PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Germline Mutations in PMS2 and MLH1 in Individuals with Solitary Loss of PMS2 Expression in Colorectal Carcinomas from the Colon Cancer Family Registry Cohort
AUTHORS	Rosty, Christophe; Clendenning, Mark; Walsh, Michael; Veriksen, Stine; Southey, Melissa; Winship, Ingrid; Macrae, Finlay; Boussioutas, Alex; Poplawski, Nicola; Parry, Susan; Arnold, Julie; Young, Joanne; Casey, Graham; Haile, Robert; Galinger, Steven; Le Marchand, Loic; Newcomb, Polly; Potter, John; DeRycke, Melissa; Lindor, Noralane; Thibodeau, Stephen; Baron, John; Win, Aung; Hopper, John; Jenkins, Mark; Buchanan, Daniel

VERSION 1 - REVIEW

REVIEWER	Leigha Senter The Ohio State University, U.S.A.
REVIEW RETURNED	11-Dec-2015

GENERAL COMMENTS	This is a description of genetic testing outcomes for colorectal cancer cases with selective loss of PMS2 protein on IHC. It is well-accepted that the primary cause of this MMR IHC profile is a germline mutation in PMS2, which is confirmed by this study. Given that not all individuals with this MMR IHC profile are found to have a germline PMS2 mutation, though, the authors analyzed MLH1 systematically and report a higher frequency of MLH1 mutations in this group than previously estimated (12%). Overall, the manuscript adds to what we understand about tumor screening for Lynch syndrome and could impact clinical approach to genetic testing. My only suggestion for edit would be to remove all reference to and description of the large CFR cohort that were not part of this paper. The reference to the 5707 CRC's is a bit distracting and I think the paper would be clearer with a focus on the tumors that showed isolated absence of PMS2 only. It is also unclear to me how MLH1 promoter hypermethylation adds to this particular subset. With retention of MLH1 protein, this would not be a typical step in Lynch screening. As such, it would be helpful to authors to briefly explain their rationale in including this analysis in indivudals with loss of PMS2 protein only on IHC.

REVIEWER	Jinru Shia Memorial Sloan Kettering Cancer Center USA
REVIEW RETURNED	13-Dec-2015

GENERAL COMMENTS	I think the study is pertinent. The cases presented expand the current knowledge and confirm pathogenicity of some MLH1 mutations that are associated with isolated PMS2 loss on IHC. Just 2 comments: 1. In the introduction, the authors “hypothesized that germline mutations in MLH1 may underlie a proportion of CRC with solitary loss of PMS2 expression”. But this is no longer a novel assumption or hypothesis. This is a well documented phenomenon (known in the field of Lynch syndrome for at least a decade now). I think it would be good if the authors acknowledge this and mention that the contribution of their current study is confirmatory with further evidence of pathogenicity of the associated MLH1 mutations. 2. Related to the comment above, I think it would be very helpful if the authors could summarize all reported cases (including the associated MLH1 mutations and InSiGHT classification) in table format (same format as the current Table 1).

REVIEWER	Sanne W. Ten Broeke, MD PhD student at Department of Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands
REVIEW RETURNED	22-Dec-2015

GENERAL COMMENTS	This is a well-performed study which is useful for clinical practice and discusses strengths and limitations very adequately. I advise acceptance pending some minor revisions which are listed below: 1. The authors write that there was no complete mutation screening of 22 PMS2 deficient CRCs in their database and that they were for this reason excluded, but do not mention why germline testing wasn’t performed. This might lead to an overestimation in mutation detection either in MLH1 or PMS2, which can be important as the yield of detected mutations is quite high. 2. I found it notable that MLH1 promotor methylation was found in these tumours with solitary PMS2 abrogation. This is an interesting finding for clinical purposes as it means that it is useful to test for methylation in these tumours to exclude the possibility of Lynch syndrome. I would suggest adding this to the discussion. 3. It would be nice to include a comparison in table 2 with tumours of MLH1 mutation with abrogation of both the MLH1 and PMS2 protein. 4. A statement on the lower penetrance of PMS2 mutations is made on page 16, but it’s missing references: ‘…; this is of particular interest clinically, where the PMS2 gene has lower penetrance than other MMR genes and family history is a suboptimal way of finding potentially high-risk families...’.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1 (Leigha Senter)

1. My only suggestion for edit would be to remove all reference to and description of the large CFR cohort that were not part of this paper. The reference to the 5707 CRC's is a bit distracting and I think the paper would be clearer with a focus on the tumors that showed isolated absence of PMS2 only.

Response: We shortened the method section to focus on the study cases and included the immunohistochemistry and molecular testing in the 'study participant' section. Two references have been removed (ref 12 and 13 from the original submission). The first sentence of the 'Results' has been changed to remove the reference to the 5707 CRC cases.

2. It is also unclear to me how MLH1 promoter hypermethylation adds to this particular subset. With retention of MLH1 protein, this would not be a typical step in Lynch screening. As such, it would be helpful to authors to briefly explain their rationale in including this analysis in indivudals with loss of PMS2 protein only on IHC.
Response: MLH1 methylation and BRAF mutation testing were performed to address the possibility of MLH1 methylation (sporadic cases) underlying some of PMS2-deficient cases. Our findings of 4 cases with MLH1 methylation of BRAF mutation indicate that isolated PMS2 loss can be seen in the context of sporadic tumours. We added a section in the discussion to address this result and put it in clinical context (see comment 2 reviewer 3 below).

Reviewer 2 (Jinru Shia)

1. In the introduction, the authors "hypothesized that germline mutations in MLH1 may underlie a proportion of CRC with solitary loss of PMS2 expression". But this is no longer a novel assumption or hypothesis. This is a well documented phenomenon (known in the field of Lynch syndrome for at least a decade now). I think it would be good if the authors acknowledge this and mention that the contribution of their current study is confirmatory with further evidence of pathogenicity of the associated MLH1 mutations.
Response: This sentence in the last paragraph of the introduction and the abstract (objectives) have been modified accordingly.

2. Related to the comment above, I think it would be very helpful if the authors could summarize all reported cases (including the associated MLH1 mutations and InSiGHT classification) in table format (same format as the current Table 1).
Response: The supplementary table 2 lists the results of the 49 individuals with a PMS2 germline mutation with a similar format as the table 1. In the revised version, we added the 6 cases with no identified mutation at the end of supplementary table 2. We rather keep these cases separate from the 11 cases with MLH1 mutation (table 1) to avoid a long table with all 66 cases in the main manuscript.

Reviewer 3 (Sanne W. Ten Broeke)

1. The authors write that there was no complete mutation screening of 22 PMS2 deficient CRCs in their database and that they were for this reason excluded, but do not mention why germline testing wasn't performed. This might lead to an overestimation in mutation detection either in MLH1 or PMS2, which can be important as the yield of detected mutations is quite high.
Response: Reason for exclusion of these 22 CRC cases are listed in the flow diagram (Figure 1). For the majority of cases, the absence of PMS2 mutation screening was due to unavailability or insufficient DNA to perform the complete analysis. We believe this is unlikely to include any bias in the overall mutation detection rate.

2. I found it notable that MLH1 promotor methylation was found in these tumours with solitary PMS2 abrogation. This is an interesting finding for clinical purposes as it means that it is useful to test for methylation in these tumours to exclude the possibility of Lynch syndrome. I would suggest adding

this to the discussion.
Response: We added a section in the discussion (last paragraph p.16) to address this result.

3. It would be nice to include a comparison in table 2 with tumours of MLH1 mutation with abrogation of both the MLH1 and PMS2 protein.
Response: This is an interesting comment. We decided to focus on cases with isolated PMS2 loss for this study and made it even more focussed in the revised manuscript to address reviewer's 1 comment. To compare with MLH1 mutation carriers with a MLH1/PMS2 deficient CRC would require substantial additional work that would be beyond the scope of this study.

4. A statement on the lower penetrance of PMS2 mutations is made on page 16, but it's missing references: '…; this is of particular interest clinically, where the PMS2 gene has lower penetrance than other MMR genes and family history is a suboptimal way of finding potentially high-risk families...'.
Response: A reference has been added (Senter et al. Gastroenterology).

VERSION 2 – REVIEW

REVIEWER	Leigha Senter The Ohio State University, USA
REVIEW RETURNED	26-Jan-2016

GENERAL COMMENTS	The authors have adequately addressed reviewer comments, in my opinion. This is a nice paper that will be a nice contribution to the literature in this area.

REVIEWER	Jinru Shia, MD Memorial Sloan Kettering Cancer, New York, NY, USA.
REVIEW RETURNED	20-Jan-2016

GENERAL COMMENTS	The reviewer completed the checklist but made no further comments.

REVIEWER	Sanne W. ten Broeke Leiden University Medical Center, Leiden, The Netherlands
REVIEW RETURNED	27-Jan-2016

GENERAL COMMENTS	The reviewer completed the checklist but made no further comments.