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.

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Author's response to reviews: see over

Response to referees:
In their assessment of the submitted manuscript, the expert referees have revealed a number of mistakes in the submitted manuscript. We are very grateful that the referees found those mistakes and that we have a chance to revise the manuscript once again. We apologize for the incorrectness of the submitted revised manuscript.
Below, we go through each point raised by the expert referees and how the manuscript has been revised to accommodate the comments.

Jing Shen:

Major revision:
The referee suggests that we should adjust for multiple comparisons. We have chosen not to adjust for multiple analyses as suggested by Perneger [1]. The three polymorphisms in COX-2 and the two in MDR1 are linked, and therefore cannot be regarded as independent tests. Since we have biological reasons for all the interactions tested, we think that it is not meaningful to adjust for multiple testing in line with the argumentation by Perneger.

Table 2: We apologize for the unclear marking. It is now clearly stated that the tend trend is perform for the adjusted risk estimates (IRR^b). The p-value reflects that same as the confidence intervals, but the trend test compares all three risk estimates to each other instead of the pair-wise comparison that is reflected by the confidence intervals, where all risk estimates of heterozygous and homozygous variant allele carriers are compared to wt carriers.

Table 3: We apologize for the unclear description of table 3, which has now been revised. In table 3, the rate of colorectal cancer risk pr additional intake of 25g red or processed meat is calculated for two genotypes (homozygous wildtype carrier or variant allele carriers). Then it is tested whether the two rates are statistically significantly different from each other. If they are statistically significantly different, then it means that there is interaction between genotype and intake of red and processed meat in relation to risk of colorectal cancer. We have previously published similar interaction analyses in relation to smoking, alcohol or intake of fruit and vegetables in relation to breast- lung- and colorectal cancer [2-9]. We have chosen the analysis to avoid the small groups and accompanying power problems, had we chosen to subdivide by genotype and meat intake, due to the limited sample size of the present study group. With the present analysis, all available information is used to generate two risk estimates, which are then compared.

Gregory Tranah:
We apologize for the inconvenience which is due to an inexperienced corresponding author.

Kiyonori Kuriki:
Risk estimates were not adjusted for habitual exercise, because physical activity was not a risk factor for colorectal cancer in the present cohort [10]. Risk estimates are adjusted for BMI.
Special comments:
1) The interactions between the 3 genes and the environmental factors are altered in abstract and background in order to make the purpose of the study clear and as suggested by the reviewer.

2) We think that the BCRP should be analysed together with MDR1 because both are xenobiotic transporters and transport potential carcinogenic substrates. We have tried to argument better for this standpoint in the introduction.
3) NSAID use was found to be associated with a non-statistically significantly lowered risk of CRC in the present cohort [11]. We have changed the background and discussion section in order to more clearly discuss the NSAID findings.
4) Risk estimates were not adjusted for habitual exercise, because physical activity was not a risk factor for colorectal cancer in the present cohort [10].
5) Since the study was matched on sex and Age was used as the time scale in the Cox regression model, all risk estimates were adjusted for age and sex. We realize that this is not clearly stated, and it is now clearly stated in the notes of tables 2-4.
6) Smoking is not a strong risk factor for CRC. We only include smoking status as the strongest predictive variable. Risk estimates for alcohol and smoking status are shown in table 1. In dept analysis of the association between different smoking variables and risk of CRC is beyond the scope of the present paper.
7) Validation of the food frequency questionnaire is published elsewhere [12;13].
8) Total dietary fibers are calculated by the AOAC methods [14]. A reference has been added in the text.
9) Processed meat does not include processed fish. The text has been changed to 'Intake of processed meat in grams per day was calculated by adding up intake of processed red meat, including bacon, smoked ham, salami, frankfurter, Cumberland sausage, cold cuts and liver pâté.'
10) table 3: We apologize for the unclear description of table 3, which has now been revised. In table 3, the rate of colorectal cancer risk pr additional intake of 25g red or processed meat is calculated for two genotypes (homozygous wildtype carrier or variant allele carriers). Then it is tested whether the two rates are statistically significantly different from each other. If they are statistically significantly different, then it means that there is interaction between genotype and intake of red and processed meat in relation to risk of colorectal cancer. We have previously published similar interaction analyses in relation to smoking, alcohol or intake of fruit and vegetables in relation to breast- lung- and colorectal cancer [3;7;9;15-19].
11) The reviewer is of course right. The estimate was not adjusted for red meat and the note has been corrected.
12) All meats were classified as either 1) white meat, 2) red meat or 3) processed meat. In table 3, red and processed meat were grouped together for reasons of statistical power.
13) A sentence about trend test has been added to the statistical methods. 'Trend test were calculated using the Wald test.' The 25 g/day is based on an evaluation of the interquartile rage of the meat variable and a realistic increase in consumption.

Reference List

1 Perneger,T.V. (1998) What's wrong with Bonferroni adjustments. BMJ, **316,** 1236-1238.

2 Hansen,R.D., Sorensen,M., Tjonneland,A., Overvad,K., Wallin,H., Raaschou-Nielsen,O. and Vogel,U. (2007) XPA A23G, XPC Lys939Gln, XPD Lys751Gln and XPD Asp312Asn polymorphisms, interactions with smoking, alcohol and dietary factors, and risk of colorectal cancer. Mutat.Res., **619,** 68-80.

3 Hansen,R.D., Sorensen,M., Tjonneland,A., Overvad,K., Wallin,H., Raaschou-Nielsen,O. and Vogel,U. (2008) A haplotype of 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. BMC.Cancer, **8,** 54.

4 Raaschou-Nielsen,O., Sorensen,M., Hansen,R.D., Frederiksen,K., Tjonneland,A., Overvad,K. and Vogel,U. (2007) GPX1 Pro198Leu polymorphism, interactions with smoking and alcohol consumption, and risk for lung cancer. Cancer Lett., **247,** 293-300.

5 Raaschou-Nielsen,O., Sorensen,M., Overvad,K., Tjonneland,A. and Vogel,U. (2007) Polymorphisms in nucleotide excision repair genes, smoking and intake of fruit and vegetables in relation to lung cancer. Lung Cancer.

6 Vogel,U., Christensen,J., Nexo,B.A., Wallin,H., Friis,S. and Tjonneland,A. (2007) Peroxisome profilerator-activated receptor{gamma}2 Pro12Ala, interaction with alcohol intake and NSAID use, in relation to risk of breast cancer in a prospective study of Danes. Carcinogenesis., **28,** 427-434.

7 Vogel U., Christensen,J., Dybdahl M., Friis,S., Hansen,R.D., Wallin H., Nexo,B.A., Raaschou-Nielsen O., Andersen,P.S., Overvad,K. and Tjonneland,A. (2007) Prospective study of interaction between alcohol, NSAID use and polymorphisms in genes involved in the inflammatory response in relation to risk of colorectal cancer. Mutation Research, **624,** 88-100.

8 Vogel,U., Christensen,J., Wallin,H., Friis,S., Nexo,B.A., Raaschou-Nielsen,O., Overvad,K. and Tjonneland,A. (2008) Polymorphisms in genes involved in the inflammatory response and interaction with NSAID use or smoking in relation to lung cancer risk in a prospective study. Mutat.Res., **639,** 89-100.

9 Vogel,U., Olsen,A., Wallin,H., Overvad,K., Tjonneland,A. and Nexo,B.A. (2005) Effect of polymorphisms in XPD, RAI, ASE-1 and ERCC1 on the risk of basal cell carcinoma among Caucasians after age 50. Cancer Detect Prev, **29,** 209-214.

10 Johnsen,N.F., Christensen,J., Thomsen,B.L., Olsen,A., Loft,S., Overvad,K. and Tjonneland,A. (2006) Physical activity and risk of colon cancer in a cohort of Danish middle-aged men and women. Eur.J.Epidemiol., **21,** 877-884.

11 Friis,S., Poulsen,A.H., Sorensen,H.T., Tjonneland,A., Overvad,K., Vogel,U., McLaughlin,J.K., Blot,W.J. and Olsen,J.H. (2009) Aspirin and other non-steroidal anti-inflammatory drugs and risk of colorectal cancer: a Danish cohort study. Cancer Causes Control, **20,** 731-740.

12 Tjonneland,A., Overvad,K., Haraldsdottir,J., Bang,S., Ewertz,M. and Jensen,O.M. (1991) Validation of a semiquantitative food frequency questionnaire developed in Denmark. Int.J.Epidemiol., **20,** 906-912.

13 Tjonneland,A., Haraldsdottir,J., Overvad,K., Stripp,C., Ewertz,M. and Jensen,O.M. (1992) Influence of individually estimated portion size data on the validity of a semiquantitative food frequency questionnaire. Int.J.Epidemiol., **21,** 770-777.

14 Prosky,L., Asp,N.G., Furda,I., DeVries,J.W., Schweizer,T.F. and Harland,B.F. (1985) Determination of total dietary fiber in foods and food products: collaborative study. J.Assoc.Off Anal.Chem., **68,** 677-679.

15 Hansen,R.D., Sorensen,M., Tjonneland,A., Overvad,K., Wallin,H., Raaschou-Nielsen,O. and Vogel,U. (2007) XPA A23G, XPC Lys939Gln, XPD Lys751Gln and XPD Asp312Asn polymorphisms, interactions with smoking, alcohol and dietary factors, and risk of colorectal cancer. Mutat.Res., **619,** 68-80.

16 Raaschou-Nielsen,O., Sorensen,M., Hansen,R.D., Frederiksen,K., Tjonneland,A., Overvad,K. and Vogel,U. (2007) GPX1 Pro198Leu polymorphism, interactions with smoking and alcohol consumption, and risk for lung cancer. Cancer Lett., **247,** 293-300.

17 Raaschou-Nielsen,O., Sorensen,M., Overvad,K., Tjonneland,A. and Vogel,U. (2007) Polymorphisms in nucleotide excision repair genes, smoking and intake of fruit and vegetables in relation to lung cancer. Lung Cancer.

18 Vogel,U., Christensen,J., Nexo,B.A., Wallin,H., Friis,S. and Tjonneland,A. (2007) Peroxisome profilerator-activated receptor{gamma}2 Pro12Ala, interaction with alcohol intake and NSAID use, in relation to risk of breast cancer in a prospective study of Danes. Carcinogenesis., **28,** 427-434.

19 Vogel,U., Christensen,J., Wallin,H., Friis,S., Nexo,B.A., Raaschou-Nielsen,O., Overvad,K. and Tjonneland,A. (2008) Polymorphisms in genes involved in the inflammatory response and interaction with NSAID use or smoking in relation to lung cancer risk in a prospective study. Mutat.Res., **639,** 89-100.