Prof. Lian-Sheng Ma
Science Editor, Company Editor-in-Chief, Editorial Office
Baishideng Publishing Group Inc
World Journal of Gastroenterology

Jun. 30, 2021

RE: World Journal of Gastroenterology Manuscript NO: 67139 – Manuscript revision
MTNR1B polymorphisms with CDKN2A and MGMT methylation status are associated with poor prognosis of colorectal cancer in Taiwan

Dear Professor Ma,

Thank you for your invitation to contribute an article to the World Journal of Gastroenterology (Invited Number ID: 03005388, Title Accepted Date: 2021-01-28, Manuscript Submission Deadline: 2021-04-28, Manuscript Submitted Date: 2021-04-15. However, your record showed that this manuscript was not an invited manuscript. Please help us resolve this problem, thank you very much) and kindly for providing us with the opportunity to resubmit a revised manuscript. We have taken into account the reviewer's in-depth comments and have carefully and extensively revised our manuscript according to the reviewer's comments. We have highlighted amendments we made in red font.
Our specific responses are as follows:

Reviewer #1:
Scientific Quality: Grade B (Very good)
Language Quality: Grade A (Priority publishing)
Conclusion: Minor revision
Specific Comments to Authors: In general, this is a well-designed and mature retrospective trial. I only have a few comments for authors.
1. The introduction of CDKN2A and MGMT genes is abrupt. Please supplement more information about them and explain why choose these two genes for combination analysis.
Responses:
We agreed with the reviewer's comments and added sentences to supplement more information about *CDKN2A*, *MGMT* and *MLH1* genes and explain why choose these genes for combination analysis. Please see the revised manuscript in Introduction on page 6.

2. Authors used Cox proportional-hazards models for to analyze the survival. The adjusted factors included age, sex, stage, adjuvant chemotherapy, tumour location, and the methylation status of the CDKN2A, MLH1 and MGMT genes. What about BMI and combined primary diseases?

Responses:

We appreciate the reviewer's comments. We conducted this retrospective cohort study and collected participates which was diagnosed in the Tri-Service General Hospital (TSGH), Taiwan, from 2006 to 2010. Data regarding registered patients—including sex, surgical age (permanent variable), adjuvant chemotherapy, histologic grade and location of the tumour, and survival—were collected from the TSGH's cancer registry database. Indeed, data on other factors related to CRC risk, such as diet, BMI and combined primary diseases were unavailable in the database. This is the limitation of this study. Please see the revised manuscript in Discussion on page 15.

3. The sample size is a concern. The wide 95%CI indicated poor stability.

Responses:

We appreciate the reviewer's comments and added one sentence to descript this limitation of this study. Please see the revised manuscript in Discussion on page 14.

Reviewer #2:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: acceptable

Responses: Thank you very much.

Reviewer #3:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Accept (General priority)

Specific Comments to Authors:

This study indicated the novel genetic biomarkers, MTNR1B, combined with CDKN2A and MGMT gene methylation statuses, maybe a predictive tool for CRC prognosis.

The researchers hypothesised that the influence of MTNR1B gene variation combined with the hypermethylation of CDKN2A and MGMT genes would predict the prognosis and provide clinical recommendations for optimal treatment of CRC.

This study offers insights into novel genetic and epigenetic biomarkers for the prediction of CRC prognosis, and the findings could be used to individualise the treatment of patients with CRC. Since CRC is a high-incidence cancer, such a clinical research that investigated the new biomarkers of CRC is helpful.

One concern is that adjusting for confounding factors such as obesity, a sedentary lifestyle, and

unhealthy dietary habits in Table 4, 5 and 6 would be better for studying the effect of MTNR1B SNPs associated with 5-year OS of CRC patients, as this study mentioned.

Responses:

We appreciate the reviewer's comments. We conducted this retrospective cohort study and collected participants who were diagnosed in the Tri-Service General Hospital (TSGH), Taiwan, from 2006 to 2010. Data regarding registered patients—including sex, surgical age (permanent variable), adjuvant chemotherapy, histologic grade and location of the tumour, and survival—were collected from the TSGH's cancer registry database. Indeed, data on other factors related to CRC risk, such as obesity, a sedentary lifestyle, and unhealthy dietary habits were unavailable in the database. This is the limitation of this study. Please see the revised manuscript in Discussion on page 15.

Secondly, the inclusion and exclusion criteria for this study seem to be unclear.

Responses:

Thank you for your comments. We recruited the CRC participants who were diagnosed in the Tri-Service General Hospital (TSGH), Taiwan, from 2006 to 2010. The TSGH Colon and Rectum Division's clinical practice guideline requires enrolees to return once every 3 months in the first year after surgical resection and once every 3–6 months thereafter. Considering the case number of this study, we used all the CRC participants' data from TSGH's cancer registry database.

Thirdly, it would be better to review format of the full text carefully, with respect to spaces between text and punctuation in the method.

Responses:

We appreciate the reviewer's comments and we have reviewed format of the full text.

Authors must revise the manuscript according to the Editorial Office's comments and suggestions, which are listed below:

(1) Science editor:

1 Scientific quality: The manuscript is a retrospective cohort observation on "MTNR1B polymorphisms with CDKN2A and MGMT methylation status are associated with poor prognosis of colorectal cancer in Taiwan". The topic is within the scope of the WJG.

(1) Classification: Grade C, C and B

(2) Summary of the Peer-Review Report:

Reviewer 1: This study indicated the novel genetic biomarkers, MTNR1B, combined with CDKN2A and MGMT gene methylation statuses, maybe a predictive tool for CRC prognosis. The researchers hypothesised that the influence of MTNR1B gene variation combined with the hypermethylation of CDKN2A and MGMT genes would predict the prognosis and provide clinical recommendations for

optimal treatment of CRC. This study offers insights into novel genetic and epigenetic biomarkers for the prediction of CRC prognosis, and the findings could be used to individualise the treatment of patients with CRC. Since CRC is a high-incidence cancer, such a clinical research that investigated the new biomarkers of CRC is helpful. One concern is that adjusting for confounding factors such as obesity, a sedentary lifestyle, and unhealthy dietary habits in Table 4, 5 and 6 would be better for studying the effect of MTNR1B SNPs associated with 5-year OS of CRC patients, as this study mentioned. Secondly, the inclusion and exclusion criteria for this study seem to be unclear. Thirdly, it would be better to review format of the full text carefully, with respect to spaces between text and punctuation in the method.

Reviewer 3: In general, this is a well-designed and mature retrospective trial.

Responses: Thank you very much. We have finished all comments of the three reviewers.

I only have a few comments for authors.

1. The introduction of CDKN2A and MGMT genes is abrupt. Please supplement more information about them and explain why choose these two genes for combination analysis.

Responses:

We agreed with the reviewer's comments and added sentences to supplement more information about *CDKN2A*, *MGMT* and *MLH1* genes and explain why choose these genes for combination analysis. Please see the revised manuscript in Introduction on page 6.

2. Authors used Cox proportional-hazards models for to analyze the survival. The adjusted factors included age, sex, stage, adjuvant chemotherapy, tumour location, and the methylation status of the CDKN2A, MLH1 and MGMT genes. What about BMI and combined primary diseases?

Responses:

We appreciate the reviewer's comments. We conducted this retrospective cohort study and collected participates which was diagnosed in the Tri-Service General Hospital (TSGH), Taiwan, from 2006 to 2010. Data regarding registered patients—including sex, surgical age (permanent variable), adjuvant chemotherapy, histologic grade and location of the tumour, and survival—were collected from the TSGH's cancer registry database. Indeed, data on other factors related to CRC risk, such as diet, BMI and combined primary diseases were unavailable in the database. This is the limitation of this study. Please see the revised manuscript in Discussion on page 15.

3. The sample size is a concern. The wide 95%CI indicated poor stability. The suggestions raised by the reviewer should be addressed.

Responses:

We appreciate the reviewer's comments and added one sentence to descript this limitation of this study. Please see the revised manuscript in Discussion on page 14.

(3) Format: There are 7 tables, 0 figure. (4) References: A total of 45 references are cited, including 10 references published in the last 3 years; (5) Self-cited references: There are 5 self-cited references. (6) References recommendations: No

2 Language evaluation: Classification: Grade B, B and A

3 Academic norms and rules: No academic misconduct was found in the Google/Bing search.

4 Supplementary comments: No

5 Issues raised:

1. Results part, “49.5% were stage III-IV”, according to table 2 and 3, it seems to me that it is “51%” instead.

Responses:

We appreciate your comments and we have revised the percentage of the stage III-IV. Please see the revised manuscript in Results on page 10.

2. Results part, for interpretation of Table 2 and 3, difficult to understand “As shown in Table 2 and Table 3, certain possible CRC risk factors—such as age, sex, tumor-node-metastasis (TNM) stage, and CDKN2A, MLH1 and MGMT genes methylation status—did not affect the MTNR1B genotype result. However, the MTNR1B polymorphism, rs1387153 C > T, and 5-year OS differed significantly.” Is it more reasonable to say “As shown in Table 2 and Table 3, certain possible CRC risk factors—such as age, sex, tumor-node-metastasis (TNM) stage, and CDKN2A, MLH1 and MGMT genes methylation status—were not significantly associated with the MTNR1B genotype. However, tumor location and survival were associated with MTNR1B polymorphism”.

Responses:

We appreciate your comments and we have revised the sentences. Please see the revised manuscript in Results on page 10.

3. Results part, the aHR and 95% CI numbers in “The variant types of rs1387153 (CC + CT vs. TT: aHR = 5.41, 95% CI = 1.85–15.9), rs2166706 (TT + TC vs. CC: aHR = 5.96, 95% CI = 2.10–16.9), rs10830963 (CC + CG vs. GG: aHR = 7.01, 95% CI = 2.50–19.7), and rs1447352 (GG + GA vs. AA: aHR = 4.04, 95% CI = 1.23–13.3) decreased the 5-year OS in patients with CRC.” do not match table 4. Please double check it.

Responses:

We sincerely appreciate your comments and we have revised the sentences of the result. Please see the revised manuscript in Results on page 10.

6 Re-Review: Required

Responses: Thank you very much.

7 Recommendation: Conditional acceptance with major revision

Responses: Thank you very much.

(2) Company editor-in-chief: I have reviewed the Peer-Review Report, full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Gastroenterology, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors.

Responses: Thank you very much.

We sincerely thanks for reviewer's comments and your editorial efforts on our manuscript. We believe that the revised manuscript is significantly improved for scientific merits.

Sincerely yours,

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