Reviewer’s report

Title: The application of nonsense-mediated mRNA decay inhibition to the identification of breast cancer susceptibility genes

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Reviewer: Fabienne Lesueur

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Reviewer’s comments:

Nonsense-mediated decay (NMD) degrades both normal and aberrant transcripts harbouring stop codons in particular context. Gene identification by nonsense-mediated mRNA decay inhibition (GINI) has proved successful in identifying nonsense mutations in several cancers but the approach has not been applied to identify new highly penetrant breast cancer susceptibility genes. In this paper, the authors applied GINI to LCLs from three multiple-case breast cancer families negative for BRCA1/2, and then used Illumina gene expression microarrays to identify transcripts stabilised by NMD inhibition by caffeine agent.

This is a well-written manuscript that addresses an important question with an interesting methodological approach, despite the limits of the sensitivity of the technique and its application to transformed cells.

Major compulsory revisions:

1. In the Abstract, it is stated that the authors applied GINI on heterozygous LCLs from high-risk breast cancer patients. Why and for what markers the selected LCLs have to be heterozygous?

I think the authors mean that they are aiming to identify patients who would be heterozygote for a truncating mutation in a novel gene. In such case, the sentence may be reformulated or the word heterozygous could simply be removed here.

Similarly, the second paragraph in the Discussion (page 15) is a bit confusing and should be re-written. Again, it is expected that pathogenic mutations in novel yet-unidentified high-risk breast cancer genes (or other autosomal dominant disorder) will be present in a heterozygous state in the affected subjects, but having heterozygote cell lines for a specific marker is not a requirement to be able to apply the GINI technique.

2. It would be interesting to show Pedigree B (for example on Figure 5) and to indicate on the Figure the family members who present an up-regulation of PPARGC1A, and who carry the intronic variant. Have the authors tried to determine which of the two alleles is up-regulated using a coding SNP (e.g. Thr612Met)?

Was the gene PPARGC1A mutation screened in Family C? Has any coding
variant/SNP been found?

3. In the Discussion, the authors argue that the NMD inhibition by caffeine may affect differently the stabilization of the two transcript of PPARGC1A. Were primers designed to amplify specifically one of the two transcripts in the qRT-PCR experiment for the validation of the Illumina results? Are the Illumina probes on the array tagging the 2 transcripts?

Minor essential revisions:

Page 7, line 4: “We then added fresh medium…” (and lines 8 and 9).

Page 10, line 3 and line 16: indicate amount of DNA rather than volume.

Page 10, line 11 and page 12, line 24 change “2MB region” for “2Mb region”.

Page 12, line 7: it is indicated that 6 candidate genes have been identified in Family C whereas Figure 3 indicate 5 up-regulated genes after caffeine treatment.

Page 12, line 24: missing word? Please rephrase.

Page 15: “LCLs established from peripheral blood mononuclear leukocytes…”

Page 16: the term SMD is not employed in the text of the manuscript.

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

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.