Title: Genotype-phenotype correlations among BRCA1 4153delA and 5382insC mutation carriers from Latvia

Authors:

Grigorijs Plakhins (grigorijs.plakhins@stradini.lv)
Arvids Irmejs (arvids.irmejs@stradini.lv)
Andris Gardovskis (andris.gardovskis@stradini.lv)
Signe Subatniece (signe.subatniece@stradini.lv)
Santa Rozite (santa.rozite@vsmtva.gov.lv)
Marianna Bitina (mary-ann@inbox.lv)
Guntars Keire (guntarskeire@inbox.lv)
Gunta Purkalne (gunta.purkalne@stradini.lv)
Uldis Teibe (uldis.teibe@rsu.lv)
Genadijs Trofimovics (trofimovic@navigator.lv)
Edvins Miklasevics (edvins.miklasevics@stradini.lv)
Janis Gardovskis (janis1105@inbox.lv)

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

In this covering letter we are describing revisions performed in this version of the manuscript.

All the suggestions and corrections of the reviewers are now included in the revised version of manuscript. We found that most of the comments of the reviewers were helpful and contribute to improve the quality of the paper.

In order to improve the quality of the manuscript we performed also two corrections, which were not suggested by reviewers:

1. We investigated the current status of patients included into survival analysis and provided an updated data about overall survival with increased median follow-up period.

2. Because the main topic of our manuscript is the investigation of genotype-phenotype correlation of two BRCA1 founder mutations: c.4034delA and c.5266dupC, we decided remove the information about the c.181T>G founder mutation from the Results and transfer estimation of prevalence of this founder mutation to the Discussion. In addition, we found that due to several reasons we are unsure about the exact prevalence data of this mutation in our population-based series, we therefore describe only estimation.

Corrections performed according to the suggestions of the reviewer Sergi Castellvi-Bel.

Reviewer: Are the discussion and conclusions well balanced and adequately supported by the data?
Discussion and conclusions are well balanced. On the other hand, in order to improve them, the authors should comment/hypothesize somehow about differential clinical/screening/therapeutic strategies for asymptomatic carriers in familial cases.

Author: In discussion: page 12 paragraph 2 and page 13 paragraph 1 we hypothesized about the differences in clinical strategies of the carriers of c.4034delA and c.5266dupC founder mutations.

Reviewer: Are limitations of the work clearly stated?
I suggest the authors to include a percentage for the three analyzed mutations among the total BRCA1 mutated alleles in breast and ovarian cancer in their population. A limitation of their study is that they only analyze three founder mutations and not the entire BRCA1 gene.

Author: As was suggested by reviewer we included short review of already published data about population prevalence of BRCA1 founder mutations in Latvia (page 8, last paragraph - page 9, paragraphs 1)
In addition we emphasize this limitation of our study in discussion (page 8, last paragraph).

Reviewer: Do the title and abstract accurately convey what has been found? I suggest the title should be “Genotype-phenotype correlation among BRCA1 4153delA and 5382insC mutation carriers from Latvia”. Abstract is fine.

Author: As was suggested by reviewer we changed the title of our manuscript.

Reviewer: Is the writing acceptable? Manuscript writing needs a general English correction.

Author: The manuscript was edited by native English speaker.

Reviewer: I would also suggest the authors to eliminate Figure 1, since it is not necessary in my opinion.

Author: As was suggested by reviewer we eliminated the Figure 1.

Corrections performed according to the suggestions of the reviewer Miguel M de la Hoya.

Major compulsory revisions

Reviewer: I miss population prevalence of c.5266dupC and c.4034delA BRCA1 mutations in Latvia. Is the data already known? The authors should address this issue in the text. This will provide a better estimation of risk for c.5266dupC and c.4034delA carriers.

Author: As was suggested by reviewer we included short review of already published data about the population prevalence of BRCA1 founder mutations in Latvia. This include the most widespread founder mutations among breast and ovarian cancer patients as well as data about the population screening which describe the estimation of the prevalence of BRCA1 founder mutations in Latvian population. (page 8, last paragraph - page 9, paragraphs 1).

Reviewer: I am not convinced by the survival analysis. In particular: age at diagnosis is relevant for survival analysis. I expect an early age at onset in carriers.

Author: In the revised version of manuscript we selected the control group for survival analysis from patients matched by age of onset, tumor size and nodal status with BRCA1 mutation carriers. The data about the median age of onset in each group is included in the Table 1.
In addition we investigated the influence of age of onset on the overall survival in multivariate Cox regression analysis.

Reviewer: ER/PR/erb2 status is relevant for survival analysis. I expect an excess of triple negative/basal-like cases among carriers. In addition, is there any difference between c.5266dupC and c.4034delA carriers?

Author: We collect all possible data about the status of ER/PR/ERBB2 and included it into the Table1. In addition we performed Cox regression analysis to investigate the association among hormone receptors status and overall survival of breast cancer patients. Unfortunately, the data about hormone receptor status was available only for 52% of patients included into survival analysis, whereas data about ERBB2 status only for 40% of patients. The main problem was that in Latvia ER/PR status have not been investigated for clinical purposes before 2002 and ERBB2 status before 2004 and the retrospective analysis was not possible due to technical reasons.

Reviewer: Most probably, mutations carriers have a distinctive clinical management, such as risk-reduction surgery or intensive surveillance programs.

Author: The information about this topic was included into discussion (page 11, line 3).

Minor essential revisions

Reviewer: The manuscript needs revision by a native English speaker.

Author: The manuscript was edited by native English speaker.

Reviewer: Page 4, line 10. Reference 4 does not seem appropriate.

Author: The reference was changed.

Reviewer: Apparently, the study population is comprised of unselected breast and ovarian cancer cases, but the patients and methods first paragraph (page 5 at the end, page 6 top) is confusing. It should be rewritten.

Author: Due to the fact that we have not found any statistical difference among population groups investigated during different time periods, we decided to show all patients as a common group. This explanation therefore was removed as unnecessary.

Reviewer: Page 13. I doubt the claim that several authors have suggested that other genes may explain BRCA1 genotype/phenotype correlations? Other genes may explain variable penetrance of BRCA1 mutations but not that two specific mutations such as c.4034delA and c.5266dupC confer different risk (unless modifier risk genes are linked to BRCA1). The authors should clarify this point.
Author: We agree with the reviewer that other genes most probably can not explain the genotype-phenotype correlation effect. Taken to consideration the new data obtained during improvement of the survival analysis, we include into discussion our hypothesis about the possible explanation of the biological mechanisms which can be responsible for genotype-phenotype correlation (page 11, paragraph 2 - page 12, paragraph 1).

Reviewer: The manuscript will improve clarity if Figures 2 and 3 use the same code for c.4034delA, c.5266dupC and control curves.

Author: We correct figures 1 and 2 (former 2 and 3) according to suggestions of the reviewer.

Corrections performed according to the suggestions of the reviewer Khalil Helou.

Reviewer: I would recommend that the authors remove the confidence intervals from the first paragraph of the results. If you observed 96 patients with mutations of 2546, that is 96. In this section no inference is made you just summarize the study population, hence no need for confidence interval. The observed number and percentages are sufficient.

Author: The confidence intervals were removed in this paragraph.

Reviewer: The second paragraph of the result section on the other hand requires a test statistic. Please present both the raw data and the associated P-value, or confidence interval.

Author: Due to the fact that we have not found any statistical difference among population groups investigated during different time periods we decided to show all patients as a common group. We therefore remove this paragraph from the revised version of manuscript.

Reviewer: Very long sentence, it is difficult to follow. Additionally, I’m unsure if here odds ratios proper. If I understood correctly the authors compare the prevalence of different mutation among breast and ovarian cancer. I would suggest that the authors present the prevalence (maybe as percentages) and test the difference. You could do the same as in point 2.

Author: We corrected this data according to the suggestions of the reviewer. The revised version of this sentence located on page 5: Results, Part 1, paragraph 2.

Reviewer: I would recommend against using the “correlation”. Maybe indicating that the cumulative incidence differs among the three mutational types is better. The Log Rank should refer to the difference in cumulative incidence and not
tendency for younger age at onset. This is an understandable typo, just move test statics and P value.

Author: We corrected this sentence according to the suggestions of the reviewer (See page 6, line 10)

Reviewer: Were you excluded the comparison with patient with no mutation, while the figure plots the incidence rate even for that. This has to be specified as readers might think otherwise.

Author: In the revised version of our manuscript we provide data of the comparison (Log-Rank test) with patients with no mutation (See page 6, line 12)

Reviewer: As a last point I would suggest to try a Cox-regression analysis and perhaps adjust for other clinical characteristics as well.

Author: As was suggested by reviewer we investigated the impact of different clinical characteristics on overall survival using Cox regression analysis (See Table 2 and 3).

Reviewer: Quality of written English: Needs some language corrections before being published.

Author: An article was edited by native English speaker.

In addition, as was suggested by Dr. Guian Paolo Declaro (letter from 1.07.2011), part of the paragraph 1 of Patients and Methods was included into the Part 4 of results, as well as 1st part of the paragraph 3 of Part 1 of results was transferred to discussion (Page 9, Paragraph 2).

We would like to thank the referees for their helpful comments and hope that we have now produced a more balance and better account of our work. We trust that the revised version of manuscript is acceptable for publication.

Yours sincerely,

Dr. Grigorijs Plakhins