Author’s response to reviews

Title: Tumour spectrum in non-BRCA hereditary breast cancer families in Sweden

Authors:

Camilla Wendt (camilla.wendt@karolinska.se)
Annika Lindblom (annika.lindblom@ki.se)
Brita Arver (brita.wasteson-arver@karolinska.se)
Anna von Wachenfeldt (anna.vonwachenfeldt-vappling@karolinska.se)
Sara Margolin (sara.margolin@karolinska.se)

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Author’s response to reviews: see over
Dear editor,

On behalf of all the authors we thank you and the reviewers for their insightful comments which will improve the manuscript. Below we have answered all the comments by the reviewers and report the changes made in the manuscript.

Reviewer Thilo Dork:

Thank you for important response on the statistics of the study. The aim was to compare the incidence of tumour types in families with hereditary breast cancer compared to the general population. The data has been collected during many years. To identify a true difference and not only changes in incidence over time, two reference years were used (1970 and 2010) and consequently a cancer diagnosis in the study population was regarded as overrepresented only if the confidence interval was above both population reference values. The population reference values were assumed to reflect a true distribution. Since the incidence of different tumour types are not direct comparable, the difference in distribution of cancer diagnoses between the study population and the general Swedish population were compared as proportions. Indirect standardisation is the statistical method that was used to compensate for differences in age and gender between the populations.

Using the words “differencies were significant” is unclear and we have now removed that sentence, thank you for pointing that out.

Comments on minor suggestions to improve the manuscript:
1. The introductory part from “Major advances…” to “…still remains unclear” is unnecessarily long and verbous, this should be condensed since known breast cancer susceptibility genes have been covered by several reviews.

The introductory part is now revised and shortened as follows (Page 3-4, line 14):

Major advances in the understanding of breast cancer susceptibility were made in the 1990s when the two major high-risk breast cancer and ovarian cancer predisposition genes BRCA1 and BRCA2 were identified {Hall, 1990 #19; Wooster, 1994 #18; Wooster, 1994 #20; Miki, 1994 #21}. Significant for all identified high-risk breast cancer predisposition genes is that they are observed in the context of breast cancer syndromes involving not only breast cancer but also an increased risk of other tumour types. Apart from the Hereditary Breast and Ovarian Cancer Syndrome caused by mutations in BRCA1 and BRCA2, these include Li-Fraumeni Syndrome (TP53) {Achatz, 2009 #15}, Cowdens Syndrome (PTEN) {Farooq, 2010 #69}, Peutz-Jeghers Syndrome (STK11) {Tomlinson, 1997 #75; van Lier, 2010 #76} and Hereditary Diffuse Gastric and Lobular Breast Cancer Syndrome (CDH1) {Pharoah, 2001 #77}. In addition, most of the identified moderate penetrance breast cancer genes also predisposes to other tumours types. Besides an intermediate increased risk of breast cancer CHEK2 mutations has been associated
with an increased risk of bladder, colorectal, prostate and kidney cancer
(Vahteristo, 2002 #31; Weischer, 2008 #40). Mutations in BRIP1 has been
associated with increased risk of breast and ovarian cancer (Seal, 2006 #82; Rafnar,
2011 #81). Carriers of deleterious PALB2 mutations has a moderate to high risk of
breast cancer and also a increased risk of pancreatic cancer and ovarian cancer
(Antoniou, 2014 #41) (Rahman, 2007 #44; Tischkowitz, 2010 #49). Inherited
deleterious ATM mutations has been associated with both breast cancer and
pancreatic cancer predisposition (Thompson, 2005 #80) (Roberts, 2012 #90). These
moderate risk genes confers a 2-4 fold risk compared to the 10% risk in the general
population. In recent years genome-wide association studies in large cohorts has
identified multiple low risk variants that each confers a modest risk though the
combined effect can be substantial (Michailidou, 2013 #48).

2. In the Discussion part, the authors may wish to be more cautious with their
statement that “there is no association with breast cancer” in Lynch Syndrome.
While this view is supported by Reference 44, others have provided evidence for
some role of mismatch repair gene mutations in breast cancer.

Good point, we have revised this part and added a reference as you suggested
(Page 9, line 14):

Two autosomal dominant genetic disorders causes increased risk of developing
dometrial cancer. One of them, Cowdens syndrome, caused by germ-line mutations in
PTEN also confers increased risk of developing breast cancer (Farooq, 2010 #69).
Hereditary non-polyposis colorectal cancer (HNPCC) or Lynch syndrome is caused by
defect mismatch repair system and causes increased risk of developing endometrial
cancer. Evidence for an association with breast cancer risk has not been shown even
though some role of mismatch repair genes in breast cancer development in these
families has been suggested (de la Chapelle, 2005 #70) (Win, 2013 #92). In our cohort
all pedigrees were assessed by genetic counsellors and in relevant cases specific high
risk syndromes were ruled out by genetic screening. It is therefore unlikely that families
with either Cowden or Lynch syndrome are included in the study.

3. The authors propose that PALB2 could partly explain the excess risk for
prostate, ovarian and pancreatic cancer. However, PALB2 mutations are quite
rare and it would be worthwhile to test whether their population frequency and
penetrance are sufficiently high to result in the excess of these cancers.
Unfortunately the data on PALB2 mutation frequency for the cohort or for Sweden is not available. We revised the changed the manuscript as follows below (Page 12, line 7):

Since PALB2 mutations are rare the contribution to the increased risk for these tumour types should be limited in the study cohort. Nevertheless, PALB2 mutations and pathogenic mutations in other genes involved in the same pathway as BRCA2 could explain a minor part of the excess of pancreatic and ovarian cancer.

4. Gene symbols should be in italics and should follow consensus nomenclature, e.g. “BRCA2" instead of “BRCA 2”. Typo in Table 1: Thyroid.

Thanks for pointing that out. Corrections have been made in the manuscript.

Reviewer Steven Narod:

Should list detailed limitations and stress that if the risk of cancer is higher in relatives than in general population it may not be appropriate to compare proportions esp if proportion is low. I.e rate could be the same but proportion will be low because of increased risk at other sites.

Thank you for the insightful comment on the statistics. Since proportions are compared, the diagnoses in the hereditary cancer population with large proportions could as a result leave other potentially overrepresented tumours types undetected. We now have clarified that issue in the manuscript (Page 11, line 13).

As for rare tumour types it is difficult to draw any conclusions since the statistical method compares proportions not incidence (a higher incidence in the study population may go undetected since proportions will be dominated by the most common cancer types).

The risk of overestimating proportions is low though. Some of the rare tumour types were not represented in the study cohort, and in the reference population these tumour types represented about 3% of the proportion. On the other hand the tumour site Abdomen unspecified was represented only in the study cohort with 3.5 % proportion. Our result therefore remains solid.

In addition, language review has been performed.

With the best regards

For the authors

Camilla Wendt MD