Author’s response to reviews

Title: Spectrum and prevalence of BRCA1/2 germline mutations in Pakistani breast cancer patients: results from a large comprehensive study

Authors:

Muhammad Usman Rashid (usmanr@skm.org.pk)
Noor Muhammad (bslab@skm.org.pk)
Humaira Naeemi (bslab1@skm.org.pk)
Faiz Ali Khan (bslab3@skm.org.pk)
Mariam Hassan (crc@skm.org.pk)
Saima Faisal (drsaimafaisal@aol.com)
Sidra Gull (gull_sidra@yahoo.com)
Asim Amin (Asim.Amin@Carolinashealthcare.org)
Asif Loya (asifloya@skm.org.pk)
Ute Hamann (u.hamann@dkfz-heidelberg.de)

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Author’s response to reviews:

Prof. Jan Lubinski & Prof. Rodney Scott
Editor-in-Chief
Hereditary Cancer in Clinical Practice

Re: Resubmission of the manuscript: HCCP-D-19-00022

Dear Editors,

Enclosed please find the revised manuscript entitled “Spectrum and prevalence of BRCA1/2 germline mutations in Pakistani breast cancer patients: results from a large comprehensive study”
(HCCP-D-19-00022) and our responses to the comments of the reviewers. We would like to express our appreciation for the input given by the reviewers, which enabled us to improve the manuscript.

We hope the revised manuscript is now acceptable for publication in Hereditary Cancer in Clinical Practice. All amendments in the text are marked in blue.

I greatly appreciate your consideration of our study. Please do not hesitate to contact me for further clarification.

Yours sincerely,

Ute Hamann

Point-by-point response to the comments of the reviewers

HCCP-D-19-00022

“Spectrum and prevalence of BRCA1/2 germline mutations in Pakistani breast cancer patients: results from a large comprehensive study”

Reviewer #1 (minor revision):

General comments

Point 1: This is a very valuable and interesting study performed to summarize knowledge about prevalence and spectrum of BRCA1/2 mutation among HBC/HBOC patients from Pakistani population. The manuscript is very well written, however the description of methods seems to be inadequate. In my understanding, screening for BRCA1/2 mutation was performed and described in earlier published studies (ref. 11 and 22). In this study Authors analyzed the available data, so in fact this study is a meta-analysis of previously published and as such should be described.
Specific comments

Description of methods should be adapted to that which is appropriate for meta-analysis.

Please note that in the present study we did not perform a meta-analysis as we have included data from two previous studies (ref. 11 and 22) as well as novel data.

• Ref. 11: Study of the contribution of BRCA1/2 large genomic rearrangements to hereditary breast cancer in 120 patients negative for BRCA1/2 small-range mutations.

• Ref. 22: Investigation whether diagnosis of triple-negative breast cancer independently increases risk of carrying a BRCA1/2 mutation in Pakistan. Four-hundred patients with or without the triple-negative breast cancer phenotype were screened for BRCA1/2 small-range mutation and large genomic rearrangements.

• Present study: Investigation of the spectrum and prevalence of BRCA1/2 mutations in 539 breast cancer patients including 400 patients previously described in ref. 11 and 22 and further screened 139 novel patients. Data from all patients were combined.

According to the reviewer’s suggestion, we now provide a detailed description of the methods (please see Methods section, page 6, 1st paragraph, lines 4-10).

Minor comments

Point 1: Figure 1 presents not only description of the participants, it is more like a flow diagram of study selection, including number of participant, used screening methods and detected mutations

According to the reviewer’s suggestion, we updated the title of Figure 1 and Figure legend (Fig. 1) (please see Figure Legends section, page 28, line 1).

Point 2: In fig. 2A and 2B is presented distribution and frequency of mutations along BRCA1/2 genes, with indicated functional domains and OCCR. It would be useful to mark which are 18 and 3 recurrent mutations, and also discuss the presented distribution. Otherwise, Fig. 2 is useless, because distribution and frequency of detected mutations can be obtained from table 2.

As per reviewer’s suggestion, we have now marked the recurrent mutations in BRCA1 (please see Figure 2A) and in BRCA2 (please see Figure 2B and Figure legend section, page 28, line 2 and 3). These mutations are also discussed (please see Discussion section, page 10, 3rd paragraph, lines 7-12 and page 11, 1st paragraph lines 1, 2).
Point 3: It is interesting if the distribution of BRCA1/2 mutation was the same in different ethnic groups, and in all are the same recurrent mutations. I am aware the in this study is overrepresentation of Punjabi and Pathan ethnic groups, but such analysis would have significant implications in clinical screening.

As per reviewer’s suggestion, we now discussed the distribution of BRCA1/2 mutations in different ethnic groups and its implications (please see Discussion section, page 10, 3rd paragraph, lines 7-12 and page 11, 1st paragraph, lines 1,2).

Point 4: Discussion, page 11, line 6: how the 152.6% increased occurrence of BRCA1 mutation in BC families was calculated?

Please note that in the current study the prevalence of BRCA1 mutations in breast and ovarian cancer families vs. breast cancer only families was 53.8% (35/65) vs. 21.3% (52/244), respectively (please see Table 1). So we observed a 2.52 fold (53.8% / 21.3%) increased occurrence of BRCA1 mutations in breast and ovarian cancer families compared to breast cancer only families (please see Discussion section, page 11, 2nd paragraph, lines 9 and 10).

To simplify further we have now deleted 152.6% from the discussion section. It was calculated as follow:

A 32.5% difference in the prevalence of BRCA1 mutations (53.8-21.3=32.5) was noted between breast and ovarian cancer families and breast cancer only families.

Overall, we observed a 152.6% (32.5/21.3 x 100) increased occurrence of BRCA1 mutations in breast and ovarian cancer families compared to breast cancer only families.

Point 5: Page 11, last paragraph: in this study the number of patients was smaller than in previously published (ref. 11 and 22), so its size is not really the main strength. In my opinion, the main advantage of this study is comprehensive analysis mutations which allowed to identify the recurrent BRCA1/2 mutations in Pakistani population and define the frequencies among high-risk families.

Please note that in the current study the number of patients (n=539) was larger than those reported in previous studies, ref 11 (n=120) and ref 22 (n=400). Hence, the main strength of the current study is its large size of a total of 539 high-risk families and the comprehensive screening of both genes for small-range mutations and LGRs using highly sensitive methods. We clarified and commented on this point (please see Discussion section, page 12, 2nd paragraph, lines 3-5).
Point 6: In conclusion should be added possible clinical implication of these results in preventive strategies and treatment.

As per reviewer’s suggestion, we have now added a statement in the conclusion section. (please see Conclusions section, page 12, 3rd paragraph, lines 2-5).

Reviewer #2: Accept