ANSWERING REVIEWERS

January 28, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 8319-review.doc).

Title: Risk of gastric cancer is associated with *PRKAA1* gene polymorphisms in Koreans

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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 reviewer 1

(1) I would like to know whether there are any other studies on different population. This information will give the valuable insight of the role of these SNPs in gastric carcinogenesis. As the frequencies of these SNPs may be varied in different population and can lead to racial difference.

➔ To our knowledge, there is no study addressed association between PRKAA1 gene and gastric cancer except in Chinese and Korean population. As reviewer's comments, SNPs frequency may different according to the population. It is need to further study in different races other than Asian. We added following sentence in Discussion.

"To our knowledge, there is no study addressed association between PRKAA1 gene and gastric cancer except in Chinese and Korean population. Since SNPs frequency may different according to the population, it is need to further study in different races other than Asian."

(2) Also, I would like to know on what basis the authors selected and compared 4 haplotype blocks only?

➔ Among haplotypes, we selected 4 haplotypes blocks which frequency over 5%. As reviewer's comments, we added following sentence in method section.

"Haplotype blocks which frequency over 5% were selected for analysis."

(3) Write the full name of *PRKAA1* gene in the introduction.

➔ We added the full name of PRKAA1 in Introduction part as follows,

"~ protein kinase, AMP-activated alpha 1 catalytic subunit (*PRKAA1*)~"

(4) State clearly in the results section for each SNP which is the dominant and recessive allele and which genotypes are associated with increase risk of gastric cancer.

➔ As reviewer's comments, we described risk allele in each SNPs in Result section as follows;

"SNPs *rs13361707* ('C' allele, OR: 1.51, 95% CI: 1.07, 2.11; $P = 0.018$; FDR $Q = 0.023$), *rs154268* ('C' allele, OR: 1.65, 95% CI: 1.22, 2.22; $P = 0.001$; FDR $Q = 0.006$), *rs6882903* ('A' allele, OR: 1.48, 95% CI: 1.09, 2.00; $P = 0.012$; FDR $Q = 0.023$), and *rs10074991* ('G' allele, OR: 1.53, 95% CI: 1.09, 2.16; $P = 0.014$; FDR $Q = 0.023$) were significantly associated with an increased risk of gastric cancer."

3 Revision has been made according to the suggestions of the reviewer 2

(1) Gastric carcinogenesis is a multifactorial and multistage process in which several factors, such as nutritional, infectious and genetic ones, play a role. To consider only the genetic aspect, without analyzing dietary habits, H.pylori infection, gene expression is not exhaustive to conclude that a polymorphic variant is associated with an increased susceptibility to gastric cancer. The authors must collect data about dietary and smoking habits and H. pylori infection in cases and controls.

➔ We absolutely agreed with reviewer's comments. It is true that more risk factors including the analysis, more clear to understand association between PRKAA1 gene and gastric cancer.

However, unfortunately we have no more information related with gastric cancer. Please consider this study as pilot study. If we collect more information, we will analyze whether some risk factors can modify the effect of PRKAA1 SNP in gastric cancer development. We mentioned this limitation in Discussion part as follows,

"Finally, because the data of environmental factor for gastric cancer such as H. pylori infection and diet was not available in this study, we could not evaluate the gene-environmental interaction. It is needed further study about it."

(2) Regarding the genotyping, the authors should state what quality control measures were made.

➔ As reviewer's comments, we revised Method section as follows,

"Genotyping was carried out by Macrogen (Seoul, Republic of Korea). The average call rate was 99.40%. And to check validity of genotypes, we performed genotyping with duplicate for 20 samples."

(3) The authors should include the results of testing the allelic difference between diffuse-type cancer cases vs. intestinal-type cancer cases and EGC vs AGC.

➔ We could not obtain detailed data on the histological tumor types for the cases of gastric cancer. This is a kind of limitation in the present study. We mentioned about this in Discussion section.

(4) Did the authors compare the SNPs frequencies with those generally appearing in other, e.g. Asian or Caucasian populations?

➔ Yes, we compared these SNPs frequencies of Koreans with those in Asian, African, and European. As result, the SNPs frequencies in Korean were similar those in Asian, but were little bit different those in African and European. This suggests that further study in other population rather than Asian is needed to evaluate the associations between PRKAA1 and gastric cancer. Regarding these facts, we described in Discussion section.

SNPs	Alleles	Frequencies			
		This study	Asian	African	European
rs6882903	C	0.752	0.826	0.773	0.672
rs10074991	G	0.456	0.434	0.617	0.747
rs13361707	C	0.459	0.434	0.633	0.747
rs154268	T	0.812	0.794	0.612	0.653
rs3805486	T	0.704	0.733	0.817	0.808

(5) In the Discussion section, the authors found that all of the tested SNPs of PRKAA1 we tested were associated with significantly increased risk of gastric cancer. The authors must collect more data to gain insights into a mechanistic hypothesis that could link PRKAA1 SNP to an increased susceptibility to gastric cancer in Korean patients.

➔ As reviewer's comments, we described possible mechanisms of association of PRKAA1 SNPs (AMPK activation) with gastric cancer development in Discussion section as follows.

"Although the biological mechanism underlying the association between *PRKAA1* and gastric cancer has not been clarified, these significant associations could potentially be explained by the ability of activated AMPK phosphorylates p53 to induce G1/S arrest. Further, the AMPK-p53 connection may represent a cell cycle checkpoint[23]. Therefore, individuals with mutant *PRKAA1* alleles, which encode inactive AMPK, may be vulnerable to gastric cancer. Anti-inflammatory action by AMPK could provide another explanation of the association between SNPs of *PRKAA1* and gastric cancer. A recent study has reported that activated AMPK can counter-regulate macrophage inflammatory function[24] and activate some anti-inflammatory agents[25]. Loss of anti-inflammatory action by AMPK in the body of individuals with mutant *PRKAA1* alleles results in more severe injury of the epithelium[26]. Bone marrow-derived cells are recruited at these sites of epithelial damage, and these cells can

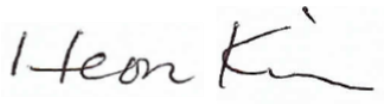
be a potential source of malignancy[27].”

(6) In addition, it is not still unclear why the authors select 5 SNPs of PRKAA1. Is there any evidences or paper describing that PRKAA1 play a role in gastric carcinogenesis.

➔ We selected SNPs of *PRKAA1* from several prominent online databases (GeneCards, HUGE navigator, NCBI; www.ncbi.nlm.nih.gov/SNP) because this gene may be related to diet risk factors for gastric carcinogenesis. To select tagging SNP, we identified functional elements from the Functional Elements SNPs Database (FESD), used the tagger pairwise method from the International HapMap Project, and finally selected SNPs with a minor allele frequency (MAF) ≥ 0.05 in JPT (Japanese in Tokyo, Japan) and CHD (Han Chinese in Beijing, China) samples. SNP (rs 3805490) that significantly deviated from the Hardy-Weinberg equilibrium was discarded. We described tis sentences in Method section.

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

Sincerely yours,



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