Reviewer's report

Title: Five recurrent BRCA1/2 mutations are responsible for cancer predisposition in the majority of Slovenian breast cancer families

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Reviewer: Patricia N Tonin

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Reviewers report

This report describes BRCA1 and BRCA2 mutation analyses in 145 Slovenian families with a personal and/or family history breast and/or ovarian cancer. Germline BRCA1/2 mutations were identified in 56 (~39%) families. The most interesting outcome of this study is the observation that 5 specific mutations (4 in BRCA1 and 1 in BRCA2) were found more than once, and accounted for 67% of the mutation-positive families. These results could significantly impact the genetic counseling of high-risk women in this population by facilitating mutation-detection of these very large and complex genes. These findings also add to the growing list of examples of recurrent BRCA1/2 mutations found in specific populations.

Major Compulsory Revisions

1. The study uses three specific inclusion criteria for mutation detection. Background section requires a clarification of rationale for the selection of specific individuals for mutation detection.

2. In the Methods section, the inclusion criteria of cases tested for mutations requires further clarification and elaboration. Three specific inclusion criteria are indicated, but these include both female and male probands tested for mutations, as well as unaffected probands. It is difficult to sift through the additional files to appreciate the cases and family history in each of the categories listed for each inclusion criteria. Indicating the number of cases in each category would help clarify the situation. Moreover, what is the rationale for including ‘patients with bilateral breast with other cancer types in the family’? What are these other types of cancer?

3. The Methods section refers to an impressive cancer registry dating back to 1950. Were all of the cancers (breast and ovarian cancers as well as cancer at other sites) mentioned in the study, particularly those listed in the additional tables, recorded in the registry? Or were some of these cases self-reported?

4. The mutation detection rate at 39% appears impressive. Likewise the observation that 5 specific mutations accounted for about 67% of mutation-positive cases. Although the mutation analysis of BRCA1 and BRCA2 was extensive, it was not comprehensive, as the largest exons of these genes were screened by PTT assay; and families with only 2 cases of breast cancer
may have been further restricted to screening for the recurrent mutations only (Please clarify statement in Methods section paragraph 4). It is possible that mutations may have been missed and that the mutation detection rate identified in the 145 families is an underestimate of the overall mutation rate. It is also possible that the Slovenian families are more genetically heterogenous than indicated by the present analysis. The possibility of missed mutations due to the methods used should be discussed. It is difficult to evaluate how many families have a high a priori probability of harboring a mutation because there is no adequate description of these mutation-negative families – only the cancer phenotypes of the positive families are shown in the additional tables. Is it not possible to summarize the phenotypes of the mutation-negative families according to the categories used in the inclusion criteria for testing?

5. Further to the above comment, in the Discussion section cites mutation detection rates of between 15% and 37% from independent reports. However, were the cited studies using the same inclusion criteria? If not then it is difficult to compare studies.

6. The authors indicate in the Results section that the probability of finding a mutation ‘correlated with the number of affected patients’. However only percentages are given and no statistical analyses are included to support this statement.

7. The Results section describing the cancer phenotypes of families is the weakest part of the manuscript. It describes relative risk estimates and penetrance. Overall the analysis is interesting but is not necessarily valid given the biases inherent in the selection of individuals for mutation detection, the small sample size used in the calculations, and the possibility of missed mutations due to incomplete mutation analyses for all cases.

a. In the first paragraph of this section, does the mean age of diagnosis of cancer include both breast and ovarian cancers? If so, why are they combined when the penetrance and age of diagnosis of breast cancer in mutation carriers is usually lower than ovarian cancer cases?

b. Paragraph two of this section describes cancer phenotypes of all cases studied as shown in Table 1. What is the purpose of doing this? The inclusion criteria are complex. Again for reasons mentioned above, the frequency of mutation-positive cases per inclusion criterion maybe more meaningful. Also included are cancers at other sites. As mentioned above, were these cases verified by the cancer registry? If the authors argue the merits of including such a table why not also include similar data for the mutation-negative cases?

c. In paragraph four of this section, although controlled for mutation type, the calculations for relative risk of cancers is not convincing given the small number of cases used in the analysis. This perhaps explains the discordant observation with previous studies (as noted by the authors) where the analysis of IVS16-2A>G mutation was only reported in families with breast cancer unlike the present study which included families with ovarian cancer.
d. Paragraph five of this section, describe penetrance estimates. This data too is not convincing given the small sample size, the possibility that not all cases within the family where tested for mutations, and biases inherent in the inclusion criteria.

8. The cancer phenotypes other than breast an ovarian cancer are shown in the additional tables and referred to the Discussion section. Where these phenotypes also observed in the mutation-negative families? (Also see comment 7b above.)

Minor Essential Revisions

1. A lot of information about family history is presented in the additional tables that would be of particular interest to genetic counselors. It might be easier to study the information in the tables if the age(s) of diagnosis of the proband where put into a column separate from the cancer status. As the penetrance for breast and ovarian cancer is much higher than any other type of cancer, the number of female first degree relatives (above age 18 years) would give a better indication of ‘penetrance’ than all such individuals. As the authors have indicated in the Discussion, it is difficult to assess risk assessment of cancers at sites other sites. However, reporting cancers that occur in mutation-positive families from other sites is interesting. Could this information be separated from breast/ovarian cancer cases (with the exception of double primary cases)? As noted above (see item 6), the reliability of the data should be clarified.

2. In the Discussion section, the authors suggested that the level of participation is ‘higher’ than reported in the literature. Perhaps authors could clarify this statement, particularly for readers unfamiliar with participation rates reported in the literature.

3. The report requires careful editing throughout. Specific examples are included below:

a. Methods section: Patients and families
   i. Delete ‘and’ in first sentence of paragraph one
   ii. Something is missing in last sentence of paragraph one
   iii. Add ‘to the’ between ‘according’ and ‘liberal’ in forst sentence of paragraph two
   iv. First sentence of paragraph 4: “Both genes were screened in full, except in families with only 2 breast cancer cases..” rather than as written.
   v. First sentence of paragraph 5: “After a mutation was found in the proband...” rather than ‘family’.

b. Results section: BRCA1 mutation analysis
   i. Rewrite the sentences describing repeatedly observed mutations: “the throughout the world widely reported”.

c. Discussion section:
i. Delete second “in” in the last sentence first paragraph.

ii. Delete ‘spontaneously’ from second paragraph, as the family members of mutation positive cases were contacted for further genetic counseling (unless the authors are referring to self referrals?)

iii. First sentence in the third paragraph requires editing (“... a search for BRCA1/2 mutations” rather than as written.) [This paragraph nicely describes the overall rationale for the study and could also be iterated in the Background section.] The sentence “However, in the Slovene breast cancer only families we found much more mutations than corresponding Belgian families” requires editing to read “However, more Slovene breast cancer only families were found mutation-positive as compared with comparable...”

iv. In paragraph seven, correct spelling of cysteines and add database to ‘BIC’ database.

v. Paragraph 8 : clarify what is meant by ‘double rate of ovarian cancers’?

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

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests