Dec 5, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 6800-topic highlight.doc).

Title: Ethnic Differences in Genetic Susceptibility to Gastric Cancer: Allele Flips of Interleukin Gene between Asians and Non-Asians

Author: Juwon Kim, Yoonjung Kim, Kyung-A Lee

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 6800

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) As this is the review article, several points may be added for the readers;

1. It will be better if real data (tables and figures) can be summarized.
   →Table 1 summarizing which cytokine polymorphisms come into play a role in the carcinogenesis of tumors arising in different anatomical sites and tumors of different histologic types has been added to the manuscript.

2. Is there any data with combined polymorphisms of IL-1B and IL-10 which show any association of gastric cancer with location specificity?
   →It would be interesting to conduct a study to see any synergistic effect of those polymorphisms. We have found some studies on the IL-1B and IL-10, but regarding combined effect of IL-1B and IL-10 polymorphisms in association with gastric cancer could not be found. This may be due to smaller size of the group having information on polymorphisms of both IL-1B and IL-10, causing limitations to power analysis.

3. It will be better if any association can be described between type specificity (diffuse and intestinal type) and specific polymorphism. In the manuscript, there is only short description about the relationship with intestinal type.
   →We did not concentrate on histologic types in the first draft since it might cause more confusion to the readers. No association specific to diffuse type gastric cancer was observed in polymorphisms of IL-1B, IL-10 nor IL-8. Diffuse type very much different from intestinal type gastric cancers that diffuse type gastric cancer is thought to arise via genetic changes that occurred in gastric cancer stem cells or epithelial precursor cells and usually lack defined premalignant lesions. Recently, several genome wide association studies have been conducted in regards to diffuse type gastric cancer and found that locus near MUC1 and PSCA are associated with diffuse types among Asians. IL-1RN and TNFA have been previously found to be associated with diffuse type gastric cancer in Caucasian population. Our recent study showed that another cytokine gene called RANTES-403A allele increased the risk of diffuse type gastric cancer in Asian male population. More study should be conducted based on the anatomical and histologic types of gastric cancers which are thought to have different pathogenesis. This information was also added to the manuscript.

4. It will be better how much real production of cytokine is increased based on the polymorphisms.
   → In a study by *Iacoviello et al* (Arteriosclerosis, thrombosis, and vascular biology. 2005; 25: 222-7), *IL-1B-511CC* (4500±800pg/mL; *P*_0.003) released more IL-1β upon stimulation of mononuclear cells with lipopolysaccharide, whereas IL-1B-511TT homozygotes showed decreased release of IL-1β (800±200 pg/mL), compatible with the results reported by *Chen et al* (Human molecular genetics. 2006; 15: 519-29) who suggested *IL-1B*-1464G/-511C/-31T was clearly associated with higher level of IL-1β in Caucasian population.

5. Is there any data about second primary gastric cancer after gastrectomy that is correlated with the polymorphisms?
   →The association study of second primary gastric cancer after gasterectomy and cytokine polymorphism could not be found.

6. In endemic area, H. pylori is controlled by treatment. In this case, how can we expect the specific polymorphisms with gastric cancer development?
   → Relation between genetic polymorphism of inteleukin and cytochrome P2C19 (CYP2C19) and triple therapy were studied among Chinese population, an endemic area of *H. pylori*, and only extensive metabolizer genotype of CYP2C19 associated with metabolism of proton pump inhibitor responded better to rabeprazole based triple therapy than omeprasole therapy. No significant differences in cure rates were observed among different IL-1RN and IL-1B genotypes. (J Clin Pharm Ther 2010;35(6):713-22)

(2) This is a well-written manuscript in which the authors summarized the up-to-date data on interleukin gene polymorphisms on the susceptibility of developing gastric cancer. However in my point of view, more than a few points should be noted and have to be discussed:

1. The manuscript describes the impact of interleukin gene polymorphisms on susceptibility to gastric cancer. Unfortunately it is not timely for the more recent studies have established the analytic methods and data for the whole genome-wide association or relation, including interleukin gene although not exclusively, for the development of cancer.
   → Recently, many genome wide association studies (GWAS) has revealed some additional gastric cancer susceptibility loci. In a Japapanese GWAS, a SNP (rs2976392) in *prostate stem cell antigen* (*PSCA*) gene encoding a glycosylphosphatidylinositol-anchored cell surface antigen was significantly associated with diffuse type gastric cancer. In addition, two SNPs (rs2070803 and rs4072037) in *Mucin 1*(*MUC1*) was also found as susceptible gene for diffuse type gastric cancer in Asian population. For non-cardia gastric cancer, rs13361707 SNP in the first intron of *protein kinase, AMP-activated, alpha 1 catalytic subunit* (*PPKAA1*) and rs9841504 in the intron of *zinc finger and BTB domain containing protein 20* (*ZBTB20*) were found to be the susceptibility loci from GWAS analysis.

2. The authors wrote that there would be differences in the susceptibility of developing gastric cancer between Westerners and Asian population. It is by far agreed by investigators worldwide, and Asian patients with gastric cancer probably have different tumor biology than Caucasians. But it is still unclear to which extent, what degree or which polymorphisms, the results give us information about the molecular pathogenesis of cancer.
   → The non-cardia/intestinal type gastric cancer follows a multistep progression that is usually initiated from chronic gastritis and progress to intestinal metaplasia and subsequently to gastric cancer, whereas cardia cancers are caused by gastroesophageal reflux and increased acid secretion. From recent molecular studies revealed that different genetic components come into play in the pathogenesis of those two subtypes of gastric cancers, and gene expression profile concretely showed different genetic components between the two. However, the genetic factors

are different between Asian and non-Asians, that genetic heterogeneity may cause different cytokine genes to play a dominant role in each ethnic group in reaction to stress on the mucosa and cause different outcomes. We have added Table 1 to briefly summarize the types of tumor by anatomical sites and Lauren classification and listed some of genes having polymorphisms, which seem to play an important role in the pathogenesis of gastric cancer.

3. I would suggest rewriting some part of the manuscript. It might be very helpful to include study results achieved by the authors themselves, in respect to Asian gastric cancer patients.
   →Previously published data and data to be published were added in the manuscript. We discovered a polymorphism of IL-1B at -1464 to be associated with increased risk of gastric cancer in Korean population (reference 29) and reported allele flip of IL-10 polymorphism in Asians vs non-Asians (reference 59). Finally, we found association of RANTES-403 and diffuse type gastric cancer in Asian male population (reference 83).

4. The overall quality of written English needs some language corrections. The authors need some editorial assistance in English.
   →The manuscript was proof read by two professional English editors.

(3) The authors address an important topic—differences in associations between cytokine polymorphisms and gastric cancer risk by population (Asian versus Caucasian). The authors make some interesting points, for example, regarding functional activity by haplotypes. Unfortunately, the way the paper is currently presented; it is very difficult to follow these points. It would useful if the authors could reorganize and clarify the paper in a number of ways.

1. From the abstract, it is not clear that this manuscript is in fact a review. I kept waiting to hear what the authors did and why it was important.
   →The abstract has been rewritten, directly explaining our goal of writing this review.
   1) We try to make clear that allele flip occurs in Asians vs non-Asians, and IL-1B and IL-10 are the main ones that were confirmed to show allele flip phenomenon in large meta-analyses, that is why we focused on those two interleukins.
   2) The allele flip seems to occur due to difference in the composition of gasric cancer types in different ethnic groups in different geographic regions since some regions are endemic area of *H. pylori* infection. Prevalence of gastric cancer in Asians and non-Asians is apparently different. Stratifying gastric cancers in to different groups by its molecular characteristics, lauren classification and anatomical sites could help resolve those controversial results respect to interleukin polymorphisms in relation to gastric cancer.

2. It would be helpful to clarify why the authors focused on these particularly genes/polymorphisms. (Did the authors just choose IL-1B and IL-10 as examples of pro-inflammatory and anti-inflammatory cytokines, or are they the only cytokine polymorphisms that show these kinds of differences by ethnic group?) Also, how did the authors decide which studies and reviews to include?

   →Actually, IL-1B and IL-10 are the main ones that were confirmed to show allele flip phenomenon in large meta-analyses, that is why we focused on those two interleukins. Allele flip seems to occur in IL-8 but more data should be accumulated to confirm this finding. In regards to IL-1RN, IL-1RN*2 seems to be involved in the pathogenesis of non-cardia gastric cancer which appears to be confined to non-Asians, but this may be due to low allele frequency of IL-1RN*2 in Asians *per se*.

3. The entire manuscript needs to be organized in a more logical manner. For example, on page 6,

the authors introduce the first study that showed an association between pro-inflammatory cytokines and gastric cancer. Then they explain that IL-1B is a pro-inflammatory cytokine involved in a variety of cellular activities, etc. Then they go back to describe the initial study in more detail. That paragraph continues for 3 pages. Explaining more clearly why the commentary focuses on IL-1B and IL-10 would help because the authors could describe their function at that time. Similarly, the description of potential explanations for polymorphism difference by population is currently buried on page 9. In any case, a 3-page paragraph is much too long and must be broken into logical pieces that the reader can follow to understand the argument the authors are trying to make. Currently, it is very difficult to pull out the authors' main point and how they got there.

→The manuscript has been reorganized as recommended by the reviewer. Partly rewritten and added more recent information as well to help readers to understand the allele flips and the significance of these findings in understanding pathogenesis of different subtypes of gastric cancers.

4. Finally, the authors claim that cardia cancer is relatively common among the Caucasians compared to Asians, when in fact, the opposite is true. What the Kamangar paper that the authors cites refer to is a change in the RANTES of non-cardia and cardia gastric cancer, not the overall proportion. In fact, noncardia gastric cancer is still more common in Western countries than non-cardia cancer, despite the fact that the rate of noncardia gastric cancer has been decreasing. (See Abrams J Clin Gastroenterol 2013 as one of many examples.)

   → We added some of the information regarding epidemiology of the gastric cancer with the reference. From our data and other association studies have shown that the non-cardia cancers definitely outnumber cardia among Asians. We rewrote those parts which assumed that non-cardia is more frequent in Asians and cardia cancers are more frequent in Caucasians.

5. Minor comment: The manuscript would benefit from review by an English editor.
   → The manuscript was proof read by two professional English editors.

3 References and typesetting were corrected

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

Sincerely yours,

Kyung-A LEE, MD, PhD
Department of Laboratory Medicine
Yonsei University College of Medicine
211 Eonju-ro, Gangnam-gu,
Seoul 135-720, Republic of Korea
Fax: +82 2 2019 4822
E-mail: Kal1119@yuhs.ac