Reviewer's report

Title: Rapid detection of carriers with BRCA1 and BRCA2 Ashkenazi founder mutations using high resolution melting analysis.

Version: 2 Date: 12 December 2007

Reviewer: Anna Jakubowska

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Major Compulsory Revisions

Takano et al. in a manuscript "Rapid detection of carriers with BRCA1 and BRCA2 Ashkenazi founder mutations using high resolution melting analysis" evaluated the use of high resolution melting analysis (HRM) methodology in detection three Ashkenazi BRCA1/2 founder mutations.

HRM analysis has been preformed for two exons of the BRCA1 gene and part of the exon 11 of the BRCA2 gene in a series of patients carrying earlier detected mutation in the tested regions. All the Ashkenazi (185delAG and 5382insC in BRCA1, and 6174delT in BRCA2) as well as other mutations were reproducibly detected and readily identified.

Authors concluded that HRM is a simple effective rapid scanning method for known and unknown BRCA1 and BRCA2 germline mutations that can dramatically reduce costs and time for mutation screening and testing. Despite I see this paper is important and useful, I do have some minor comments and suggestion which could improve it.

Comments:

I do not agree with statement that "Currently, these three mutations are identified using sequencing". The founder mutations are routinely detected not only by sequencing but also by other, very sensitive methods based on PCR (e.g. ASA-PCR), real-time PCR (taqman, simpleprob or hybprobe analyses) or just normal hybridisation (ASO).

Methods and result

How many samples with known mutations have been tested by HRM?

All tested mutations should be listed and number of patients with a target mutation should be given. There is nothing written about results of ex 20 BRCA1 and ex11 BRCA2. The figures helped to see what was done but this should be also written in the result section.

Discussion

The discussion should be improved:

1. Giving a general statement, authors should always include an appropriate reference, eg. last sentence, 2nd paragraph: "Both DHPLC and DGGE are
both operator and interpreter sensitive and probably miss about 5% of sequence
detectable mutations## the word ##probably## should be removed; 2nd
sentence, 3rd paragraph: ##For germline mutations in the BRCA1 and BRCA2
genes, all the mutations are expected to occur in heterozygotes as the mutant
homozygotes are lethal## and so on##;

2. if authors are writing about costs, the overall prices and comparisons to the
expenses in applying other methods should be given

3. Authors suggest that routinely the three Ashkenazi mutations are identified
using sequencing, this is not correct (see major comment)

4. Regarding the primer design, does it matter position of mutation in amplicon?
How big can be ampicons for effective HRM analysis?

As HRM, similarly to DHPLC, is a method based on analysis of melting profile of
heteroduplexes and both are a screening methods followed be sequencing of
detected variants, the simple description of advantages of HRM technique over
DHPLC would be useful.

What next?: Unable to decide on acceptance or rejection until the authors have
responded to the major compulsory revisions

Level of interest: An article of importance 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