Author's response to reviews

Title: NMD and microRNA expression profiling of the HPCX1 locus reveal MAGEC1 as a candidate prostate cancer predisposition gene

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

Thank you for Your e-mail of June 20th 2011, informing that our manuscript (MS: 7467044945066462) is acceptable for publication in the BMC Cancer, pending some revision. We have considered carefully the comments of the reviewer and our detailed responses to the issues raised are as follows.

1. The reviewer pointed out that the role of the HPCX1 locus in HPC is not concerned much in this study and suggested that we should remove one sentence from the last paragraph of the introduction section. We have changed the sentence as follows: “Here, we present a study with Finnish multiplex HPCX1 linked families in which we have characterized the HPCX1 locus by NMD and miRNA microarray methods and evaluated the role of HPCX1 in the causation of familial prostate cancer.”

2. The reviewer noticed that there is inconsistency in number of families used for the miRNA analysis between the text and Table 1. This has now been corrected. The original number of families in NMD microarray analysis was five and the number was increased by seven for the miRNA array analysis. The part in the text has been modified accordingly.

3. The reviewer was curious to know how the boundaries of HPCX1 were defined. Baffoe-Bonnie et al. (2005) has refined the HPCX1 locus to a 150-kb region on Xq27-q28 between markers D3S2390 and bG82i1.0. By knowing the complex genomic structure of the HPCX1 locus and the ways miRNAs regulate gene expression, we also included interesting genes from Xq25, Xq26, and Xp11.

4. The reviewer required that the link between variation sites found in candidate genes and miRNAs should be more thoroughly described in the Results section. The results of miRNA target detection analysis were considered more carefully only in the case of MAGEC1 and the miRNAs for validation were selected partially based on these results. We have modified part of the text in the Results section (third paragraph) as follows and also modified Table 5 accordingly: “The miRanda algorithm produced 1211 different variant-miRNA combinations with a total score value above the cut-off value. From
the 29 differentially expressed miRNAs between patients and healthy individuals twelve miRNAs for validation were selected based on that they supposedly had a target site in MAGEC1 gene (Table 5).”

5. A) As the reviewer mentioned it would be extremely interesting to know if and how the nonsense-mediated mRNA decay has occurred in the case of a translational start failure as NMD is a eukaryotic system to remove transcripts with premature termination codons. Investigation of this would require such further experiments that are in authors’ opinion beyond the focus of this paper.

B) The reviewer pointed out that a damage prediction programme, which does not consider start codons as potentially relevant features for generating proper proteins is not worth mentioning at all. Therefore, this sentence has been removed from Discussion (third paragraph): “The structural damage of this variant to protein functionality was analyzed by PolyPhen ([http://coot.embl.de/PolyPhen/](http://coot.embl.de/PolyPhen/)), which predicted the change to be benign (PSIC score difference=1.350). However, the program does not take into account the fact that the mutation is in the start codon [29].”

6. As the reviewer correctly mentioned, lymphoblastoid cell lines may not resemble the whole set of active genes in prostate tissue, and therefore, the authors might have missed relevant variants. The following piece of text has been added to the manuscript (Discussion, fourth paragraph): “In addition, relevant variants might have been missed by being limited to use lymphoblastoid cell lines, as they may not resemble the whole set of active genes in prostate tissue. Although there is substantial amount of evidence that lymphoblastoid cells encompasses a variety of metabolic pathways that are specific to individuals where the cells originated, making these cell lines suitable for individuals where the cells originated, making these cell lines suitable for molecular and functional studies [30].”

We thank the reviewer for the constructive criticism and believe that our detailed responses have strengthened the paper. We hope that the manuscript is now acceptable for publication in the BMC Cancer.

We look forward to hearing from you.

Sincerely,

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