Author's response to reviews

Title: High resolution melting analysis for a rapid identification of heterozygous and homozygous sequence changes in the MUTYH gene

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

We are now submitting a revised version of the manuscript entitled “High resolution melting analysis for a rapid identification of heterozygous and homozygous sequence changes in the MUTYH gene” by Rossella Tricarico et al.

We thank the distinguished reviewers for their helpful comments and suggestions, which have allowed us to improve the quality of the manuscript. These have been addressed as shown at the bottom of this letter.

As requested, we have now explicitly stated that the study was conducted in accordance with the principles of the Helsinki declaration and in accordance with guidelines from the local ethical committee.

We hope that the manuscript is now acceptable for publication on BMC Cancer.

Yours sincerely,

Maurizio Genuardi, M.D.

RESPONSES TO REVIEWERS’ COMMENTS

Reviewer 1. As requested, we have now specified in the text that the same primers have been used for HRMA and sequencing.

Reviewer 2.
1. An algorithm for HRMA-based mutation detection is now proposed in the Conclusions Section.

2. We have now added inclusion criteria for the initial and validation sets. The initial group of cases had been selected because of the previous finding of MUTYH variants using other methods (cases) or because of a negative personal and family history of polyps and colorectal cancer. This explains why the rate of positive cases was much higher in in the subgroup of patients of the first set; therefore we think that specification of the inclusion criteria for the first set could be misleading.

3. While we appreciate that clinical information can be of interest to some readers, the focus of this manuscript is essentially on a novel HRMA-based molecular screening approach for the detection of MUTYH mutations. Therefore, we think that addition of clinical details would be out of scope.

4. We modified the designation of the c.1023_1024insGA and c.[1187-27C>T(+1258C>A)] variants in Table 1, as requested. We have also specified that the phase is unknown in footnote 3 of the Table.

5. Three references (Nielsen et al., 2009; Terdiman, 2009, Vogt et al., 2009: reported as ref. 5-7) have been added to provide documentation of phenotypic variability of MAP, in the Introduction (third paragraph) and Discussion (last paragraph). Subsequent reference numbers have been modified accordingly.

6. The sensitivity of HRMA without mixing with reference DNA in the initial and validation sets is now reported in the sixth and ninth paragraphs of the Results section.
7. While we appreciate that classification of the variants based on their pathogenicity/neutrality would be of interest, the focus of the manuscript is on the technical approach to variant identification. We do not discuss this issue in the text, if not marginally, and we think that addition of further information would be out of scope.

8. We thank the reviewer for pointing out this mistake, which has now been corrected.

9. Reference to the MUTYH gene has now been added in the second paragraph of the Abstract, as requested.

10. We have now added references on phenotypic variability (see response to point 5 above).

Minor issues:

1a. the comma after “Primers” has been removed from the text.

1b. the sentence has been changed into: “All variants were identified by SSCP, with the exception of the c.64G>A and c.1014G>C substitutions; these were detected in the heterozygous and homozygous state, respectively, only by sequencing.”

1c. the sentence has been changed into: “In particular, HRMA allowed the detection of heterozygous and homozygous sequence changes (c.64G>A and c.1014G>C) that had not been identified by SSCP.”

2. We have now changed the sentence into: “The remaining fraction of MUTYH variants identified in MAP patients is highly heterogenous and can be located along the whole coding sequence.”

3. A comma has been deleted, and this is the new version of the second sentence of the paragraph: “In addition to the c.1187G>A mutation, other sequence variations in exon 13 were recognized (Figure 2).”

4. The initial sentence of the legend specifies that experiments were performed without mixing.