Author's response to reviews

Title: Single nucleotide polymorphisms of the APC gene and colorectal cancer risk: a case-control study in Taiwan

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Author's response to reviews: see over
To answer to Reviewer: Mef Nilbert

Q1 The methods section needs to be clarified. Were family histories of cancer available? What were the tumor locations; i.e. the distribution of proximal and distal tumors? The section on sex and age distributions should be moved from the Results to the Materials section.
The family histories of cancer were available, and every patients in the study didn’t has family histories of colorectal cancer. We add a sentence to clarify this point in the methods section of the revision.
We add a section to describe the tumor locations in the methods section of the revision.
We move the section on sex and age distributions from the Results to the method section of the revision.

Q2 How was the size of the study sample determined? Was a power analysis performed?
In the study design, we make a assumption that interesting allele frequency of specific site is about 0.5. When collecting 80 samples in each case and control group, the study will has more than 82% power to detect a true OR of 2.5 by power estimation based on the Mantel-Haenszel test (stratum number = 1). Actually we only collected 74 subjects in the control group, but it still gets more than 80% power to detect a true OR of 2.5. In the table 3 of this revision, to report the ORs for all genotypes relative to the most common genotype reduce our sample size in each test, but it still gets more than 80% power for each significant result because of high OR. However, we didn’t present the power analysis on the article.

Q3 All gene names should be italicized. Also, the international nomenclature should preferably be used for the labelling of the SNPs.
We italicize all gene names. We label SNPs by international nomenclature and the refSNP IDs from NCBI RefSNP Clusters are listed under reported SNPs in Table 1.

Q4 The last sentence could be omitted. It draws a very far-fetched conclusion.
We agree and omit the last sentence in the conclusion section of the revision.

Q5 The sequence figure could be omitted since it adds no new information.
We agree and omit the Figure 1 and the figure legends in the revision.
To answer to Reviewer: Gregory Tranah

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

Q1) Several clarifications need to be provided regarding the colorectal cancer cases and controls:

Q1a) Please describe the exam used to screen for colorectal tumors.
We screened for colorectal tumors by colonoscopy. We add it to the methods section of the revision.

Q1b) Were the controls also screened? This is not clear.
Yes, the controls were also screened. We add it to the methods section of the revision.

Q1c) Please list the location of the tumors when reporting mutations (proximal, distal, rectal).
We change original Table 2 to Table 3 and add new Table 2 to list the location of the tumors when reporting mutations.

Q1d) Is there any information on tumor grade (Duke’s stage)?
Yes, we add tumor grade by Duke’s stage of patients in the methods section of the revision.

Q2) The logistic regression analysis is not acceptable as it is currently presented.

Q2a) In the methods section please describe your logistic regression analyses - were there matching factors (age, gender?) and were any additional variables adjusted for in the model (smoking, BMI, etc.)? The sample size is probably too small to adjust for many covariates but adjusting for gender and age (~5 year groups) should be tolerable.
We didn’t adjust for any other variables because of the small sample size. For reporting the ORs for all genotypes relative to the most common genotype, the sample size is probably too small to adjust for other covariates. Thus, we still don’t adjust for any other variables in the revision. We clarify this point in the methods section.

Q2b) In the results and in Table 2 please report the ORs for all genotypes relative to the wild-type/common genotype. It is more informative to see the ORs for each genotype even if the rare allele prevalence requires collapsing heterozygotes and homozygous variant genotypes into a single category. Knowing the number of cases and controls would also be helpful for interpreting the ORs and this information could be moved to Table 2 from Table 1.
We now report the ORs for all genotypes relative to the most common genotype and add the number of cases and controls. We update Table 3 (original table 2) for these changes.
Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1) In two places within the discussion the authors mention that a particular mutation may alter function of APC without giving a reference. Are the authors suggesting this or are there studies that demonstrate functionality of the mutations in question? The authors should be more cautious when suggesting that a particular mutation is functional if there are no studies to support these claims.

   a) page 10, second paragraph - "Such substitutions may result in disruption of the putative cell signaling function of the APC protein".
   We add "...; however, this needs to be demonstrated experimentally".

   b) page 11, second paragraph - "...B-catenin binding repeat (amino acids 1840-1866) and may disrupt the function of the APC gene product".
   We omit the sentence.

   If these statements do not have a reference then the author's should revise these comments by adding, "...; however, this needs to be demonstrated experimentally".

2) In the discussion, the authors refer to two papers (Slattery et al. 2001 and Tranah et al. 2005) suggesting that each of these studies report genetic modification (gene*environment interaction) between the APC D1822V polymorphism and dietary fat intake. Slattery et al. did report a significant interaction between fat intake and the APC D1822V polymorphism; however, this was not replicated in the Tranah et al. study. One major difference between the studies was the retrospective ascertainment of diet in the Slattery et al. study and the prospective ascertainment of diet in the Tranah et al. study, suggesting that the Slattery et al. is potentially more prone to recall bias. This needs to be clarified in the discussion.

   Also, Tranah et al. observed a significant interaction between the APC D1822V polymorphism and postmenopausal hormone use.

   We have reviewed again and revise these sections.
Besides, some type error are also revised in this revision:

In the original Table 1, the SNP at exon 9 of codon 450 are revised to 350. The same errors in other place of the manuscript are also revised.

In the original Table 1, the CT genotype number in the patient group of the distribution at the V1125A variant site was 2. It is revised to 3. The same errors in other place of the manuscript are also revised.