

Interleukin-8 Promoter Polymorphism Increases the Risk of Atrophic Gastritis and Gastric Cancer in Japan

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Abstract

Host genetic susceptibility may influence gastric carcinogenesis caused by *Helicobacter pylori* infection. We aimed to clarify the relationship of interleukin (IL)-8 polymorphism with the risk of atrophic gastritis and gastric cancer. We examined IL-8 -251 T > A, IL-1B -511 C > T, and IL-1RN intron 2 polymorphisms in 252 healthy controls, 215 individuals with atrophic gastritis, and 396 patients with gastric cancer. We also investigated the effect of the IL-8 polymorphism on IL-8 production and histologic degree of gastritis in noncancerous gastric mucosa. Although no correlation was found in the analysis of the IL-1B and IL-1RN polymorphisms, IL-8 -251 A/A genotype held a higher risk of atrophic gastritis [odds ratio (OR), 2.35; 95% confidence interval (CI), 1.12-4.94] and gastric cancer (OR, 2.22; 95% CI, 1.08-4.56) compared with the T/T genotype. We also found

that the A/A genotype increased the risk of upper-third location (OR, 3.66; 95% CI, 1.46-9.17), diffuse (OR, 2.79; 95% CI, 1.21-6.39), poorly differentiated (OR, 2.70; 95% CI, 1.14-6.38), lymph node (OR, 2.50; 95% CI, 1.01-6.20), and liver metastasis (OR, 5.63; 95% CI, 1.06-30.04), and p53-mutated (OR, 1.91; 95% CI, 1.13-3.26) subtypes of gastric cancer. The A/A and A/T genotypes were significantly associated with higher levels of IL-8 protein compared with the T/T genotype. Neutrophil infiltration score was significantly higher in the A/A genotype than in the T/T genotype. In conclusion, we showed that the IL-8 -251 T > A polymorphism is associated with higher expression of IL-8 protein, more severe neutrophil infiltration, and increased risk of atrophic gastritis and gastric cancer. (Cancer Epidemiol Biomarkers Prev 2005;14(11):2487-93)

Introduction

Despite the decreasing incidence and mortality rates observed worldwide, gastric cancer still ranks second as the cause of cancer-related deaths (1). Many epidemiologic studies have revealed a strong association between *Helicobacter pylori* infection and gastric cancer (2, 3), and in 1994, the IARC classified the bacterium as a definite biological carcinogen. *H. pylori* colonizes persistently in the gastric mucosa and leads to chronic mucosal inflammation, atrophic gastritis, and finally, gastric cancer (4). However, there are distinct differences in the extent of gastric inflammation among *H. pylori*-infected patients, and only a small group of them develop gastric cancer, indicating that gastric carcinogenesis may be under the combined influence of bacterial pathogenicity, host genetics, and environmental factors.

As one candidate for the host genetic factors, recent reports have revealed that pro- and anti-inflammatory cytokine [interleukin (IL)-1B, IL-1RN, tumor necrosis factor (TNF) A, and IL-10] polymorphisms are associated with a risk for atrophic gastritis and gastric cancer (5-8). Proinflammatory cytokines such as IL-1 β , TNF- α , and IL-8 are up-regulated during chronic *H. pylori* infection (9, 10), and play a crucial role in inflammation of gastric mucosa. In addition, T helper cell 1 phenotype-predominant immune response, generally observed in *H. pylori*-positive gastritis (11), is possibly associated with the development of cancer (12).

IL-8, a member of the CXC chemokine family, was originally identified as a potent chemoattractant for neutro-

phils and lymphocytes (13, 14). Subsequent studies confirmed that IL-8 could also induce cell proliferation (15) and migration (16), as well as angiogenesis (17). Some studies have reported that IL-8 -251 T > A polymorphism in the promoter region is associated with respiratory syncytial virus bronchiolitis (18), prostate cancer (19), enteroaggregative *Escherichia coli* diarrhea (20), and colorectal cancer (21). Furthermore, the IL-8 -251 A allele tended to be associated with increased IL-8 production by lipopolysaccharide-stimulated whole blood (18). Concerning the role of IL-8 polymorphisms in gastric carcinogenesis, one recent study has reported that the IL-8 -251 A allele increases the risk of noncardia and intestinal-type gastric cancer (22). From these findings, we hypothesized that the IL-8 -251 T > A polymorphism could affect each stage of gastric carcinogenesis, the extent of atrophic gastritis as a precancerous lesion (23-25), and the risk of development and the different growth of gastric cancer.

In this case-control study, we determined IL-8 -251 T/A genotype, as well as IL-1B -511 C/T genotype and IL-1RN variable number of tandem repeat region in intron 2, and elucidated the relationship of these genetic variants to the risk of atrophic gastritis and to the risk of gastric cancer, including its subtypes and clinicopathologic features. We also evaluated the effects of IL-8 polymorphism on IL-8 production in the *H. pylori*-infected gastric mucosa and on histologic degree of gastritis in the noncancerous gastric mucosa adjacent to cancer of surgical specimens.

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Materials and Methods

Study Population. A total of 863 subjects were enrolled in this study, including healthy controls ($n = 252$), individuals with atrophic gastritis ($n = 215$), and patients with gastric cancer ($n = 396$). The healthy controls (mean age, 51 years; range, 26-86 years; male/female, 188/64) and individuals with

atrophic gastritis (mean age, 56 years; range, 26-81 years; male/female, 162/53) were recruited consecutively from health checkup examinees who had undergone gastroscopy and/or double contrast radiography as part of a screening program for gastric cancer from January to March 2000 at Aichi Prefecture Health Care Center, Japan. These two groups were discriminated by the presence of gastric atrophy, defined as the spread of atrophic mucosa to the cardia in the lesser curvature of the stomach by gastroscopy, and/or double contrast radiography.

Patients with gastric cancer (mean age, 62 years; range, 30-91 years; male/female, 291/105) had been diagnosed histologically and treated at Nagoya University Hospital (Nagoya, Japan) between January 1999 and January 2002. Gastric cancers were histologically classified according to Lauren's classification (26) and the Japanese Classification of Gastric Carcinoma (27); detailed information about TNM staging, anatomic location, venous and lymphatic invasion, lymph node and distant metastasis, and peritoneal dissemination was available. Furthermore, in noncancerous gastric mucosa adjacent to cancer from 194 surgical specimens, the degree of neutrophil infiltration, mononuclear cell infiltration, atrophy, and intestinal metaplasia were assessed according to the updated Sydney system (28), and were scored as follows: normal, 0; mild, 1; moderate, 2; marked, 3.

All subjects were Japanese and were surveyed about their history of any illness, and smoking habits. Individuals with past history of gastrectomy were excluded from this study. The Ethics Committee of the Nagoya University Graduate School of Medicine approved the protocol, and prior, written informed consent was obtained from all participating subjects.

Detection of *Helicobacter pylori* Infection. *H. pylori* status was assessed by serologic analysis. Peripheral blood was collected from each subject and serum samples separated by centrifugation were stored at -20°C until analysis. The anti-*H. pylori* IgG antibody titer was determined by HM-CAP IgG EIA assay (Kyowa Medex, Tokyo, Japan), and ELISA values >2.2 were regarded as *H. pylori*-seropositive.

Genotyping of Cytokine Gene Polymorphisms. Genomic DNA was isolated from peripheral blood using a standard phenol/chloroform extraction method. The *IL-8* polymorphism was genotyped by PCR-RFLP. Primer sequences for PCR were as follows: forward primer, 5'-TTCTAACACCTGCCACTC-TAG-3'; reverse primer, 5'-CTGAAGCTCCACAATTTGGTG-3'. PCR was carried out in a volume of 10 μL containing 40 ng of genomic DNA, 1 \times reaction buffer, 0.125 mmol/L deoxynucleotide triphosphates, 1.5 mmol/L MgCl_2 , 0.75 $\mu\text{mol/L}$ of each primer and 0.5 units of Platinum Taq DNA polymerase (Gibco BRL, Gaithersburg, MD). The DNA was denatured at 94°C for 4 minutes, followed by 35 cycles at 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds with a final extension at 72°C for 7 minutes. The enzyme digestion using 5 units of *MfeI* (New England Biolabs, Inc., Beverly, MA) was done to analyze the *IL-8* -251 T > A polymorphism and yielded a product of 108 bp (-251 T) and 76 + 32 bp (-251 A). The digestion was incubated overnight at 37°C and then its products were visualized on a 5% agarose gel stained with ethidium bromide.

The *IL-1B* -511 C > T polymorphism was distinguished by 5' nuclease PCR assay (TaqMan) using TaqI for -511. For the TaqMan assay, sequences of primers and probes were courtesy of Dr. Emad M. El-Omar. Thermal cycling of optical plates was done in GeneAmp PCR System 9700 and end point analysis was done in the ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, CA). For *IL-1RN*, genomic DNA was amplified using PCR encompassing an 86-bp variable number of tandem repeats in intron 2. The PCR products were separated by electrophoresis on 2%

agarose gels and stained with ethidium bromide. The alleles were coded conventionally as follows: allele 1, four repeats; allele 2, two repeats; allele 3, five repeats; allele 4, three repeats; and allele 5, six repeats. Because alleles 3, 4, and 5 were very rare, these alleles were classified into the short (allele 2: *2) and long alleles (alleles 1, 3, 4, and 5: L) for the purpose of statistical analysis in accordance with the recent study (7).

Detection of *p53* Mutational Analysis. In 226 of 396 patients with gastric cancer, DNA was extracted from frozen tumor tissue by the standard phenol/chloroform method. Complete coding sequences and splice junctions for exons 5 to 8 of *p53* gene were screened for mutations by PCR-based single-strand conformational polymorphism analysis as previously described by us (29). The sequences of the used primers were: forward, 5'-TCTGTCTCCTTCTCCTTCCTG-3'; reverse, 5'-TCTCTCCAG-CCCCAGCTG-3'; forward, 5'-CTGATTCCTCACTGATTGCTC-3'; reverse, 5'-GAGACCCCAGTTGCAAAC-3'; forward, 5'-CTTGGGCCTGTGTGTCTC-3'; reverse, 5'-AGGGTGGCAAGTGGCTCC-3'; and forward, 5'-GCTTC-TCTTTTCCTATCCTGA-3'; reverse, 5'-GCTTCTTGTC-CTGTTGC-3' for exons 5 to 8, respectively. PCR was carried out with Platinum Taq DNA polymerase (Gibco BRL) for 1 cycle at 94°C for 4 minutes followed by 35 cycles at 94°C for 30 seconds, 46°C to 61°C for 30 seconds, and 72°C for 30 seconds with a final 7-minute extension at 72°C in the presence of 0.2 mCi of [^{32}P]dCTP. PCR-single-strand conformational polymorphism was done using MDE (FMC BioProducts, Rockland, ME) gels. The DNA fragments that showed mobility shifts were excised from the gels and reamplified using the same primers. The PCR fragments were purified using the Microcon-100 microconcentrator (Amicon, Stonehouse, United Kingdom) and sequenced using the ABI Prism Big-Dye Terminator Cycle Sequencing Reaction Kit (Applied Biosystems).

No significant differences were found between cases without analysis of *p53* mutations and cases with analysis with respect to sex, age, distribution of *IL-8* polymorphism, histologic type, tumor location, staging, and other clinical features.

IL-8 Protein Measurement. In 50 patients with gastric cancer, two biopsy specimens were obtained from the greater curvature of the upper gastric body mucosa during gastroscopy and were immediately placed in 3 mL of RPMI 1640 (Gibco BRL) at 4°C . After 6 hours, samples were mechanically homogenized and aliquots of homogenate supernatants, obtained by centrifugation (1,000 $\times g$ for 10 minutes), were stored at -80°C until use. Total protein in the biopsy method was assayed using the Bradford method. *IL-8* protein was measured by chemiluminescent immunoassay using commercially available assay kits (Research and Diagnostic Systems, Minneapolis, MN) according to the manufacturer's instructions. The mucosal *IL-8* levels were expressed as picograms of cytokine per milligram of biopsy protein (pg/mg protein). All donors were serologically *H. pylori*-positive and anatomic location of the tumor was distant enough from the upper gastric body to avoid the influence of inflammation induced by the tumor.

Statistical Analysis. Statistical analyses were done with Fisher's exact probability test or χ^2 test for the comparison of *IL-8*, *IL-1B*, and *IL-1RN* genotype frequencies between cases and controls. The odds ratios (OR) with 95% confidence intervals (CI) were computed using unconditional logistic models, adjusting for sex, age, and *H. pylori* seropositivity. Differences among groups in the gastric mucosa levels of *IL-8* protein and in the histologic score of gastritis were determined using the Mann-Whitney *U* test or Kruskal-Wallis rank test. $P < 0.05$ were considered statistically significant.

Table 1. Characteristics of subjects

	Healthy control	Atrophic gastritis	Gastric cancer	P
Subjects (n)	252	215	396	
Sex [male/female (%/%)]	188/64 (74.6/25.4)	162/53 (75.3/24.7)	291/105 (73.5/26.5)	0.87
Mean age \pm SD (y)	51.1 \pm 10.1	55.6 \pm 9.7	62.2 \pm 11.7	<0.001
Anti- <i>H. pylori</i> IgG antibody seropositivity (%)	30.6	87.0	53.3	<0.001
Smokers (%)	59.1	56.7	63.3	0.30

NOTE: Smokers, current and ex-smokers.

Results

Subjects. A total of 252 healthy controls, 215 individuals with atrophic gastritis, and 396 patients with gastric cancer participated in this study. Demographic comparison of healthy controls, individuals with atrophic gastritis, and patients with gastric cancer are summarized in Table 1. There was no significant difference among these groups in the distribution of sex and smoking habits. The average age increased successively among healthy controls, individuals with atrophic gastritis, and patients with gastric carcinoma (51.1, 55.6, and 62.2 years, respectively; $P < 0.001$). The percentage of *H. pylori* infection was highest in individuals with atrophic gastritis, followed by patients with gastric cancer, and in healthy controls (87.0%, 53.3%, and 30.6%, respectively; $P < 0.001$).

Association Between Cytokine Gene Polymorphisms and the Risk of Atrophic Gastritis and Gastric Cancer. Genotype frequencies of *IL-8* -251 T > A, *IL-1B* -511 C > T, and *IL-1RN* intron 2 polymorphism in the healthy control group did not deviate significantly from those expected under the Hardy-Weinberg equilibrium ($P = 0.99, 0.75, 0.76$, respectively), and did not have any significant difference from the genotype distribution of the cytokine gene in other studies of the Japanese population (30, 31).

In the group of individuals with atrophic gastritis, we found that the *IL-8* -251 A/A genotype, considered as the high IL-8-producing genotype (18), was significantly associated with elevated risk of atrophic gastritis (OR, 2.35; 95% CI, 1.12-4.94). *IL-1B* -511 and *IL-1RN* polymorphisms were not associated with the risk of atrophic gastritis (Table 2).

Comparison of genotype frequency between the healthy control group and patients with gastric cancer, the *IL-8* -251 A/A genotype, was more frequent in patients with gastric cancer than in the healthy control group (OR, 2.22; 95% CI,

1.08-4.56). Similarly, *IL-8* -251 A carriers had a significantly higher risk of gastric cancer (OR, 1.50; 95% CI, 1.00-2.25; Table 2). There was no significant difference in the *IL-8* -251 allelic frequencies between individuals with atrophic gastritis and patients with gastric cancer. We could not find any significant correlation between the risk of gastric cancer and *IL-1B* -511 or *IL-1RN* polymorphisms.

We further investigated whether the *IL-8* -251 polymorphism might affect the clinicopathologic features of gastric cancer. Tumor location, staging, histologic classification, lymphatic and venous invasion, lymph node metastasis, peritoneal dissemination, liver metastasis, other distant metastasis, and *p53* mutations were included in this stratification analysis. Among these clinicopathologic features, we found that *IL-8* -251 A/A genotype increased the risk of upper-third location (OR, 3.66; 95% CI, 1.46-9.17), diffuse type (OR, 2.79; 95% CI, 1.21-6.39), poorly differentiated type (OR, 2.70; 95% CI, 1.14-6.38), lymph node metastasis (OR, 2.50; 95% CI, 1.01-6.20), liver metastasis (OR, 5.63; 95% CI, 1.06-30.04), and *p53*-mutated type (OR, 2.95; 95% CI, 1.18-7.39). *IL-8* -251 A carriers also had an association with diffuse type (OR, 1.88; 95% CI, 1.16-3.04), poorly differentiated type (OR, 1.84; 95% CI, 1.11-3.05), and *p53*-mutated type (OR, 1.91; 95% CI, 1.13-3.26; Table 3).

Effects of the *IL-8* -251 T > A Polymorphism on IL-8 Protein Levels in Gastric Mucosa. Mucosal IL-8 levels in gastric biopsy specimens were measured in 50 *H. pylori*-infected patients with gastric cancer. The *IL-8* -251 A/A genotype and the A/T genotype were significantly associated with higher IL-8 levels than T/T genotype ($358.7 \pm 78.8, 404.8 \pm 301.1$, and 180.6 ± 147.9 pg/mg protein, respectively; $P = 0.003$). Similarly, the *IL-8* -251 A carrier had significantly higher IL-8 levels than the T/T genotype (397.3 ± 276.9 pg/mg protein; $P = 0.02$; Fig. 1).

Table 2. Association between cytokine gene genotypes and risk of atrophic gastritis and gastric cancer

Genotype	Healthy controls, n (%)	Atrophic gastritis			Gastric cancer		
		n (%)	OR (95% CI)	P	n (%)	OR (95% CI)	P
<i>IL-8</i> -251							
A/A	22 (8.7)	26 (12.1)	2.35 (1.12-4.94)	0.02	44 (11.1)	2.22 (1.08-4.56)	0.03
A/T	105 (41.7)	99 (46.0)	1.35 (0.87-2.11)	0.18	191 (48.2)	1.38 (0.91-2.11)	0.13
A carrier	127 (50.4)	125 (58.1)	1.50 (0.98-2.23)	0.06	235 (59.3)	1.50 (1.00-2.25)	0.05
T/T	125 (49.6)	90 (41.9)	1.0 (Reference)		161 (40.7)	1.0 (Reference)	
<i>IL-1B</i> -511*							
T/T	49 (19.6)	46 (21.4)	1.40 (0.76-2.61)	0.28	81 (21.7)	1.69 (0.92-3.08)	0.09
T/C	133 (53.2)	104 (48.4)	0.93 (0.57-1.52)	0.77	188 (50.4)	1.08 (0.67-1.73)	0.77
T carrier	182 (72.8)	150 (69.8)	1.04 (0.65-1.66)	0.86	269 (72.1)	1.22 (0.77-1.92)	0.40
C/C	68 (27.2)	65 (30.2)	1.0 (Reference)		104 (27.9)	1.0 (Reference)	
<i>IL-1RN</i> †							
*2/*2	2 (0.8)	0 (0)	0	1.00	3 (0.8)	1.09 (0.09-13.70)	0.95
*2/L	21 (8.8)	17 (8.2)	1.08 (0.49-2.41)	0.84	25 (6.8)	0.71 (0.32-1.57)	0.39
*2 carrier	23 (9.7)	17 (8.2)	0.83 (0.43-1.60)	0.58	28 (7.7)	0.78 (0.44-1.38)	0.39
L/L	215 (90.3)	191 (91.8)	1.0 (Reference)		337 (92.3)	1.0 (Reference)	

NOTE: A carrier, A/A + A/T; T carrier, T/T + T/C; L, alleles 1, 3, 4, and 5. *2 carrier, *2/*2 + *2/L. ORs are adjusted for sex, age, and *H. pylori* seropositivity.

*Two healthy control and 23 gastric cancer samples could not be genotyped.

†Fourteen healthy-control, 7 atrophic-gastritis, and 31 gastric cancer samples could not be genotyped.

Table 3. Association between *IL-8* –251 polymorphism and clinicopathologic features of gastric cancer

Variables (<i>n</i>)	<i>IL-8</i> –251 genotype				A/A vs. T/T		A carrier vs. T/T	
	A/A	A/T	A carrier	T/T	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>
Healthy controls (252)	22	105	127	125				
Overall gastric cancer (396)	44	191	235	161	2.22 (1.08-4.56)	0.03	1.50 (1.00-2.25)	0.05
Tumor location								
Cardia (27)	4	12	16	11	2.71 (0.69-10.58)	0.15	1.28 (0.53-3.08)	0.58
Non-cardia (369)	40	179	219	150	1.99 (0.94-4.22)	0.07	1.44 (0.95-2.19)	0.09
Upper third (82)	15	36	51	31	3.66 (1.46-9.17)	0.006	1.56 (0.89-2.83)	0.12
Middle third (116)	8	59	67	49	1.26 (0.47-3.38)	0.65	1.37 (0.82-2.28)	0.23
Lower third (198)	21	96	117	81	1.95 (0.84-4.50)	0.12	1.41 (0.88-2.26)	0.15
Staging								
Early (250)	29	118	147	103	2.06 (0.95-4.47)	0.07	1.41 (0.91-2.19)	0.12
Advanced (146)	15	73	88	58	1.90 (0.77-4.69)	0.16	1.47 (0.89-2.45)	0.14
Lauren's classification								
Intestinal type (221)	22	100	122	99	1.59 (0.69-3.63)	0.27	1.13 (0.71-1.79)	0.60
Diffuse type (175)	22	91	113	62	2.79 (1.21-6.39)	0.02	1.88 (1.16-3.04)	0.01
Japanese classification								
<i>tub1</i> (113)	10	55	65	48	1.49 (0.57-3.93)	0.42	1.23 (0.73-2.01)	0.44
<i>tub2</i> (108)	12	45	57	51	1.68 (0.66-4.28)	0.27	1.04 (0.61-1.77)	0.89
<i>por</i> (145)	18	75	93	52	2.70 (1.14-6.38)	0.02	1.84 (1.11-3.05)	0.02
<i>sig</i> (23)	3	13	16	7	3.75 (0.81-17.37)	0.09	2.35 (0.89-6.22)	0.08
<i>muc</i> (7)	1	3	4	3	2.54 (0.24-26.83)	0.44	1.38 (0.30-6.44)	0.68
Lymphatic and venous invasion								
Positive (151)	18	76	94	57	2.23 (0.94-5.30)	0.07	1.50 (0.91-2.48)	0.11
Negative (195)	21	96	117	78	1.95 (0.86-4.41)	0.11	1.42 (0.89-2.25)	0.14
Lymph node metastasis								
Positive (108)	14	53	67	41	2.50 (1.01-6.20)	0.05	1.59 (0.93-2.72)	0.09
Negative (286)	30	138	168	118	1.87 (0.86-4.06)	0.12	1.39 (0.90-2.14)	0.14
Peritoneal dissemination								
Positive (22)	1	13	14	8	0.98 (0.11-8.80)	0.99	1.71 (0.66-4.43)	0.27
Negative (372)	43	178	221	151	2.07 (0.98-4.38)	0.06	1.42 (0.93-2.16)	0.10
Liver metastasis								
Positive (12)	3	5	8	4	5.63 (1.06-30.04)	0.04	1.83 (0.51-6.54)	0.35
Negative (382)	41	186	227	155	1.96 (0.92-4.16)	0.08	1.43 (0.94-2.17)	0.10
Other distant metastasis								
Positive (16)	2	9	11	5	3.00 (0.50-17.94)	0.23	2.07 (0.67-6.40)	0.21
Negative (378)	42	182	224	154	2.00 (0.94-4.22)	0.07	1.42 (0.93-2.16)	0.10
<i>p53</i> mutations								
Positive (143)	19	69	88	55	2.95 (1.18-7.39)	0.02	1.91 (1.13-3.26)	0.02
Negative (83)	8	42	50	33	2.06 (0.72-5.85)	0.18	1.73 (0.97-3.01)	0.07

NOTE: A carrier, A/A + A/T. All data are adjusted for sex, age, and *H. pylori* seropositivity.

Effects of the *IL-8* –251 T > A Polymorphism on Histologic Degree of Gastritis in Noncancerous Gastric Mucosa Adjacent to Cancer. Using samples of noncancerous gastric mucosa adjacent to cancer from 194 surgical specimens, we assessed the histologic degree of gastritis according to the updated Sydney system (28) and scored them as follows: normal, 0; mild, 1; moderate, 2; marked, 3. A significant difference of neutrophil infiltration score was found among *IL-8* –251 A/A, A/T, and T/T genotypes (1.91 ± 0.44 , 1.58 ± 0.78 , 1.47 ± 0.78 , respectively; $P = 0.02$). The *IL-8* –251 A carrier tended to score higher than the T/T genotype (1.64 ± 0.74 ; $P = 0.09$; Table 4). Other scores were not significantly different among genotypes.

Discussion

It has been reported that *IL-8* production in *H. pylori*-infected gastric mucosa is influenced by the presence of the *cag* pathogenicity island of *H. pylori* (32), in which major virulence factors are included. However, as most clinical isolates of *H. pylori* are similar within Japan (33), it is suggested that host genetic factors may play an important role in the differences in *IL-8* expression in *H. pylori*-infected subjects. In the present study, we showed that the *IL-8* –251 T > A polymorphism was associated with increased risk of atrophic gastritis and gastric cancer. The –251 A/A genotype showed a 2-fold risk of atrophic gastritis and gastric cancer, and a similar tendency was observed in the analysis of the

–251 A carrier. These epidemiologic findings were confirmed by the differences in *IL-8* expression in gastric mucosa among genotypes; the –251 A allele was associated with significantly higher levels of *IL-8* protein compared with the –251 T/T genotype.

IL-8 stimulated by *H. pylori* infection induces the recruitment of neutrophils, which secrete proinflammatory cytokines such as TNF- α , IFN- γ , and IL-1 β . The cytokine response in gastric mucosa is thought to be T helper cell (Th) 1–predominant, characterized by the accumulation of IFN- γ , not of IL-4–expressing T lymphocytes (11, 34). Chronic inflammation with a Th 1–predominant immune response in the gastric mucosa of mice has been reported to cause gastric atrophy, whereas Th2 cytokines are protective against gastric inflammation (35, 36). In addition, proinflammatory cytokines play an important role in cellular proliferation and gastric mucosal damage (37). Our study shows that the –251 A/A genotype is associated with increased risk of both atrophic gastritis and gastric cancer, suggesting that a high producer of *IL-8* may induce a Th 1–predominant immune response, lead to more severe gastric atrophy, and be more susceptible to gastric cancer than a low producer of *IL-8*.

On the other hand, the *IL-1B* and *IL-1RN* polymorphisms were not associated with the risk of atrophic gastritis or gastric cancer in the present study. It was reported that the *IL-1B* –511 T and *IL-1RN* *2 alleles were associated with increased IL-1 β production in *H. pylori*-infected gastric mucosa (38), and increased the risk of atrophic gastritis (6)

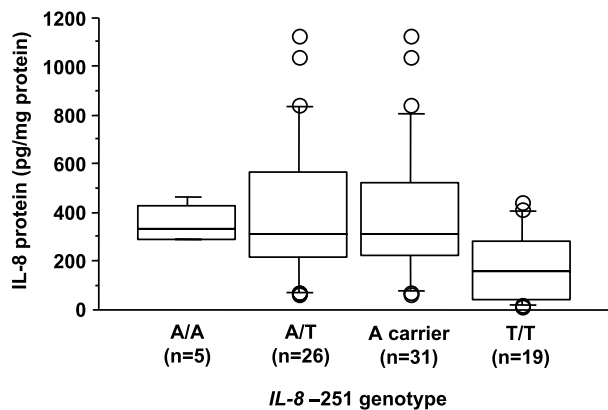


Figure 1. Mucosal IL-8 levels of the gastric body in relation to the genotypes at *IL-8* -251. A significant difference was found among A/A, A/T, and T/T genotypes ($P = 0.003$, assessed by the Kruskal-Wallis rank test). In comparison with the A carrier and T/T genotypes, IL-8 levels in the A carrier were significantly higher than in the T/T genotype ($P = 0.021$, assessed by the Mann-Whitney U test). Columns, 25th and 75th percentiles; horizontal lines in columns, 50th percentile (median); bars, 10th and 90th percentiles; circles, data outside the 10th and 90th percentiles.

and gastric cancer (5). However, some opposite studies have recently been reported. The *IL-1B* -31 T allele, being in almost complete linkage disequilibrium with the *IL-1B* -511 C allele, was associated with increased mucosal IL-1 β levels, and increased risk of intestinal-type gastric cancer in Korea (39). In addition, two studies in Japan reported that the *IL-1B* -511 T allele decreased the risk of gastric atrophy (40) and intestinal metaplasia (41), and was not associated with increased risk of gastric cancer (41). As above, because the functional roles of *IL-1B* polymorphisms in the risk of atrophic gastritis and gastric cancer vary among the different studies, further investigations are necessary to resolve this controversy.

Next, we investigated the effect of the *IL-8* -251 T > A polymorphism on the progression of different gastric cancer subtypes by stratification analysis. It was revealed that the -251 A/A genotype holds a higher risk of upper third location, diffuse type of Lauren's classification, poorly differentiated adenocarcinoma of Japanese classification, lymph node metastasis, liver metastasis, and *p53*-mutated gastric cancers. According to Correa's cascade (23), beginning with chronic gastritis, followed by atrophy, metaplasia, and intestinal type of gastric cancer, we expected that the -251 A/A genotype would be associated with a higher risk of intestinal type gastric cancer in agreement with the recent study (22). However, interestingly, the -251 A/A genotype is related to a higher risk of diffuse type and poorly differentiated adenocarcinomas. A recent study

reported that IL-8 was more strongly expressed in diffuse than in intestinal type gastric cancers (42). We did not investigate the effect of *IL-8* polymorphisms on the expression of IL-8 protein in gastric cancer tissue, but it is possible that the -251 A allele might affect the production of IL-8 protein even in gastric cancer tissue. IL-8 is shown to decrease expression of the epithelial cell adhesion molecule E-cadherin by autocrine or paracrine mechanisms (43). In gastric cancer, low or absent E-cadherin expression is associated with disintegration of tissue architecture and leads to the progression of the diffuse-type gastric cancer (44). Thus, a high IL-8 producer genotype may be associated with elevated risk of diffuse-type and poorly differentiated adenocarcinoma, frequently developing in the upper third location.

The *IL-8* -251 A/A genotype also correlated with a higher risk of lymph node and liver metastasis. These results may be due to tumorigenic and angiogenic functions of IL-8, modulating the growth and invasive behavior of malignant tumors (45). It has been reported that the IL-8 mRNA level in gastric cancer directly correlated with the vascularity of the tumors (46), and transfection of gastric carcinoma cells with the *IL-8* gene enhanced their tumorigenesis and angiogenesis in the gastric wall of the nude mouse (47). Although these findings suggest that IL-8 induces metastasis by autocrine mechanisms, exogenous IL-8, derived from macrophages or neutrophils, also mediated cell migration and angiogenesis (16, 17). We also revealed that the -251 A/A genotype were associated with more severe neutrophil infiltration in noncancerous gastric mucosa adjacent to cancer, therefore, our results suggest that IL-8 increases the metastatic potential of gastric cancer cells by both autocrine and paracrine mechanisms, and suggests that genetic variants of *IL-8* may have some potential to affect the prognosis of gastric cancer.

With regard to the association between the *IL-8* -251 T > A polymorphism and *p53* mutations, the -251 A/A genotype was associated with increased risk of *p53*-mutated gastric cancer. It is speculated that neutrophils induced by IL-8 synthesize active radicals such as nitric oxide (48). These radicals have a mutagenic potential, which could cause mutations in gastric epithelial cells (49). Specifically, nitric oxide deaminates intact DNA, methylated cytosine in particular, at physiologic pH and leads to cytosine-to-thymine transition (50, 51), which is known as the most common base substitution of *p53* (52). Moreover, some studies have reported that aberrant *p53* expression is strongly associated with IL-8 mRNA expression in non-small cell lung cancer (53) and mutated *p53* may up-regulate IL-8 expression by up-regulation of nuclear factor κ B transcription activity (54). These findings suggest that *p53* mutation induced by high IL-8 expression may enhance IL-8 expression in itself, leading to more altered *p53* accumulation. Because *p53* alterations are found even in precancerous gastric mucosa, and is considered as an early event in gastric carcinogenesis (55, 56), and these oxidative stresses in the gastric mucosa are attenuated by *H.pylori*

Table 4. Association between *IL-8* genotype and histologic scores in noncancerous gastric mucosa adjacent to cancer

<i>IL-8</i> -251 genotype (n)	Histologic score			
	Neutrophil infiltration	Mononuclear cell infiltration	Atrophy	Intestinal metaplasia
A/A (21)	1.91 \pm 0.44*	1.67 \pm 0.91	2.00 \pm 0.95	1.67 \pm 1.07
A/T (100)	1.58 \pm 0.78	1.68 \pm 0.72	1.67 \pm 1.02	1.44 \pm 1.09
A carrier (121)	1.64 \pm 0.74 [†]	1.68 \pm 0.76	1.73 \pm 1.01	1.48 \pm 1.08
T/T (73)	1.47 \pm 0.78	1.69 \pm 0.74	1.69 \pm 0.97	1.41 \pm 0.93

NOTE: A carrier, A/A + A/T. Scores shown are mean \pm SD.

* $P = 0.02$, compared among A/A, A/T, and T/T genotype by the Kruskal-Wallis rank test.

[†] $P = 0.09$, compared with A carrier and T/T genotypes by the Mann-Whitney U test.

eradication (57), it is expected that *H.pylori* eradication can suppress *p53*-mediated gastric carcinogenesis in subjects with the *IL-8* -251 A/A genotype.

In conclusion, we showed that the *IL-8* -251 A allele is associated with higher expression of the *IL-8* protein, more severe neutrophil infiltration in gastric mucosa, and increased the risk of atrophic gastritis and gastric cancer, especially diffuse type, poorly differentiated adenocarcinoma, lymph node and liver metastasis, and *p53* mutations. We investigated the *IL-8* -251 T > A polymorphism in only a limited area in central Japan. It has been shown that the allelic frequency of the *IL-8* -251 T > A polymorphism is different between Japanese and Western people (19, 21, 30). Thus, further studies are needed in a larger and ethnically different population to confirm these genetic influences on gastric carcinogenesis.

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BLOOD CANCER DISCOVERY

***Interleukin-8* Promoter Polymorphism Increases the Risk of Atrophic Gastritis and Gastric Cancer in Japan**

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