The COX-2-1195AA Genotype Is Associated with Diffuse-Type Gastric Cancer in Korea


*Department of Internal Medicine, †Laboratory for the Study of Gastroenterology and Hepatology, and ‡Department of Pathology, Hallym University College of Medicine, Seoul, Korea

Background/Aims: The potential role of the cyclooxygenase (COX)-2 polymorphism has been reported in relation to the risk of gastrointestinal tract malignancies. Therefore, we investigated whether COX-2 polymorphisms are associated with the risk of gastric cancer (GC) in Korea, one of the areas with a high prevalence of this condition.

Methods: We evaluated the genotypic frequencies of COX-2-765 and -1195 in 100 peptic ulcer patients, 100 GC patients, and 100 healthy controls. The polymorphisms of the COX-2-765 and -1195 genes were analyzed by polymerase chain reaction and restriction fragment length polymorphisms. Results: The frequencies of the COX-2-1195 GG, GA, and AA genotype were 20%, 60%, and 20% in intestinal-type GC and 8%, 48%, and 44% in diffuse-type GC, respectively (p=0.021). There were no significant differences in the frequency of COX-2-765 genotypes between intestinal-type GC and diffuse-type GC (p=0.603). Age- and sex-adjusted logistic regression analysis showed that the COX-2-1195 AA genotype was the independent risk factor of diffuse-type GC compared with the COX-2-1195 GG genotype (p=0.041; odds ratio, 6.22; 95% confidence interval, 1.077 to 35.870).

Conclusions: The COX-2-1195 AA genotype may render subjects more susceptible to diffuse-type GC.

Key Words: Stomach neoplasms; Diffuse type; COX-2; Polymorphism

INTRODUCTION

Although Helicobacter pylori infection has been generally accepted as the main risk factor for gastric cancer (GC), carcinogenesis of GC is still unclear. H. pylori infection alone is not enough to explain the gastric carcinogenesis because GC develops in only a small portion of infected subjects. It has been suggested that GC may have a more complex mechanism involving bacterial, dietary, and host factors that are intimately interconnected. Among the host factors, single nucleotide polymorphisms (SNPs) of interleukin (IL)-1, IL-10, and tumor necrosis factor (TNF)-α have been thought to play an important role in Caucasians. The impact of polymorphisms of IL-1, IL-10, and TNF-α on the development of GC, however, is still controversial in Asians including our previous studies. Recently, several studies suggested that cyclooxygenase (COX)-2 polymorphisms are related with the high risk of various human malignancies, predominantly in gastrointestinal tracts including GC. As well known, COX is a rate-limiting enzyme that converts arachidonic acid to prostaglandins. As of today, three isoenzymes COX-1, COX-2, and COX-3 are found in human. Among them, COX-2, only expressed by various stimuli such as cytokines, growth factors, and mitogens, is responsible for inflammatory process and carcinogenesis. In particular, increased COX-2 expression is linked to progression of gastric pre-malignant lesions and gastric carcinogenesis by activating angiogenesis, inhibiting apoptosis, and accelerating invasion and metastasis. Moreover, it has been reported that SNPs in the promoter region of the COX-2 encoding gene have a direct effect on COX-2 expression and its functional activity. That is, COX-2-765 C allele increased the production of PGE2 and PGD2 in asthma, and COX-2-1195 A allele is associated with increased COX-2 expression in esophageal cancer. In GC, to our knowledge, few studies have been published on the SNPs of the promoter region -765 (rs689466) or -1195 (rs20417) of the COX-2 encoding gene. Moreover, it is still inconclusive although it is probable that those polymorphisms are helpful to identify the high-risk subjects for GC.
GC can be subdivided into two pathologic entities with intestinal and diffuse types, which have distinct epidemiological and clinical features. It is generally accepted that the carcinogenesis of intestinal type GC shows a multi-step progression of gastric mucosal lesions from atrophic gastritis to adenoma, followed by dysplasia and then eventually GC. To date, few data about polymorphisms of sporadic diffuse type GC has been reported. GC is highly prevalent in the Far East, especially in Korea, Japan, and China. According to a recent survey, in South Korea, GC incidence in men and women reaches 62.8 and 25.7 cases per one hundred thousand, respectively. Such a high incidence of GC in South Korea remains an etiologic and treatment challenge. Therefore, in this study, we focused whether COX-2 polymorphisms are associated with risk of GC, H. pylori infection, and pathologic types of GC in South Korea, one of the most prevalent areas of GC in the world.

MATERIALS AND METHODS

1. Patients and samples

A total of three hundred cases with peptic ulcer diseases (PUDs) (n=100), GC (n=100), and healthy controls (n=100) were enrolled and analyzed. Healthy controls were recruited voluntarily, who were conducted a routine medical check-up at Hallym University Kangdong Sacred Heart Hospital, Seoul, South Korea. All enrolled subjects underwent endoscopy, and the endoscopic findings were reviewed by two experienced endoscopists with a blind fashion. The control group has superficial gastritis or normal appearance of the gastric mucosa endoscopically. PUD and GC were diagnosed by endoscopic findings combined with histology. GC was subdivided into two distinct pathologic entities according to the Lauren’s classification by an experienced pathologist blindly. The tumor, node, metastasis (TNM) stages were assigned according to the 6th American Joint Committee on Cancer TNM staging system. GC was grouped into cardiac and non-cardiac cancer by the locations of GC. H. pylori infection was evaluated by histologic examination, rapid urease test, and/or anti-H. pylori immunoglobulin G quantification (GCRL Co., Seoul, Korea; sensitivity, 98.10%; specificity, 90.82%). We defined H. pylori infection as being positive when at least one test of them showed positivity. All the subjects had no past history of H. pylori eradication. Buffy coat was separated from the whole blood and stored immediately at -70°C until use. The study protocol was approved by Institutional Review Board at Hallym University Kangdong Sacred Heart Hospital.

2. Analysis of COX-2 gene polymorphism

Genomic DNA was extracted from the buffy coat using a commercialized kit (QIAamp; QIAGEN, Valencia, CA, USA). Following polymerase chain reaction (PCR) amplification using the primers as listed in Table 1, the COX-2 polymorphism was analyzed by restriction fragment length polymorphism (RFLP) method. Briefly, PCR conditions for COX-2-765 were as follows: 94°C for 2 minutes, then 35 cycles of 94°C for 1 minute (denaturation), 60°C for 1 minute (annealing), 72°C for 1 minute (extension), and finally 72°C for 10 minutes. And then, the PCR products were digested with AciI (Promega, Madison, WI, USA) at 37°C for 4 hours and separated by electrophoresis on a 2% agarose gel. A fragment containing the AciI polymorphic site at position -765 of COX-2 gene was separated as follows: the G

![Fig. 1. Restriction patterns for cyclooxygenase (COX)-2-765 with AciI and COX-2-1195 with PvuII. (A) The genotypes of COX-2-765 were designated as follows: GG, 2 bands of 100/210 bp; GC, 3 bands of 100, 210, and 310 bp; and CC, a single band of 310 bp. A 100-bp ladder is displayed in Lane 1 (Marker). Lanes 2, 3, 4, 5, and 6 are GC, GG, GC, GG, and GG, respectively. (B) The genotypes of COX-2-1195 were designated as follows: GG, 2 bands of 50/220 bp; GA, 3 bands of 50, 220, and 270 bp; and AA, a single band of 270 bp. A 100-bp ladder is displayed in Lane 1 (Marker). Lanes 2, 3, and 4 are AA, GG, and GA, respectively.

Table 1. Primers Used for the Polymerase Chain reaction

<table>
<thead>
<tr>
<th>Position</th>
<th>Primer set</th>
<th>Sequences</th>
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<tr>
<td>-765 COX-2</td>
<td>Sense</td>
<td>5’-AGG CAG GAA ACT TTA TAT TGG-3’</td>
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<tr>
<td></td>
<td>Antisense</td>
<td>5’-ATG TTT TAG TGA CGA CGC TTA-3’</td>
</tr>
<tr>
<td>-1195 COX-2</td>
<td>Sense</td>
<td>5’-AGT TTT TAG TGA CGA CGC TTA-3’</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>5’-CCC TGA GCA CTA CCA ATG AT-3’</td>
</tr>
</tbody>
</table>

COX, cyclooxygenase.