Author's response to reviews

Title: Variations in the NBN/NBS1 gene and the risk of breast cancer in non-BRCA1/2 French Canadian families with high risk of breast cancer.

Authors:

Sylvie Desjardins (sylvie.desjardins@crchul.ulaval.ca)
Charles Joly Beauparlant (charles.joly-beauparlant@crchul.ulaval.ca)
Yvan Labrie (yvan.labrie@crchul.ulaval.ca)
Geneviève Ouellette (genevieve.ouellette@crchul.ulaval.ca)
INHERIT BRCAs (jacques.simard@crchul.ulaval.ca)
Francine Durocher (francine.durocher@crchul.ulaval.ca)

Version: 4 Date: 27 March 2009

Author's response to reviews: see over
Quebec City, March 26th, 2009

Mrs Melissa Norton, MD
Editor-in-Chief, BMC Series
BioMed Central Ltd,
Middlesex House,
34-42 Cleveland Street, London W1T 4LB, UK

Object : Revised version of the manuscript #1951956212237778 entitled: Variations in the NBN/NBS1 gene and the risk of breast cancer in non-BRCA1/2 French Canadian families with high risk of breast cancer.

Dear Doctor Norton,

Thank you for the response we received on March 24th from BioMed Central Editorial including the editorial decision as well as pertinent comments from both reviewers regarding our manuscript «Variations in the NBN/NBS1 gene and the risk of breast cancer in non-BRCA1/2 French Canadian families with high risk of breast cancer» by Desjardins et al. (MS: 1951956212237778).

Following the editorial requests and the reviewers’ suggestions, we enclose a revised version, which we have amended in the light of the referee’s comments. Please find in the following pages our specific point-by-point responses to the reviewers’ comments detailing the changes made.

We would like to thank again the BioMed Central Editorial and the reviewers for their comments and the time they spent on our manuscript.

If you need any further information, please feel free to contact us at any time.

Regards,

Francine Durocher, Ph.D.
Associate Professor, Department of Anatomy and Physiology, Laval University
Investigator, Cancer Genomics Laboratory, CHUQ Research Centre, CHUL
francine.durocher@crchul.ulaval.ca

Response to reviewers:
Minor Revisions requested from Dr Thilo Dork

1. «A 4bp deletion was identified at a putative NBN promoter region with an about 3-fold increased prevalence in cases compared with controls, a difference that was marginally significant, and the authors define a risk haplotype carrying this deletion. They also provide evidence that luciferase constructs harboring the deletion had less activity than wild-type to stimulate NBN expression in 2 out of 4 different cancer cell lines tested. The effects were small, but it remains possible that they are further induced by irradiation or other stimuli, and this possibility should be discussed. … One should also include the caveat in the discussion that no binding experiments have yet been performed to confirm the hypothesis.»

To address the reviewer’s comments, the following sentences have been added:

P. 18 « On the other hand, one could hypothesize that NBN (or transcription factors affecting its expression) may be regulated following irradiation or other genotoxic stress. Additional research will however be needed to address this possibility as well as binding experiments to confirm ATFB1 binding to this region of the promoter.»

«The more responsive lines appeared to have a higher expression of ATBF1, one of four candidate transcription factors with putative binding to the deletion site. Primers to quantitate ATBF1 expression should be provided in the Methods section.»

The sequences of the primers used to quantitate ATBF1 now appear in the Methods section.

2. «A number of report are cited that seem to show an association of I171V with cancer, but they are missing a larger study that has not confirmed such findings for breast cancer (Breast Cancer Res Treat 2008; 112 :75-79).»

We thank the reviewer to point to us this obvious oversight on our part. We included this information in the revised form of this manuscript.

P.15 «However, the impact of the p.Ile171Val substitution remains to be clarified as a large study, including cases from Germany and the Republic of Belarus, did not find an association with breast cancer in those populations.»

3. «The authors also included a brief section on alternative splice forms that have been detected in cDNA samples from a subset of the patients. An in-frame skipping of three exons was observed at apparently low abundance. There seems to be no correlation with any genotype drawn from the genomic sequencing data. However, this part of the results should be elaborated further as the authors neither say how many samples were analysed nor which tissues were under investigation.»

We agree that further precisions are useful in this instance. These analyses were performed on the cDNA extracted from the lymphoblastoid cell lines of a subset of 10 breast cancer individuals and we obtained very low copy numbers. Relevant details have been added to the text.
P.9 «However, QRT-PCR of this specific form was performed on cDNA samples from a subset of 10 breast cancer cases from our cohort and showed a very low expression relative to the main isoform (data not shown), which is consistent with previous work.»

4.  «I am reluctant to believe in odds ratios and confidence intervals with three decimal places. In view of the limited study size, this may suggest a much higher level of accuracy than the present data set actually can provide.»
In accordance with Dr Dork’s comment, we adjusted the number of decimals for odds ratios and confidence intervals.

Minor Revisions requested from Dr Eva Seemanova

5)  «There are mentioned practically all relevant papers dealing with the related problematic. I would recommend also to discuss the results of Someya et al. paper ‘association of ionizing radiation-induced foci of NBS1 repression’.»
We agree with the reviewer that the results presented in the paper from Someya and collaborators bring additional information pertinent in the context of our study.
P.14 «In this regard, a recent study by Someya et al. showed a correlation between persistent radiation-induced NBN foci and both chromosomal instability and sporadic breast cancer risk.»

7)  «According to my knowledge, the authors did not forgot any recent and relevant publications, which are connected to this problematic with only one exception. I would see useful to discuss also the paper by Vineis P. et al. ‘A field synopsis on low-penetrance in DNA repair genes and cancer susceptibility’ in JNCI from Jan 7 2009.»
The new paper by Vineis et al. provides useful information about the association of an NBN variant with risk of cancer and we agree with Dr Seemanova that these results are important in the context of our paper.
P.16 «… and a recent meta-analysis of this variant in bladder cancer found a significant association after controlling for potential bias.»

Editorial Requests:
To address the Editorial requests, the following changes have been made to the format of the text:
1. A Methods subheading and relevant information now appear in the Abstract.
2. The Methods section has been moved after the Background section, a heading now appears for the conclusion, and a list of abbreviations has been provided.