Novel interleukin 1β polymorphism increased the risk of gastric cancer in a Korean population

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Background. Polymorphisms in the gene for interleukin 1β (IL-1B) have been found to increase the risks of gastric cancer and its precursors in response to Helicobacter pylori infection in white populations. However, there has been no independent confirmation of the role of IL-1B markers in gastric cancer patients from Asian populations. Moreover, there have been conflicting data regarding the effect of IL-1B-511/-31 on the risk of gastric cancer or its precursors in Asian populations. Therefore, we assessed an additional polymorphism in the promoter region of IL-1B at position-1473 with the IL-1B-511/-31 polymorphisms in a Korean population.

Methods. In a case-control study, including 331 gastric cancer cases and 433 controls, we assessed the association between the three polymorphisms and the risk of gastric cancer. All genotyping was performed in duplicate. To assess the DNA-binding activity of IL-1B-1473 in vitro, we performed an electrophoretic mobility shift assay (EMSA).

Results. When cases were divided according to the histologic type of the tumor, a significant difference in genotype frequencies for IL-1B-1473 was observed only between intestinal-type cases and controls (odds ratio [OR], 1.8; 95% confidence interval [CI], 1.0–3.5 and OR 2.1 and 95% CI, 1.1–4.2 in the CG and GG genotypes, respectively). In the cases, there was a deviation from Hardy-Weinberg equilibrium in the IL-1B-511/-31 loci confined to the intestinal type, due to the excess of heterozygotes. The IL-1B-1473G allele showed decreased binding to nuclear extract, indicating a weaker promoter activity on EMSA.

Conclusions. We identified a novel single-nucleotide polymorphism, 1473C→G, in the IL-1B promoter that was significantly associated with gastric cancer among Koreans. Our results also suggest that the association between IL-1B polymorphism and an increased risk of gastric cancer may depend on the histologic type of gastric cancer.

Key words: interleukin 1β, promoter, polymorphism, gastric cancer, histologic type

Introduction

Interleukin (IL)-1 beta is an important proinflammatory cytokine with profound inhibitory effects on gastric acid secretion.1 The IL-1 receptor antagonist is an anti-inflammatory cytokine and competes with IL-1 beta for binding to IL-1 receptors.2 El-Omar et al.3 have recently reported that proinflammatory genotypes of the IL-1 gene cluster (IL-1B-511/-31 and IL-1RN*2/*2) were associated with an increased risk of gastric cancer and its presumptive precursors, gastric atrophy and hypochlorhydria, in white populations from Poland and Scotland. More recently, Machado et al.4 confirmed the same associations with gastric cancer in a population from northern Portugal.

There is substantial international variation in gastric cancer incidence, with the highest rates reported from eastern Asia, including Korea and Japan.5 However, there has been no independent confirmation of the role of IL-1B markers in gastric cancer patients from Asian populations. Moreover, there have been conflicting data regarding the effect of the IL-1B511/-31 cluster on the risk of gastric cancer or its precursors in Asian populations.6,7 In our preliminary study (unpublished data), we found no significant association between the IL-1B-511/-31 genotypes and gastric cancer, and IL-1RN*2/*2 was very rare among Koreans (0.50% and 0.46% in cases and controls, respectively), rendering its use unhelpful. Therefore, we assessed an additional polymorphism, in the promoter region of IL-1B at position-1473,
representing a C-G transversion with the IL-1B-511/-31 polymorphisms. We selected this polymorphism based on the results of a statistical analysis for the prediction of transcription start sites within a span of about 2.5kb in the promoter region of the IL1B gene, using the McPromoter program (http://genes.mit.edu/McPromoter.html) (Fig. 1). The IL-1B-1473 (rs no. 1143623) is the first polymorphism in the promoter of IL-1B and has been mapped within 2kb of an mRNA transcript for IL-1B; its allele frequency data were validated by noncomputational individual genotyping (www.ncbi.nlm.nih.gov/SNP).

We examined the association between three polymorphisms, IL-1B-1473 and IL-1B-511/-31, in the promoter region of IL-1B and the risk of gastric cancer among Koreans. We also investigated to determine whether a previously suggested relationship with the histologic types of gastric cancer was present with a larger sample size of subgroups.

**Subjects and methods**

**Subjects**

Peripheral blood samples were obtained from 331 patients with gastric cancer (cases; male-female ratio, 2:1:1; average age, 55 ± 11.6 years; range, 24–81 years) and 433 healthy controls (male-female ratio, 9:3:1; average age, 53 ± 8.9 years; range, 18–91 years). Blood samples from the case and control groups were collected after institutional review board (IRB) approval was granted and after informed consent was obtained. Gastric cancers were diagnosed at Samsung Medical Center, Seoul, Korea. According to the Lauren classification, cases were classified as diffuse (n = 188), intestinal (n = 133), and mixed type (n = 10). All of the histopathologic slides were reviewed by one pathologist initially and confirmed by one pathologist (S. Y. S.) independently. Genomic DNA was isolated from peripheral blood leukocytes using a Wizard Genomic DNA Purification kit (Promega, Madison, WI, USA).

**IL-1B genotyping**

All genotyping of 331 cases and 433 control samples was performed in duplicate, by DNA sequencing and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). IL-1B polymorphisms were distinguished by PCR-RFLP, using the primer pairs and restriction enzymes listed in Table 1. Amplification was performed in a volume of 25µl, containing 2.5µl of 10× PCR buffer (100mM Tris-HCl, 15mM MgCl₂, and 500mM KCl, pH 8.3), 200nM each dNTP (Roche Diagnostics Korea, Seoul, Korea), 1µM each primer (Bioneer, Daegeon, Korea), 1 U Taq DNA polymerase (Roche Diagnostics Korea, or Takara Shuzo, Otsu, Japan), and 100ng of genomic DNA. The thermocycling conditions were as follows: 95°C for 5min; then 35 cycles of 95°C for 30s, 58°C–60°C for 30s, and 72°C for 1min; then 72°C for 10min. Fifteen microliters of the reaction mixture was treated with 5U of appropriate restriction enzymes (NE Biolabs, Beverly, MA, USA) at 37°C for 12h and subsequently analyzed on 3% agarose (2% Nusieve [BioWhittaker Molecular Applications, Rockland, ME, USA] and 1% agarose) gel. PCR-RFLP was conducted using the control samples with known restriction enzyme recognizing sequences for each analysis.

DNA sequencing was performed using an ABI prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

**Electrophoretic mobility shift assay (EMSA)**

To assess the DNA-binding activity of IL-1B-1473 in vitro, we performed an EMSA. Nuclear extracts were prepared from freshly isolated human monocytes. Complementary single-stranded oligonucleotides were synthesized (Bioneer) as follows: IL-1B-1473, 5’-GCTCACTCCCTTG G/C ATAATGCAGAGC-3’.

Complementary strands were annealed by combining 2µg of oligonucleotide and 6µg of 10× annealing buffer (500mM Tris, 100mM MgCl₂, and 50mM dithiothreitol) in a 60-µl aliquot of reaction mixture, placing the mixture in a boiling water bath for 5mins, and allowing it to cool to room temperature. The complementry strands were end-labeled using T4 polynucleotide kinase with 3000 Ci/mmol [r-³²P] ATP (Amersham Pharmacia Biotech Korea, Seoul, Korea). The DNA-protein bind-

![Fig. 1. McPromoter results for promoter prediction (input sequences; Chr2:114532148–114534677, Apr. 5, 2002). IL, interleukin; SSM, stocastic segment model](image-url)