Reviewer's report

Title: Simplifying the detection of MUTYH mutations by high resolution melting analysis

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Reviewer: Maartje Nielsen

Reviewer's report:

Dear editor,

This manuscript present some interesting results. It describes the high resolving melting analysis for detecting MUTYH mutations. This relatively new technique is being used especially for more complex genes such as BRCA1/2 and APC. The text is not very well structured en the research question is not well posed. Is the question to test whether HRM analysis can detect MUTYH mutations well enough or is the question how many mutations are detected in a cohort of polyposis patients or both? In discussion also an association analysis is presented (relation MUTYH mutations and CRC and polyps and family history), which was also not mentioned as a research question in the introduction.

Major comments:

• It would be better if the HRM data were compared with sequence data of the used samples, to verifyicate the mutations found (and not found). I.e. what is the detection rate using sequencing as the golden standard? The authors mention that all samples were sequenced, but do not clearly show the comparison between sequence data and HRM data.

• For detection of homozygote mutations it is essential to mix with wild type PCR, because otherwise detection can be below 85%, which is not acceptable in a diagnostic setting. The approach used by authors is not clearly described in the methods section.

• Also, with melting analysis of only three exons, a number of mutations is being missed (about 17-33% depending on the country of origin).

• The MUTYH gene is a relatively simple (small) gene to analyse, which leaves the questions whether melting analysis is really more efficient than normal sequence analysis (the authors state a reduction sequence reactions of up to 80%, but what does this mean in cost?).

Abstract: authors state that the technique is about to show its effectiveness in detecting homozygous mutations, but this is not further clarified.

Introduction:

• The introduction is not clearly structured; the order of the sentences seems not logic.
• In the introduction the authors should clarify in what respect their used technique differs from the ‘normal’ application HRM-PCR. Why are the authors able to identify homozygote mutations, when previously this was not the case (according to the authors)?

Results
• Authors describe phenotype-genotype features, and state for example that biallelic carriers are associated with being a carrier of synchronous polyps. But was this not one of selection criteria in the first place? And why is this finding of importance? This comment also applies to table 7.

Minor comments
General:
• The gene name should be written in Italic (MUTYH instead of MUTYH). Sometimes the authors still use MYH instead of MUTYH. Because MYH is already in use for another gene (Myosin Heavy chain) this term should not be used anymore.
• The numbers shown have two numbers behind the point. This preciseness does not add to the relevance of the outcomes, so it would be better to round it up to one number or no numbers after the point.
• In numbers a point instead of a comma should be used.
• The authors use the abbreviation p. (indicating that the mutation shown is amino acid notation) not consequently throughout the text.

Introduction:
• It would make more sense if the sentence “the importance of identifying these mutations…pattern” was placed directly after “however, mutant homozygotes…MUTYH gene”
• ‘Thus this application provides an easy platform to detect germline mutations.in MUTYH ” Which application? How is this an easy platform?
• “In fact, these are responsible…..in the APC gene”. What do the authors mean with “these? A reference is also missing.

Methods
• What were the selection criteria of the analysed 82 patients?

Results
• ‘Patients carriers” patients carrying?
• The sentence “the melting profile of a wild type …sensitivity 0,3” is more appropriate for the methods section.

Discussion
• ‘However, we believe it feasible ….. in the 82 cases’ What do the authors mean with however?
The following text is not completely clear and should be reformulated.

‘By discriminating between distinctive individuals based on their features high resolution melting curves, one of the most challenging tasks is the discrimination of amplicons differing by homoduplexes, not only between heteroduplexes and homoduplexes. HRM is a rapid and informative toll but requires a fine and conscientious post-PCR analysis, in the case of differing by homoduplexes; but not in the case between homoduplex and heteroduplex.’

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

**Quality of written English:** Needs some language corrections before being published

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