Author's response to reviews

Title: A novel pathogenic MLH1 missense mutation, c.112A>C, p.Asn38His, in six families with Lynch syndrome

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Author's response to reviews: see over
Dear dr. Sijmons,

Please find enclosed the revised manuscript “A novel pathogenic MLH1 missense mutation, c.112A>C, p.Asn38His, in six families with Lynch syndrome” by E. van Riel, MGEM Ausems, FBL Hogervorst, I Kluijt, ME van Gijn, J van Echtelt, K Scheidel-Jacobse, EFAM Hennekam, RP Stulp, YJ Vos, GJA Offerhaus, FH Menko and JJP Gille. The manuscript has been revised according to the comments of the reviewers.

We would like to comment to the reviewers suggestions:

Reviewer1 (PP):
1. We indicated in Table 1 which individuals were directly tested for the UV and for which individuals complete sequencing of the MLH1 gene was performed.
2. Just as the reviewer, we were surprised by the results of the immunostaining. With immunochemistry, we look at the presence of protein regardless of functionality of the concerning protein. A missense mutation can give a positive immunostaining, when the antibody is able to bind to a nonfunctional protein. Another explanation could be that this missense mutation influences the stability of the MLH1-PMS2 complex, with loss of PMS2 staining as a result. This has been earlier described by Plotz et al (Nucleic Acids Res. 2006 December; 34(22): 6574–6586). Why PMS2 staining in absence of MLH1 staining occurs is difficult to explain. In theory, it could be that the MLH1 protein is still able to bind to PMS2 and form a complex, while the epitope recognized by the antibody has been lost. It is possible that second hits determine if there is staining or not. The process of second hit can differ between tumors, which could explain different pattern of immunostaining in tumors from individuals with the same missense mutation. Also, the possibility of an artefact can not be completely ruled out in this specific case.
3. The size of the conserved disease haplotype is 3.9 Mb. We did not
investigate if the haplotype extends beyond this area.

Reviewer2 (HM):
Table 1 and the figures are revised according to the comment of the reviewer. We also included a legend of the used abbreviations in the revised manuscript.

We would like to thank the reviewers for the useful suggestions. We trust that this revised manuscript will meet the wish of the reviewers and hope that you will find it suitable for publication in Hereditary Cancer in Clinical Practice.

Yours sincerely, also on behalf of the co-authors,

Ms. dr. E. van Riel
Genetic Counselor