Author's response to reviews

Title: Association between cigarette smoking, APC mutations and the risk of developing sporadic colorectal adenomas and carcinomas

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

Please find enclosed our response to the reviewers’ comments.

**Reviewer: Walter Giaretti**

His conclusion was: "That the present study was well conducted and well described and it is surely of relevant interest to cancer research". There were not any specific comments to respond to.

**Reviewer: Kirsti Hausgafvel-Pursiainen**

**Major Compulsory Revisions** (that the author must respond to before a decision on publication can be reached) and specific points:

1. The CRC cases were recruited into the study using a different study design as compared to adenoma patients and the controls. The authors should appropriately discuss justification of this, and estimate whether the inclusion of these different populations may somehow affect the analysis and results.

   **Response:**
   Page 8, 3rd sentence - The controls used as a reference group in this study of adenomas and carcinomas are matched to the cases by region (Telemark). Page 11, 2nd sentence - The fact that the CRC cases and controls have not been matched by age may affect the result of this analysis, but the result is comparable to other studies published in recent years (Giovannucci 2001).

   - In this case-control study, which is based on the NORCCAP cohort, the reference group (controls) is drawn from the same cohort (Telemark) as the adenomas and the CRC cases. Sporadic CRC cases tend to be older and this may be a problem when calculating relative risk using younger controls. However, our controls have been screened and found to be polyp free and the risk of any of them having colon cancer at the time of inclusion is not very likely. In our opinion, this makes these controls better than controls with no screening for CRC, but they are not the optimal match due to age differences. When cases are divided based on APC mutational status this is not likely to cause any problem since the focus in this case is to investigate if smoking is related to specific subgroups of tumors identified as having an APC mutation or not. This was also tested for by using case-case comparisons. We came to the conclusion that combing these groups in the study may have an affect on the overall analysis of smoking and CRC, but would not have a major affect on the analysis of the APC mutation status and smoking.

2. The study is focused on sporadic cases, but the paper does not indicate which were the criteria used for exclusion of possible hereditary ones. Were there any data on eg. microsatellite instability positivity
Response:
Page 6, 2nd sentence - The questionnaire contained information on a family history of cancer and the included CRC cases had no known personal history of cancer.

- We have screened the cases for microsatellite instability but these data have not been published yet. Our preliminary results do not indicate that any of the cases are HNPCC.

3. The authors should discuss more thoroughly which might be the explanations for their main findings indicating that the various smoking parameters were mostly associated with APC truncation mutation negative cases. Is it likely that there are other factors (e.g. dietary, hereditary, tumour subsite, other genes), that may play a major role in development of these tumours? This would be important, in particular as there are literature data suggesting that MSI positive cases were more likely to smoke =20 cigarettes (Slattery et al., JNCI, 2000), or that smoking may play a role in p53 negative tumours and be associated with tranversion mutations (Diergaarde et al. Carcinogenesis, 2003).

Response:
Page 12, 6-11th sentence - As previously discussed, this may imply the involvement of other genes in association with cigarette smoking and colorectal carcinogenesis. Sporadic CRC is a complex disease and several environmental factors in combination with genetic make up may have an impact on CRC development. The chromosomal instability (CIN) pathway involves several genes including APC, k-ras and TP53 (Vogelstein, Fearon et al. 1988; Groden, Thliveris et al. 1991; Powell, Zilz et al. 1992). It has been reported that smoking may be associated with k-ras transversion mutations and play a role in TP53 negative tumors in CRC (Diergaarde, Vrielings et al. 2003). The other major pathway for CRC development involves microsatellite instability (MSI) (Haydon and Jass 2002). Slattery et al reported that MSI positive cases are more likely to smoke more than 20 cigarettes a day (Slattery, Curtin et al. 2000) and CRC tumors displaying MSI have been positively associated with cigarette smoking.

4. I would also like to suggest that the authors wrote a bit more about what might be the mechanisms through which 'starting smoking ≥40 yrs ago' (associated to increased truncation mutations in CRC) may function in colorectal tumorigenesis. Would it rather be just 'early initiation' (and thus long constant exposure to the variety of tobacco carcinogens and a long time span for accumulation of alterations), as mentioned now, or is it likely that there might be some other (perhaps site specific) biological mechanisms involved?

Response:
Page 13, 6th sentence - This parameter (starting smoking ≥ 40 years ago) highlights the importance of the particular point in time when starting smoking. There were no difference between the two CRC subgroups when comparing dose and duration.

- Since this is a pilot study, we find it somewhat difficult to speculate on this. A larger study would be needed in order to discuss the effect of initiation on CRC development as a function of doserate.

5. In my opinion, the results presented in Table 5 do not fully justify the conclusions as presented in the abstract and on page 13 (last few sentences), but the role for this one
smoking parameter (starting smoking ≥40 yrs ago) indicating an association to truncation mutation positive CRCs is too strongly underlined.

Response:
To the abstract and page 13 (last few sentences):
Abstract (Conclusion): Our data suggest an association between smoking and adenoma and CRC development. This association was strongest for cases without APC truncation mutation. This may implicate other factors in development of these tumors. The association detected between smoking and CRC cases with APC mutation was in relationship to the smoking parameter “starting smoking ≥ 40 years ago”, a time period long enough to proceed CRC initiation.

Conclusion page 13: Our results indicate that there is an association between cigarette smoking and adenoma and CRC development. For cases divided based on APC truncation mutation status this association was strongest for cases without mutation. This indicates that there are other factors that may play a major role in development of these tumors. The exception was for a smoking history with a time span of 40 years or more since first starting smoking. This smoking parameter was only associated with cases with APC mutations, though this association was not statistically significant. This may suggest that smoking can contribute to CRC development through mutations in the APC gene if smoking starts prior to CRC initiation. A larger study would be required to clarify this issue.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. There are a few unclear sentences (e.g. Abstract: 3rd sentence of the 'results' section; page 5: 1st sentence; page 9: the two last sentences appear repetitive; page 11: 2nd para, 6th sentence, starting with 'For adenomatous polyps…'); these should be clarified. In general, language revision would be beneficial for the paper.

Response:
The sentences have been clarified and the paper will be sent to copyediting by www.abdn.ac.uk/mps as recommended by the BioMed Central Editorial Team.

2. The recent IARC monograph on tobacco smoking (vol. 83, 2004) should be cited for current data on smoking and colorectal cancer.

Response:
The IARC monograph has been cited in relation to smoking data.

3. In Tables 4 and 5, it should be indicated in the title that the mutation status refers to truncation mutations. Also, in all of the tables, I sometimes found that the superscripts for the footnotes could be replaced closer to the figure/item they refer to, for easier reading.

Response:
Colorectal cancer, smoking history and APC truncation mutation status (Tables 4 and 5). The footnotes are written as close as the double line (as requested by BMC) allows.

Regards,
Elin H. Kure