Author's response to reviews

Title: Evidence against PALB2 involvement in Icelandic Breast Cancer Susceptibility

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**Regarding MS: 1844609475189384**  
Evidence against PALB2 involvement in Icelandic Breast Cancer Susceptibility  
Haukur Gunnarsson, Adalgeir Arason, Elizabeth M Gillanders, Bjarni A Agnarsson, Gudrun Johannesdottir, Oskar Th Johannsson and Rosa B Barkardottir  

Dear Sir  

Enclosed is a revised version of our paper “Evidence against PALB2 involvement in Icelandic Breast Cancer Susceptibility”  

In your e-mail dated 12th of May you requested changes according to the reviewers comments. We have attempted to address the reviewers comments in the attached revised manuscript. In the accompanying letter we have listed the changes made in accordance to the suggestion of the reviewers, to whom we are most grateful.  

We hope this is adequate, otherwise please let me know.  

Yours sincerely,  

___________________________  
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**Reviewer 1**

**Major points:** The authors should clarify the reasons why they have chosen to perform a linkage study as opposed to, for example, sequencing of PALB2. The sample size in the linkage study is not very large and would, therefore, not require extensive sequencing efforts. In addition, PALB2 mutations are generally considered to be intermediate penetrance alleles. They have not so far been detected through linkage approaches possibly due to relatively low prevalence and lower risks compared to e.g. BRCA1 or BRCA2.

We agree that sequencing analysis is a good choice but since we were looking for a highly penetrant mutation in high-risk breast cancer families, a linkage analysis is also a logical method to apply, given our presumption that there also might exist highly penetrant PALB2 mutations. Furthermore, a linkage analysis is faster and less expensive than sequencing. It may also be argued that having found linkage to be absent, the advantage of also performing sequencing would, most probably, at best be confined to detecting very few carriers which would not be the major explanation of the high breast cancer burden of their relatives.

We also agree that PALB2 mutations are generally considered to be of moderate risk. However, although a rare event, PALB2 mutations have also been detected in high risk breast cancer families as reported both by Tischkowitz et al [9] and by Foulkes et al [7], suggesting that some PALB2 mutations could also cause high penetrance.

At the time we decided to perform linkage analyses we reasoned that the general picture of PALB2 as of low-moderate risk determination was in no way proof that no high-risk PALB2-mutations were still to be found, e.g. in such a geographically confined population as in Iceland. This argument was not clearly put forward in our previous manuscript, thus perhaps leading the reader to assume we were in search of low-moderate alleles by our linkage analysis or otherwise mislead in our experiment design. We apologise for this shortcoming. We have tried to amend the introduction accordingly without expanding the text too much and also inserted a short addition in the abstract (see also notes to reviewer’s 2 comments). We are grateful for this opportunity and hope we are right that the manuscript has been considerably improved by this.

**Minor points:** Gene names are not in italics as they should be. This must be corrected.

This has been corrected and the gene names are now in italics.

**Discretionary Revisions:** The manuscript could be substantially improved by inclusion of some population genetics aspects such as the population history of Iceland. This would also make the paper of more interest to people who are not from Iceland. It is understandable to want to make the paper brief but the genetics perspective should not be ignored.

In an effort to comply with this suggestion without taking to much space we have added in a few sentences in the introduction about possible historical explanations of the extreme frequency difference between the only two BRCA mutations found in the Icelandic population. We have also moved a sentence from the results chapter, partly rewritten it and
included in the introduction chapter together with a few sentences about the indication that the Finnish and the Icelandic BRCA2 999del5 mutation might be of a common ancient origin. After these amendments the paragraph about population genetics and population history is as follows:

- “Only two BRCA1 and BRCA2 mutations have been found in the Icelandic population, BRCA2 999del5 and BRCA1 G5193A, both being founder mutations explaining a large proportion of familial breast cancer in Iceland [10]. The BRCA2 999del5 mutation is much more frequent, accounting for around 40% of the hereditary cases and found in about 8% of unselected breast cancer cases and 0.4% of population based control [11]. A BRCA2 999del5 mutation is also the most frequently occurring BRCA1/2 mutation in Finland [12], and haplotype analysis of Finnish and Icelandic BRCA2 999del5 families did not exclude the possibility of a common ancient origin of the mutation [13]. The BRCA1 G5193A mutation however is very rare, found in less than 2.5% of hereditary breast cancer families and in 0.2% of unselected breast cancer cases [14]. The extreme frequency figures of these two founder mutations reflect low genetic diversity in the Icelandic population. The Icelandic population is a very young one, the country being settled by a few thousand founders about 1100 years ago. Low genetic diversity is probably explained by the relatively homogeneous group of settlers, and genetic drift resulting from repeated population bottlenecks due to diseases and famines [15, 16].”

**Reviewer 2**

**Comment:** The aim of the study was to find out whether the PALB2 gene has a role in breast cancer susceptibility in Iceland. The study is limited to linkage and the Finnish 1592delT founder mutation analysis in order to test whether PALB2 would be a highly penetrant gene in Iceland.

So far, PALB2 has not been a high-penetrant gene in any population studied and therefore the original hypothesis is a bit constrained.

This comment by reviewer 2 is in the line with one of the comments of reviewer 1. As said earlier we agree that the current data about PALB2 mutations and breast cancer susceptibility indicate that the majority of PALB2 mutations are of moderate risk. However, as stated above, PALB2 mutations have also been detected in high risk breast cancer families (by Tischkowitz et al [9] and by Foulkes et al [7]), suggesting that some PALB2 mutations could also cause high enough penetrance to be detected by linkage analyses.

The presumption that there also might exist highly penetrant PALB2 mutations is made clearer by adding to the manuscript, or modifying, the following sentences:

- In the abstract: “Although most often the risk is moderate, it doesn’t exclude families with high-risk mutations to exist and such observations have been reported. To see if high-risk PALB2-mutations may be present in the geographically confined population of Iceland...”

- In the introduction section: “Although predisposing PALB2 mutations generally appear to cause moderate risk of breast cancer [8], mutations have also been found in strong hereditary breast cancer families [7,9] and might thus be worthwhile searching for by linkage analysis in e.g. geographically confined populations.”