patient population should be consistently and accurately defined throughout. The authors have used multiple ‘sample sets of convenience’. Additionally, it is not at all clear at all that the missense mutations are pathogenic, as the calls are based on very limited analysis, which is not even consistent. Finally, one of the truncating mutations is in the last coding exon and due to its location, it is a variant of uncertain significance, not a deleterious mutation, without additional evaluation. Thus, the conclusions of the paper about PALB2 mutations are vastly overstated.

Major Comments

1) SIFT and Polyphen2 are insufficient to classify the PALB2 missense variants as “predicted pathogenic” and to make the statement “While the variants are possibly pathogenic functionally...”. Predicted pathogenic reflects a posterior probability of likelihood of disease, which is not demonstrated by the authors. The conclusions are particularly problematic given that c.1846G>C (p.D616H) does not segregate with disease or show LOH in the tumor and c.3418T>G (p.W1140G) does not show LOH in the tumor. Their analysis need to be redone using additional pathogenicity softwares, including ones that have been validated as likely more predictive than SIFT and Polyphen2, such as MutationTaster and Condel. Their conclusions regarding the missense mutations are overstated.

2) What is the reasoning for testing only the c.3287A>G (p.N1096S) variant in the additional case population? The rationale should be clarified.
3) The description of the different sample sets is very difficult to follow. The authors appear to have included every convenient sample set that they get their hands on. Have they excluded patients with BRCA1/2 mutations? – it is not stated in the manuscript.

4) The discussion of tumor morphology is confusing – the authors point out six references that did not find associations of PALB2 mutations with HR negative breast tumors but then say that TNBC is overrepresented in PALB2 cancers as per reference 4. The last sentence of this paragraph that it is interesting that the probands have 24% TNBC is also unclear.

5) Discussion page 13: The last sentence of the last full paragraph is too much of a stretch given the small data set.

6) The evidence that the second truncating mutation is truly deleterious is weak, as I am sure the authors are aware. The mutation would not lead to nonsense mediated decay, but add an extra 19 amino acids. Although it may be pathogenic, and the authors provide evidence suggesting that it could be, there are examples of such changes (albeit in other genes) that are no pathogenic. The authors are stretching it, classifying it as a class 4 variant (VUS, likely pathogenic) is likely more accurate.

Minor Comments
1) Abstract: “…but not in other tumors from that family or in tumors from carriers of other mutations” – this statement is misleading as in the text tumors from two of the variant carriers were not available

2) Abstract: “In our cohort, all clearly pathogenic…” Even with the n defined at the end of the sentence, “all” is misleading as it is only two mutations, would replace “all” with “both”. In addition, the final statement is too strong of a recommendation given the small data set. Same for Conclusion page 16 “Each of the families” would be better clarified as “Both”

3) The HGVS full name is given for the variants in some locations and only the cDNA change is given in other locations; when referring to the missense mutations, it is more clear to give the full name at all times.

4) Discussion page 14: There is no proof that the 11yo daughter with melanoma carries the PALB2 mutation

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I have no competing interests.