Author’s response to reviews

Title: Somatic Variants of Potential Clinical Significance in the Tumors of BRCA Phenocopies

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To the Managing Editor:

On behalf of all the authors, I would like to thank the reviewers for their valuable comments regarding our manuscript entitled “Somatic Variants of Potential Clinical Significance in the Tumors of BRCA Phenocopies”. We now offer a revised version for publication in Hereditary Cancer in Clinical Practice. Our responses to reviewers’ comments are below (reviewer’s comments are italicized):
Reviewer 1: Major revisions required

The first sentence: 'Cancer predisposition in hereditary breast and ovarian cancer (HBOC) syndrome is usually caused by pathogenic germline mutations in the BRCA1/2 genes (gBRCA mutations) and is inherited in an autosomal dominant pattern.' is problematic because it introduces the concept HBOC as undefined but 'usually caused by'. As a starting point for a scientific paper this is not acceptable: logical concepts are to be defined.

Response: The modifier, “usually” was intended to include the current observations of BRCA phenocopies, showing symptoms of HBOC but lacking the familial germline mutations. Admittedly, other inherited mutations may be present. The modifier has been deleted.

The other problem is that inherited variants are not mutations, an inherited variant demonstrated to cause disease is by convention denoted a pathogenic variant.

Response: The term “mutation” has been replaced with “variant” or “genetic alteration” where appropriate.

The novelty 'gBRCA mutations' is not acceptable.

Response: “gBRCA” has been replaced with “germline BRCA variant”

Further in the first section of the intro the risk for carriers of pathogenic variants of both BRCA1 and BRCA2 are pooled together, but they are different and they cause two different biological forms of breast cancers which need different treatments and have different prognoses.

Response: BRCA1 and BRCA2 variants are combined with regard to increased risk of breast and ovarian cancer within the current study families. A reference to differences in risk in the presence of BRCA1 and BRCA2 genetic variants has been added to the introduction.

In the next section phenocopy is defined as 'affected by an HBOC-associated cancer', which illustrates the problems above: pathogenic variants of BRCA2 may cause prostate and pancreatic cancer, but not so for pathogenic variants of BRCA1. Is a prostate cancer in a family with a pathogenic variant of BRCA1 a phenocopy??

Response: No. The prostate cancer is a phenotype of the genetic alteration. If the prostate cancer occurred in the absence of the familial alteration, then the patient would be a phenocopy.
“HBOC-associated” refers to the participants in the current study. To avoid confusion, “HBOC-associated” was deleted from the definition.

The rest of the intro is to this referee speculative. This might have been OK but is not when the major problem is overlooked: In the early days and still today, most families/cases were selected for genetic testing because of aggregation of cancers: Seemingly very high penetrance for HBOC and/or many cancer cases in general, and some had cancers in both the maternal and paternal lineages. All these selection models indicate that many of the families might have more than one cancer predisposing inherited genetic variant which may both cause cancer in the absence of the major gene (BRCA1/2) and modify the penetrance of the major gene (cfr CIMBA reports). Not only is these arguments lacking, there are a large number of references which should have been indicated, but which are lacking.

Response: Previous studies describe modifiers that affect the penetrance and phenotypic manifestation of defined BRCA variants or unaffected BRCA variant carriers, but not phenocopies. The effect of modifiers of BRCA is not fitting in this study as the familial BRCA variant is not present. It is stated in the introduction that “Explanations offered for HBOC malignancies in BRCA phenocopies include sporadic cancer related to familial lifestyle and/or environmental factors, germline mutations in other, possibly, not yet identified genes that cause HBOC.” Furthermore, the possibility of more than one cancer predisposing inherited genetic variant (genetic background) in the absence of the familial BRCA variant is addressed in the discussion, as well as the potential effects of somatic variants.

Methods:’ tested negative for a known familial mutation’ is not good enough: was the carrier with the pathogenic variant (not 'familial mutation' and which should have been the gBRCA mutation in the wrongful nomenclature defined above) the mother, or a distant relative, and to which degree did the family histories allow for more than one disease-causative genetic variant segregating in the family?

Response: Family histories, reported in our previous study (Mitchell et al., 2018), show variant carriers were parents and/or siblings of the study patients. Four of the phenocopy patients were further tested for up to 49 cancer genes with no variants detected. Whole genome analysis of family members to detect additional variants was not performed (nor clinically relevant as these tests were performed for diagnostic purposes).

Results: Diagnoses in the selected patients studied are not results, they were selection criteria, endometrial cancer is not a phenocopy of HBOC.
Response: The selection criterion was the absence of the familial variant in cancer patients. The one case of endometrial cancer was included due to the phenocopy status. The fact that it is non-HBOC is noted. Further details regarding this case have been placed in the discussion.

Discussion: 'The familial mutations in 6/11 cases' is unclear - do they mean the ROS1 variant found in 6/11 cases studied? If so it is a variant frequently seen in the tumours, but there is no information that this was inherited? If it was, what is the prevalence of this variant in the population studied: Is it a de-novo mutation in the tumours, or an inherited variant. Without contrast information it is difficult to consider the impact of this finding.

Response: As indicated in the results section: “The most frequently observed mutated gene was ROS1 (6/11 cases), displaying variants p.S1109L and p.I537M, neither of which predict a deleterious effect on protein function, and p.S1109L, which may affect protein function (Polyphen score 0.908).” The allele frequencies fall within the range of potentially inherited variants described in the table legend: “Putative somatic variants are identified as those with <1% allele frequency in the 1000 genomes population and alternate allele frequency >70% or <30%, or those annotated in COSMIC.” The nature of the variant and the role of ROS1 as an oncogene, rather than a tumor suppressor in cancer, gain-of-function variants of which would not be inherited.

'It is quite possible that a complex cancer phenotype is not driven solely by a single genetic or epigenetic variant, even in the presence of a highly penetrant familial mutation' is an understatement: HBOC in carriers of pathogenic BRCA1 variants are caused by the combination of 46XX and the BRCA1 variant. The CIMBA reports have presented many interacting genetic variants. The discussion is, as indicated above, out of frame with current knowledge.

Response: As previously stated, many studies address additional mutations in BRCA variant carriers. The current study (and discussion) addresses BRCA non-carriers from families with pathogenic BRCA variants. We describe the polygenic model with regard to the observation of BRCA phenocopies, not BRCA variant carriers. The sentence cited was made stronger to the point with references added: “A complex cancer phenotype is probably not driven solely by a single genetic or epigenetic variant, even in the presence of a highly penetrant familial mutation.”

Conclusions: 'An initial hypothesis that BRCA phenocopies were secondary to chimerism was not confirmed in our previous study.' is OK. For the rest, there are some findings of possibly de-novo mutations in tumours which are difficult to interpret because there is neither the relevant
family histories, testing of relatives, testing of blood/normal tissue, nor information of population prevalences of the variants if they were to be inherited.

Response: The authors agree that the availability of normal tissue would allow more definitive designation of inherited vs. somatic mutations. The rarity of the phenocopy status and use of archival tissue was a limiting factor. Family histories are available in the previous study as indicated above.

Reviewer 2: Acceptable

This study examined the possible genetic causes of a small cohort of BRCA phenocopies, patients who demonstrate a cancer phenotype similar to that of their BRCA-positive relatives, but do not present with the known familial germline mutation. They examined a panel of 572 cancer-associated genes and identified potential clinically significant variants.

The research question is very interesting and valid, the study is scientifically sound, but the conclusions drawn fall down slightly due to a lack of experimental data. Testing only cancer-associated genes was limiting, in that it couldn't identify novel causative somatic mutations, particularly considering it is only a small sample size. Furthermore, conclusions surrounding the methylation effect are interesting but also only speculative. This paper could have been a lot stronger by performing methylation arrays, for example, to determine the methylation patterns between patients, or between BRCA-negative phenocopies and their BRCA-positive family members, if possible, and also if there are any correlations with their identified genetic variants.

The authors agree that the use of more comprehensive array and/or sequencing studies would provide insight on novel variants, however, this was not the intent of the current study. Since the familial variant was already known, the question addressed was whether this variant was in the phenocopy tumors, while not in other tissues (blood). The gene panel used was limited to known cancer-associated alterations in selected genes in efforts to find a possible common genetic alteration or altered pathway among the phenocopies.

Instead of targeted sequencing, could whole exome sequencing provide a greater understanding of the genetic differences between BRCA phenocopy patients? This may provide more depth to identify additional novel causative gene mutations, or novel candidate susceptibility genes, which may explain the phenotype.
Whole genome/exome sequencing would certainly provide more information in this regard, but we were not looking for novel cancer pathways in these patients. Such a study would require a much larger patient populations, and the phenocopy condition is very rare.

Of the patients who had somatic genetic variants, how many had multiple genetic mutations across the genes discovered? This wasn't clear from Table 1. Could you include a Table that shows which patients had a ROS1/NUP98/BRCA2 mutation or those that had some but not the others? Could there be some sort of polygenic risk attributed to patients who had multiple mutations in the same genes? This was discussed but not clearly presented in the results.

The genetic variants were filtered and annotated by bioinformaticists. The presence of multiple mutations in the same gene were noted based on the allelic frequencies.

Have the genetic variations described in this paper been validated by sanger sequencing? Do you know if these genetic variants are also present in the familial BRCA-positive tumour tissues?

Sanger sequencing was not performed to confirm the variants, which are mostly somatic. There are many studies on the presence of mutations in BRCA carriers, e.g. (Hamdi Y 2017).

More discussion is needed as to the possible reasons why BRCA non-carriers develop these similar phenotypes. Could it be due to other, unknown driver germline mutations?

Explanation for the phenotypes observed were offered in the polygenic model as well as in the epigenetic study of BRCA gene promoter methylation. A number of more speculative explanations are possible, but the hypotheses mentioned are considered the most likely and testable.


We feel the manuscript is greatly improved with the incorporation of edits and suggestions.

Respectfully yours,

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