Author's response to reviews

Title: RNA Profiling Reveals Familial Aggregation of Molecular Subtypes in non-BRCA1/2 Breast Cancer Families

Authors:

Martin J Larsen (martin.larsen@rsyd.dk)
Mads Thomassen (mads.thomassen@rsyd.dk)
Qihua Tan (gtan@health.sdu.dk)
Anne-Vibeke Lænkholm (anlae@regionsjaelland.dk)
Martin Bak (martin.bak@rsyd.dk)
Kristina P Sørensen (kristina.sorensen1@rsyd.dk)
Mette K Andersen (Mette.Klarskov.Andersen@regionh.dk)
Torben A Kruse (torben.kruse@rsyd.dk)
Anne-Marie Gerdes (anne-marie.gerdes@regionh.dk)

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Author's response to reviews: see over
Dear Editor

Thank you for the positive feedback and constructive criticism of the manuscript. We are very thankful for the deep and thorough review. We have revised the present manuscript in the light of the suggestions and comments brought up and hope the revision has improved the paper to a level of satisfaction.

Please find enclosed:
- Revised manuscript
- Rebuttal letter with response to the reviewers comments

Yours sincerely,

Martin J. Larsen
martin.larsen@rsyd.dk
Response to Reviewer’s Comments:

Reviewer: Lorenzo Melchor

Comment: 1. Discussion section titles are repetitive with the Result section ones and may lead to confusion. The authors could take advantage of these section titles in the Discussion to deliver a much clearer message based on their results and conclusions.

Response: The section titles in the result section have been modified in order to make it less repetitive and make the conclusions stand out.

Comment: a. Discussion p.15. Sentence starting “The aim of this study…” Replace ‘homogenous’ for ‘homogeneous’

Response: The misspelling has been corrected

Comment: b. Discussion p.18, third paragraph. “On the contrary, a study analysed tumors using noted that different tumors within the same family…” Consider revising the structure or meaning of this sentence.

Response: The sentence has been revised

Reviewer: Florentine Hilbers

Comment: Although the number of families with multiple samples is small, the clustering of molecular subtypes within families is an interesting observation. The authors argue that selection of families based on the clustering a specific subtype could aid the discovery of new high risk breast cancer genes in for example next generation sequencing studies. However, the high number of non-BRCA1/2 families (8 out of 11) in which a specific subtype seems to cluster suggests that within these groups of families, there is still a considerable amount of etiological heterogeneity. Multiple segregation studies show that mutations in additional high penetrance genes are likely to be very rare and explain only a small proportion of non-BRCA1/2 families, while most familial clustering is likely caused by a polygenic risk. Do the authors think moderate or low risk variants can be responsible for the observed clustering of molecular subtypes? Or do they have another explanation for the difference in suggested frequency of high penetrance mutations in non-BRCA1/2 families suggested by their study and that of most segregation studies (Antoniou et al., Cui et al.)

1. Discussing this might give some depth to the Discussion section which currently contains maybe too many details from the results.
Response: A discussion about rare high-penetrant genes and polygenetic risk in relation to our observation has been added to the discussion section as suggested by the reviewer.

Comment: Another argument against the usefulness of this approach to select more genetically homogeneous non-BRCA1/2 families is the fact that tumor RNA of sufficient quality can rarely be collected for more than one individual per family. It will therefore be very hard to collect enough families with the same clustering subtype to gain enough power to detect an association with rare variants within high risk gene.

Response: We acknowledge that fact that frozen tumor specimens are rarely available. However, the molecular breast cancer subtypes can be determined using other methods that do not require access to frozen tissues. A 50 genes qPCR FFPE assay has been developed based on the PAM50 classifier to robustly classify tumors according to their molecular subtypes and has been commercialized (Parker et al, 2009). IHC stainings can also to some extent be used as surrogate markers in order to determine the molecular subtypes.

Comment: 2. The authors claim that they are the first to “…systematically demonstrate that members of the same family often share the same tumor subtype…”. However, Didraga et al. (Breast Cancer Research and Treatment 2011) shows that a specific tumor array CGH profile also clusters within a subgroup of non-BRCA1/2 families. (While a follow-up WES study was unable to find mutations in a high penetrance gene in these families).

Response: We acknowledge that the study by Didraga et al. have shown clustering of the specific arrayCGH profile. We have therefore changed the wordings of that sentence to emphasize that the our statement were related to the well-known (intrinsic) molecular subtypes determined by RNA profiling. Reference to the mentioned study has also been included in the discussion.

Minor Essential Revisions

Comment: 3. In Figure 4 three individual in Family 029 are colored green, suggesting that the tumors of these three individuals have been analyzed and show the same molecular subtype. However, Table 2 includes only two individuals with the same subtype for family 029. Please check if either one contains a mistake or make clear why one individual was not included in Table 2.

Response: Table 2 (now Table 3) has been corrected to include all three individuals.

Comment: Page 18, last paragraph: “On the contrary, …” a word seems to be missing from this sentence.

Response: The sentence has been revised.

Comment: Page 15, first sentence of the last paragraph: also some missing words.
Response: The sentence has been revised

Discretionary Revisions

Comment: 5. Since the BRCA1, BRCA2, Sporadic and non-BRCA1/2 group differ a lot in size, it would be helpful to add percentages to Table 1.

Response: Table 1 now include percentages as suggested by reviewer

Reviewer: Ana Osorio

Minor essential revision:

Comment: My only concern to this study is the heterogeneous and somehow incomplete way in which the patients have been tested for mutations in BRCA1 and BRCA2. The authors mention a mixture of techniques used for the screening that include the Protein Truncation Test which could have lead to some false negatives. It is necessary to specify the percentage of cases tested with each of the techniques to be sure about the reliability of the results.

Response: The phrasing of the section describing the procedure for genetic mutation testing of the BRCA1 and BRCA2 genes were imprecise. We have re-phrased it so it is clearer that the different methods described were used in concert and no single method stand alone in the genetic screening analysis. Two different setups have been applied in our laboratory for clinical diagnostic BRCA1/2 screening. During the period from 2000 to 2005 genetic mutation screening were conducted using a setup consisting of DHPLC, PTT and Sanger sequencing analysis in addition to MLPA for detection of larger copy number abnormalities. From 2006, we replaced the DHPLC method with TGCE, being the only difference between the two setups. Percentages of cases tested with the two setups have been added to the manuscript as suggested by the reviewer.

Comment: A table showing the distribution of the different families with respect to the inclusion criteria should be also added.

Response: A table showing the distribution of different family characteristics has been added to the manuscripts (Table 2)

Regarding the non-BRCA1/2 tumours that the authors find to be BRCA1 or BRCA2-like, using the BRCA1 and BRCA2-signatures prediction, they should specify how this cases were tested (which was the technique used) to be sure that they don’t harbour a mutation that could have been missed.

Response: As described above, the genetic testing of all patients, including the patients from whom tumors predicted as BRCA1-like and BRCA2-like came from, were conducted very homogenously and thorough. We acknowledge that there is a risk for false negative tests, although we are convinced that the number of missed positive mutations is low. This issue is also discussed in the manuscript.