April 12, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (ESPS2102_Revision.doc).

Title: *CYP1A1*, *GSTM1*, *GSTT1* and *NQO1* polymorphisms and colorectal adenomas in Japanese men

Author: Tadamichi Hamachi, Osamu Tajima, Kousaku Uezono, Shinji Tabata, Hiroshi Abe, Keizo Ohnaka, Suminori Kono

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 2102

The manuscript has been improved according to the suggestions of reviewers:
1   Format has been updated.

2   Revision has been made according to the suggestions of the reviewers as described below. Please note that changes are shown in red except for References and Tables. When not changed, we explained a reason or reasons in the response.

Reviewer 02459305
The study subjects are so special that they were all male officials in the Self Defense Forces. Despite smoking, they may have similar risk factors such like drinking, food habits, etc. Although you have lifestyle questionnaire, I hope you give us more proof that other factors did not affect your final results.

[Response]   Indeed, dietary factors are important in colorectal carcinogenesis. Dietary factors were not considered because data were not available. This point was added as a weakness in the Discussion (page 12).

Reviewer 02459573
The Abstract, Introduction, and Methods are nicely written, but the Results section is currently quite problematic in terms of how it is written and how findings are presented both in the text as well as tabularly. As such, I recommend major revision.

ABSTRACT
1) "Polyp" and "odds ratio" should be pluralized ?
[Response]   We changed to "polyps", but odds ratio (OR) remained as such because it was a methodological term.

2) The first sentence of "RESULTS" should be changed to "None of the five polymorphisms showed a measurable univariate association with…"?
[Response]   "Singly" meant "individually" (not in combination), not referring to a "univariate" association. We deleted "singly" to avoid confusion.

3) In the RESULTS, the authors should avoid comparing increases and decreases in ORs in statistical terms (i.e., the odds ratio increased or decreased) and should rather rewrite focusing on the practical importance of these relationships (e.g., the odds of X increased twofold if Y). ?
[Response] We modified the sentence. The OR was shown in parenthesis, and a quantitative explanation was not done.

4) In the CONCLUSION, what is "wild type"?
[Response] We deleted the words.

5) In the CONCLUSION, that the sample was composed of Japanese men should be mentioned in the METHODS if it is going to be mentioned here.
[Response] We deleted the words "in Japanese men".

INTRODUCTION
6) Please standardize throughout the manuscript whether genotypes/polymorphisms will be italicized or not.
[Response] As a rule, non-italic words indicate enzymes (proteins), and italic words are used for genes. Although confusing, italic and non-italic are mixed for the distinction between gene and protein.

7) Generally, the INTRODUCTION is well written and has a nice flow. Perhaps a bit more on the originality of the study's contribution would be nice.
[Response] We modified the last paragraph of the Introduction (page 6).

METHODS
8) Please explain what Self Defense Forces are?
[Response] This is an official term for the so-called defense forces (military forces), but we cannot say "Defense Forces" for the nation's constitution. However, we replaced "the Self Defense Forces Study" with "in Japanese men" in the title.

9) Pluralize "polyp"?
[Response] Yes, we did.

10) Specify that all participants were Japanese in ethnic heritage.
[Response] We added a sentence to this effect (line 2 from the bottom on page 6).

11) In second paragraph of SUBJECTS, please add percentages to N values where appropriate ?
[Response] We added % values in part (page 7).

12) We were unclear what "cigarette-years" meant (cigarettes per year)?
[Response] To make clear the way of calculation, we inserted words "which were calculated as" in the sentence (page 7).

13) Please explain how BMI and alcohol use categories were established?
[Response] Categories were arbitrary but the same as used in our previous studies. This was explained in the text (bottom 2 lines on page 7).

14) Please explain how hospital (a categorical variable) was controlled for in analyses.
[Response] The word 'dichotomous variable' was added in parenthesis (bottom line on page 8).

RESULTS ?
15) Need to report standard deviations wherever there are means.
[Response] We added a new table describing the subject characteristics as suggested by Reviewer 02472140 (see below). SD was reported in the table (Table 1).

16) The first paragraph of the results is very difficult to read and understand. An odds ratio of 2.11 would not typically be described as a very "strong" association.
[Response] Because the subject characteristics were presented in Table, the text became clear (page 9).

17) Not singly, but univariately?
[Response] We deleted "singly" to avoid confusion. See comment 2) to ABSTRACT.

18) There is no flow in this section, and the results are not discussed in a clear manner that mirrors the clarify of the METHODS ? Why when some significant findings are discussed are odds ratios and confidence intervals provided but then otherwise not?
[Response] According to Reviewer 02472140, we revised the section.

REFERENCES
19) Please be aware that there are several references where the journal title is NOT italicized (e.g., CA Cancer J Clin and Cancer Epidemiol Biomarkers Prev) ?
[Response] We appreciate pointing out these typographical errors, which were corrected.

20) It would be helpful for interested readers to have DOIs reported for all and not just selected studies.
[Response] DOI was added whenever available.

TABLES
21) It needs to be made clear in all titles, that was is being presented are the results of investigations into THE ASSOCIATION between genotypes and adenomas, rather than simply saying "genotypes and adenomas"?
[Response] Titles were modified by including **"The associations"** in Tables 2-5.

22) Please define "referent" throughout.
[Response] Actually we had indicated the referent category. Please note "(referent)" in each comparison.

23) Be careful of using two long hyphens in a row rather than one.

[Response]   This is not a double-hyphen, but "en-dash" which is used for the range or as minus sign. This matter can be solved at the Editorial Office.

24)   In Tables 2 and 4, the first two columns are difficult for the reader to understand. If they were perhaps labelled with different subheadings (e.g., Genotype 1, Genotype 2), this may be helpful.

[Response]   Yes, we followed to the advice (Tables 3 and 5, now).

25) Look at the footnotes of all tables. The font size gets larger and then smaller again.

[Response]   Once again, we appreciate meticulous reading. They were corrected.

Reviewer 02454589

I carefully read this manuscript, and found that it was interesting and well written. I have no other comment.

[Response]   Thank you very much for the appraisal.

Reviewer 02493079

1) What is the connecting link among CYP1A1, GSTM1, GSTT1 and NQO1 besides the fact that they have been reported? What was your hypothesis that they would be associated with [adenomas in] men?

[Response]   Carcinogens are activated by CYP1A1 (phase I enzyme) and detoxified by GSTM1 and/or GSTT1 (phase II enzyme). NQO1 is a phase II enzyme, but may be act a phase I enzyme for certain substances. We thought that these things had been explained clearly (as appraised by reviewers **02459573** and **02454589**). The last paragraph of the Introduction was modified to mention our aim/hypothesis more explicitly (page 6).

2) From the introduction, it appears that you chose the SNPs in each gene based on prior literature. You should state your SNP selection criteria.

[Response]   We focused on functional SNPs, and this study was not a genome-based study. Thus we did not have to decide SNP selection criteria. No correction was made.

3) Please show HWE value for each SNP.

[Response]   We had presented P values for the HWE regarding CYP1A1 and NQO1 (the second paragraph of the Results in the previous version). Non-null and null genotypes of GSTM1 and GSTT1 were not applicable to the HWE.

4) Authors claimed that "A nearly significant interaction was observed for the combination of CYP1A1*2C and NQO1 (P = 0.051)." But, four SNPs were tested. Thus, Bonferroni correction is needed to be performed for this association.

[Response]   This is not an expedition-type study, and Bonferroni correction will not always be needed. Also please not that P=0.051 was among the gene-gene interactions. We referred to the problem of 8 statistical tests in connection with this finding (page 12).

5) In terms of sample size, this study was conducted in 455patients which is middle number. Please provide the power of a statistical test in the manuscript. This may improve the quality of this study.
[Response]   We referred to the powers in the Discussion (page 13).

6) Discussion is not up-to-date.
[Response]   Recent meta-analyses were newly referred to regarding the CYP1A1 polymorphisms in the first paragraph of the Discussion (references 32 and 33; page 10).

Reviewer 02472140
1) A regular Table 1 is missing from this manuscript. Such table usually reports sample characteristics by case-control status. It is important to include this table.
[Response]   We included a table describing subject characteristics (Table 1).

2) The authors should explain why the mean age in both groups was 52.4 years. Were the observations age-matched, or was it just a coincidence?
[Response]   This study was not age-matched. The subjects were in a narrow range of age, and the same mean ages were a coincidence. The SD was shown in the above-mentioned table, and we did not explain a reason for the coincidence.

3) The odds ratios (and CI's) reported in paragraph 1 of the results section are out of place! Report association of polymorphisms first (main effects)- adjusted or/and unadjusted - before you assess the interaction effect of smoking.
[Response]   We moved the OR for smoking to the place as suggested (page 10).

4) Check epidemiological journals on the standard way to report results of logistic regression - usually one reports odds ratios of categories relative to some reference.
[Response]   The referent category had been indicated in the tables.

5) Results on Hardy-Weinberg equilibrium tests should come immediately after Table 1 (sample characteristics) results are reported - not after reported association results.
[Response]   We followed to the advice (page 9).

6) Persistent use of the word 'prevalence' is problematic. Remember, this is case-control study and not a cross-sectional study.
[Response]   The method was a case-control approach, but actually the study was based on adenomas diagnosed at the time of colonoscopy, i.e., prevalent cases. However, we avoided use of "prevalence" in accordance with the comment.

7) On page 8 last paragraph, instead of using the term 'nearly,' use the term 'borderline.'
[Response]   Done as suggested (page 9). This was applied also in the Abstract (page 3).

8) Instead of using the term "measurable" association, use "significant" association.
[Response]   Done as suggested (page 9) and also in the Abstract (page 3).

9) On page 8, second last paragraph, last line, 2 cases were excluded! Why? Was it to make

sure that the polymorphisms were in complete linkage disequilibrium? This should be explained.

[Response]   We had intended to mean "except for 2 cases", and this departure was probably due to an error in genotyping. We added an explanation on this point (page 9).

10) In general, the results section needs to be re-written to reflect standard reporting of logistic regression results in case-control studies.

[Response]   Please see the response to comments 3 to 5 of this reviewer.

Reviewer 00186451

The paper deserves publication if some necessary corrections are made.

1) The roles of these polymorphisms in genes have not been shown in this manuscript. The authors should mention that point in Discussion and should try to clarify the genetic effects and their function to colorectal cancer.

[Response]   We modified the conclusive sentences to clarify the message (page 13).

2) To ensure optimal presentation of your studies, please make sure that you have your paper edited by a native English speaker or otherwise by a professional editing agency, if you have not already done so.

[Response]   The revised manuscript was edited by a native English speaker, as certified by an attached document (file name: ESPS2102_Certificate.pdf).

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology.*

Sincerely yours,

Suminori Kono, MD, PhD