Author's response to reviews

Title: Contribution of germline BRCA1 and BRCA2 sequence alterations to breast cancer in Northern India.

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Version: 2 Date: 15 May 2006

Author’s response to reviews:

Dear editor,

Please find attached our responses to the reviewers' comments and the manuscript and tables revised accordingly. Table 4 still needs minor revisions but since we were encroaching the dead line we have uploaded this also. We would replace it with the revised one shortly. We appreciate your consideration of our manuscript and are happy to respond to any additional comments or questions you may have.

Sincere regards,

Reviewer's report

Title: Contribution of germline BRCA1 and BRCA2 sequence alterations in to breast cancer in Northern India.
Version: 1 Date: 17 February 2006
Reviewer: Fernando Schmitt

Reviewer's report:

General
Identification of BRCA1 and BRCA2 has led to major changes in the treatment and follow up of patients with an inherited predisposition to breast and ovarian cancer. The genetic approach has allowed the identification of high risk patients what makes this study biologically and medically relevant. However the authors should clarify the points that I addressed in the review.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. The authors claim that both BRCA1 and BRCA2 genes have been found more prevalent among early onset cases compared to older onset and familial breast cancer patients, however it's important to stress that the study included 105 early onset cases and only 34 cases with a family history of breast and/or ovarian cancer. So it is not surprising that we observe more mutations in the early onset cases than in the ones with a family history (also the difference is not that striking, 15 out of 105 compared to 4 out 34);

2. In the case selection section, the 204 breast cancer cases are well described, still the authors did not mention the control group. Actually I could only find the number of controls in Tables 2 and 3. I assumed it was 65, was that correct (and which criteria was used to select them ?)? If so, it was a very small number of controls compared to the breast cancer population studied. The authors should include a larger number of controls (bigger than the population studied) and try to match it with the population in analysis in terms of,
for example, age.

3. The description made in the missense mutations in BRCA1/2 genes is not precise. The authors should refer that although they haven’t found this alterations in the control group, some of them are considered, according to the BIC database, polymorphisms, like for example S1613G and A2951T. In the case of this last alteration the explanation given for the substitution of a non polar hydrophobic amino acid for a polar hydrophobic one is, in the opinion of this reviewer, a little wishful thinking for a possible deleterious mutation. And what about D47E? Do the authors have any possible explanation for a probable role?

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. - Reference [2] used for the estimation of breast cancer deaths in India is a bit old (1996). The authors should find a more recent statistic (for example in the International Agency for Research on Cancer; www.iarc.fr);
2. - How many times was the sequencing performed? How did the authors ruled out the possibility of PCR fidelity artefacts?
3. The journals in the references are not in the BMC requested form. They should be presented in italic.

Discretionary Revisions (which the author can choose to ignore)

What next? Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of limited interest
Quality of written English: Needs some language corrections before being published
Statistical review: No
Declaration of competing interests:
'I declare that I have no competing interests'

Fernando Schmitt

1. We have modified the discussion of the percentage of BRCA1/2 gene mutations identified in early-onset cases compared to familial breast cancer patients

2. The study included 65 age-matched controls however we have further added 55 additional controls to be confident of detecting variants observed in the breast cancer cases if they are present at similar frequencies in the general population. Corresponding corrections have been made in tables 2 and 3

3. S1613G has been found in two of the controls, although the frequency is not as high in the Indian population as has been reported elsewhere. However, the frequency of A2951T, which is reported as a common polymorphism in BIC, is not present at an appreciable frequency in North-Indian population. The strong evolutionary sequence conservation suggests that it may be worthwhile exploring the potential functional significance of this variant in further detail. However, we have modified the discussion of this mutation to indicate that it has uncertain disease relevance.

4. D47E is an error. The current descriptor of this mutation is 22C>G, a change in the 5'UTR region of BRCA1 whose explanation for a probable deleterious role had already been discussed in the manuscript.

5. Reference [2] used for the estimation of breast cancer deaths in India has been replaced by GLOBOCAN 2002, IARC(http://www.sunmed.org/incidence.html)

6. To rule out the possibility of PCR fidelity artifacts, both PCR amplification and gel based heteroduplex analysis was done twice for the samples that showed altered mobility on HDX gels before doing sequence analysis. Samples were both forward and reverse sequenced to corroborate the findings.

7. We have changed the journals in references from normal to italic according to BMC format.

Reviewer's report
Title: Contribution of germline BRCA1 and BRCA2 sequence alterations in to breast cancer in Northern India.
Version: 1 Date: 14 February 2006
Reviewer: Mary-Claire C King
Reviewer's report:
General
This manuscript presents heteroduplex analysis of BRCA1 and BRCA2 from 204 patients from Northern India with either early-onset breast cancer or familial breast cancer. BRCA1 and BRCA2 have not previously been evaluated in such a large series from this population, so the information is a valuable addition to the cancer genetics literature.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. It is misleading to state that "genetic susceptibility to breast cancer due to BRCA1/BRCA2 mutations was noticed in 11.3% (23/204) patients." This is not correct, because most of the variants noted in these 23 patients are very likely to be neutral, albeit rare, SNPs. The proportion of patients in this series with genetic susceptibility to breast cancer due to BRCA1/BRCA2 mutations is actually 6/204, or 2.9%. The abstract, Table 1, and the text should be corrected to reflect the frequency only of mutations known to be deleterious.

2. The BRCA1 mutation 4476(+2)T>C, which is probably the same as ivs13(+2)T>C although noted in two different ways on Table 4, was observed by these authors in their previous, smaller study in 2002 and again (independently?) in this series. It is very possible that this mutation alters BRCA1 splicing and is deleterious. This possibility should be tested by evaluation of BRCA1 message from these patients.

3. Please discuss the limits of heteroduplex analysis in this project. Specifically, what fraction of mutations of various classes were detected by heteroduplex analysis in the hands of these experimentalists in blind testing of known mutations?

4. For all families in whom the history of the mutation BRCA1.185delAG has been explored, its ancestry has proven to be Jewish, regardless of the geographic locale of current residence of the family. Furthermore, all haplotypes surrounding this mutation determined so far are the same, suggesting a single occurrence. This does not preclude the possibility of an independent occurrence of this allele, but thus far none has been demonstrated. Text should be corrected for this point.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

In the title, the word "in" in the phrase "sequence alterations in to breast cancer" should be removed.

Other errors of English in the text should be corrected.

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Discretionary Revisions (which the author can choose to ignore)
What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions
Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Not suitable for publication unless extensively edited
Statistical review: No
Declaration of competing interests: I declare that I have no competing interests

Mary-Claire C King

1. Corrections have been made in the abstract, table 1 and discussion as per recommendations.

2. IVS13 (+2) T>C in exon 13 BRCA1 that we have found in the present study was also observed in the previous pilot study in independent samples from individuals not known to be related. This splice site variant could be deleterious, but evaluating the BRCA message to support its functional significance is not possible for us at this time because tissue samples for the same patients are available only in the form of paraffin embedded blocks. However, a study is undergoing at present in our lab to further characterize mutations in the BRCA1/2 genes in the Northeastern population, for which we are also collecting tissue samples along with blood. If we identify this splice mutation again in the newly collected samples, the evaluation of BRCA message would be confirmed. We will also try to recontact the original patients in order to try to obtain a new blood sample for this purpose.

3. In the study, scanning for the presence of sequence variation was performed by analyzing PCR amplicons using gel based heteroduplex analysis. Although its sensitivity is low compared to other mutation
analysis techniques its cost effectiveness and technical simplicity dictated this approach as the one most readily amenable to technology transfer, given the resource availability in our institute. Training in the technique was carried out under the supervision of investigators with extensive experience in these methodologies, and subsequent visits by these investigators helped to trouble-shoot the technique in our own laboratory. Detection of known polymorphisms, including relatively rare alleles identified through the use of more sensitive methods, such as DHPLC (ex. BRCA2 1593 A>G; BRCA2 7470A>G; and BRCA2 A2951T) suggest that our technique permits detection of single base-pair substitutions with good sensitivity, and these are relatively less efficiently detected by heteroduplex analysis than frame shift alterations. It is estimated that the mutation detection sensitivity of the heteroduplex method utilized is approximately 80% (Eng, et al., 2001). To increase the efficacy of mutation detection, the screening for mutations in both the genes was repeated by HDX in all familial cases. Moreover no currently available technique can guarantee 100% detection of pathogenic mutations in BRCA1 and BRCA2 gene. We have included a discussion of these considerations in the manuscript text.

4. 185delAG is a common Ashkenazi founder mutation but it has also been reported on a different haplotype in northern England by Neuhausen et al., 1996 and Xu, et al., 1997. This suggests that although by far the most prevalent form of this mutation derives from the ancestral occurrence in the Jewish population, the same mutation has arisen independently at least once. Given that it has been reported in all the BRCA1/2 studies done on different Indian populations by different workers, and the multi-ethnic origins of the present day Indian population, it would indeed be interesting to determine whether this mutation has different origins in the different regional Indian populations where it has been reported. We plan to undertake haplotypic analysis of this mutation in all the cases reported by different Indian workers showing this mutation. We have modified the text of the manuscript to better reflect this rationale.

5. In the title "in" is removed from the phrase.