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

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Reviewer: Christiane Maier

Reviewer's report:

The manuscript by Mattila, et al. is focused on an X-linked prostate cancer risk locus, designated HPCX1, that was previously identified by classical approaches of genetic epidemiology. The causal gene is still unknown. The present study implemented two alternative, rather sophisticated strategies in order to identify causal variants within this region. The ingenious GINI assay for capturing premature stop-mutations in coding genes by chemical blocking of the cellular nonsense-mediated mRNA decay was used in addition to miRNA profiling of the candidate HPCX1 locus. Both methods are more sensitive when applied to X-chromosomal loci, and were carried out on lymphoblastoid cell lines from hereditary prostate cancer cases with evidence of linkage to HPCX1. The highlighted finding is an uncommon, presumably deleterious mutation in the translation start site of the MAGEC1 gene. The variant was further found to be enriched in cohorts of hereditary and unselected prostate cancer cases as compared to several large control groups. Differentially expressed miRNAs also point at a relevance of MAGEC1. In summary, the choice of promising methods together with the evidence for the emphasized candidate gene merits publication.

Discretionary revisions:

1) Aim

Introduction last paragraph, reads “… we have characterized the HPCX1 locus by NMD and miRNA microarray methods and evaluated the role of HPCX1 in the causation of HPC.” The role of the locus in HPC seems not concerned much in this study. Part of sentence could be omitted.

2) number of families used for miRNA analysis

Materials and Methods, Study population, second paragraph states six plus five families were analysed. This seems not consistent with Table 1.

3) candidate region

For considering candidate gene / miRNA selection by location, it would be interesting to know how the boundaries of HPCX1 were defined.

4) miRNAs targeting MAGEC1 (and other candidate genes at HPCX1)

For miRNA target detection, as described in Materials and Methods, sequences were considered where genetic variants alter potential annealing sites. This is a
commendable approach in the context of seeking causes for HPC, and thus, this important information could be given/repeated in other prominent parts of the manuscript as well. Especially in the results section, the link between variation sites found in candidate genes and miRNA is not described very well and would be worth presenting. Perhaps by cross-references between Tables 3 and 5 (?), or even in an additional Table (?).

5) on the MAGEC1 variant p.Met1?

a) Is it expected that a translational start failure would be capable by the GINI approach, as NMD depends on one initial round of ribosomal reading? Anyway, the results suggest so and we should believe. For curiosity however, it would be interesting to learn if there are alternative start sites or open reading frames in the mutated transcript, and hence, how the NMD may have occurred.

b) A damage prediction programme, which does not consider start codons as potentially relevant features for generating proper proteins may not be worth mentioning at all (discussion, third paragraph). The authors’ attentive review of literature concerning exceptional start codons seems much more reliable. Be confident about this!

6) study limit lymphoblastoid cell lines

Lymphobastoid cell lines may not resemble the whole set of active genes in prostate tissue, thus, relevant variants might have been missed. It is absolutely clear, for the purpose of the GINI assay that requires drug treatment, no other cells than lymphocytes could be taken into culture from these highly selected probands. Nevertheless, mentioning this study limit for the sake of integrity would not derogate the scientific merit of the paper.

Level of interest: An article of outstanding merit and interest in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests.