Author’s response to reviews

Title: Genetic variants of prospectively demonstrated phenocopies in BRCA1/2 kindreds

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Author’s response to reviews:

Dear Editor,

Thank you for the positive and constructive reviews of the manuscript above. We have revised the manuscript in line with the reviewers’ suggestions and we respond to reviewers as follows:
Reviewer #1

- Comment: While the patient population is very specific, then in this study is small. And I felt like information was missing regarding were there other women who were not included in this analysis. More information regarding selection of participants would be helpful. On my first read-through of the paper, it was difficult to figure out who the patient population was. It was written, at multiple points, that the women were those who had tested negative for a familial BRCA1 or BRCA2 mutation, and then had developed breast and/or ovarian cancer. But then in the abstract it says "We identified 10% (5/48) of the cases to carry pathogenic variants in ATM, BRCA2, MSH6 and MUTYH genes, of whom two were from families with a known path_BRCA1 (15% of 13 cases) and three from a demonstrated path_BRCA2 (9% of 35 cases)" which does not seem to echo the same patient population. I would encourage consistency in writing across the paper in terms of who the patient population is.

Response: The description of the study population has been improved, as suggested. In addition, we included a flow chart about the selection of the participants (Figure 1) (Abstract section, page 2 and Results section, page 7).

- Comment: I question whether the recommendation to test all individuals with family history of a known pathogenic BRCA1 or BRCA2 mutation is necessary. In the cases of the patients who were found to have Lynch Syndrome through this study (not including the MSH6 mutation), it sounds as if the participants met criteria and should have been offered this testing prior to their diagnosis. If an individual is having a comprehensive family history obtained when they are being tested, this could reduce the need for further testing in many scenarios. I wonder if this was performed for the participants in this study? I think including the family histories of the other participants who tested positive would be helpful in assessing this.

Response: We agree in principle, but this is simultaneously the underlying basic problem. Until now, genetic testing has been expensive and availability limited, and most of them have been based on family history, and when a pathogenic variant was found, the testing most often stopped.

Family criteria are insensitive (https://www.ncbi.nlm.nih.gov/pubmed/17574839) and may overlap, and may function as self-fulfilling prophecies: When restricting testing to families meeting the family criteria, all carriers of pathogenic variants will meet these criteria. There may be more than one pathogenic variant segregating in families that are highly selected because of aggregation of cancers. Such variants may act as modifiers of penetrance of the ‘major gene’ causing disease in the families.
Our study examined for such variants under the assumption that they may increase probabilities for disease in persons not carrying the ‘major gene’ = phenocopies. We see no other way to sort this out than to test as many family members as possible for all genes/variants in question. When done, we may get information enough to say this would be no longer necessary – but until then we don’t know and has to examine the problem. We now mention this aspect in the Discussion.

- Comment: Additionally, many of the genes included on the panel do not have concrete associations with breast/ovarian cancer (MAP3K1, RAD50B, CDK2, POLE) so while there is no recommendation to test these genes, I wonder why they were included in the analysis?

Response: We made a test panel of the most interesting genes at a certain point to be applied to all families to get out of the logical circle of only testing for what you expect to find, and through that never find anything else. The 44-gene panel used in this study includes genes that, for the most part, are associated with a greater than twofold increased risk of breast cancer, colorectal cancer, melanoma, ovarian cancer, endometrial cancer, prostate cancer, gastric cancer and pancreatic cancer (https://www.ncbi.nlm.nih.gov/pubmed/28608266). In addition, gene panel testing is less expensive than single gene testing (https://www.ncbi.nlm.nih.gov/pubmed/28196074). POLE has for instance been described as relevant for ovarian cancer (https://www.ncbi.nlm.nih.gov/pubmed/25860647). More recently, other panels have been developed that are more comprehensive than ours, also from commercial providers of sequencing panels.

Importantly, recent next generation sequencing (NGS) studies have described pathogenic variants in genes that are not traditionally associated with their phenotype. In breast cancer, emerging data using multigene panels have described pathogenic variant in mismatch repair genes (i.e. MSH6) (https://link.springer.com/article/10.1007%2Fs10549-016-3948-z). On the other hand, approximately 18% of patients diagnosed with colorectal cancer (CRC) younger than 50 years had pathogenic variants in genes not traditionally associated with CRC (i.e.ATM, CHEK2, BRCA1, BRCA2, CDKN2A and PALB2) (https://jamanetwork.com/journals/jamaoncology/fullarticle/2593042, https://www.ncbi.nlm.nih.gov/pubmed/28135145).

Reviewer #2:

- Comment: Cases from MU were from BRCA2 families only. Were they selected or only families with BRCA2 mutation were available
Response: The cases selected were prospectively detected phenocopies in path_BRC2 carriers described in an earlier report (https://www.ncbi.nlm.nih.gov/pubmed/24285840) to be more frequent than expected, and which we hypothesized might be due to additional genetic factors in these families. The text is now specifically mentioning this.

- Comment: Pedigree in figure 1 was chosen not very fortunately. Patient with CC and LC / father of patients with ovarian cancer (all of them are carriers of the BRCA1 mutation), is not a relative of a patient with EC - a carrier of the MSH6 mutation. Therefore, the term that the pedigree of a patient with EC corresponds with Lynch syndrome is exaggerated (he is not a relative of a patient with CC, relationship with patients with OC, is far - III degree), unless MSH6 carrier would be confirmed in a DNA sample of one of the patients with OC. MSH6 testing in OC patients should be performed or suggestion about LS should be withdrawn.

Response: We agree, and we have therefore modified the phrasing and deleted the figure (Abstract section, page 2, Results section, page 8, Discussion section, page 9). What we meant and mean is that inherited ovarian cancer may be caused by pathogenic variants of MLH1, MSH2 and MSH6, as well as BRCA1/2, and family history has low sensitivity, especially for path_MSH6 families (https://www.ncbi.nlm.nih.gov/pubmed/20587412). Ovarian cancer cases in general, and in families with cancer cases, specifically, should be tested for MSH6. On reflection on previous results on this aspect, and in observation that the results are in agreement with this, it is to us all about the emerging international agreement on testing all ovarian and endometrial cancers for MSI and/or MMR genes.

In general, as panel testing is increasingly becoming cheap and available, one should examine as many cases as possible in cancer kindreds, and not stop when a first pathogenic variant is detected in one family member.

With these alterations, we hope that the manuscript may be acceptable for publication in Hereditary Cancer in Clinical Practice journal.

On behalf of the authors

Mev Dominguez-Valentin