Author's response to reviews

Title: Polymorphisms in the xenobiotic transporter Multidrug Resistance 1 (MDR1) gene and interaction with meat intake in relation to risk of colorectal cancer in a Danish prospective case-cohort study.

Authors:

Vibeke Andersen (va9791@gmail.com)
Mette Østergaard (mette.oestergaard@viborg.rm.dk)
Jane Christensen (jane@cancer.dk)
Kim Overvad (ko@dce.au.dk)
Anne Tjønneland (annet@cancer.dk)
Ulla Vogel (ulbvo@food.dtu.dk)

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Author's response to reviews: see over

Please read below a list of corrections to our manuscript according to the recommendations by the reviewers

Reviewer: Jing Shen

Major revisions

1. Regarding HRT (Table 1): We agree with the reviewer that there is no difference in the use of HRT between cases and controls, and the sentence stating this in Results has had been removed accordingly. We have now shown the IRR with 95 % CI in Table 1. This means that potential differences in frequencies would be better revealed.
2. Regarding the results in former Table 5 (now Table 4) on NSAID use, SNPs and CRC risk: The description of the results has been changed according to the reviewers comments.
3. Results and discussion have been changed to provide a more conservative interpretation of the results.
4. The reason for combining variant genotypes has now been described: Variant genotypes were combined to obtain sufficient power to interaction analyses and since we observed no gene-dose effects.
5. MDR1 C3435T and risk of CRC in relation to the previous findings is now more thoroughly dealt with in the Discussion.
6. We have discussed the risk of chance findings in more detail in the Discussion. The fact that we find interaction between MDR1 polymorphisms and meat intake makes a chance finding less likely.
7. We have corrected the inconsistent or incorrect descriptions of the genotypes. Especially, "homozygous wild type carries" has been changed to "homozygous wild type allele carriers" or "homozygous carriers of the wild type allele".

Minor revisions

1. We have now consistently used the term case-cohort study for our study.
2. We have now indicated the reproducibility of the repeated genotype analyses (100%).
3. Only risk estimates regarding women were adjusted for HRT. This is now clearly stated.
4. A description of the interaction analyses has been added to the Methods section.
5. The 'c' of P-value was supposed to denote that P-value was for trend. This is now described in the notes of both Table 2 and Table 3.
6. We have used the CC (MDR1 C3435T) as reference group in Table 5 because this is what is used in the literature. However, in our sample the TT genotype is the most frequent, and we have therefore used this genotype as reference in Table 3, as described in the notes.
7. The limitation for our study has now been more thoroughly described in the Discussion.

   Quality of written English: the whole manuscript has been edited in order to improve the written English.

Reviewer: Kiyonori Kuriki

1. IRRs and p-values have been added to the abstract.
2. A better description of the functions of the three genes has been included. In contrast to the suggestion by the reviewer we have retained the analyses of the BCRP gene SNP because we think these analyses are relevant, with BCRP representing a second xenobiotic transporter.

3. The role of NSAID in relation to CRC is now commented in the Background.
4. We have added the analyses of the interaction between intake of red and processed meat with MDR1 and BCRP polymorphisms (Results, Table 3).
5. It is now clearly stated in the Methods section, which variables were treated as continuous variables and which were categorized.
6. Physical activity has been shown not to be a risk factor for colorectal cancer in the present study group and was therefore not adjusted for (Johnsen NF, Christensen J, Thomsen BL, Olsen A, Loft S, Overvad K, Tjønneland A., Physical activity and risk of colon cancer in a cohort of Danish middle-aged men and women. Eur J Epidemiol. 2006;21(12):877-84).
7. The risk estimates are now adjusted for NSAID use as suggested.
8. The limitation due to limited power has been commented in the Discussion.
9. We have added IRRs in Table 1.
10. The purpose of the haptotype analyses (former Table 3, now supplemental Table 5 and 6) was to see which polymorphisms contributed to the increased risk. We believe these analyses are still justified, and therefore we have retained these analyses.
11. The title has been changed, so that focus is on the MDR1 gene and the association to meat intake.
12. The keywords ATP-binding cassette transporters and xenobiotics have been removed.

Minor comments

1. A description of gene functions has been added to the Abstract.
2. The sentence "So far, the SNP has no known functional effects" has been changed into "So far, the functional effect of this SNP is unknown".
3. A note [c]P-value for trend has been added to the table (Table 2).
4. Abbreviations are now shown, including for NSAID.
5. We have reduced the numbers of references, however, as we have been asked to elaborate on other matters, we have only been able to reduce the number of references by ten.
6. The volume number has been added to reference no. 25.

Reviewer: Gregory Tranah

1. The enumeration of the different sections has been removed, in accordance with the journal style.
2. The statement of a protective effect of NSAID in connection with COX-2 T8473C has been removed. The evaluation of this data has been changed, in accordance also with the comments of reviewer Jihn Shen, as stated above.
3. The word 'use' has been added after NSAID, as requested.

Minor revisions

1. We agree with the reviewer on his point and have commented on single-point collection of life style factors in the Discussion accordingly.
2. We appreciate the suggestion by the reviewer, however, we find that there are too few MDR1 studies and they are too small for a meta-analysis.
3. We have now included IRR for the lifestyle factors in Table 1, and it is apparent that HRT use is associated with a lowered risk of CRC in the cohort. We have chosen not to include menopausal status in Table 1, since we do not want to put too much emphasis on the distribution of menopause status because we already know that cases were older than members of the comparison group, and as such, more likely to be postmenopausal.

4. We will not be able to answer this question as we have not investigated adenomas.
5. There was no interaction between the polymorphisms in Table 3 (now supplemental Table 5). The purpose of Table 3 was to clarify whether the observed associations for one of the polymorphisms could be explained by linkage with the other polymorphism and not whether there was interaction between the polymorphisms.