Author’s response to reviews

Title: BRCA1 and BRCA2 mutation spectrum - an update on mutation distribution in a large cancer genetics clinic in Norway

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Version: 1 Date: 19 Dec 2017

Author’s response to reviews:

Thank you for your insightful comments, and the opportunity to make a better paper.

Reviewer 1:

Specific comments

1. Throughout the manuscript, different ways of addressing the frequency and spectrum of germline mutations in the study cohort are mixed: the number of unique BRCA mutations (207), the number of families (i.e. the number of apparently unrelated index patients) with BRCA mutations (981), and the overall numbers of mutation carriers (3522). To avoid confusion, I would recommend that the authors clearly mention and differentiate between these three categories. The number of mutation carriers for a given (founder) mutation might be heavily biased through the methods applied and the fact that in former times only founder mutation screening was performed which makes it more likely to have identified more mutation carriers by predictive testing in those old families with founder mutations (see results), as stated in the discussion.

Thus, the number of carriers might be less meaningful than the number of affected families to describe mutation frequencies. Based on this considerations it might be important to always
describe the frequency of the founder mutations related to the number of families (index patients). E.g., in the abstract it seems more relevant to mention the fraction of families rather than the fraction of carriers (86%) with frequent mutations.

Reply: We have included a paragraph called “Mutation frequencies” explaining the categories applied, in Methods p. 7. We have rewritten result section stating both frequencies of families and carriers, instead of using “mutation carrier rate per family”. The measure “Mutation carriers per family” were included to illustrate the selection bias described by reviewer 1 in his comment. The number of mutation carriers per family are still listed in the table 2 & 3, and discussed briefly on p. 15.

2. Are there any attempts being made to re-analyse the old cases, where only targeted founder mutation screening was performed, with up-to-date methods (sequencing of the whole coding region and MLPA) to uncover more BRCA1/2 germline mutations in the unexplained old families?

Yes, as a consequence of the work presented in this paper, and the results presented in a paper recently published in BMC Cancer, “Current guidelines for BRCA testing of breast cancer patients are insufficient to detect all mutation carriers” by Grindedal et al (2017), our section is currently evaluating ways of addressing this issue, but specific resources must be allocated to this work as it includes re-contacting patients/families with such an offer.

3. Page 6, Classification, the authors state that all identified variants were re-evaluated but they don’t describe, how this was done. I would suggest to describe the procedure in more detail since this is essential to assess the quality of variant interpretation.

Reply: We have extended the description on variant classification, with specific comments on the classification on splicing and missense mutations.

4. Page 20, Table 4: it should be indicated that the 17 assumed missense mutations all are regarded as pathogenic (class 4 and 5), if this is true. Overall, the number of missense mutations is quite low (8% of all BRCA1/2 mutations). Do the authors have any explanation for this? How many VUS were identified in the cohort as a whole?
Reply: Regarding the missense mutations, we detected a mistake in the original table. Two seemingly missense mutations (c.5434 C>G and c4484 G>A) which have been shown to lead to serious altered splicing had been counted as missense instead of splicing mutations. This has now been corrected in the tables. The percentage of missense mutations is still similar. We find the numbers in accordance to other reports and have added this information to the manuscript.

A correct percentage for VUS in the cohort as a whole is difficult to give, as strategies for testing, variant classification and reporting has changed over time, therefore it was outside the scope of the study. However, we have included a sentence and reference about the VUS percentage (4.9%) after 2014.

Minor issues:

1. Page 7, last sentence: It is not clear to me in which cases "more than one mutation is suspected"?

Do the authors refer to index patients with more than one pathogenic BRCA1/2 germline mutation?

How many index patients/families met this criterion?....

Reply: This is referring to families with more than one mutation possible for cascade testing, yes.

This was the case for fourteen families, and this information is added to the manuscript.

2. Page 8, Results: to describe it more clearly: "There were 120 unique (or different) BRCA1 variants and 87 unique (or different) BRCA2 variants …"

Answer: This suggestion is integrated in this paragraph.

Reviewer 2:

Comment: Since no correlation is made to any phenotypic information, it is mainly population genetic. Therefore a direct link to clinical practice is weak.

Answer: In discussion section (which have been somewhat shortened) we have tried to clarify our view on the link to clinical practice. See also last comment on NGS/foundertesting.
Comment: Given the fact that de novo mutations in BRCA1 and BRCA2 are supposedly very rare, I don't quite understand the relevance of the distinction between founder mutation and (high, moderate, less) frequent mutation. It's just a continuum.

Answer: The reason for dividing into three categories, was mainly for systematic purposes, but also due to an expectation of finding new founder candidates this way. The BRCA1 founder mutations in our study are confirmed to be founders through a haplotype study. Some define founder mutations as variants found in a different number of families, i.e. more than three.

Comment: The study population consists of 981 families of which 68% had a BRCA1 and 32% had a BRCA2 mutation. But nowhere I can find how 'family' has been defined. We know that separate nuclear families eventually show to be branches of large pedigrees, i.e. families.

Answer: Family definition is now included: Index patient is given a separate family number if the patients do not already have family members registered in the clinic. Hence a family may contain only one mutation carrier. This is now stated on p.5/6

Comment: What is the scientific message behind the given number of mutation carriers per family in the results section? When we don't know family size nor phenotype, nor referral/testing criteria, this doesn't contribute much I think.

Answer: We stated number of mutation carriers per family mainly to illustrate selection bias. As Reviewer 1 mentions, addressing the mutation frequency as fraction of mutation families as well as fraction of carriers may be a more intuitive way of presenting the data. These two issues have now been addressed together by rewriting result sections, stating both frequency of a variant in carriers and in families, instead of number of mutation carriers per family. The number of mutation carriers per family is still listed in table 1 & 2, and discussed briefly on p. 15

Comment: P10 line 56: 2 out of 5 is too small a number to say it's 40% P11 line 56: what is ment by 'quite extreme in both ends'? I see only one end.

Answer: The sentences are changed.

Comment: The discussion part could be way much shorter.

Answer: We have tried to shorten the discussion somewhat.
Comment: Given the fact that founder mutations are present in Norway, it would be interesting what their contribution is to the carrier frequency. This may affect clinical choices as to whether in presymptomatic setting, DNA testing should be more comprehensive than just for the familial mutation (as in Ashkenazi Jewish populations). This is hardly touched upon in the paper. (top of page 8 ’.......when more than one mutation is suspected…)

Answer: The founder mutations showed a lowered contribution to the overall carrier frequency compared to previous study 15 years ago. This the main message, and is now more clearly stated in results p. 10 and discussion p 13. The sentence on top of page 8 is mainly to explain that in some families, there were actually more than one pathogenic BRCA1/2-mutation.

Comment: In general, the clinical relevance of this paper is rather restricted: not only are we shifting towards NGS, but also towards breast cancer gene panel testing. Moreover, initial founder mutations, based on historic population structures and geographic/ethinic or religious boundaries are quickly deluting due to migration. Ethnicity and/or genetic background are rapidly becoming more diverse, which means that also in Norway, the relevance of this data is becoming less pronounced.

Answer: Yes, we certainly agree that there is a development towards selecting NGS and gene panel testing, at least in diagnostic testing. We think the results of this paper support a shift towards more comprehensive testing, but in terms of penetrance there are important differences between the genes, and therefore it is important to consider how the patient/public will benefit the best from the cancer prevention potential of BRCA1/2 testing. We show that, by testing only for founders you will over time loose a substantial amount of BRCA ½ mutation carriers, even in traditional founder mutation populations. Specific data on mutation frequenices are necessary as background information if a broader testing approach for other breast cancer genes with lower penetrance are applied, and at least if voluntarily, presymptomatic, population screening should be discussed as it has been for Ashkenazi Jews.

We have rewritten some passages in discussion to make these implications throughout discussion, especially on 15 / 16.