Reviewer's report

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

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Reviewer: Patricia Tonin

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The study describes the frequency of germ-line CHEK2 mutations in Pakistani breast and/or ovarian cancer families found negative for mutations in the BRCA1 and BRCA2 breast-ovarian cancer susceptibility genes. The rationale being that less is known about the contribution of CHEK2 cancer susceptibility gene in various Asian populations as compared with other “caucasian” populations.

To address this question in the context of the Pakistani population, two cancer affected cohorts were investigated: the first was subjected to a comprehensive genetic analysis (by DHLPC) of CHEK2 and the other used to investigate the frequency of protein coding variants identified in the first group. DNA from controls was also investigated for all variants identified in the first group to evaluate their frequency in cancer unaffected population. Established in silico methods were used to infer the biological consequences of the variants identified. Though functional assays are lacking (as pointed out by the authors), compelling evidence is presented for the identification of a new pathogenic CHEK2 variants predicted by in silico analyses. The study makes the case for a thorough genetic analysis of the CHEK2, where previous independent studies have usually focused their mutation screening analyses on identifying the common c.1100delC pathogenic mutation that has been reported in a various populations. The study also reaffirms the low frequency of CHEK2 pathogenic variants in hereditary breast and/or ovarian cancer families consistent with what has been reported by other groups that have investigated other geographically (“ethnic”) defined populations. This study contributes to the growing list of cancer susceptibility genes implicated in hereditary cancers found in diverse populations.

With few exceptions, this is a well-written paper and thoroughly researched paper. However, this report could be improved with the following modifications of which only the first item is considered a major compulsory revision:

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.

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?

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?

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/

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

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests