Author's response to reviews

Title: Constitutional CHEK2 Mutations are Infrequent in Early-Onset and Familial Breast/Ovarian Cancer Patients from Pakistan

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Author's response to reviews: see over
Re: Resubmission of the manuscript (MS: 2275536829108433)

Dear Dr. Cheang,

enclosed please find the manuscript "Constitutional CHEK2 Mutations are Infrequent in Early-Onset and Familial Breast/Ovarian Cancer Patients from Pakistan" by Rashid and colleagues to be reconsidered for publication upon revision.

In accordance with the comments of the reviewers we changed the manuscript in order to meet their recommendations. We addressed each of the requested points. All amendments/changes in the text are marked in blue.

Thank you very much for giving us the opportunity to revise and resubmit our manuscript.

With these changes, we hope that the manuscript is now acceptable for publication.

I am looking forward to hearing from you.

Yours sincerely,

Ute Hamann
Point-by-point Response to MS: 2275536829108433

Constitutional CHEK2 Mutations are Infrequent in Early-Onset and Familial Breast/Ovarian Cancer Patients from Pakistan by Rashid et al.

Reviewer's report: 1

Minor essential revisions:

Point 1. “The index cases included 111 female breast cancer patients, 11 male breast cancer patients, and 27 ovarian cancer patients. As this does not sum up to 145, some 6 (correct four) index patients appear to have both breast and ovarian cancer but this should be specified and is not immediately obvious from Table 1”.

According to the comment of reviewer 1 and (also of reviewer 2, point 1) we have restructured Table 1 and clarified this point. The explanation for the difference in the numbers is that breast and ovarian cancer in the same patient were counted as two independent cases of cancer. In Group 1 there are 4 patients and in Group 2 there are 3 patients, who presented with both cancers (see Table 1, page 22,23; Study subject section, page 5, lines 1-9; Result section, page 7, 3rd paragraph, lines 1-3).

Point 2. “It might be appropriate to provide a figure for the novel p.P92R mutation as a supplemental file”.

According to the reviewer’s suggestion a new figure with the electropherogram of the novel p.P92R was added (Supplementary Figure 1, page 25).

Point 3. “It is stated for p.P92R that “the mutation was predicted to be likely pathogenic in two of the four tools”, this result should be specified”.

We have specified this result by changing the sentence as follows:

“The mutation was predicted to be likely pathogenic by PolyPhen2 and SNAP” (see page 7, 3rd paragraph, line 14,15).

Point 4. “It is discussed that “CHEK2-linked breast tumors (c.1100delC, c.IVS2+1G>A, del5395) were predominantly ER-positive and of non-lobular type”. But the authors report missense variants. While the ER-positive phenotype is clear, the predominance of non-lobular breast cancer is under debate for such a mutation type (e.g. Huzarski 2005, Domagala 2012) and the authors may argue more cautiously in this regard”.

We agree with the reviewer and more cautiously discussed these results by adding the following sentences:

“The breast tumor of the patient harboring the p.P92R missense mutation was positive for ER, which is consistent with previous findings that breast tumors linked with CHEK2 frame shift and missense mutations (c.1100delC, c.IVS2+1G>A, del5395, p.I157T) are predominantly ER-positive [48-50]. Additionally, an association of the CHEK2 p.I157T missense mutation has been reported with lobular carcinoma [50,51]. This link was not observed with the novel p.P92R missense mutation in the present study given the only p.P92R-associated tumor was invasive ductal carcinoma. Given the solitary finding, no interpretation could be rendered.” (see page 11, 3rd paragraph).
Reviewer's report: 2

Major revision:

Point 1. “More precisely define the cancer cases (individuals) specifically investigated by genetic analysis in Groups 1 (n=145) and 2 (n=229). All that is listed in Table 1 or referred in the Methods and Materials and Results section appear to be the cancer phenotypes of associated family members. This information could be added to Table 1 and commented upon in the Results section. This information would provide a more accurate description of the estimates of frequency of CHEK2 variants found with respect to the cancer phenotypes actually tested for germline mutations”.

According to the comment of reviewer 2 and (also of reviewer 1, point 1) we have restructured Table 1 and clarified this point. The explanation for the difference in the numbers is that breast and ovarian cancer in the same patient were counted as two independent cases of cancer. In Group 1 there are 4 patients and in Group 2 there are 3 patients, who presented with both cancers (see Table 1, page 22,23; Study subject section, page 5, lines 1-9; Result section, page 7, 3rd paragraph, lines 1-3).

Minor revisions:

Point 2. “Is it possible to provide figures of the pedigrees of the CHEK2 mutation-positive families, which include other members tested as described in the Results section?”

According to the reviewer’s suggestion a new figure showing the pedigrees of the p.P92R and p.R406C mutation carrier families was added (Supplementary Figure 2, page 26, 27).

Point 3. “Comment on the specificity/sensitivity the initial mutation screen using DHPLC (versus direct DNA sequencing) in the Discussion section. This gene is complex and some regions are difficult to assess in genetic tests. Is it possible that some variants were missed using this assay?”

According to the reviewer’s suggestion, a paragraph was added in the discussion section.

“Mutation screening was performed using the combined approach of DHPLC analysis followed by DNA sequencing of variant fragments. DHPLC has been commonly used for mutation screening of various genes as it is rapid, cost-effective and highly sensitive detecting 93 to 100% of mutations that were observed by DNA sequencing [47]. However, since the DHPLC mutation detection rate is not 100% for each gene, it cannot be ruled out that some mutations were missed. CHEK2 mutation analysis is hampered by the presence of multiple homologous copies for exons 10-14 and requires an adapted primer design and amplification conditions. In the present study we employed a nested PCR strategy that specifically amplified the functional copy of the CHEK2 gene [40].” (see page 10, 2nd paragraph).

Point 4. “Consider also reviewing (and including) the NHLBI Exome Sequencing Project – Exome Variant Server for the presence and evaluation of CHEK2 variants identified. The database includes additional information regarding the frequency of some of the variants identified and the results of other in silico analysis. See http://evs.gs.washington.edu/EVS/.”

We have reviewed the NHLBI Exome Sequencing Project data base and added the following sentences:

“Three of these (c.252A>G, c.319 +43_319+44insA and c.592 +50A>T) have also been identified in the NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/EVS/).”(see page 8, 3rd paragraph, lines 3, 4).
Reviewer's report: 3

Point 1. “It is my impression that the article is too long and should be shortened so that it is a short report, not a full paper”.

Since the other two reviewers requested additional information, we refrained from shortening the article.