Author's response to reviews

Title: Association between Variations in the Fat Mass and Obesity-Associated Gene and Pancreatic Cancer Risk: A Case-Control Study in Japan

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Author's response to reviews: see over
Dear editor,

Thank you for consideration of our revised manuscript for publication in your journal.

We have added author contribution in the revised paper. We actually used the editing services of Edanz before submitting this manuscript. We have sent our revised paper for edit again. We can send you the certificate of editing quality to BMC cancer if it is necessary.

Below are the responses to the reviewers’ comments. All the changes are highlighted in red in the revised paper.

Response to Reviewer (Dr. Jianjun Zhang)’s comments

We appreciate the reviewer’s comments on our study. We also thank the reviewer for pointing out several issues that need to be addressed.

Minor Essential Revisions:

1. rs9939609 is a variant located in the first intron of the FTO gene. The functionality of this polymorphism largely remains unclear. The rationale for selecting this variant should be addressed in more detail.

1. The reviewer is correct. The functionality of rs9939609 in the FTO gene is largely unknown. It remains a challenge to determine the significance of the intronic SNPs, including rs9939609, in the FTO gene. Of obesity-related FTO
variants, rs9939609 has been most studied, and has been shown to be associated with obesity in both Western and Asian populations. Furthermore, rs9939609 is in strong linkage disequilibrium with other SNPs in the FTO genes, such as rs8050135, rs3751812, rs17817449, which have also been reported as BMI-related variants. Therefore, we genotyped rs9939609 in the FTO gene of 360 cases and 400 control subjects.

We have added the following sentences in the revised paper.

Page 6, Line 9-14

‘In a search of the literature for obesity-related genetic variants, we found that FTO rs9939609 was the most widely studied single nucleotide polymorphism (SNP), and has been found to exert strong effects on BMI, as well as diabetes. Furthermore, it showed a strong linkage disequilibrium with other SNPs in the FTO gene, such as rs8050135 and rs17817449.’

2. As mentioned by the authors, at least 30 loci (including rs9939609) have been linked to BMI and obesity in genome-wide association studies. Did the authors evaluate the effects of some or all remaining SNPs on pancreatic cancer risk? If yes, what were the results? Were there any significant interactions between rs9939609 and other SNPs? If not, why not?

2. We did not evaluate the effect of other SNPs in the FTO gene for two reasons. First, we are continuing data collection from case patients and control subjects in our ongoing case-control study. The final goal is to conduct a genome-wide association study (GWAS) of pancreatic cancer in the Japanese population. As a first step, we selected 30 SNPs located in several candidate genes, including tobacco carcinogen metabolizing genes (4 SNPs), alcohol metabolizing genes (5 SNPs), folate metabolizing genes (3 SNPs), diabetes-related genes (7 SNPs), and 2 SNPs reported from recent GWAS of pancreatic cancer, because very few molecular epidemiologic studies exist in Japan, and it is still not clear whether SNPs reported in the studies of Western populations are also associated with the risk in Japanese populations. Second, when we searched the literature for obesity-related genetic variants, we found that FTO rs9939609 is the most widely studied polymorphism, and exerts strong effects on BMI, as well as diabetes. A 2012 meta-analysis showed that rs9939609 is significantly associated with diabetes risk in Asian populations independently
of obesity. As the reviewer pointed out, the functionality of rs9939609 remains largely unknown, raising the question of whether it is a causal variant. It seems possible that this polymorphism is linked to other yet unidentified causal variants in the FTO gene. We plan to evaluate the effect of multiple SNPs in the FTO gene using pathway analysis in the future studies.

We have revised the manuscript as follows.

Page 13, Line 6-9

‘Given that the function of the FTO gene is largely unknown, further studies are needed to comprehensively evaluate multiple SNPs in the FTO gene and elucidate the mechanisms by which FTO rs9939609 influences pancreatic cancer risk.’

3. A response rate of 85% among cases was reported in this study. This is quite high for pancreatic cancer. Were the cases who died before the interview considered in the calculation of the response rate? It was also stated that a rapid case ascertainment system was used to recruit patients diagnosed with pancreatic cancer. What were the median and/or mean numbers of days between the diagnosis of pancreatic cancer and the interview of cases?

3. We agree. We achieved a high response rate using a rapid case ascertainment system, one important feature of which was that the physicians were co-investigators in this study and they were directly involved in enrolling case patients. Unfortunately, the exact statistics of the median and mean number of days were not available, but almost all of the cases were approached within a week after they were diagnosed with pancreatic cancer, and very few cases had died before they were invited to participate in our study.

We have added the following sentences in the revised paper.

Page 7, Line 1-4

Almost all of the cases were approached within a week after the diagnosis of pancreatic cancer, and very few cases died before they were invited to participate in our study.

4. Controls were recruited from inpatients and outpatients. What were the diseases for which those patients sought medical care in the study hospitals? Were those diseases associated with BMI or obesity (risk factors examined in
this study)?

4. We appreciate the reviewer’s concern about control selection. Less stringent criteria were used because our main interest was to look at the association between genetic variants and pancreatic cancer risk. The controls had a variety of diseases, such as anemia, digestive diseases (gastric ulcer, irritable bowel syndrome). It is possible that some of the diseases could have been associated with obesity. However, the OR of pancreatic cancer among the obese people in this study was comparable with other studies of Japanese subjects, suggesting that bias due to control selection did not seriously bias our study results.

We have added the following sentences in the revised paper.

Page 7, Lin 10-12
Control subjects had a variety of diseases, such as anemia, gastric ulcer, and irritable bowel syndrome.

Discretionary Revisions:

1. A potential interaction between rs9939609 and diabetes was detected in relation to pancreatic cancer risk. It should be more cautious to mention “the presence of a multiplicative interaction ...” (page 10) as p for interaction was 0.28.

1. The reviewer is correct. We have removed the phrase ‘the presence of a multiple interaction’ throughout the revised paper.

2. It is better to combine the TA genotype with AA genotype in the evaluation of gene effect as only three cases and one control were homozygous for the A allele among subjects with BMI ≥25 (Table 3).

2. In accordance with the reviewer’s comment, we have combined the TA genotype with AA genotype in Table 3 of the revised manuscript.
Response to the Reviewer (Dr. Wenqing Li)’s comments

Major compulsory revisions
1. This is a case-control study with a moderate sample size. Overweight or obese subjects were few (less than 100 in cases or control), which limited the statistical power to test the hypothesis. The results could be due to chance, so I would like to see words like "further replication in other independent samples is required" in the limitation and in the conclusion.

1. The reviewer is correct. We cannot rule out the possibility that the results may be due to chance. We added the following sentence in the Abstract and Discussion (limitation and conclusion)

Abstract; Page 3, Bottom line
‘Further investigation and replication of our results in other independent samples is required.’

Page 14, Line 2-3
‘Finally, it is possible that the results could represent a chance association and therefore replication in other independent samples is required.’

Page 14, Line 9-10
‘Because of the limited statistical power, our results need replication in other independent samples.’

2. Since BMI was a major variable focused in this study, only those with information on BMI should be included in the final analyses. We typically reserve the missing data only if the variables were covariates.

2. We appreciate the reviewer’s comment. It is correct that only those with information on BMI should be included in the final analysis if BMI is a major variable.

Because FTO is an obesity-related gene and obesity is a well-established risk factor in studies targeting the Western populations, it is difficult to address the association between FTO genotype and pancreatic cancer risk without
investigating the BMI-pancreatic cancer association. As we wrote in the Discussion, case-control studies are prone to selection and recall bias. In the case of pancreatic cancer, another added difficulty is weight loss arising from tumor development. Compared with case-control studies, prospective cohort studies are more appropriate to evaluate BMI-pancreatic cancer associations. However, given that our main interest was to evaluate the association between the FTO genetic variants and pancreatic cancer risk, we did not exclude those with missing information on BMI.

3. The recruitment of cases and controls is unclear. How to interpret the response rate (85% for cases and 98% for controls)? Is this the percentage of the final included subjects divided by all the people that have been approached for attending this study? If so, please specify the numbers. Data on usual weight means the weight before this study entry or before the pancreatic cancer diagnosis? Why didn't evaluate the association with BMI at age 20 and describe the distribution of BMI at age 20? I think highly likely most subjects had BMI at age 20 < 25 which made further investigation impossible, right?

3. Regarding the response rate, we apologize for not making this clear. Our case-control study is ongoing and the response rate we showed in the paper was the final included subjects divided by all patients who had been approached to participate in our study during the specified period. We have added the specific numbers in the revised paper. The usual weight means the weight 1 year prior to the study entry. Thanks to a rapid case ascertainment system, almost all of the cases could be invited to participate in our study within 1 week after the diagnosis, so we considered that ‘usual weight before the study entry’ is similar to ‘weight before pancreatic cancer diagnosis’. We actually have examined the association between BMI at age 20 and pancreatic cancer risk. One concern with using data for age 20 is recall bias. Because similar results were observed, we did not show them in a Table, but wrote ‘Similar results were obtained in an additional analysis in which BMI at age 20 was used (data not shown).’ (Page 9, bottom line)

The revision is as follows.
Page 6, Line 22-23
‘The response rate among cases was approximately 85% (441/516) as of July
Recruitment of controls was accomplished by approaching eligible participants in the hospitals who satisfied the study requirements, and the response rate was approximately 98% (525/534).

4. It's uncommon to show the OR in the table 1 when aiming to present the characteristics of the participants, or the authors should edit the table title.

4. We agree with the reviewer that if BMI were the main interest, it would be uncommon to show the ORs in Table 1. However, presenting ORs in Table 1 can be seen in case-control studies focusing on genotype-cancer association (one example: Tang H, et al. Cancer Epidemiol Biomarkers Prev 2011;20(5) 779-92). From these ORs, we believe the readers can interpret whether the risk estimates for lifestyle factors are consistent or contradictory with previous studies.

5. This is not the first study on this topic, as the authors have refereed the MD Anderson study, although this might be the "first in Japanese", but I would not describe this as an advantage.

5. We have deleted the phrase 'first in Japanese' in accordance with the reviewer's comment.

6. How did the authors conduct and interpret the interaction analyses? I did not see interaction between this SNP and history of diabetes (P-interaction=0.28). Among those with diabetes, the exact effect of TA/AA genotype should be 3.7/1.7=2.2. Since the number of those with diabetes is small, I would not expect any heterogeneity between this OR (2.2) and the OR in those without diabetes (1.4). If no effect modification by diabetes, the Results and Discussion section need to be revised.

6. We apologize for not interpreting the interaction analysis appropriately. To test for multiplicative interaction, we performed logistic regression with and
without the interaction terms and a chi-square test for -2 times the differences of the log likelihood values. P for interaction was 0.28, indicating no multiplicative interaction between genotype and history of diabetes. We agree with the reviewer that the findings should be interpreted more cautiously, given the small number of patients with diabetes. As the reviewer pointed out, there was no effect modification by diabetes. We have revised the Results and Discussion in accordance with the reviewer’s comment.

Abstract
‘There were no significant interactions between TA/AA genotypes and body mass index.’

Results (Page 10, Line 18-22)
‘We found no significant interaction between FTO rs9939609 and BMI (Table 4). Individuals with both a TA/AA genotype and a history of diabetes had a 3.7-fold increased risk of pancreatic cancer compared with those with a TT genotype and no history of diabetes (Table 5), but a test for the interaction was not statistically significant.’

Discussion (Page 13 Line 1-4)
We found that individuals with a TA/AA genotype and a history of diabetes were at a 3.7-fold increased risk of pancreatic cancer. However, a test for the interaction was not statistically significant.

7. Why precretic cancer cases were excluded if the onset of diabetes was within 2 years prior to the diagnosis of pancreatic cancer? My understanding is that there are bi-directional relationship between diabetes and pancreatic cancer, right? Right now I only noticed the description of diabetes as a risk factor for pancreatic cancer.

7. The association between diabetes and pancreatic cancer is complex because the development of diabetes and pancreatic cancer may involve similar pathogenesis and share common risk factors such as obesity, smoking and insulin resistance. Moreover, while epidemiologic studies have shown that long-standing diabetes is associated with approximately a 2-fold increased pancreatic cancer risk, clinical studies found that new-onset diabetes is a
manifestation or a result of pancreatic cancer. There was a lack of data on the proportion of pancreatic cancer that can be attributed to long-term diabetes or the prevalence of pancreatic cancer-induced new-onset diabetes. Our study was not designed to estimate the prevalence of pancreatic cancer-induced new-onset diabetes. In case-control or cohort studies, it is common to exclude cases with the onset of diabetes within 2 or 3 years to limit the effect of ‘reverse causation’, i.e., diabetes caused by pancreatic cancer.

Minor essential revisions

1. The manuscript is generally clear but further English edits would be warranted. For example, “statistically insignificant” is very odd use (not statistically significant)

   1. We actually used the editing services of Edanz before submitting this manuscript. We have sent our revised paper for edit again. However, in accordance with the comment, we have changed this phrase to ‘not statistically significant’.

   2. Please specify the adjustment of covariates (continuous or categorical variable), particularly for BMI in the Statistical analysis.

   2. We have specified the adjustment of covariates (continuous or categorical variable) in the revised paper.

Page 8 Line 24-Page 9 Line 1-3

‘All analyses were adjusted for age (continuous), sex (continuous), BMI (<20, 20-22.4, 22.5-24.9, ≥25.0), history of diabetes (yes or no), and cigarette smoking (current, former, never smokers).’