Author’s response to reviews

Title: Prevalence and Spectrum of MLH1, MSH2, and MSH6 Pathogenic Germline Variants in Pakistani Colorectal Cancer Patients

Authors:

Muhammad Rashid (usmanr@skm.org.pk)
Humaira Naeemi (bslab1@skm.org.pk)
Noor Muhammad (bslab@skm.org.pk)
Asif Loya (asifloya@skm.org.pk)
Jan Lubiński (lubinski@sci.pam.szczecin.pl)
Anna Jakubowska (aniaj@sci.pum.edu.pl)
Muhammed Yusuf (aasim@skm.org.pk)

Version: 1 Date: 02 May 2019

Author’s response to reviews:

Prof Rodney Scott
Editor-In-Chief

Dr Gabriela Moeslein
Associate Editor

Hereditary Cancer in Clinical Practice

Re: Resubmission of the manuscript: HCCP-D-18-00030

Dear Sir/Madam,

Enclosed please find the revised manuscript entitled "Prevalence and Spectrum of MLH1, MSH2, and MSH6 Pathogenic Germline Variants in Pakistani Colorectal Cancer Patients" (HCCP-D-18-00030) and our responses to the comments of the reviewers. We would like to express our appreciation for the input given by the reviewers, which enabled us to improve the manuscript.

We hope the revised manuscript is now acceptable for publication in Hereditary Cancer in Clinical Practice. All amendments in the text are marked in blue.

I greatly appreciate your consideration of our study. Please do not hesitate to contact me for further clarification.

Yours sincerely,
Muhammad Usman Rashid

Point-by-point response to the comments of the reviewers (Acceptance after minor revision)
HCCP-D-18-00030

“Prevalence and Spectrum of MLH1, MSH2, and MSH6 Pathogenic Germline Variants in Pakistani Colorectal Cancer Patients”

Reviewer #1:

General comments

Point 1: The term hereditary nonpolyposis colorectal cancer (HNPCC) was coined to distinguish familial aggregation of CRC from the polyposis phenotypes. The HNPCC subset of CRC families is heterogeneous and broadly consists of the 4% linked to LS (which may or may not fulfill the Amsterdam Criteria), <1% with a Lynch-like syndrome, and 2-4% classified as familial colorectal cancer type X (FCCTX).

According to the reviewer’s suggestion, we have incorporated the required details (see Background section, page 4, 1st paragraph, lines 6-12).

Point 2: It would be more appropriate to use the term LS, when a defective mismatch repair (MMR) system due to the presence of pathogenic variants in at least one of the MMR genes (path_MLH1, path_MSH2, path_MSH6 and path_PMS2) or due to deletions of the 3’ portion of the EPCAM gene is the cause.

As per reviewer’s recommendation, we have incorporated the required details (see Background section, page 4, 1st paragraph, lines 13-14).

Point 3: It would have been more appropriate to use the term "variant" throughout the manuscript, which is in line with the standards and guidelines for the interpretation of sequence variants [e.g. Richards et al, 2015]. The term "variant" should replace the term "mutation" or "polymorphism" with the following modifiers: (i) pathogenic, (ii) likely pathogenic, (iii) uncertain significance, (iv) likely benign, or (v) benign.

As per reviewer’s recommendation, we have used the term “variant” throughout the manuscript including the title. Furthermore, 5-tier classification is also included (see Method section, page 7, 3rd paragraph, lines 10-20).

Point 4: Genetic analysis have not included PMS2 gene, which can be briefly mentioned.
We have briefly mentioned about PMS2 gene (see Discussion section, page 18, 2nd paragraph, lines 4-7).

Please note that PMS2 gene screening was not performed in our study due to several reasons. First, About 95% of all reported LS germline pathogenic variants are identified in MLH1, MSH2 and MSH6 genes (Kantelinen et al. 2011; Plazzer et al. 2013; Peltomäki P 2016; Lynch et al. 2003; Woods et al. 2007). Conversely, PMS2 variants have been rarely reported (Truninger et al. 2005; Hendriks et al. 2006; Steinke et al. 2014). Hence, it is usually...
appropriate to first analyze patients for MLH1, MSH2 and MSH6 genes. Second, comprehensive PMS2 gene screening is difficult due to the presence of 15 highly similar pseudogenes that closely flank the PMS2 gene (De Vos et al. 2004).

Specific comments

-Introduction

Include the general comments
The general comments are now included as mentioned above in Points 1-4.

-Methods

Point 5: Molecular analysis: The results from DHPLC, have been validated by another method?

Yes, the results from DHPLC have been validated on an independent sample by bidirectional genomic DNA sequencing (see Methods section, molecular analysis, page 7, 1st paragraph, lines 1-4).

Point 6: In silico analyses: VUS are relatively common, and complicate the interpretation of gene test results. There is a strong need to classify these VUS as either benign or deleterious. The use of bioinformatics tools combined with novel functional assays will be important for the interpretation of next generation sequencing analyzes of high numbers of genes, and the diagnostic assessment of cancer associated genetic variants

Please note that 4 VUS were identified in our study. Of these, 3 VUS were classified as Benign based on in silico analyses. One VUS is classified as likely pathogenic based on the use of bioinformatics tools combined with functional assay. (see Method section, In silico analyses, page 7, 4th paragraph, lines 22-25; page 8, 1st paragraph, lines 1-13 and Table 5).

Point 7: The links (Align_GVGD, SNAP, MaxEntScan, NNSPLICE) are not working.
The links are updated (see Methods section, In silico analyses, page 8, 1st paragraph, lines 1,5,9 and 10).

Point 8: A section about the classification of the genetic variants found in the study (e.g. 5 tier-classification, etc) should be included.

According to the reviewer’s suggestions, we have included a section about the 5 tier-classification of the genetic variants found in the study (see Methods section, Classification of MMR gene variants, page 7, 3rd paragraph, lines 10-20).

- Results

Point 9: MSH2 germline mutations: Cannot rule out that the nonsense MSH2 variant is unique to the Pakistani population. Need to confirm/validate in a larger number of cases.

Pathogenic MSH2 germline variant, c.2656G>T, is likely to be specific to the Pakistani population as it has not been reported in other populations (see Discussion section, page 14, 2nd paragraph, lines 20-21).
Point 10: Suggestion to change the headline MLH1, MSH2 and MSH6 sequence variants to Novel and VUS MMR variants. VUS need to be previously defined.

As per reviewer’s suggestion, we have now changed the headline to “Other MMR gene variants: novel and previously reported” (See Results section, page 12, 2nd paragraph, lines 6; page 13, 1st paragraph, lines 1-8).

We have also defined VUS (see Method section, page 7, 3rd paragraph, line 12; Table 5; and discussed VUS (see Discussion section, page 16, 1st paragraph, lines 6-20).

- Discussion

Point 11: In general, the results presented in the present study highlight the challenge associated with using family history for detecting families with pathogenic MMR variant. There is a recommendation on the application of population-based screening protocols for all CRC and endometrial cancers diagnosed below age 70 using IHC of the MMR proteins. Nonetheless, patients with a young age of onset and/or a positive family history of LS-associated cancers without an identified pathogenic MMR variant, may suggest the involvement of pathogenic variants in as yet undiscovered genes.

According to the reviewer’s suggestions, we have incorporated the details about the study cases with undetected MMR gene variants and the possibility of the involvement of pathogenic variants in as yet undiscovered genes (see Discussion section, page 18, 2nd paragraph, lines 8-11).

Reviewer #2:

Point 1: Hereditary nonpolyposis colorectal cancer (HNPCC) refers to patients and/or families who fulfill the Amsterdam criteria. Lynch syndrome refers to patients and families with a germline mutation in one of the DNA mismatch repair genes (MLH1, MSH2, MSH6, PMS2) or the EPCAM gene. Those can’t be used interchangeably. Not all HNPCC may be named Lynch syndrome.

You wrote "HNPCC is caused by germline mutations in DNA mismatch repair” look on definition what is HNPCC. Lynch syndrome is caused by mutation of MMR genes. In manuscript it have to be corrected in all places

We thank the reviewer for this important point. We have included the valid terminologies of HNPCC and Lynch syndrome (see comments to Reviewer’s 1, Points 1-2; see Background section, page 4, 1st paragraph, lines 7-10 and 13-14).

Point 2: Interesting is novel substitution c.116+3A>T. wasn’t it possible to make cDNA and check the impact of the mutation on mRNA sequence. In this case it will clarify the doubts

We thank the reviewer for this important suggestion but could not clarify it due to lack of blood sample. Further evidence for the impact of c.116+3A>T variant on aberrant mRNA splicing could not be provided because of unavailability of blood sample from the carrier for RNA isolation and subsequent cDNA analysis. This statement is added in the manuscript (see Discussion section, page 15, 2nd paragraph, lines 20-22).
References


