Title: Molecular Genetics Analysis of Hereditary Breast and Ovarian Cancer Patients in India

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Author's response to reviews: see over
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To,

The Editor,

Hereditary Cancer in Clinical Practice.

Sub: Manuscript ID: MS: 9475271652729587
Titled: Molecular Genetics Analysis of Hereditary Breast and Ovarian Cancer Patients in India

Sir,

At the outset we would like to thank you and the Reviewers for their constructive comments.

I am herewith enclosing the revised manuscript and the response to the Reviewers comments.

Kindly acknowledge receipt.

Thanking you,

Yours sincerely,

Dr.T.Rajkumar
RESPONSE TO REVIEWERS COMMENTS

REVIEWER 1

Reviewer: Annemarie Hedwig H van der Hout
Reviewers report: In this manuscript mutation analysis of BRCA1 and 2 and clinico-pathological characteristics of one of the largest groups of patients with familial breast/ovarian cancer in India is described.

Major Compulsory Revisions

1. The authors provide a lot of data about IHC analysis, clinico-pathological features and statistical analysis. These are presented in tables and figures. However, a discussion on the meaning of these data, is almost completely lacking. The discussion section should be considerately extended, or the manuscript should be rewritten as a survey of mutations in BRCA1 and -2 in Indian families with familial breast/ovarian cancer.

RESPONSE: The discussion section has now been extended and the IHC results and their correlation with mutation analysis is mentioned now in the discussion section.

2. It is stated that all missense variations in table 2. are defined as polymorphisms in the BIC database. This is not true, some are not mentioned at all in the BIC database, others are mentioned with the comment that the clinical importance is unknown.

RESPONSE: We apologize for the error. This has now been corrected.

Minor essential revisions

1. is the crude incidence rate of breast cancer in Chennai really as low as 30.1/100,000? Is there an explanation for the large difference with Western Europe, where the incidence is around 1/9?

RESPONSE: The breast cancer incidence had been only around 12/100,000 when the population based registry was started in Chennai in 1982. However, over the past two decades, it has progressively increased. This is not due to under-reporting as the data collection is active and the data from the MMTR is considered to be the best in the country by the IARC, Lyon, France. The general risk factors such as age at first child birth, nulliparous status and age at menopause have been different from the West. Till the early nineties, most women had married by around 20 years of age and had their first child birth by 22, completed their family by 25 and breast fed their children, some upto a year or more. Additionally the traditional life style of the women has been protective with a balanced diet tilted towards vegetarianism. In fact the overall incidence of cancer is around 100/100,000 in the urban region and it is only half (around 50/100,000) in the rural areas of India.
However, currently, in the urban population across the country, this factor seems to be changing, with more women delaying their first child birth till after 30 years, in view of their careers. The diet has also been changing to high calorie, high fat diet with improving economic status. These and other factors now seem to be responsible for the progressive rise in the incidence of breast cancer among urban women of India.

2. what are the inclusion criteria?

**RESPONSE:** The inclusion criteria were as follows: Early onset of breast cancer (≤35 years of age) or ovarian cancer (≤40 years of age); Two cases of breast cancer diagnosed under the age of 50 years in a family (first and second degree relatives); Three or more cases of breast cancer diagnosed at any age; Presence of breast and ovarian cancer in the family or in the same individual; Male breast cancer with a relative (of either sex) with breast cancer; Family history of Prostate or pancreatic or colorectal cancer or sarcomas, with breast cancer in the family.

This has been added in the Materials section.

3. nomenclature in table 1. is not according to HUGO recommendations as is stated on page 6. Ileu is Leu or lle (case 14 and 15)? In the long notation on the protein level first affected residue should be mentioned, not the residue in which the first DNA change occurs. e.g. p.Ser2072SerfsX4 in case 11 can not be correct.

**RESPONSE:** The error is regretted. It has been corrected now.

4. on page 9. it is stated that p.A392V being a pathogenic mutation can be confirmed by segregation analysis. This analysis, however, is not shown.

**RESPONSE:** We had only mentioned that it could be confirmed by segregation analysis. We have not done it and hence it is not shown.
REVIEWER 2

Reviewer: Theresa Larriba Harboe

Reviewer's report:
Major and Minor Compulsory Revisions
The paper is interesting and the data is worth publishing but there are some revisions needed in my opinion

1. Table 1: Why the use of different accession numbers for the same gene?

RESPONSE: We had submitted each new novel mutation identified separately and hence an Accession number for each of the novel mutation.

2. Table 2 and 3: It would make it easier to read if the tables contained numbers instead of percentages.

RESPONSE: This has been changed to include the numbers and the percentages given in brackets.

3. Table 4: It would benefit the table if the authors results were added. Would make it easier to compare.

RESPONSE: As suggested this has been added.

4. Figure 1: The figure seems redundant

RESPONSE: In our opinion, it serves to provide the readers the sequence information and the effect of the mutation on the secondary structure.

5. Why was not the whole CHEK2 gene examined?

RESPONSE: Our objective was to assess the incidence of the 1100delC alone, and hence the whole CHEK2 was not examined.

6. In general the writing of the paper needs to be reworked. Some parts of the discussion are very difficult to follow due to the language. Very long sentences makes it hard to read. The Discussion needs more discussion of the obtained results compared to the litterature. Also there lack a conclusion of the authors results.

RESPONSE: We have tried to correct the language wherever it was obvious to us. The discussion has been expanded and a Conclusion has been added.
Reviewer's report
Reviewer: Irene Konstantopoulou

Reviewer's report:
Population-based studies of BRCA1/BRCA2 mutations can have a wide publication impact for scientific (differences in mutation spectra) as well as clinical reasons (migration of people). In this paper the authors have screened 91 families from the Chennai (Madras) region of India with characteristics of hereditary breast and/or ovarian cancer for germline mutations in the complete coding region of BRCA1 and BRCA2, as well as for the most common CHEK2 predisposing mutation 1100delC. They have discovered 15 pathogenic mutations (12 in BRCA1 and 3 in BRCA2) of which 6 are novel BRCA1 mutations reported for the first time here. The authors also attempt to associate 5-year survival and other clinico-pathological features with mutation status.
Overall, the data presented are decent and complementary to those previously reported by other Indian groups. The study includes the largest cohort so far of Indian familial breast/ovarian cancer cases. The percentage of carriers found (16%) is in accordance to most international studies performed in groups of similar characteristics.

In more detail:

Major compulsory revisions:
None

Minor essential revisions:
1. (In Materials & Methods:) A clearer description of the 91 cases included in the study should be given. Were they enrolled in a single hospital or several hospitals from the Chennai region? How many have family history? What are the exact criteria for classification as ‘family history’? How many were early-onset and what is the age limit for that classification? This section should include all the above information.

RESPONSE: As suggested, additional information has been provided. The Hereditary Cancer Detection and Prevention program includes patients seen in the Hereditary cancer clinic, run once a week at the Cancer Institute (WIA) and patients identified to have a family history in the Population based hereditary cancer registry covering the Chennai metropolitan area.

2. (In Results:) Table 1 should have an indication of the mutations that were previously reported by the authors (with a reference), since the current data is summed with that already published.

RESPONSE: This has been included now.
3. (In Results:) It should be noted here or in the discussion section that mutation c.68_69delAG (187delAG in BIC nomenclature) of BRCA1, a founder in various populations worldwide, is the only recurrent in their patients’ sample. It is reported here 5 times, as c.66_67delAG (187delAG in BIC nomenclature) in sample no. 13 is the same mutation; the confusion appears often in literature as nucleotides c.66-69 are AGAG. Mutation naming should be consistent in all 5 cases of this variant’s carriers.

RESPONSE: The error has been corrected. The mutation c.68_69delAG; p.Glu23Val fsX16 was seen in four cases (two in HBOC families and two in early onset breast cancer cases without family history) and is the only recurrent mutation seen in this study. However haplotype analysis was not performed in these four families to check for common ancestor. These four families are unrelated and are from different parts of South India, and ethnically different.

4. (In Results:) It is not mentioned whether the two CHEK2 variants reported here are novel or previously described. Actually, R406H was reported once previously (Novak et al. 2008, BMC Cancer 8:239) with results that support its neutral effect, consistent to the authors’ findings. This should be discussed somewhere in the manuscript, stressing that (novel?) variant A392V is likely to be pathogenic. Personal and family history of the carrier should be given.

RESPONSE: A note has been added as suggested.

Discretionary revisions:

5. (In Introduction:) More recent references could be mentioned where estimated risks are given (refs 6 & 7). Also, it is recommended that other genes recently implicated in breast cancer risk should be mentioned.

RESPONSE: This has been added now.

6. (In Results:) In the 1st paragraph, page 6, Gene Bank should be changed to GenBank (a url link is recommended).

RESPONSE: This has been included.

7. (In Results:) As the paper is focused on BRCA1/2 mutational analysis, my feeling is that in the statistical analysis results presentation (results section, page 7), the mutation status association with clinico-pathological features should be at least mentioned, if not stressed, even if no correlation was found. This is mentioned in discussion, but it is also useful to include it as a result here.

RESPONSE: This has been included.
8. (In Discussion:) First paragraph, page 8: The authors’ finding is not only similar to the work by Wagner et al in an Austrian population, but to numerous other studies worldwide that give a frequency of BRCA1 and BRCA2 mutations around 20% when studying high-risk families in heterogeneous populations. This discussion should be made in a broader context, as it concerns the major finding of this work: BRCA1/2 mutation prevalence in the population studied.

**RESPONSE:** This has been added now.

9. (In Discussion:) The second paragraph (page 8) discusses two different aspects of the authors’ results: (a) that breast-ovarian cancer families tend to show a greater prevalence of BRCA1 rather than BRCA2 mutations, and (b) present results compared to results from other Indian groups. These separate subjects should be better handled in separate paragraphs, where the point should be made clearer. Table 4 is helpful in that direction.

**RESPONSE:** This has been done now.

10. (In Discussion:) Concerning the missense variants, it would be helpful if Table 2 included the number of cases reported in the BIC database, as an indication of their overall frequency.

**RESPONSE:** We have not changed this, as we did not think that it would add much to the paper.