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Lack of association between pro-inflammatory genotypes of the interleukin-1 (IL-1B -31 C/+ and IL-1RN *2/*2) and gastric cancer/duodenal ulcer in Korean population

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Abstract

IL-1β is a pro-inflammatory cytokine with multiple biological effects and is a potent inhibitor of gastric acid secretion, and IL-1RN has been shown to be associated with enhanced IL-1β production in vitro. Recently, it was reported that the pro-inflammatory genotypes, IL-1B -31 C/+ and IL-1RN *2/*2, were associated with an increased risk of gastric cancer in a Caucasian population. We tested the association between the polymorphisms and 190 gastric cancer, 117 duodenal ulcer, and 172 healthy subjects as controls in the Korean population. The allele frequency of IL-1B -31 C was more prevalent in Korean (51%) than in Caucasian (30%), while the frequency of IL-1RN *2 allele was less in Korean (6%) than in Caucasian (27%). Using the IL-1B TT genotype as a reference group, the CC genotype was not associated with an increased risk of gastric cancer or duodenal ulcer in the Korean population (odds ratios (OR) = 0.90, 95% confidence interval (CI) = 0.50–1.64; OR = 0.72, 95% CI = 0.36–1.46, respectively). Similarly, IL-1RN*2 was not a risk genotype for either gastric cancer or duodenal ulcer. No association was recognized on the haplotype analysis of the two genes, either. Our results did not support the previous report that IL-1B -31 C/IL-1RN*2 polymorphisms were associated with an increased risk of gastric cancer. The lack of association with duodenal ulcer also suggested that the polymorphisms were not directly related to the acid-secreting capability.

Keywords: Duodenal ulcer; Gastric cancer susceptibility; IL-1B; IL-1RN; Polymorphism

1. Introduction

Gastric cancer is one of the most common malignant diseases worldwide. Gastric cancer is a disease of complex etiology involving infectious, dietary, environmental, and genetic factors that are intimately interconnected [1]. *Helicobacter pylori* infection is strongly associated with gastric cancer as well as duodenal ulcer [2,3]. Intriguingly, epidemiological studies have shown that the two diseases are inversely associated [4,5]. The two diseases have been reported to have distinct patterns of gastric acid secretion: duodenal ulcers tend to be associated with high acid-secreting capacity whereas gastric cancers are associated with low acid-secreting capacity due to diffuse loss of parietal cell mass [6,7]. It is to be elucidated how individuals respond to *H. pylori* infection in such contrary ways. Host factors related to the acid-secreting capacity may play an important role in the distinct pathogenesis of the two *H. pylori*-associated diseases.

The interleukin-1 (IL-1) gene cluster on chromosome 2q contains three related genes within a 430 kb region: IL-1A, IL-1B, and IL-1RN, which encode the pro-inflammatory cytokines IL-1α, IL-1β as well as their

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endogenous receptor antagonist IL-1ra, respectively [8]. IL-1β is up-regulated in the presence of *H. pylori* [9] and is a potent inhibitor of gastric acid secretion [10]. Three diallelic polymorphisms in IL-1B have been reported, all representing C–T base transitions, at positions -511, -31, and +3954 bp. IL-1RN has a penta-allelic 86 bp tandem repeat polymorphism in intron 2. IL-1RN allele 2 has been shown to be associated with enhanced IL-1β production in vitro [11]. Recently, El-Omar et al. [12,13] reported that pro-inflammatory genotypes of the interleukin-1 loci (IL-1B -31 C+, IL-1B -511 T+, and IL-1RN*2/*2) were associated with a significantly increased risk of a chronic hypochlorhydric response to *H. pylori* infection and gastric cancer in a Caucasian population, presumably by altering IL-1β levels in the stomach. The molecular mechanism involved in the possible increase of IL-1β expression was not clear.

Subsequent studies of the association have been controversial. In the Portugese population, IL-1B -511 T and IL-1RN*2 alleles were associated with increased risk of gastric cancer [14]. However, in the Japanese population, the proposed association has not been confirmed [15]. The discrepancy could reflect the genotypic differences in the study populations. According to the proposal by El-Omar et al., the gastric cancer risk-genotypes IL-1B -31 C+ and IL-1RN*2/*2 were associated with decreased acid-secreting capacity. We presumed that it might be tested by analyzing the allelic frequencies in gastric cancer and duodenal ulcer subjects in a population. Because subjects with duodenal ulcer tend to have elevated acid-secreting capacity, the gastric cancer and duodenal ulcer groups would be expected to show different allelic frequencies, presumably in the opposite way compared to the controls.

In this study, we tested whether the pro-inflammatory genotypes of IL-1 were associated with an increased risk of gastric cancer and/or duodenal ulcer in the Korean population in which the prevalence of *H. pylori* infection and gastric cancer incidence are extraordinarily high [16]. We have also analyzed the association according to the topographical distribution and histopathologic classification of gastric cancers. It has been suggested that the topographical location of *H. pylori* gastritis was implicated in the pathogenesis of gastric cancer [6,7]. Gastric cancers have been classified as either diffuse or intestinal types according to Laurén's classification [17]. In the Portugese population, the IL-1RN*2 allele was associated with intestinal type gastric cancer [14].

2. Results

A total of 190 gastric cancer (153 diffuse types, 28 intestinal types, 9 unclassified), 117 duodenal ulcer, and 172 healthy controls were studied (Table 1). As shown in Table 1, all tested cancer cases and controls were frequency matched for sex and age ($P = 0.82$ and 0.61, respectively), however, duodenal ulcers and controls were not matched well ($P < 0.05$). The data were analyzed with odds ratio adjustment for age and sex. The distribution of the genotypes of IL-1B and IL-1RN among the control subjects was in agreement with that predicted under the conditions of Hardy–Weinberg equilibrium ($P = 0.45$ and 1.00, respectively). The overall frequency of IL-1B -31 C allele in Korean population was 51% compared with 30% in a Polish population [12]. The frequency was similar among gastric cancer, duodenal ulcer, and controls (Table 2). In the Korean population, IL-1B -31 and -511 promoter polymorphisms showed linkage disequilibria (-31 C and -511 T/ -31 T and -511 C) (data not shown).

Table 1
Characteristics of study subjects

	Controls ($n = 172$)		Gastric cancer ($n = 190$)		Duodenal ulcer ($n = 117$)	
	N	(%)	*N*	(%)	*N*	(%)
Sex						
Male	103	(59.9)	116	(61.1)	93	(79.5)
Female	69	(40.1)	74	(38.9)	24	(20.5)
Age (years)						
≤45	58	(33.7)	61	(32.1)	61	(52.1)
46–55	54	(31.4)	62	(32.6)	35	(29.9)
56–65	45	(26.2)	50	(26.3)	18	(15.4)
>65	15	(8.7)	17	(8.9)	3	(2.6)
Mean ± SD	50.3 ± 11.5		51.3 ± 10.5		46.3 ± 10.3	

The frequency of CC homozygote of IL-1B -31 was not statistically different between the cases and controls (23.3% in controls, 24.7% in gastric cancer, and 25.6% in duodenal ulcer). Using the IL-1B TT genotype as a reference group, neither CT nor CC genotype was associated with an increased risk of gastric cancer (adjusted odds ratio (OR) = 0.76, confidence interval (CI) = 0.45–1.26; adjusted OR = 0.90, CI = 0.50–1.64, respectively) or duodenal ulcer (adjusted OR = 0.63, CI = 0.35–1.15; adjusted OR = 0.72, CI = 0.36–1.46, respectively). The frequency of IL-1RN*2 genotype was 6% in the Korean controls compared with 27% in the Polish population [12]. Only three tested subjects had IL-1RN*2/*2 genotypes. The IL-1RN*2 genotype was not associated with an increased risk of either gastric cancer or duodenal ulcer (Table 2).

The haplotype analysis of IL-1B and IL-1RN revealed that the majority of the population had either T/1 or C/1 haplotypes (45.9 and 45.1% of the control population, respectively). The pro-inflammatory haplotype C/2 was 5.5% of the control. No haplotype showed association with either gastric cancer or duodenal ulcer (Table 3).

The association of the proposed genotypes with each pathologic type or topographical location of gastric cancers was analyzed. The diffuse and intestinal type cancers were 153 (80.5%) and 28 (14.7%) cases, respectively. The allele frequency of IL-1B -31 C in diffuse type gastric

Table 2
Logistic regression analysis of IL-1B and IL-1RN polymorphisms and risk of gastric cancer and duodenal ulcer

	Controls		Gastric cancer			Duodenal ulcer		
Loci	*N*	(%)	*N*	(%)	Adjusted OR (CI)[a]	*N*	(%)	Adjusted OR (CI)[a]
All subjects	172	(100)	190	(100)		117	(100)	
IL-1B (-31)[b]								
TT	38	(22.1)	50	(26.3)	1.00	33	(28.2)	1.00
CT	94	(54.7)	93	(48.9)	0.76 (0.45–1.26)	54	(46.2)	0.63 (0.35–1.15)
CC	40	(23.3)	47	(24.7)	0.90 (0.50–1.64)	30	(25.6)	0.72 (0.36–1.46)
TT	38	(22.1)	50	(26.3)	1.00	33	(28.2)	1.00
CT/CC	134	(77.9)	140	(73.7)	0.80 (0.49–1.30)	83	(71.8)	0.66 (0.38–1.16)
Allele frequency (C)	0.51		0.49			0.49		
IN-1RN (intron2)[c]								
11	142	(82.6)	165	(86.8)	1.00	101	(86.3)	1.00
12	20	(11.6)	18	(9.5)	0.78 (0.40–1.54)	12	(10.3)	0.82 (0.37–1.81)
13	5	(2.9)	3	(1.6)	0.52 (0.12–2.20)	0		NC
14	4	(2.3)	3	(1.6)	0.67 (0.15–3.06)	2	(1.7)	0.62 (0.11–3.60)
22	1	(0.6)	1	(0.5)	0.92 (0.06–14.99)	1	(0.9)	0.81 (0.05–13.38)
23	0		0		NC	2	(0.9)	NC
Allele frequency (*2)	0.06		0.05			0.06		

[a] Adjusted for age and sex.
[b] There was no difference in the IL-1B -31(C/T) distributions between gastric cancer and controls ($P = 0.53$) or duodenal ulcer and controls ($P = 0.33$).
[c] There was no difference in the IL-1RN VNTR distributions between gastric cancer and controls ($P = 0.83$) or duodenal ulcer and controls ($P = 0.35$). NC, not calculated.

cancer was 0.49 compared with 0.51 in control. The CC genotype was not associated with an increased risk of gastric cancer regardless of the histopathologic type (Table 4). Sixty-six (34.7%) and 89 (46.8%) gastric cancers were in the antrum and body, respectively. The allele frequencies of IL-1B -31 C in antral and body cancers were 0.48 and 0.49, respectively. The CC genotype was not associated with the increased risk of either gastric antral or body cancer (Table 4). Taken together, no particular pathologic type or topographical location of gastric cancer was associated with the IL-1B -31 C genotype.

3. Discussion

In this study, no association was present between the IL-1B -31 C/IL-1RN*2 and gastric cancer. The result was consistent with the Japanese population [15] but not with the Caucasians [12,14]. The discrepancy may reflect the genetic difference between the Caucasians and Asians. We have reported that the SNPs may differ considerably among the populations [18]. The different genetic backgrounds were reflected in the allele frequencies of the control populations: the IL-1B -31 /CC genotype was more frequent in the Korean population (23.3%) than in the Caucasian population (10.7%), while the IL-1RN*2 genotype was less frequent in the Korean (6.4%) than in the Caucasian (26.9%). The frequencies of IL-1B -31 C and T alleles in the Japanese population were similar to those of the Korean population, 0.45 and 0.55, respectively [19].

Recently, it was reported in the study of Japanese non-cancer outpatients who had participated in a *H. pylori* eradication program [19] that the TT homozygote of IL-1B -31 was a higher risk genotype for *H. pylori* infection and more vulnerable to persistent infection. It suggested that host genetic factor determines susceptibility and inflammatory responses to *H. pylori* infection, which is an important etiologic factor for gastric cancer. Further study is required to check whether the TT genotype is also a risk genotype to *H. pylori* infection in the Korean population.

Our results showed that allele frequencies of pro-inflammatory IL-1B and IL-1RN were not significantly different between gastric cancer and duodenal ulcer. Indeed, the frequencies of all alleles were very similar among the gastric cancer, duodenal ulcer, and controls. Because the two groups were from the same ethnic background, it was suggested that the gastric carcinogenesis was not directly dependent on the hypochlorhydric

Table 3
Haplotype analysis of IL-1

Haplotype[a]	Controls (%)	Gastric cancer (%)	Duodenal ulcer (%)
IL1B-IL1RN			
T/1	45.9	49.2	48.3
C/1	45.1	43.9	44.0
C/2	5.5	4.1	4.7
T/3	1.5	0.4	0.4
T/4	1.2	0	0.9
T/2	0.9	1.2	1.7
C/4	0	0.8	0
C/3	0	0.4	0
P[b]		0.10	0.74

[a] Haplotypes of IL-1 were predicted from EMPLUS program based on Expectation-Maximization algorithm.
[b] Chi-square test between controls and cases.

Table 4
Logistic regression analysis of IL-1B polymorphism and risk of stratified gastric cancer

	Controls		Diffuse type gastric cancer			Gastric antral cancer			Gastric body cancer		
Genotype	*N*	(%)	*N*	(%)	Adjusted OR (CI)[a]	*N*	(%)	Adjusted OR (CI)[a]	*N*	(%)	Adjusted OR (CI)[a]
All subjects	172	(100)	153	(100)		66	(100)		89	(100)	
IL-1B (-31)											
TT	38	(22.1)	40	(26.1)	1.00	20	(30.3)	1.00	23	(25.8)	1.00
CT	94	(54.7)	75	(49.0)	0.77 (0.45–1.33)	29	(43.9)	0.57 (0.29–1.14)	45	(50.6)	0.80 (0.42–1.50)
CC	40	(23.3)	38	(24.8)	0.92 (0.49–1.73)	17	(25.8)	0.84 (0.38–1.86)	21	(23.6)	0.86 (0.41–1.81)
TT	38	(22.1)	40	(26.1)	1.00	20	(30.3)	1.00	23	(25.8)	1.00
CT/CC	134	(77.9)	113	(73.9)	0.82 (0.49–1.37)	46	(69.7)	0.65 (0.34–1.24)	66	(74.2)	0.82 (0.45–1.49)

[a] Adjusted for age and sex. There were no statistical significances between stratified gastric cancer cases and controls.

effect of the pro-inflammatory IL-1 polymorphisms at least in the Korean population. However, because the gastric secretory capacity was not measured, the actual relationship between the IL-1B -31 C/IL-1RN*2 polymorphism and the gastric acid secretion is still to be elucidated in Asian populations. Further investigations are required. We are searching for the factors inversely associated with duodenal ulcer and gastric cancer.

4. Materials and methods

4.1. Study subjects

A total of 190 gastric cancer patients, 117 duodenal ulcer patients, and 172 controls were included in this population-based case-control study. Gastric adenocarcinomas were diagnosed based on biopsies between November 30, 1999 and November 29, 2001 at Asan Medical Center, Seoul, South Korea. Duodenal ulcers were diagnosed by gastroduodenoscopy; most of them had active gastritis at the antrum as well. No duodenal ulcer patients had gastric cancer simultaneously. The mean age for the development of gastric cancer was 51.3 years (range 27–74 years) and duodenal ulcer was 46.3 (range 20–75 years). The controls were cancer-free individuals stratified and frequency matched with age and sex from the Health Promotion Center of the hospital where routine physical check-ups are done. The mean age of control group was 50.3 years (range 28–74 years). Gastric cancers were classified as diffuse, and intestinal types according to the Laurén's classification [17]. Subjects were also categorized according to the topographical location of cancer. This study was approved by the Ethical Committee of Asan Medical Center.

4.2. Analysis of IL-1 polymorphism

DNA was isolated from blood samples using standard procedures with sodium dodecyl sulfate (SDS)-proteinase K-RNases digestion and phenol–chloroform extraction. To analyze the IL-1B promoter polymorphism, a 253 bp fragment was PCR amplified using primers 5′-CCCTTCCATGAACCAGAGAA-3′ and 5′-GAGCAATGAAGATTGGCTGA-3′ and sequenced. The variable number tandem repeat (VNTR) polymorphism in intron 2 of IL-1RN was PCR amplified (272–627 bp) with forward primer 5′-CCCCTCAGCAACACTCC-3′ and reverse primer 5′-GGTCAGAAGGGCAGAGA-3′ and was separated by electrophoresis on a 2% agarose gel. Alleles of VNTR were coded as: allele 1 = 4 repeats, allele 2 = 2 repeats, allele 3 = 5 repeats, and allele 4 = 3 repeats. Thermocycling conditions were as follows: initial denaturation at 95 °C for 12 min, 35 cycles of denaturing at 95 °C for 30 s, annealing at 56–60 °C for 2 min, and extension at 72 °C for 40 s, followed by a final extension step at 72 °C for 5 min on the Gene-Amp PCR System 9700 (Applied Biosystems, Norwalk, CT). Sequencing reaction was performed as previously described [18].

4.3. Statistical analysis

The genotype frequencies were checked for consistency among controls with those expected from Hardy–Weinberg equilibrium using GENEPOP program (http://www.biomed.curtin.edu.au/genepop/). For the analysis of association between genotypes and the gastric cancer risk or duodenal ulcer risk, the unconditional logistic regression analysis adjusted for age and sex was used to calculate odds ratios and their 95% confidence intervals. The analysis included examining association of IL-1 polymorphisms with histological type and topographical location at the time of diagnosis as well as comparing genotype frequencies between cases and controls. These variables were included in logistic regression analysis and used to analyze the risk genotype for gastric cancer. The data were analyzed using the SAS Version 8.01 (SAS Institute, Cary, NC).

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