Author's response to reviews

Title: Associations Between Variants on ADIPOQ and ADIPOR1 with Colorectal Cancer Risk: a Chinese Case-control Study and updated Meta-analysis

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Dr. Sergi Castellvi-Bel. Hospital Clinic, Centre Esther Koplowitz, Spain

RE: Associations Between Variants on ADIPOQ and ADIPOR1 with Colorectal Cancer Risk: a Chinese Case-control Study and updated Meta-analysis

Dear Dr. Sergi,

Thank you for your letter dated on September 16, 2014 regarding the review of our aforementioned manuscript. We appreciate the comments from you and the reviewers, and have found the comments and suggestions helpful in preparation of the revised manuscript. We are glad to hear that our reports could be accepted for publication on BMC Medical Genetics if we carefully revised our manuscript. According the suggestions of you and the reviewers, we have thoroughly revised our manuscript and hope these could meet your and the reviewers’ criteria. The following are our responses to the comments and suggestions.

Responses to Reviewer #1:

1. Under statistical methods, it is stated that Association testing was performed under an unconditional logistic regression model with or without adjustments for covariates sex, age, drinking habit and smoking status. All four variables are
included in the model as covariates as stated in Table 2. However, Table 1 states that only age and alcohol intake are statistically significant between cases and controls. How do you explain the inclusion of the other variables in the model, considering this fact could make the model slightly overfitting? Do the p-values change if sex and smoking status are removed from the regression model?

Response: We agreed with the reviewer that the covariants age and the alcohol intake level are significantly distributed in the cases and the controls, but not for the sex and the smoking status. There could be a possibility of over-fitting in the unconditional logistic regression model to determine the associations between the SNPs and the colorectal cancer risk if the covariants sex and the smoking status were included in the statistical model for the current epidemiological study. Because sex hormones level (Clin Gastroenterol Hepatol, 2013;11(4):419-424.; J Natl Cancer Inst, 2010;102(23):1746-7) and smoking status have important impacts on the development of colorectal neoplasia, even larger than the family history (an important indicator for the genetic factors) as proposed by Michael et al. (Clin Gastroenterol Hepatol. 2010;8(10):870-6.), we would like to assess the associations between the SNPs and the colorectal cancer risk with the adjustments of the factors sex and smoking status. These were also usually performed by other studies to include the potential covariants that may affect the colorectal cancer risk in the statistical analyses (J Natl Cancer Inst. 2013;105(24):1852-61; JAMA. 2008;300(13): 1523-31.). In addition, when we excluded the sex and smoking status from the statistical model, no significant change of the estimates for the associations between the SNPs and the
colorectal cancer risk was found (For rs1342387, Before: p = 0.123, After: p = 0.134 for CT vs. CC; Before: p = 0.013, After: p = 0.019 for TT vs. CC; Before: p = 0.028, After: p = 0.034 for CT/TT vs. CC).

2. The patient characteristics section in this section is not a result by itself, but a description of the features of the cohort. Therefore it should be included in the materials & methods section.

Response: The reviewer gave valuable suggestions. As we have performed the statistical analyses to compare the covariants including the sex, age, alcohol level and smoking status of the participants, it will be more suitable to gave a complete description of the features for the participants in the results section of the manuscript.

3. Under results for genotyping, the authors declare that the T allele carriers at the rs1342387 marker have an 24% decreased risk of CRC with respect to the controls. Please explain how that decrease was calculated.

Response: We apologized for the wrong typing of the number and we have corrected it in the manuscript. Under the dominant model, the adjusted OR was 0.74 (95% CI = 0.57-0.97) for the allele T carriers compared to the CC carriers for rs1342387. As the OR was usually estimated as the relative risk (RR) in the epidemiological studies when the prevalence of the diseases is relatively lower, we estimated that a 26% reduction of the colorectal cancer risk for the allele T carriers (CT or TT) when compared to the common CC carriers.

4. Although the number of markers is reduced, no corrections for multiple testing are performed to validate the results from the hospital-based study. Please comment on
Response: The reviewer is correct that multiple testing correction should be performed in the study when multiple markers were included. The FDR (false discovery rate) and Bonferroni methods are widely used in the genome-wide association studies (GWAS) and other epidemiological studies to reduce the false positive rate of the results. We found no significant association between the other variants including rs266729, rs2241766, rs822395, rs1501299 or rs12733285 and the colorectal cancer risk, and they have little effects on the association between the rs1342387 and the colorectal cancer risk. When the unconditional logistic regression with all the other SNPs as covariants, we still found a marginal association for the rs1342387 and the colorectal cancer (P = 0.252 for CT vs. CC and P = 0.081 for TT vs. CC, P-trend = 0.068). However, there are linkage disequilibrium between the rs1342387 and the other SNPs, the unconditional logistic regression was possibly overfitted. The meta-analysis of the published epidemiological studies also suggested a significant association between the variant and the CRC risk. Thus, we did not perform the multiple testing correction for our study as limited biomarkers were included.

5. In results of the meta-analysis studies, a pooled OR is given for the association between rs1342387 and CRC risk in the published studies. However, no p-value for this association is given to ascertain the significance of this association.

Response: According to the reviewer’s suggestion, we have added the P-values for the meta-analysis studies in the manuscript. We have moved the Supplementary Table
8 as Table 3 in the revised manuscript to provide the summary data of the meta-analysis results for the associations between the selected variants and CRC risk.

6. In Table 1, measure as percentages for each of the subtypes of the covariates should be given. Also, description of samples per hospital should also be included, given that the samples were collected at three different centers.

Response: As suggested by the reviewer, we have added the percentage data for each subgroup in Table 1. We also provided the percentage of the samples for each hospital in the revised manuscript. All the cases were from Chongqing or the surrounding regions (including the Sichuan, Yunnan, and Guizhou provinces in the southwest of China), there was no sign of the population stratification for the participants.

7. Overall, there is much insistence on variant rs266729, and although there is no evidence of Association with CRC risk, a figure for the meta-analysis is provided, along with a long paragraph in the discussion. I do not understand the need for such long explanations provided that there are 4 other variants that are not treated in such detail. Please provide any explanations to support the inclusion of this data or otherwise I believe variant rs266729 should be treated like the rest of the markers for which there is no strong evidence for Association with CRC risk.

Response: The reviewer gave valuable suggestions. The variant rs266729 has been reported to be associated with higher circulating adiponectin levels (Eur J Endocrinol. 2010;163: 251-7.; J Intern Med. 2010;268: 194-205.), higher plasma total antioxidant status (Eur Heart J. 2009;30: 1263-9.), lower plasma oxidized-LDL levels (Eur Heart J. 2009;30: 1263-9.), and reduced risk for type 2 diabetes (Diabetologia. 54:
These data suggested that rs266729 could be a functional variant that confer the susceptibility of these diseases. Kaklamani et al. firstly reported a significant association for the rs266729 and CRC risk (JAMA. 2008;300: 1523-31.), which suggested a genetic relationship for the association between the obesity, diabetes and the colorectal cancer risk. The locus has drawn more attention than the other loci by the experts in the field. Many epidemiological studies have repeated the results for Kaklamani et al. (JAMA. 2008;300: 1523-31.); however, none of them found a significant association between the locus and the CRC risk. For other loci, less studies have determined their associations between the CRC risk. For our current study, we provided strong evidence that rs266729 may not confer the susceptibility of CRC risk with the meta-analysis methods. No clear presumption was proposed for the association between the other loci and the CRC risk. Thus we discussed more about the association between the rs266729 and the CRC risk in our manuscript.

8. Meta-analysis of the associations between the selected variants with CRC risk. In paragraph 1, it is mentioned that for meta-analysis purposes with overlapping samples, only the most complete study was included. Please clarify what “most complete” means in this context.

Response: We apologized for the unclear statement in the manuscript. If overlapping samples were identified between the studies, the study with the largest sample size and/or provided the detailed information about the genotype information for the participants was included in the meta-analysis studies. We have revised these in our manuscript.
9. Under results of the meta-analysis study, the first sentence says: "of the four included studies, five individual studies...". This seems a little off. Do the four studies have more than one stage and there are thus more “individual” studies? I suggest clarifying this or dropping the “four” altogether to avoid ambiguity.

Response: The reviewer is correct. The studies performed by Kaklamani et al. (JAMA. 2008; 300: 1523-31.) and Keku et al. (Cancer Causes Control. 2012; 23(7):1127-38.) have separate subgroup studies and they were recognized as individual studies in the meta-analysis. According to the suggestions by reviewer, we have revised our manuscript and hope it will meet the reviewer’s criteria.

Response to reviewer #2:

1. page 3 line 46 inclusive should be replaced with inconclusive; page 5 first sentence; page 6 line 106-111; page 9 line 182-183; page 13 line 263-265; page 15 line 300-303 need language correction; page 11 line 230 risk is stated as 24% the same risk is stated as %21 on page 15 line 308; Figure 3 is not referred/mentioned in the text.

Response: According to the suggestions of the reviewer, we have revised the manuscript and hope these will meet the reviewer’s criteria.

2. The study investigated rs2221766, 1501200, 266729, rs822395 in ADIPOQ and rs12733285, rs1342387 in ADIPOR1. There is no information on why rs2241766 and rs1501299 were chosen in the introduction. However there is information on rs822396 and rs1063585 which are not investigated in this study. Relevant
information on rs2241766 and rs1501299 should be included in the introduction.

Response: According to the suggestion of the reviewer, we have added relevant information about the rs2241766 and rs1501299 and their associations with the CRC risk in the introduction section.

3. In the methods section it is stated that a semi-quantitative food frequency questionnaire was filled by cases and controls. However in the manuscript there is no information/observation related to this analysis. Thus there is no results supporting the statement on page 15 sentence starting on line 303 and page 16 sentence on line 318-320. The authors either needs to provide data supporting their statement or they should remove these statements.

Response: According to the suggestion by the reviewer, we have revised our manuscript and hope it will meet the reviewer’s criteria.

4. Supplementary Table 8 should be transformed into a Table 3.

Response: As suggested by the reviewer, we have transformed the supplementary Table 8 as Table 3 for the manuscript.

Thank you for your considering, and we hope the responses will meet your and the reviewers’ criteria.

Please send all correspondences to me at the following address:

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Sincerely,

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