Author's response to reviews

Title: Association between an 8q24 locus and the risk of colorectal cancer in Japanese.

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Author's response to reviews: see over
Dear Dr. Norton,

We are submitting a revised manuscript entitled, “Association between an 8q24 locus and the risk of colorectal cancer in Japanese.” by Matsuo et al. We appreciate if the manuscript would be considered for possible publication in BMC Cancer as a Research Article. We revised the manuscript according to comments by reviewers. Our responses to each comment are in the separate sheets.

In this study we evaluate possible impact of 8q24 locus, rs6983267, which is consistently reported its association with colorectal cancer in whole genome association study, among Japanese population. Finding was that rs6983267 is significantly associated with colorectal cancer risk in Japan without any interaction with potential confounders. We believe the finding would be important information to understand colorectal cancer genesis and interest information to readers of BMC Cancer.

We look forward to hearing from you at your earliest convenience.

Yours sincerely,

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Responses to Dr. Tenesa:

First of all, I would like to express sincere appreciation for Dr. Tenesa’s comprehensive and constructive comments. We revised the manuscript according to his comments and our responses are shown below.

1. Careless mistakes in the manuscript.
   Missing table 1. → Added.
   Rs4693267 → rs6983267

   Although the paper by Tenesa et al. was referenced, it was not referred as information source of Japanese population. We revised description in introduction section as follows.
   “Most of these CRC GWASs were conducted in Caucasian populations, however, and the data available for Asian populations is limited especially about possible gene-environment interaction [6, 14].” in Page 4, last line of paragraph 2.

3. Wrong information about 8q24.
   The reviewer pointed out wrong information in the first sentence of the second paragraph in introduction. To remove wrong information and make it clear, we revised the sentence as below.
   “Recently, several a number of genome-wide association studies (GWAS) have revealed an association between variants on chromosome 8q24 and several sites of cancer, including CRC [2-11]”.

4. Description about rs10090154.
   We evaluated this locus based upon significant result in Haiman et al (Nat Genet, 39;954-956. In table 1 in the manuscript, it showed that rs10090154 was associated with Colorectal cancer in Japanese American in Hawaii. I appreciate checking of the reference paper.

5. Rationale for evaluating non-significant confounders.
   We agree the point raised by the reviewer is very important point. In this case-control study, we did not see any significant association other than family history. Basically, the factors included as confounders are commonly accepted risk factors in the usual epidemiologic studies. Even without statistically significant association, we observed suggestive distribution of these factors. For example, higher alcohol drinker is more common among cases compared with controls. Therefore, we expected if significant gene-environment interaction might exist, we would see the enhanced effect of the locus.
   We would add the sentence as one of the limitation to be considered in interpretation.
   “Lack of interaction needs careful interpretation because confounders assessed in this study showed no association with CRC risk by themselves.”

As recommended by the reviewer, we removed dominant/recessive models from the manuscript. We think the allelic model suggested by the reviewer is equivalent with the per allele model we’ve done. We added genotypic model according to suggestion. Table 2 were revised based on it and corresponding texts were also revised.
Responses to Dr. Kono:

First of all, I would like to express sincere appreciation for Dr. Kono’s comprehensive and constructive comments. We revised the manuscript according to his comments and our responses are shown below.

1. Careless mistakes.
   - Table 1 .--→ added.
   - Typo and English errors: Suggested parts were corrected.

2. Redundant description of colon cancer and rectal cancer cases.
   - We removed the sentence in result section.

   - The reviewer recommended to specify the ORs in table 2-4. According to other reviewer’s suggestion, the ORs estimated were described as ORs in allelic model throughout the text.

4. Footnote of Table 2.
   - As suggested, we added a footnote for UK.

5. Interaction for site of cancer.
   - As we conducted conditional logistic regression, we can estimate this mathematically. However, we don’t think the evaluation of this term is valuable because of the point raised by the reviewer. So, we removed this part from table 3.

   - We conducted duplicated genotyping for 5% of random samples. Therefore, we described it in the method section.

7. Specification of description in Discussion.
   1) The reviewer requested to clarify whether the reference 26 study is in-vitro or in-vivo study. This study is in-vitro study using library of stem cells. They conducted correlation analysis using data of expression in these cell lines to identify genes strongly correlated with Oct4. To make it clear, we revised the part as follows:
      “OCT4, a transcript of POU5F1, plays a role in maintaining stem cell pluripotency, self-renewal and chromatin structure in stem cells[26]”

   2) The reviewer requested to clarify whether Tomlinson et al. showed gene expression in primary CRC of humans. Tomlinson et al. reported that they confirmed expression of POU5F1/POU5F1P1 in cell lines and in human primary CRC. Therefore, the corresponding sentence was kept unchanged.
3) The reviewer requested to clarify whether Suo et al. also confirmed expression of oct4 in cancer cell lines as well as tumor tissues. We revised the corresponding sentence as follows:

“while Suo et al. similarly reported the expression of these genes in cancer cell lines and cancer tissues [25].”

8. We added some discussion about lack of association of rs10090154 in our study as follows.

“We did not observe any association with rs10090154 (OR=0.90) on the contrary to the results from Multi-ethnic cohort study[6]. The point estimate for minor allele in the previous study was 1.41 (95%CI: 1.14-1.75). Following case-control study for Japanese American in Hawaii showed lack of association (OR=1.07, 95%CI: 0.78-1.48)[6]. Inconsistency across studies might come from the finding in the original GWAS was by chance although threshold in statistical significance was high enough. Or, statistical power in following studies including ours were not good enough. By all means, more evidence is needed to clarify significance of the locus.”
Responses to Dr. Berndt:

First of all, I would like to express sincere appreciation for Dr. Berndt’s comprehensive and constructive comments. We revised the manuscript according to his comments and our responses are shown below.

Major comments:
1) The reviewer pointed out wrong description of 8q24 region in the introduction. We revised the part as follows to avoid misunderstanding.

   “Recently, several a number of genome-wide association studies (GWAS) have revealed an association between variants on chromosome 8q24 and several sites of cancer, including CRC [2-11].”

2) The reviewer questioned about authors’ assumption about linearity of the potential confounders in the models. We revised our statistical analysis applying these factors as indicator variables as suggested. Therefore, results in table 2-4, and corresponding texts.

3) The reviewer requested to discuss rs10090154. We added following sentences in the discussion.

   “We did not observe any association with rs10090154 (OR=0.90) on the contrary to the results from Multi-ethnic cohort study[6]. The point estimate for minor allele in the previous study was 1.41 (95%CI: 1.14-1.75). Following case-control study for Japanese American in Hawaii showed lack of association (OR=1.07, 95%CI: 0.78-1.48)[6]. Inconsistency across studies might come from the finding in the original GWAS was by chance although threshold in statistical significance was high enough. Or, statistical power in following studies including ours were not good enough. By all means, more evidence is needed to clarify significance of the locus.”

4) The reviewer raised several points to be considered in discussion of limitation. Based upon the suggestions, we revised the paragraph as follows.

   “Several potential limitations of the present study require consideration. First, use of hospital-based control in this study for potential cause of selection bias. We used non-cancer patients at our hospital as controls, given the likelihood that our cases arose within this population base. Moreover, we previously showed that individuals selected randomly from our control population were similar to the general population in terms of baseline characteristics [20]. Given the similarity in minor allele frequency between our controls and that in the HapMap database for Japanese, it is reasonable to
assume the external validity of our study results to the general population. Second, as with other
case-control studies, this study may have suffered from information bias: although the questionnaires
were completed before the diagnosis in our hospital, some patients referred from other institutions
might have known their diagnosis. Lack of interaction needs careful interpretation because
confounders assessed in this study showed no association with CRC risk by themselves.”

Minor revisions:
1) Typo and mistakes.
   Rs698326 in abstract was changed to rs693267.
   “Expected and observed haplotype” was changed to “Expected and observed genotype.”
   Rs4693267 was changed to rs6983267.
   As we removed recessive and dominant models from the manuscript because of
   recommendation by another reviewer, the comment for the p-value was not reflected.
   Digits for some of odds ratios were corrected in Table 3.

2) Missing table 1.
   We added Table 1.

3) The reviewer requested to update Table 4.
   We identified three studies by Li et al, Schafmayer et al, and Curtin et al.

Discretionary revision:
1) We revised table 2 to present ORs for each genotype.
2) Table 3 was revised to add number of cases and controls.
3) Table 4 was revised to clarify adenoma estimates.