Author's response to reviews

Title: Haplotype analysis suggest common founders in carriers of the recurrent BRCA2 mutation, 3398delAAAAG, in French Canadian hereditary breast and/ovarian cancer families

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Author's response to reviews: see over
Dear Editor;

We have revised the manuscript to incorporate changes that reflect the comments brought to our attention by the reviewers. The most significant change in the manuscript includes the frequency of alleles of carriers of the 3398delAAAAG mutation which is now included in Table 2, alongside the frequency of alleles of unaffected, non-carrier female cases. Along with this change, we have estimated the frequency of haplotypes deduced from genotypes of carriers that segregate with the disease. We have also clarified the text in this regard and incorporated other minor changes as suggested by the reviewers and detailed below.

Response to Reviewers Comments:

Re: Comments from Bohdan Gorski

Major Comments:

1. The reviewer indicated that the number of tested breast and ovarian cancer cases in the study is small. In the present study we have used three independently ascertained series of cancer cases: 60 breast cancer cases diagnosed before age 41 years, 127 cases of breast cancer diagnosed before age 80, and 80 cases of epithelial ovarian cancer (no age restriction). In total, this accounts for 267 female breast/ovarian cancer cases. Perhaps (as also eluded to by the second reviewer - see below) there was a confusion as to how many breast cancer cases were included in the study. We have used these series of cancer cases unselected for family history to estimate the frequency of other recurrent mutations in the French Canadian population. Nevertheless, the infrequent occurrence of the BRCA2 3398delAAAAG mutation found in these series is consistent with low frequency of carrier-positive families observed in previous analysis of high-risk families wherein which this mutation was initially identified. We have clarified the Methods sections in this respect.

2. We have not provided an extensive discussion of the BRCA2 OCCR cluster region described in the literature as our study alone does not permit us to draw any new conclusions. Our intention was to point out that the 3398delAAAAG mutation occurs within a region identified as conferring increased risk to ovarian cancer as determined by independent reports. To clarify this intention, we have included the frequency of two other recurrent BRCA2 mutations, 6503delTT and 8765delAG, that occur in high –risk similarly ascertained French Canadian breast/ovarian cancer families, one of which, 6503delTT, occurs in the purported OCCR region. As now indicated in the revision, there was no significant differences in proportion of high-risk, similarly ascertainment French Canadian cancer families with ovarian cancer cases harboring the 3398delAAAAG mutation with those harboring other recurrent BRCA2 mutations that occur within or outside of the OCCR region.

Minor comments:

1. We have corrected the comment that families are not related to each other based on similarity of cancer phenotype.
2. We agree with the reviewers comments and have calculated the frequencies of alleles in 3398delAAAAG index cases and have added this information to the Table 3 alongside the frequency of the alleles in the unaffected individuals.

3. We agree with the reviewers comments, and have corrected the Results and Discussion section by adding that under the assumption of linkage disequilibrium, the frequency of either haplotype associated with the 3398delAAAAG mutation is estimated at 0.001.

Re: Comments from Jean-Pierre Fricker

Minor Essential Revisions:

1. We agree with the reviewer and have modified the title to include the specific name of the mutation (BRCA2 3398delAAAAG) studied in the present manuscript.

2. We have now included the Methods section and the legend to Table 2, that two to nine family members of index cases of nine of 11 families harboring the 3398delAAAAG mutation were genotyped for haplotype analysis and segregation of disease associated allele.

3. To estimate the frequency of the disease-associated haplotype we have calculated the frequency of the alleles in 11 carriers and added this alongside the frequency of alleles in affected individuals in Table 2. We have not used the haplotypes for from non-affected chromosomes from other related families (“intrafamilial” genotypes) members as controls because of the inherit bias in doing so.

4. We agree with the reviewers comment and have corrected the all references to “prevalence” to that of establishing and/or estimating the frequency of the BRCA2 3398delAAAAG mutation carriers.

5. As mentioned in the response to the first reviewers comment (see above), we have clarified that three independently ascertained series of female breast cancers (breast/ovarian cancer cases) not selected for family history for breast/ovarian cancer were investigated in this study.

6. The reviewer wishes to know the contribution of the 3398delAAAAG mutation to the BRCA families identified in the Province of Quebec or in the area of Montreal. This was, in part determined in the initial study which reported this mutation (Oros KK, Ghadirian P, Greenwood CM, Perret C, Shen Z, Paredes Y, Arcand SL, Mes-Masson AM, Narod SA, Foulkes WD et al.: Significant proportion of breast and/or ovarian cancer families of French Canadian descent harbor 1 of 5 BRCA1 and BRCA2 mutations. Int J Cancer 2004, 112:411.) and as described in the Introduction to the present study. However, for clarification we have also included in the Methods section, that all of the known cases harboring this mutation was identified through the only genetic testing/counseling clinic of Montreal area. At the time we performed the study we were not aware of any other cases such as through the only other testing centre in the Province (located in Quebec city). The genetic testing and counseling centres capture the majority of cases in the Province and all cases within the Montreal and near-by region as these are the only such centres in the area.
7. As mentioned above, we have provided more detail about the OCCR region with respect to the French Canadian high-risk cancer families investigated in the present study and a previous study. We agree that the comment about other atypical cancer sites could be eliminated but have chosen to retain the information in the Table 1 for those readers interested in spectrum of atypical cancer sites that have also been reported in hereditary breast and/or ovarian cancer families.

8. We have also modified the text according to minor points brought to our attention.

We hope that we have satisfactorily addressed the reviewers comments and look forward to your response.

Respectively,

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