Author's response to reviews

Title: Polymorphisms in ASE-1, RAI and ERCC1 and the effects of tobacco smoking and alcohol consumption on risk of colorectal cancer: A Danish prospective case-cohort study

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Author's response to reviews: see over
Letter to assistant editor Lolu da-Silva

Thank you for considering our manuscript for publication in BMC and thank you for the reviewers’ comments to our manuscript: “Polymorphisms in ASE-1, RAI and ERCC1 and the effects of tobacco smoking and alcohol consumption on risk of colorectal cancer: A Danish prospective case-cohort study”. We address each comment, giving a point-by-point response, on the following three pages.

Best regards,

Rikke Dalgaard Hansen
(on behalf of Ulla Vogel)
Answer to reviewers report, Federica Gemignani

Ad 1) We have explained the enrolment procedure with more details in “Methods” page 6, lines 10-13. We hope this clarifies who are the selected cases and the sub-cohort as comparison group. Page 6, line 19 now states that the cases identified for the present study were diagnosed between 1994 and 2003 and thus are incident cases.

Ad 2) A power calculation is now included in the manuscript at page 9, paragraph 2. In the present study a risk ratio of 1.34 could be detected with a power of 81.8% among variant carriers of the RAI polymorphism, a risk ratio of 1.28 could be detected with a power of 80.3% among variant carriers of the ERCC1 polymorphism, and a risk ratio of 1.35 with a power of 81.0% among variant carriers of the ASE-1 polymorphism. For homozygous carriers of the haplotype a risk ratio of 1.47 could be detected with a power of 80%. All at a significance level of 5%.

We thank the reviewer for the comment on reference #4. The reference is now corrected.
**Answer to reviewers report, Kiyonori Kuriki**

Ad 1) We have shortened the manuscript as suggested. However, very few studies have been published of the three individual SNPs and the haplotype and gene-environment interactions, of which only one small study (with 156 colorectal cancer cases) are published on the relation to risk of colorectal cancer. We include gene-environment interaction analyses in the present study, of which very few studies previously have been published. The results are new to the field of SNP analyses and therefore demand some space for presentation. Thus, in our opinion we still need an introduction to the matter and presentation of the results.

The two other reviewers did not comment on the length of the manuscript.

The reviewer suggested more detailed descriptions on several matters. Thus, after a shortening of the manuscript and when having considered all the suggestions from the three reviewers and made linguistic revisions, we did not find it possible to condense the manuscript further into a short report without losing too much potentially valuable informations.

Ad 2) The hypothesis and predicted results are now included in the manuscript at page 5, paragraph 3.

Ad 3) We have shortened the manuscript, the “Background” chapter in particular, as suggested.

Ad 4) A summary of the hypothesized mechanisms are now included in the manuscript at page 5, paragraph 3. We have not included an illustration of the gene-effects since we expect only one SNP to be biologically relevant by inhibiting apoptosis.

Ad 5) The haplotype and the reference genotypes are now defined in the text (page 3, paragraph 2) and as footnote in Table 2 and 4.

Ad 6) The results of previous studies are now introduced in a more focused fashion.

Ad 7) We have deleted repeated sentences.

Ad 8) We are not sure if we understand the comment from the reviewer. The two XPD polymorphisms are not included in the previous defined haplotype in the studies mentioned (references 2-5) and in the present study. Previously, a small effect was observed on the risk estimates when adjustments for the XPD effect were made (ref.3), but in the present study virtually no changes in the risk estimates was observed when making similar adjustments. Thus, we did not include the two XPD SNPs in the present study. The two SNPs are included in a study published elsewhere (Mutation Research, 2007).

Ad 9) The definition of former smokers and calculation of their smoking intensity are now included in the manuscript at page 8, paragraph 2.

Never-smokers are not excluded from the analyses. When analyzing interactions of smoking intensity we have included an indicator variable of ever/never smoking (adjustment for ever/never smoking), so the effect of smoking on risk of colorectal cancer was estimated among smokers only. We have included a sentence on the matter at page 9, line 1-2.
The reviewer suggested to show results according to pack-years in addition to smoking intensity/duration/status at enrolment when analyzing the gene-environment interactions. The analyses of the smoking duration and status did not contribute with more information, why we chose not to include the results in Table 3 and 4. A paper on different approaches in modeling of smoking history published in the American Journal of Epidemiology (Am J Epidemiol 2002; 156: 813–823) recommends the use of smoking intensity and duration rather than pack-years, why we chose that approach in the present study. If the results according to pack-years still are wanted, we will include them in Table 3 and 4.

Ad 10) In the Cox regression model, we stratified according to gender allowing separate underlying risk for men and women, respectively. Hence, adjustment for HRT in the analyses affects the risk estimates for women only. We have applied a comment on the matter on page 9, line 2-4.

Ad 11) We have genotype data on 394 cases and 791 members of the subcohort and environmental variables from all persons included in the study (as now mentioned at page 6, line 23). Hence, the interaction analysis between polymorphisms and alcohol consumption and the smoking factors include all 1185 individuals distributed in the three groups (homozygote wild type, heterozygote and homozygote variant).
The reviewer mentions, that many genes are known to act in a tissue-specific context, why it’s questioned whether previous findings of association of the haplotype with risk of one type of cancer warrants for further investigation on other types of cancer. We have found associations for the studied SNPs or the haplotype for breast, lung and skin cancer. Thus, we believe, that we are dealing with a very general mechanism for carcinogenesis. Furthermore, a small Norwegian study has recently reported a tendency for association of the haplotype with risk of colorectal cancer among women. The results of previous studies are presented in short at page 3, paragraph 1. We expect that the haplotype or the individual SNPs are linked to a biologically effective polymorphism, which may probably be located in RAI. RAI are required for induction of apoptosis (Laska et al., 2007), an essential mechanism of all cells. Furthermore, we have made a number of gene-environment interaction studies of SNPs, being able to reproduce findings from one type of cancer to another type of cancer. Two examples are the observed association of SNPs in the genes GPXI and PARP-gamma with risk of breast, lung and colorectal cancer. Thus, we hypothesize that some genes (and possibly the haplotype) are involved in mechanisms affecting many cancer forms, including colorectal cancer.

According to the discussion of tissue-specific actions of many genes, and the reviewer’s comment on not referring to an article on RAI expression in lymphocytes, we have now deleted the reference from the manuscript.

We have included the aliases for RAI and ASE-I in the manuscript, page 3 paragraph 1.