Reviewer's report

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.

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Reviewer: Thilo Dork

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This manuscript describes the result of a genetic study of the major Nijmegen Breakage Syndrome gene, NBN, in French breast cancer cases. Sylvie Desjardins and colleagues scanned the whole coding and flanking untranslated sequences of NBN for mutations in 97 patients with familial breast cancer and 74 healthy controls. This seems to be a state-of-the-art analysis in a reasonable number of individuals for a sequencing project. Unfortunately, the authors did not take the next step of a larger scale association study to better define the risks associated with the most interesting variants detected.

Minor revisions:

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 harbouring 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 could be discussed. 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. One should also include the caveat in the discussion that no binding experiments have yet been performed to confirm the hypothesis.

2. Apart from the 4 bp deletion, none of the gene alterations appeared at significantly higher frequency in cases than controls. The I171V substitution was found in a single case only, and the authors speculate about a possible disease-associated nature of this variant. I believe this part of the discussion is biased. A number of reports 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).

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.

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.

Altogether, the study provides useful information and describes a new and potentially interesting association of a putative regulatory variant in NBN with breast cancer. It also confirms that classic NBN mutations are rare in French multiple-case breast cancer families. The manuscript is well written and adds to our knowledge about NBN gene alterations. Whether the 4bp-deletion indeed contributes to breast cancer risk must await its screening in larger case-control association studies.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests