Author's response to reviews

Title: BACH1 Ser919Pro variant and breast cancer risk

Authors:

Pia Vahteristo (pia.vahteristo@helsinki.fi)
Kristiina Yliannala (kristiina.yliannala@ktl.fi)
Anitta Tamminen (anitta.tamminen@hus.fi)
Hannaleena Eerola (hannaleena.eerola@fimnet.fi)
Carl Blomqvist (carl.blomqvist@hus.fi)
Heli Nevanlinna (heli.nevanlinna@hus.fi)

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Author's response to reviews: see over
Dear Sir,

Thank you for your encouraging response to and the constructive comments by the reviewers on our manuscript entitled “BACH1 Ser919Pro variant and breast cancer risk” (manuscript no: 1497578677791403). The reviewers’ comments were very helpful, and we followed the suggestions essentially as recommended. The changes made are indicated in the following as well as by red in the revised manuscript. We hope that the revised manuscript now fulfills the high standards of your Journal, and could be considered for publication.

We are looking forward to your kind response.

Pia Vahteristo, PhD
Department of Medical Genetics
Biomedicum Helsinki, Rm B520a
PO Box 63 (Haartmaninkatu 8)
FIN-00014 University of Helsinki
Finland
Tel: INT+358-9-19125600, Fax: INT+358-9-19125105
email: pia.vahteristo@helsinki.fi
Answers to the Reviewer #1’s comments:

1-1) As suggested by the reviewer, a new table with primers used in the mutation analysis has been added in the materials and methods section (table 1). Numbering of the other tables in the manuscript has been changed accordingly. Reference to the CSGE method and used modifications has also been added (2nd paragraph on page 6).

1-2) The obtained odds ratios, being very close to one in all subgroups, suggest no increased breast cancer risk. However, as pointed out by the referee, a possible subtle increase in risk caused by the Ser919Pro variant cannot be excluded as detecting such small effect would require a very large sample material. This has now been added to the results and discussion section, in the 1st paragraph on page 8.

1-3) According to reviewer’s suggestion, we have analyzed whether the cancer risk is increased after subgrouping the patients based on the family history of cancer. We compared Ser/Pro heterozygotes and Ser/Ser homozygotes individually and combined to Pro/Pro homozygotes, but no difference in risk was observed in any of the analyses. This has now been added to the results and discussion section, in the 1st paragraph on page 8.

Answers to the Reviewer #2’s comments:

Minor Essential Revisions:

2-1) As suggested by the reviewer, the conclusions has been modified such that the results regarding the Ser919Pro variant apply to the Finnish population (abstract and 1st paragraphs on pages 8 and 9). However, as there are now several studies where no association with the observed BACH1 variants and increased breast cancer risk has been observed, we feel it appropriate to conclude that all these results suggest that the contribution of the germ line BACH1 alterations to familial aggregation of breast cancer seems rather marginal.

2-2) Methods and results in the abstract have been modified according to reviewer’s suggestions.

2-3) All the analyzed samples are DNA samples derived from peripheral blood. This has now been added to the methods section, in the last paragraph on page 5. The Val193Ile variant has been screened in randomly selected series of 346 familial breast cancer patients and 183 healthy population controls. Also this has been inserted in the text, in the last paragraph on page 5.

Discretionary Revisions:

2-1) The sentence referring to the likely neutral nature of the observed silent polymorphisms has now been better rephrased in the 2nd paragraph on page 7.

2-2) Unfortunately the 1935+22delT has later turned out to be a PCR artifact due to the long stretch of T-alleles (11) in the sequence. The variant has now been omitted from the results throughout the manuscript.

Answers to the Reviewer #3’s comments:

3-1) We agree with the reviewer that the cancer risk of the low-penetrance alleles may be modified by other risk factors, and a sentence referring to a possible joint effect of the Ser919Pro variant and other epidemiological risk factors has been added in the last paragraph on page 8. However, as the Ser919Pro alteration did not seem to have any effect on breast cancer risk overall or in any of the studied subgroups after patients were divided according to the age at cancer onset, estrogen and progesterone receptor status, family history of cancer etc. Further stratified analyses would have led to very small sample sets here, and analysis of such modifying effects will require very large international collaborative study materials.

3-2) We agree with the reviewer that haplotype analysis might be used to better delineate the effect of the whole gene. In this study, we have performed a detailed analysis of the observed non-synonymous coding variants present in our study material as these variants are the most likely to have functional effect on BACH1 protein. However, no effect of the alterations on breast cancer risk was observed. We believe that this data, together with several previous
studies, suggest that the \textit{BACH1} gene is not a major gene contributing to familial aggregation of breast cancer.