

Author's response to reviews

Title: The association between genetic variants in hMLH1 and hMSH2 and the development of sporadic colorectal cancer in the Danish population

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Author's response to reviews: see over

Comments to Reviewers report and specific details requested by the BMC editorial office.

Reviewer 1:

1. Page 9: Likewise the Bonferroni corrected p-value of the cohort of sporadic cases was $0.05/15=0.0038$. --> $0.05/15=0.0038$ should be $0.05/13 = 0.0038$.

Author comment: correction has been made

Reviewer 2

1. It is not clear why the HNPCC cohort was tested and what is the relevance of the results obtained on it in the context of the main aim of the study. No information is provided on mutation and MSI/immunohistochemistry status in this cohort. A few variants were present only in this group, but these could be pathogenic, as also indicated in the text and in Table 2. It would be appropriate to omit this data, which perhaps could be handled in a separate manuscript provided accurate phenotypic characterization is available.

Author comment: We acknowledge that the term HNPCC-suspected cohort is misleading. We have changed the name of the cohort to familiar CRC. This cohort comprises HNPCC families (based on the Amsterdam II criteria) where no conclusive mutation has been identified in MLH1, MSH2 and MSH6. In addition, the cohort also contains families not fulfilling the Amsterdam II criteria but with a clear accumulation of individuals with CRC (this description has been added to the Methods section). We have included this cohort to analyze whether a common variant could explain the elevated cancer susceptibility in these families (has been added to page 6 in the methods section). We do think that data on this cohort is important but it can be omitted if it is the wish of the reviewers.

2. The introduction is too long and difficult to read.

Author comments: The introduction has been shortened (before app. 4500 characters, after app 3000) and subdivided into paragraphs.

3. Page 9 Statistics, Bonferroni correction

Author comment: correction has been made

4. Page 10: Number of HNPCC suspected individuals.

Author comment: 311 has been changed to 285 as in the “material and methods”

5. Page 13: last paragraph of the Results section: “...several of the variants either abolish or introduce ...”; it would be appropriate to add

potentially ##, since, as also stated by the authors, programs currently used for ESE prediction are not highly reliable.

Author comment: potentially has been added.

6. The authors state that functional analyses could be helpful to establish the role of two MSH2 missense variants. MSI and immunohistochemistry data could be used as a surrogate to this purpose, and it would be important to know whether they have been performed on tumor samples from the variant carriers.

Author comment: We are currently performing functional analysis on these and other missense mutations. These analyses also include a detailed study on the families where the missense mutations have been identified. Consequently, IHC and MSI analysis are not available at the moment, but will of course be included in a future publication on the results of the functional analysis.

Reviewer 3

1. The authors state that they searched for mutations in MLH1 and MSH2 in "patients suspected to have HNPCC". There is no further information in the manuscript which criteria they have used and on whether the patient's tumour tissues have been analyzed for microsatellite stability as well as expression of the MMR proteins by immunohistochemistry. To assess the functional relevance of a germline variant/mutation in MLH1 and MSH2 these are essential information that needs to be provided.

Author comment: The variants included in the present study have been included independent on whether IHC or MSI analysis had been performed on tumor tissue from patients carrying the variant. There are several reasons for that approach: 1) missense mutations increasing cancer susceptibility do not necessarily cause lack of protein in tumor tissue, 2) the variant may not be disease causing in it self but could eventually be a marker of another disease causing mutation. 3) one common missense mutation has been identified (ref 20 in the paper) causing increased cancer susceptibility without causing MSI in the tumors. If the present study had identified a variant causing increased cancer susceptibility, information on IHC and MSI would of course have been necessary to characterize this variant in detail.

Specific details requested by BMC editorial office

1. Change of abstract according to guidelines

Author comment: The abstract has been changed according to the guidelines provided.

2. Approval by ethical committee

Author comment: The approval of appropriate local ethical committee is stated in the Methods section page 5-6.

3. Informed consent

Author comment: informed consent has been given by the individuals in the familiar CRC cohort and this has been added at page 6