Author's response to reviews

Title: No germline mutations in supposed tumour suppressor genes SAFB1 and SAFB2 in familial breast cancer with linkage to 19p.

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Author's response to reviews: see over
To the BioMed Central Editorial Team

Dear Mr/Mrs,

Please find enclosed our revised manuscript entitled “No germline mutations in supposed tumour suppressor genes SAFB1 and SAFB2 in familial breast cancer with linkage to 19p”

In compliance with the reviewer’s recommendations we have reworked the manuscript. The major revisions are:

i) As a complement to the mutation analysis, the genes have been analysed for deletions undetectable by PCR. This analysis was performed by multiplex ligation-dependent probe amplification (MLPA) using in-house designed probes.

ii) The discussion has been shortened substantially and adjusted according to referee suggestions.

In addition we intended to analyze the common promoter region of SAFB1/2 for germline mutations. Unfortunately the region is very GC-rich which complicated the PCR amplification and unable us to complete this experiment within the time limit given for the resubmission of the article. If the Biomed central editorial team considers this analysis essential for the study we are willing to complete the experiment. The reviewer’s comments are adressed in detail below.

On behalf of all co-authors
Yours sincerely,

Annika Bergman, PhD
Reviewer: Larissa Arning

1. **Comment:** I would recommend shortening the discussion section.
Answer: The discussion section has been shortened substantially.

2. **Comment:** Since the genes are ordered in a head to head state with a probable shared promoter it would be interesting to cover this region with an additional PCR-system (only 500bp).
Answer: DNA sequencing of SAFB1/2 promoters are in progress, but could not be completed due to the limited time. If the editor/reviewer considers this study highly essential we are willing to complete this study within a limited time period.

Reviewer: Tadahide Izumi

1. **Comment:** …the authors describe that the families were linked to the 19p13 deficiency in a past study (citation to this study is essential).
Answer: The reference for the linkage to 19p13 is cited at several places in the manuscript and is now also added to the material and methods section: Bergman et al., Genome-wide linkage scan for breast cancer susceptibility loci in Swedish hereditary non-BRCA1/2 families: suggestive linkage to 10q23.32-q25.3. Genes Chromosomes and Cancer 2007, 46 (3):302-309.

2. **Comment:** However, the mutation analyses were done only on the exon regions of the two genes and epigenetic changes were not considered either… Probing the expression of the genes by different strategies, i.e., analysis of methylation, levels of transcripts by RT-PCR, or tissue staining/immunoblot of the patients specimen should strengthen their conclusion.
Answer: The current study is based on the hypothesis of an inherited germline mutation that predisposes for breast cancer. For this purpose we wanted to screen for mutation in normal material, i.e. leukocytes from affected women. The analyses were performed in a non-tumorous material since any inherited genetic alterations would be present in all cells. An analysis of altered gene expression by quantifications of mRNA or protein is indeed of interest and we agree that knowledge of Safb1/2 levels may affect tumorigenicity. However, in our opinion this type of study would be more appropriate to perform on the target tissue in stead of blood. A finding of altered levels (or unaltered) of Safb1/2 in blood could probably neither support nor reject the hypothesis of SAFB1/2 genes acting as tumor suppressors. The only tumor tissue that is available from the affected women in the present study is unfortunately archived paraffin-embedded tissue and would not be suitable for expression analysis.

3. **Comment:** The authors discussed the possibility of STK11/LKB1 gene mutation instead of SAFB. It would be necessary to include similar genetic analyses on STK11/LKB1 genes.
Answer: A germline mutation screening of the STK11/LKB1 gene has already been performed in another Swedish hereditary breast cancer study (Chen et al., 2000). In this study no alterations in coding DNA sequence of the STK11/LKB1 gene was found. Based on these facts we decided not to investigate this gene further. This issue is addressed in more detail in the discussion section of the manuscript.

4. **Comment:** Another concern is that PCR might not detect possible deletions of the locus…. Discussion and precaution to exclude this possibility is missing.
Answer: MLPA is a robust and highly sensitive technique for studying entire or partial gene deletions. We performed MLPA analysis of SAFB1 and SAFB2 on one affected individual in
each family (n=14) included in the study. However, no entire or partial deletions could be detected.

Reviewer: ARUNA S. JAISWAL

1. **Comment:** Authors should use alternative strategy to rule out any technical issues.
   **Answer:** All PCR fragments were sequenced in both directions to minimize the risk of undetected heterozygote nucleotide positions. As mentioned above (Answer to comment 4 of Tadahide Izumi) MLPA analysis was used as an alternative technique to rule out potential undetected deletions in SAFB1 and SAFB2.

2. **Comment:** There is no mention of breast cancer incidence and mortality rate in the introduction section which leaves the reader to underestimate the severity of the problem being addressed.
   **Answer:** The incidence and mortality rate is now mentioned in the introduction section, page 3, line 2-4.

3. **Comment:** Last four lines of introduction overstates about the proposed study where findings are just limited in identifying the mutational status of SAFB1 & SAFB2 genes without any mention of their functional aspect of these gene products.
   **Answer:** We are not sure if we understand the comment correctly. The function of the genes is mentioned in the introduction section. As we have previously identified linkage to 19p in the 14 families included in this study, we intended to investigate whether an inherited mutation in SAFB1/2 could be the cause of the observed linkage. In our opinion, the number of families is in this case of less importance.

4. **Comment:** Did the author take the identical number of individually matched controls.
   **Answer:** Since no pathogenic mutations were detected in this study we did not consider it necessary to screen any controls. In the case of an identified mutation we planned to screen twice the number of control individuals as are included in the present study.

5. **Comment:** Authors have reported that there is linkage to chromosome 19p where SAFB1 and SAFB2 are located and while consequence and significance of mutation on the functional aspect needs to be discussed.
   **Answer:** These aspects are now addressed in the discussion section, page 8, last eight lines.

6. **Comment:** What is clinical pathobiological difference in patients 1 vs others?
   **Answer:** The clinical characteristics of the families are described in our previous publication: Bergman et al., Genome-wide linkage scan for breast cancer susceptibility loci in Swedish hereditary non-BRCA1/2 families: suggestive linkage to 10q23.32-q25.3. Genes Chromosomes and Cancer 2007, 46 (3):302-309.
   We have now added a reference to this citation in the material and methods section, page 5, line 5.