Reviewer's report

Title: Polymorphisms in NFkB, PXR, LXR, interaction with meat, tobacco smoking, NSAID use, and risk of colorectal cancer in a prospective study of Danes

Version: 2 Date: 6 April 2010

Reviewer: Kiyonori Kuriki

Reviewer's report:

Dear Authors;

In a nested-case-control study within a Danish prospective cohort study, the authors examined interactions between the six gene polymorphisms of transcription factors and nuclear receptors, i.e., nuclear factor kappa-B (NFkB), pregnane X receptor (PXR) and liver X receptor (LXR-beta), and heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs) derived from red and processed meat, and tobacco smoking, for risk of colorectal cancer. The findings, however, were not clearly shown and appropriately discussed.

Major comment

1. Numbers of the study subjects were not clearly shown in the text (including Abstract) and Tables, so that the data and the findings should be checked. In Method section 359 cases and 765 sub-cohort members were selected, as well as the data in your previous study regarding the MDR1 gene polymorphism (reference No. 20; BMC Cancer 2009;9:407). The findings in the current study, however, were based on 383 cases and 763 sub-cohort members and shown in Abstract, Result section, and all of Tables. Why?

2. Family history of colorectal cancer is one of important risk factors for colorectal cancer. This reviewer strongly suggests that the risk should be also adjusted for this factor.

3. As one of potential confounding factors, moreover, the risk should be adjusted for physical activity (or habitual exercise), even if this factor was not related to the risk in this population. In the second expert report based on World Cancer Research Fund/American Institute for Cancer Research, “physical activity” is suggested as a convincing protective factor for the risk. In general, therefore, the majority of readers believe that “physical activity (or habitual exercise)” is related to intestinal active-movement, appropriate defecation and shorter bowel transit time of xenobiotic compounds along with dietary fiber.

4. Furthermore, the risk should be adjusted for total energy intake as one of confounding factors.

5. To facilitate understanding by readers, please kindly state the validity (e.g.,
Spearman’s rank correlation coefficients between diet records and your food frequency questionnaire) for consumption of red and processed meat, dietary fiber and total energy intake in Method section.

6. There were some issues in each Table. In Table 2, were the combined genotypes of the six polymorphisms (e.g., ID + DD) included to calculate the p-values of each interaction? In footnotes “B” in Tables 2, 3 and 5, were age and sex not included? Were the risk not adjusted for age and sex, in Table 4? What was “Crude” meant in Table 5?

7. Why was not the risk adjusted for use of non-steroidal anti-inflammatory drugs (NSAIDs) in Tables 2, 3, 4 and 5? In Method section and the text, NSAIDs use was described as one of confounding factors.

8. In the text and Table 3, this reviewer suggests that the risks and the interactions should be categorized and shown by low, middle and high intakes of red and processed meat consumption, but not “intake of additionally 25 g red and processed meat”.

9. In Table 3, the risks for the two genotypes of the NFκB -94ins/del gene according to red and processed meat intake were not significantly increased or decreased, and the values were almost 1.00. Therefore, the significant interaction might be by a chance.

10. To facilitate understanding by readers, in the text, please kindly illustrate what were differences between your previous (reference No. 20) and the current study. Considering contents in Background and Discussion sections, the MDR1 gene seems to be multiply related to the NFκB, PXR and LXR genes, and all of them may be activated by xenobiotic substances, such as HCAs and PAHs. In the current study, the NFκB gene might be just liked to the MDR1 gene. This reviewer, therefore, is wondering the following issue; “what was a new finding?” or “why was not the MDR1 gene included in the current study?”.

11. Conclusion section should be clearly mentioned. For the risk, why was the NFκB gene polymorphism related to xenobiotic substances derived from red and processed meat, but not tobacco smoking. Likewise dietary intake of red and processed meat, oxidative stress and reactive oxygen spices are also generated by tobacco smoking. Therefore, the data related to fatty meat (i.e., meat rich in fat, but not hem-iron) and “smoking intensity (as described in Method section)” should be shown in the text.

12. Please introduce how high is dietary intake of red and processed meat and tobacco smoking in European countries and the World (in Discussion section). How about was fatty meat intake?

Minor comment
1. In Abstract, the following sentences were confused; variant allele carriers …, whereas “variant” allele carriers … (p=0.03).

2. In Table 4, “smoking” should be shown as “smoking status”. Why was
“smoking status” included in the footnote A? What was “fully” meant in footnote B?

3. In Conclusion section, “NFkB polymorphism” was shown as “the NFkB -94ins/del gene polymorphism”.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests.