Author's response to reviews

Title: Identification of a novel CHEK2 variant and assessment of its contribution to the risk of breast cancer in French Canadian women

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Author's response to reviews: see over
Responses to referee #1 – Mieke Schutte

We would like to offer our sincerest thanks and gratitude to Dr. Schutte for her in-depth analysis of our manuscript, and for her well thought out and constructive suggestions. We have itemized our responses below in bold, to the specific comments by the reviewer, written in italics.

Major Compulsory revisions:

1. The conclusion of the paper is too far stretched. The conclusion that R406H is a non-pathogenic polymorphism appears justified (see comment #2). Yet, the authors can not conclude that 1100delC is the only other CHEK2 variant of importance in their population. Specifically, the cohort of 25 breast cancer cases is too small to identify all CHEK2 alleles present in their population. This is exemplified by the fact that 1100delC had not been detected in the 25 breast cancer cases, while screening of 149 cases identified the mutation in 2.0% of cases (which certainly is not ignorable). The authors can not exclude that they have missed other variants as well and they therefore should rephrase their conclusion.

We agree completely with your comment. Thank you for providing your perspective on this. We previously attempted to address this issue by structuring the paper to convey that we were attempting to identify “common founder” variants with a reasonably high frequency (>2%) within this specific population. We realize that this could have been stated more clearly. For clarity, we have re-phrased the conclusion to the abstract to read: The novel CHEK2 missense variant identified in this study, R406H, is unlikely to contribute to breast cancer risk in French Canadian women. Notably, 1100delC, while important in northern Europe, seems to be less frequent in North America, and this may be why we did not find it in our initial series. We have removed any mention of 1100delC from the conclusion to the abstract.

2. The prevalence of the R406H variant among the different cohorts of cases is a bit suspicious. The variant was present in 1 of 25 cases of group 1, in 1 of 124 cases of group 2 and in 1 of 543 cases from group 3. They either have been quite lucky to detect the variant in the first two groups, or they have missed a few in group 3. This raises questions on the mutation detection methods, which were different for each of the three groups: sequencing for group 1, allele-specific PCR for group 2, and restriction assay for group 3. To answer the questions raised, the authors should indicate in the methods section which and how many positive controls have been used for each method. They should also indicate whether the wild-type or mutant allele is being cut in the restriction assay and how the quality of digestion was controlled.

Thank you for these very relevant questions. The “+” prior to the enzyme denotes that it cuts the variant strain, however, this has now been explained in greater detail within the methods. Further, additional information regarding controls has been included. Please see the revised version of the methods.

3. The overview on CHEK2 literature is disturbingly inaccurate. Currently, a breast cancer risk has been shown for FIVE variants of CHEK2: 1100delC, I157T, IVS2+1G>A, the 5.4 kb deletion and the Jewish S428F mutation. The I157T mutations is completely ignored by the authors (except for its colorectal cancer risk in the Discussion). The 1100delC mutation, which is the most important variant, is underrepresented. For example, the fifth para in the discussion starts with “To date, THREE interesting CHEK2 founder alleles have been identified,...”.

I157T and 1100delC are discussed minimally in this manuscript, as we are trying to identify founder variants which present with a disproportionately higher frequency within a specific (or very few) ethnic group(s), thus the more detailed discussion of IVS2+1G>A, the 5.4kb deletion and S428F. However, we agree that both 1100delC and I157T have previously been attributed to a single founder, and thus we have amended our text to include 1100delC and I157T. However, as CHEK2 and its associated variant alleles are not novel, and as the current paper is presenting an essentially negative conclusion, an extended discussion of these variants...
has not been included. This is in the interest of keeping our manuscript short and focused, without including redundant information.

Also, the references in the Background section are inaccurate. At the end of the 2nd para of the Background, the references that CHEK2 has been confirmed as a low-penetration breast cancer susceptibility allele should be refs 3 and 9 in stead of ref 8.

Change has been implemented

Next line, in the 3rd para, ref 9 should be replaced by ref 3.

Ref 3 has been included in addition to ref 9.

Next line, add to ref 3 also refs 9 and 8.

Change has been implemented

Last line of 3rd para, refs 10-12 should be significantly extended (currently near 25 papers touch on this subject) or should be replaced by ref 8 (which was the first to state the variation in population frequencies).

Ref 8 has been included as they are the first to suggest population variation; however ref 10-12 remains to provide more recent examples of frequency variation amongst specific populations, in addition to presenting populations where 1100delC contributes minimally to susceptibility, which is not well illustrated in ref 8. The addition of further references would be of little benefit as the concept is illustrated well by these alone.

In the discussion, 1st line of the 4th para, ref 30 is weird and ref 8 should be included.

Ref 8 has been included

Same para, 2nd line, replace "many Eastern European populations" with "many other populations".

Change has been implemented

Same para, 3rd and 4th lines are inaccurate and should be deleted or rephrased: The five CHEK2 variants mentioned above under comment #1 have all convincingly been shown to confer a breast cancer risk. Each of these variants consistently confers a breast cancer risk in different populations, PROVIDED they are present in that population. Please change the text. Fifth para, first sentence, again, there are five interesting variants, so please include 1100delC and I157T.

Please see the revised discussion. Amendments have been made.

Minor essential revisions:

1. In the Materials section, please add details on the family history of the group 2 Cases
   The selection criteria for the Group 2 cases have now been elaborated on, providing more detail on the clinical history of the patients included in this group.

2. Also in the Materials section, indicate whether the group 3 cases have been screened for BRCA1 and BRCA2 mutations.
Responses to referee #2 – Petr Pohlreich

We would like to thank Dr. Pohlreich for taking the time to review our manuscript, his accurate and concise report and favorable comments. Please find our response in bold below corresponding to the suggested revision in italics.

Discretionary Revisions

1. Some papers have suggested that the frequency of mutations identified in low penetrance genes was increased in familial but not in sporadic breast cancer. Could the authors please test this in their data-set?

This is indeed a very interesting question which certainly deserves attention. However, our sample sets are too limited in size and are largely an ensemble of familial cases. Thus, unfortunately due to the limited
sample size, we do not have the statistical power to conduct a meaningful analysis. An excellent follow up study in the future would indeed be to screen specifically for R406H in a large sample of both familial and sporadic cases.

Responses to referee #3 – Kathleen Claes

We would like to thank Dr. Claes for taking the time to review our manuscript and her accurate and concise report. Furthermore, we would like to thank Dr. Claes for her excellent and relevant comments to aid us in constructing a stronger manuscript. Please find our response in bold below corresponding to the suggested revisions in italics.

Minor essential revisions

pg6: how were the "healthy French Canadian women with unknown BRCA1/2 mutation status" selected? The authors should provide more information how these people were selected. Do the authors have detailed information about the familial anamnesis, etc. Same suggestion for the controls of group 2.

Selection procedure for both control groups has been included.
The neonatal group is matched with respect to ethnicity. The benefit of using this group as a control is that we were able to investigate the presence of R406H in a large sample of the French Canadian population, providing us with an accurate representation of the general R406H carrier frequency in this population. Although the proposition that some of these children may develop cancer at a later stage in life is very valid, the group remains just as informative as a randomly selected control cohort. We agree that unaffected women of a similar age to the cases would have been a better choice, but we do not have access to a large enough group of these women to generate statistically meaningful results.

In the result section the prevalence of the 1100delC mutation described in patients from group 3 and group 1. Based on the numbers tested, I think this should be group 2 instead of group 3???

Yes, this is absolutely correct, thank you very much for catching this. The frequency provided in Table 2 & results is in fact the prevalence of 1100delC in Group 1 and Group 2. This has been corrected.

In the discussion section no further comments are given on this prevalence. E.g. is this in agreement with other Caucasian populations (Europeans: eg correlation with the frequency in the French population, Americans,...)? Wouldn't it be valuable to determine the prevalence of the 1100delC mutation also in group 3?

This is a very fair point. While we were working on this manuscript we became aware that another group had amassed a considerable amount of data on 1100delC in various populations and wished to include their 1100delC data on French Canadians in that work. So that we would not publish the same data twice, we did not pursue the question of the frequency of 1100delC further than groups 1 and 2. In fact, we did not contribute any data on French Canadians to that paper. What we have done is compare the frequency in our cases from groups 1 and 2 with that seen in the neonatal controls (ref 30 in our ms). By doing so, we suggest that the 1100delC allele is also important in the French Canadian population. We have added some text as a footnote to Table 2. However, since another group has already done this work and published it, we have not emphasized this in the body of the text as well. Also, it should be noted that that the main focus of this paper was on new alleles.

In the conclusion of the abstract I would also mention the frequency of the 1100delC mutation rather than giving a vague indication that the 1100delC mutation is present in French Canadians.

In view of the above paragraph, instead, we have removed 1100delC from the conclusion to the abstract.

The silent variant E84E was observed at similar frequency in cases and controls "suggesting against the possibility that this variant may affect an exonic splicing enhancer". I would suggest to also add data about in silico predictions.

Within the three references pertaining to E84E, they have conclusively shown, both through functional analysis and in silico predictions that E84E is not pathogenic. Thus, in the interest of keeping the focus of our manuscript short and concise, we have decided to refrain from including this previously published data.

Typing error

Thank you very much for pointing this out. It has been rectified.