Author's response to reviews

Title: ATM variants and cancer risk in breast cancer patients from Southern Finland

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

Thank you for the encouraging response and the constructive comments by the reviewers on our manuscript: (1710557626100339) entitled “ATM variants and cancer risk in breast cancer patients from Southern Finland” by Drs. Johanna Tommiska, Laila Jansen, Outi Kilpivaara, Hege Edvardsen, Vessela Kristensen, Anitta Tamminen, Kristiina Aittomäki, Carl Blomqvist, Anne-Lise Børresen-Dale and myself.

We have considered carefully the comments of the reviewers and our detailed responses to the reviewers’ comments are indicated in the following and the changes made in the revised manuscript are shown by bold text.

We believe these revisions have strengthened our manuscript and hope that the revised manuscript now could be accepted for publication.

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ATM variants and cancer risk in breast cancer patients from Southern Finland

We thank the reviewers for their constructive comments and believe that our responses have strengthened the paper.

Reviewer: Janet Hall

Minor essential revisions:

There should be some comment on the statistical power of the study to draw conclusions about ATM haplotypes when these are determined in only 47 breast cancer patients.

Although the number of cases studied for determining the haplotypes was only 47 (94 chromosomes), the 95% confidence intervals (0.000 – 0.039) for undetected haplotypes (frequency 0.000) suggest a maximum combined frequency of 3.9% for undetected haplotypes with a 95% probability. This suggests also that any undetected haplotypes would have been rare and unlikely to account for the proposed association of ivs38-8T>C with bilateral breast cancer. This has been added on page 12.

Discretionary Revisions:

Were a proportion of the results confirmed by sequencing?

All the samples found positive for 998C>T with RFLP were confirmed by direct sequencing. For the ivs38-8T>C variant, all the positive cases were re-analysed by RFLP and a sample of them were also confirmed by direct sequencing. For the 5557G>A variant, 50 samples were genotyped by two independent methods (minisequencing and Amplifluor™ fluorescent genotyping). All the re-analysis and confirmation results were consistent. This has been added on page 7.

The 5557 variant allele were found in a total of 6 haplotypes, 3 of which also contained the variant allele of ivs38-8, perhaps the text on page 9 could be edited so that this is clearer.

We have edited the text on page 9 so that this is clearer.
Reviewer: Thilo Doerk

Major Compulsory Revisions

1. The manuscript should include a critical discussion of the study power to detect low, moderate or high risks in the respective sample sizes given the observed allele frequencies and/or the expected AT heterozygote frequency in the general population. One could refer to a previous article by Haber’s group (FitzGerald et al. 1997) and the discussion of these data (Bishop and Hopper 1997). Power estimates might also be informative for the readers in regard of any association of ivs38-8T>C with familial or bilateral disease.

In this study, we aimed to evaluate defined common ATM variants for breast cancer risk and bilateral breast cancer especially. In addition, full mutation screening was carried out in a set of families to define haplotypes and search for possible ATM mutations in breast cancer families. Our results do not support increased risk for the variants studied in any of the subgroups although small increases in risk cannot be excluded. Power calculations for associations of ivs38-8T>C have been added on page 12 and in the methods section page 8.

Combined with previous results, our results also suggest that ATM mutations are rare in breast cancer families. However, whether rare (heterozygous) ATM mutations, estimated to be present in about 0.4%-1% of the population, confer an increased breast cancer risk in the population, is beyond the scope of this study and has not been evaluated at a large scale in other studies yet either. Due to the rarity of such variants, very large population-based case-control studies will be required to detect small or even modest risk effects (Bishop and Hopper, 1997). Discussion on this has been added on page 15.

We also modified the sentence preceding this discussion to read now:
Altogether, these results suggest that possible breast cancer associated ATM mutations are very rare in breast cancer families from Southern Finland.

2. The authors report that none of the identified variants segregated with cancer in these families (p.10) but results are not provided. As far as segregation data can contribute to the characterisation of the identified variants, they need to be discussed in more detail, at least the number of diseased carriers and non-carriers per variant should be mentioned.

Segregation data are now described more in detail on page 10.

3. The second most common ATM variant, 735C>T (5/47 families), was not investigated further in the case and control series. This is not clear to me because a previous study from some of the authors had reported this synonymous variant as a splicing mutation that leads to exon 9 skipping in AT patients (Laake et al. 2000, Table 5). If true, the present study appears incomplete, and the authors
should either include 735C>T into their screening or comment on whether more recent evidence or exclusion criteria argue against screening for this variant.

The ATMex9 735C>T (V245V) variant was reported by Laake et al. (2000) to co-occur with skipping of exon 9 in some A-T patients, but this is not observed in all cases with the variant. Furthermore, it is not the causative A-T mutation in these families, other causative A-T mutations have been identified more recently in these families (Børresen-Dale, unpublished). In addition, the reported skipping of exon 9 was detected only on cDNA and is seen occasionally probably because the degradation of non-proofread transcripts has not occurred, or it may be an artifact caused by mRNA instability (Børresen-Dale, unpublished). This silent change was also reported to be more frequent in controls than in breast cancer cases from Northern Finland (Heikkinen et al. 2005). Based on these, this variant was not screened further in our study. This has been added on page 14.

**Minor Essential Revisions**

**Tables 1 and 2 seem to be partly redundant and should be combined.**

The tables are not very easily combined as Table 1 presents the genotype distributions in different groups of cases and controls whereas Table 2 presents cancer history (uni- or bilateral BC, multiple cancers or BC only) of different genotype carriers. Also, as OR:s in Table 1 are calculated compared to population controls and in Table 2 compared to unilateral and BC only cases, we feel that the presentation is more clear when the tables are separate.

In adddition, we have included Rainer Fagerholm in the acknowledgments.