

## Format for ANSWERING REVIEWERS



Nov 28, 2013

Dear Editor,

Enclosed is the edited manuscript in Word format (file name: 5327-review.doc).

Title: Association of CD14/-260 polymorphism with gastric cancer risk in Highland Tibetans

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**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO:** 5327

The manuscript has been improved according to the helpful suggestions of the reviewers:

1 Format has been updated.

2 Revisions have been made according to the suggestions of the reviewers.

3. All changes are marked in red.

1). Interesting and well done study. A survival analysis graph showing a possible fair/good correlation with the findings would improve the impact of the paper.

Thank you for your comments. We agree with your comment; however, patient prognostic data was not available, and a survival curve could not be generated. We would certainly consider inclusion of such data in a future study.

## 2). Major points

1. In Table 2, they showed the result of genotype and allele frequencies of CD14 -260C/T and -651C/T in cases and controls. According to the result, CT genotype of CD14 -260C/T is the significant risk factor but not TT genotype. It looks rather strange since it cannot be interpreted with dominant, additive or recessive model. To obtain this result, the authors performed the logistic regression. It seems that they did not adjust any related confounding factors such as age, sex, smoking or *H.pylori* infection. If the authors adjust these factors, the association result could be different.

We acknowledge this point. Originally, we had not used logistic regression analysis to adjust for related confounding factors such as age, sex, smoking or *H. pylori* infection. Thank you for pointing out the error, and we have made appropriate corrections in the revised manuscript.

2. The result of Table 2 is not consistent with that of haplotype analysis in Table 3. According to Table2, T allele of -260 C/T is the risk factor and C is the non-risk factor. In -651 C/T, both C and T alleles are non-risk factors. If we look at Table 3, the combination of non-risk and non-risk (CT) turned out to be the most associated haplotype in gastric cancer (OR =1.58, p=0.0007). This result seems not understandable.

Thank you for pointing out the error. In Table 3, our haplotype analysis result described as -651C/T, -260C/T was a minor error, and was inconsistent with the title "Haplotype distribution of CD14 -260C/T and -651 C/T in gastric cancer and healthy controls." The title of Table 3 in the revised version the paper has been changed to -260C/T, -651C/T. There was also a minor error in calculating the OR value in Table 2, which has been corrected in the revised version. According to the results, the CT and TT genotype of CD14 -260C/T is a significant risk factor, and the T allele of -260 C/T is also a risk factor for gastric cancer. Haplotype analysis showed the -260C/T--651 C/T T-C haplotype was associated with an increased risk of gastric cancer.

3. In their CD14 expression result, the authors found that T allele homozygote enhances CD14 expression on circulating monocytes. If we consider the result of Table 2, this also seems not consistent. The authors needs to explain about this discrepancy.

Thank you for your comment. We have asked a statistician to re-analyze the data which we reported in Tables 2 and 3. The -260 C/T CT and TT genotype was associated with an increased risk of gastric cancer. The -260 C/T and T allele was also associated with an increased risk of gastric cancer. There was no significant difference in the distributions of -651 C/T genotypes and alleles between control subjects and gastric cancer patients. Haplotype

analysis showed the -260C/T--651 C/T T-C haplotype was associated with an increased risk of gastric cancer and the -260C/T--651 C/T C-C haplotype was associated with a decreased risk of gastric cancer.

#### Minor points

1. The authors should be more concerned about grammars and typographical errors.

The language was edited by Medjaden Bioscience Limited, Hong Kong, China

2. In introduction, “Tibetans have one of the highest prevalence of gastric cancer in China, being infected with *H. pylori* more frequently and the prevalence is higher in Tibet compared with the average level of China.” The authors should be more specific in this sentence with numbers and reference.

Thank you for your suggestion. We have changed the sentence “Tibetans have one of the highest prevalence of gastric cancer in China, being infected with *H. pylori* more frequently and the prevalence is higher in Tibet compared with the average level of China” to “Tibetans have one of the highest prevalences of gastric cancer in China, and the prevalence in Tibet is higher than the average prevalence in China.” As you kindly pointed out, we have added the following reference: 19 Dan Z, Li K, Wang ZH, Xiangba ZX. Epidemiological features of gastric cancer in a community population in Lhasa. *World Chinese Journal of Digestology*. 2013; 21(21): 2104-08.

3). Minor Comments: 1. Introduction (page 4, last paragraph): Please clarify the statement "... it (*H. pylori*) has not been established as an etiology of gastric cancer"

Thank you for your helpful comment. We have changed the sentence “*H. pylori* is a micro-aerophilic Gram-negative bacterium and has been recognized as a Group I carcinogen for gastric cancer and lymphoma of gastric mucosa-associated lymphoid tissue (MALT) by the World Health Organization, although it has not been established as an etiology of gastric cancer” to “*H. pylori* is a micro-aerophilic Gram-negative bacterium recognized by the World Health Organization (WHO) as a Group I carcinogen for gastric cancer and lymphoma of gastric mucosa-associated lymphoid tissue (MALT).

2 Introduction (page 5, last paragraph): The following statement should be transferred to the "Material & Methods"-section. "Subsequently, we utilized the luciferase reporter assay to measure different genotypes (C and T) of CD14 on the regulation

As you kindly pointed out, we have transferred the statement "The luciferase reporter assay was used to examine the effects of different genotypes (C and T) of CD14 on regulation of CD14 expression in gastric

cancer cells.” to the "Materials & Methods" section.

4). 1) There are no pathological findings in this article. The authors should add the pathological findings such as immunohistochemistry and/or in situ hybridization. 2)The authors analyzed CD/-260 polymorphism in Highland Tibetans. How about the relationship CD/-260 polymorphism and gastric cancer in other race? 3)Is there any correlation between the CD/-260 polymorphism, the expression of CD14, and the stage and the prognosis of gastric cancer?

1) Thank you for your comments. We only collected peripheral blood from patients and the control population for genotyping purposes; therefore, investigations for pathology using immunohistochemistry and in situ hybridization could not be performed.

2) Thanks for your comments. We have searched available information and found that gastric cancer patients in a Chinese Han population have a genotype polymorphism in the CD14/-260 loci. This finding has been described in the third paragraph (Reference 18) of the “Discussion” section. At present, we are not aware of similar studies conducted with other ethnic groups.

3) Thank you for your suggestion. Prognostic data for the patients was not available, and surgical removal of the tumor was not performed in most cases. Therefore, we only collected peripheral blood for the purpose of genotyping, and studies on CD14 expression, tumor grade, and patient prognosis could not be performed.

5). 1. In the part of introduction, what is the evidence of the following sentence “Tibetans have one of the highest prevalence of gastric cancer in China, being infected with H. pylori more frequently and the prevalence is higher in Tibet compared with the average level of China”? 2. Considering that the human races may affect their SNP characteristics and the previous report that the Tibetan population are more susceptible to gastric cancer than the Chinese, what is the difference between them, the frequency and the position? 3. The figures and tables are relatively confused that should be reorganized. 4. The statistical method should be described more concisely and the language should be polished.

1) Thank you for your comments. We have changed the sentence to “Tibetans have one of the highest prevalences of gastric cancer in China, and the prevalence in Tibet is higher than the average prevalence in China.” In addition, we have added the reference for the modified sentences as follows: 19 Dan Z, Li K, Wang ZH, Xiangba ZX. Epidemiological features of gastric cancer in a community population in Lhasa. World Chinese Journal of Digestology. 2013; 21(21): 2104-08

2) Thank you for your suggestions. We have changed the sentence “Tibetan population are more susceptible to

gastric cancer than the Chinese” to “Tibetans have one of the highest prevalences of gastric cancer in China.” In addition, no difference was found between the frequency and position of a particular mutation in the Chinese Han population and the Tibetan population.

(3). The figures and tables are relatively confused that should be reorganized.?

Thank you for your suggestion. We have carefully revised the figures and tables.

(4). The statistical method should be described more concisely and the language should be polished.

Thank you for your suggestion. We have asked a statistician to re-analyze the data, and the language has been edited by Medjaden Bioscience Limited, Hong Kong, China.

3 References and typesetting were corrected

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

Editor Note: I suggest saying “Thank you again for considering our manuscript for publication in the *World Journal of Gastroenterology*.”

Sincerely,

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