

Interleukin-1 β gene in esophageal, gastric and colorectal carcinomas

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Abstract. Interleukin (IL)-1 gene polymorphisms are associated with development of gastric atrophy and with increased risk of gastric carcinoma. A -31C to T base transition in the promoter region of this gene is involved in carcinogenic changes within the stomach, especially in *Helicobacter pylori* infected individuals. We examined association between IL-1 locus polymorphisms and risk of esophageal, gastric and colorectal carcinomas in Japanese patients with *H. pylori* infection. IL-1B and IL-1RN polymorphisms were analyzed in 136 controls, 75 patients with esophageal carcinoma, 186 patients with gastric carcinoma, 69 patients with colorectal carcinoma, and 18 patients with ulcerative colitis (UC). For IL-1B-511 and -31 polymorphisms were determined by fluorescence-based polymerase chain reaction single-strand conformation polymorphism analysis. For IL-1 receptor antagonist gene (IL-1RN), penta-allelic variable number of tandem repeats (VNTR) was determined by PCR-standard agarose gel electrophoresis. For gastric carcinoma, IL-1B-511 heterozygotes (OR, 0.48; 95% CI, 0.3-0.9; p=0.0115) and T carriers (OR, 0.52; 95% CI, 0.3-1.0; p=0.0185) had a significantly reduced risk of carcinoma. For colorectal carcinoma, IL-1B-511 heterozygotes (OR, 0.34; 95% CI, 0.2-0.7; p=0.0028) and T carriers (OR, 0.43; 95% CI, 0.2-0.9; p=0.0015) had a significantly low risk of carcinoma. No significant difference was observed in the frequencies of IL-1B-31C/T and IL-1RN genotypes between controls and the esophageal carcinoma patients. Our results shows that IL-1B-511C/T and T carrier state may indicate less risk for gastric and colorectal carcinoma in the Japanese population.

Introduction

Gastric carcinoma remains the second most common carcinoma, having been superceded only by lung carcinoma in the late 1980s on a global scale (1). Gastric carcinoma shows substantial international variation in incidence, with the highest rates reported in Japan and eastern Asia. Other high incidence areas include Eastern Europe and parts of Latin America, whereas Western Europe and the United States generally have low incidence rates (1). Discovery of *Helicobacter pylori* (*H. pylori*) in the early 1980s has proved a turning point in understanding the pathogenesis of this carcinoma. *H. pylori* infection, a well-known risk factor for gastric carcinoma, depends largely on sanitary conditions, especially in childhood (2-4). Substantial differences persist in the prevalence of childhood infection between developed and developing countries, ranging from 4 to 82% (3). Although exposure to the bacterium is an important determinant of infection rate in a population, host susceptibility also is an important factor. The *H. pylori* infection rate in Japan is higher than in other populations, especially those in Western countries (5-7). According to previous reports, 60-70% of the populations over 60 years of age in Japan are infected with *H. pylori*, this rate is close to the *H. pylori* positivity rate of gastric carcinoma cases in Japan (5-7). Epidemiological studies have identified risk factors for gastric carcinoma include sex, *H. pylori* infection, high salt intake, low ascorbic acid intake, exposure to exogenous or endogenously induced nitroso compounds, and gastric musosal changes representing a precancer to cancer sequence (8-10).

Recently, genetic polymorphisms of interleukin (IL)-1, which is a 100-fold more potent inhibitor of gastric secretion than proton-pump inhibitors, have been linked to physiologic differences between individuals in the effect of IL-1 on gastric secretion (11). IL-1 is a potent proinflammatory cytokine (12) that is upregulated in the presence of *H. pylori* and is important in initiating and amplifying the inflammatory response to this infection (13,14). The IL-1 receptor antagonist (IL-1ra) is a naturally occurring anti-inflammatory cytokine that binds competitively to IL-1 receptors, thus blunting potentially injurious effects of IL-1 (15). Polymorphisms of IL-1B and IL-1RN (encoding IL-1ra) were reported to be associated with gastric carcinoma risk in a Polish population (16).

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Table I. Characteristics of controls and carcinoma cases.

	Control subjects (%)	Patients with			
		Esophageal carcinoma (%)	Gastric carcinoma (%)	Colorectal carcinoma (%)	UC (%)
No.	136	75	186	69	18
Age year (range)	60 (35-80)	68 (55-82)	67 (49-88)	70 (55-84)	42 (28-65)
Gender					
Males	95 (70)	60 (80)	130 (70)	41 (59)	9 (50)
Females	41 (30)	15 (20)	56 (30)	28 (41)	9 (50)
Drinking habits					
Non-drinkers	80 (59)	11 (15)	117 (63)	39 (57)	16 (89)
Drinkers	56 (41)	64 (85)	69 (37)	30 (43)	2 (11)
Mean alcohol intake (g)/day	15	63	17	17	12
Mean total amount of alcohol (kg)	160	715	230	221	80
Smoking habits					
Non-smokers	94 (69)	11 (15)	114 (61)	42 (61)	14 (78)
Smokers	42 (31)	64 (85)	72 (39)	27 (39)	4 (22)
Brinkman index					
0	94 (69)	11 (15)	114 (61)	42 (61)	14 (78)
0 to $\leq$ 400	20 (15)	7 (9)	18 (10)	6 (9)	3 (17)
<400	22 (16)	57 (76)	54 (29)	21 (30)	1 (5)

No., number of patients; UC, ulcerative colitis.

IL-1 β has three diallelic polymorphisms located at -511, -31 and +3954 base pairs (bp) from the transcriptional start site. Although the differences were not statistically significant, persistent *H. pylori* infection was observed in 60% of individuals harboring the C/C genotype (n=58) at -31 of IL-1 β , in 73% of those with the C/T genotype (n=67), and in 79% of those with the T/T genotype (n=24) in a Scottish population study (16). The T allele at -31 forms a TATA box that is suspected of enhancing gene expression. The study reported that the polymorphism at -511 was linked tightly with the polymorphism at -31. Accordingly, quite similar findings on the risks of gastric carcinoma and *H. pylori* infection were obtained for IL-1BC-511T. IL-1RN has a penta-allelic 86-bp tandem repeat polymorphism in intron 2, which was found to affect IL-1 production *in vitro* (17) but not to be associated with *H. pylori* infection (16).

While El-Omar *et al* studied a Polish population (16), we investigated a Japanese population. We aimed to determine whether the putative association between IL-1 loci polymorphisms and risk of carcinoma varies according to the specific gastrointestinal organ (esophagus, stomach and large intestine).

Materials and methods

Patients. The study included 466 untreated Japanese patients, with no hereditary predisposition of carcinoma, who consulted our hospital in Tokyo between 1994 and 2002. The control subjects were selected from patients visiting at our hospital, and these subjects were consecutive patients attending for routine endoscopy. Thus, no asymptomatic healthy volunteer was included in this study. There was no subject having past history of any carcinoma or peptic ulcer. The subjects without *H. pylori* infection were excluded from our study. Groups of subjects included a control group (n=136) and patients with esophageal carcinoma (n=75), gastric carcinoma (n=186), colorectal carcinoma (n=69), and ulcerative colitis (UC) (n=18) (Table I). The patients with colorectal carcinoma had no past history of UC. In the control group, the average age was 66 years (range, 32-82) and the male:female ratio was 7:3. In the esophageal carcinoma group, they were 68 years (55-82) and 8:2; in the gastric carcinoma group, 67 years (49-88) and 7:3; in the colorectal carcinoma group, 70 years (55-84) and 6:4; and in the UC group, 42 years (28-65) and 5:5. No significant differences in the mean age and gender

SPANDIDOS[®] noted between the control subjects and patients with esophageal carcinoma, gastric carcinoma or colorectal carcinoma.

Assessment of *H. pylori* status in carcinoma patients and controls. Control subjects and all patients with esophageal, gastric, colorectal carcinoma cases, and UC cases confirmed *H. pylori* infection by the use of ¹⁴C-urea breath test (18), culture, and histopathological examination. Both culture and histopathological examination were performed in all 466 subjects at the time of endoscopy. Specimens of tissue for culture were obtained from corpus of stomach, and those for histopathological examination were obtained from both corpus and antrum. ¹⁴C-urea breath test was performed in ~40% of all subjects within a few days before or after endoscopic examination. *H. pylori* infection was considered positive if results of at least one among the three tests were positive. During endoscopic examination, biopsy specimens were obtained from the antral region within 2 cm of the pyloric ring and from the body region of the stomach between 50 and 55 cm along the greater curvature. *H. pylori* infection was defined to be negative if the results of all three tests were negative, and these patients were excluded from the study. The degree of inflammation and atrophy in the corpus and antrum were assessed according to the updated Sydney system (19), since patients with extensive corpus gastritis develop hypochlorhydria and gastric atrophy, which are presumptive precursors of gastric carcinoma (8,20).

Record of daily consumption of alcohol and/or cigarettes. Daily amount (g) and periods (years) of alcohol intake were recorded in individual patients and control subjects. The amount of alcohol drinking was exchanged into weight (g/day and kg/total) of 100% ethanol. Daily number and periods of cigarette smoking were also recorded. Frequencies of alcohol intake and cigarette smoking in the esophageal carcinoma, gastric carcinoma, and colorectal carcinoma groups were compared with those in the controls. Daily amount of alcohol or cigarettes, total amount of alcohol intake and Brinkman index (B.I.) in the esophageal carcinoma, gastric carcinoma, and colorectal carcinoma groups were compared with those in the controls.

Tissue processing. Diagnosis of carcinoma was confirmed histopathologically in biopsy specimens using hematoxylin and eosin staining. Genomic DNA from the control and carcinoma groups (esophagus, stomach and large intestine), and UC group were isolated from frozen samples of non-neoplastic mucosa by standard proteinase K-phenol/chloroform extraction methods (21), after obtaining the patients informed consent. The study protocol was approved by the Human Ethics Review Committee of the Showa University School of Medicine. All non-neoplastic tissues were confirmed histologically to contain normal mucosa.

Genotyping of IL-1B-31, -511 and IL-1RN VNTR. We evaluated the IL-1B-31, -511 biallelic and IL-1RN penta-allelic variable number of tandem repeats (VNTR) polymorphisms. The IL-1B-31, -511C/T biallelic polymorphism was genotyped by fluorescence-based polymerase chain reaction single-strand conformation polymorphism (PCP-

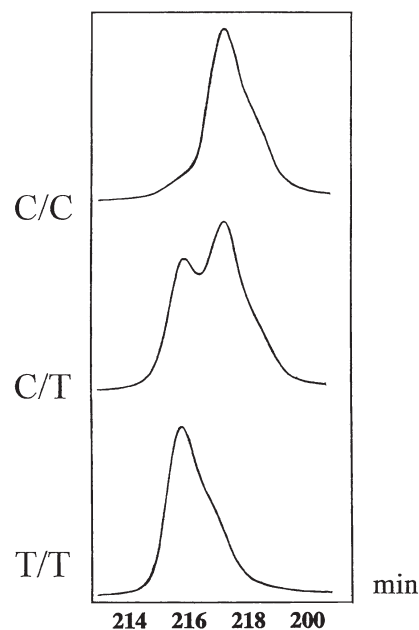


Figure 1. Genotyping of the IL-1B-31C/T: PCR-SSCP analysis of the IL-1B-31C/T polymorphism showing the C/C, T/T, C/T genotypes.

SSCP) analysis (21,22) and the IL-1RN penta-allelic VNTR by PCR-standard agarose gel electrophoresis. Primers used for PCR were based on those reported by El-Omer *et al* (16). The PCR reaction mixtures were carried out in a 20- μ l final volume, containing 0.5 units of Takara ExTaq (Takara, Shiga, Japan), 1 μ mol of each primer, 2.5 mM of each deoxyribonucleoside triphosphatase, and 10 x ExTaq buffer containing 100 mM Tris-HCl pH 8.3, 500 mM KCl, 15 mM MgCl₂ (Takara). For amplification of DNA fragments in frozen tissue, 50 ng of DNA was subjected to 30 cycles of 94°C for 30 sec, 55°C for 30 sec and 72°C for 1 min. To purify and visualize PCR products, the PCR products were electrophoresed on 2% agarose gels, and stained with ethidium bromide. SSCP was performed using an ALFexpress automated DNA sequencer (Amersham Pharmacia Biotech, Uppsala, Sweden) with a temperature-controlled cooling waterbath. Peak patterns were analyzed using the ALFwin Fragment Analyser program (Amersham Pharmacia Biotech). Samples showing peak shifts were considered polymorphic (Fig. 1). Nucleotide sequences of fragments showing peak shifts were determined using the ABI PRISM[®] BigDye[™] Terminator Cycle Sequencing Kit (Applied Biosystems, CA), using an ABI PRISM[®] 310 Genetic Analyzer (Applied Biosystems).

The IL-1RN gene has a penta-allelic 86-base pair tandem repeat in the second intron (23), and the alleles were coded conventionally as follows: allele 1, 4 repeats; allele 2, 2 repeats; allele 3, 5 repeats; allele 4, 3 repeats; and allele 5, 6 repeats (Fig. 2). Given the rarity of alleles 3, 4 and 5, this polymorphism was treated as biallelic for statistical analysis by dividing alleles into short and long categories, with a short allele having two repeats (allele 2) and a long allele having three or more repeats (alleles 1, 3, 4 and 5).

Statistical analysis. Differences in genotype frequencies between cases and controls were assessed by chi-square test. Student's-t test, odds ratios (ORs) with 95% confidence

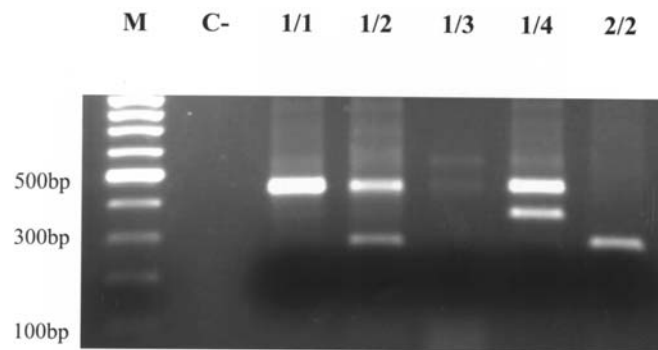


Figure 2. PCR/agarose gel electrophoresis of the IL-1RN VNTR polymorphism (M, size marker; C-, negative control).

intervals (CIs) and unconditional logistic regression models were computed using the SPSS software program (SPSS Science, Chicago, IL). Haplotype frequencies for pairs of alleles were estimated and Linkage disequilibrium coefficients $D' = D/D_{\max}$ and χ^2 values were calculated using the SNPalyze (Dynacom Co., Ltd.). P-values <0.05 were considered significant.

Results

Estimated haplotype frequencies and linkage disequilibrium coefficients. Table II shows estimated haplotype frequencies and linkage disequilibrium coefficients of the IL-1B and IL-1RN. There was marked strong linkage disequilibrium ($D' \sim 0.5$, $P=0.0001$) between IL-1B-31 and IL-1B-511 in the patients and the control subjects. Also there was strong linkage

disequilibrium between IL-1B-31 and IL-1RN in patients with esophageal carcinoma and colorectal carcinoma.

Genotype frequencies in control cases. Tables III and IV list the genotype frequencies in control cases. A near-complete linkage disequilibrium was observed between polymorphisms at -31 and -511 of IL-1B; 22.8% (31/136) for C/C at -31 and T/T at -511, 58.8% (80/136) for C/T and C/T, 17.6% (24/136) for T/T and C/C, 0.7% (1/136) for T/T and T/T, and none for the other combinations. Participants harboring the 1/1 genotype of IL-1RN represented 93% of the total.

IL-1B-31C/T. In patients with esophageal carcinoma, there was no significant difference in genotype frequency between patients and the control subjects ($\chi^2=0.691$, $p=0.708$, Table III). Genotype frequencies in patients with esophageal carcinoma were similar to control subjects and genotypic specific risks were not high. A significant difference in genotype frequency was observed between patients with gastric carcinoma and control subjects ($\chi^2=6.437$, $p=0.040$). A modest increase in risk of gastric carcinoma was observed for IL-1B-31T homozygotes, but this was not statistically significant (OR, 1.52; 95% CI, 0.8-2.9; $p=0.214$). Comparison of genotype frequencies showed a significant difference between patients with colorectal carcinoma and control subjects ($\chi^2=9.033$, $p=0.011$). A modest increase in risk of colorectal carcinoma was observed for IL-1B-31T homozygotes, but this was not statistically significant (OR, 1.43; 95% CI, 0.6-3.1; $p=0.383$). Genotype frequencies in patients with UC were similar to control subjects ($\chi^2=0.042$, $p=0.979$) and genotypic specific risks were not high (Table III).

Table II. Estimated haplotype frequencies and linkage disequilibrium coefficients.

Population	Loci	Haplotype ^a						Disequilibrium		
		1-1	1-2	2-1	2-2	1-3, 4, 5	1-3, 4, 5	D'	χ^2	P
Control	IL-1B-31/1B-511	0.011	0.467	0.518	0.004			-0.98	256.3	0.0001
	IL-1B-31/1RN	0.505	0.009	0.455	0.024	0.007		0.21	3.124	0.2097
	IL-1B-511/1RN	0.446	0.024	0.513	0.009	0.001	0.007	0.24	3.177	0.2042
Esophageal carcinoma	IL-1B-31/1B-511	0.014	0.433	0.526	0.027			-0.94	126.9	0.0001
	IL-1B-31/1RN	0.513	0.040	0.433	0.001	0.001	0.012	0.47	7.391	0.0248
	IL-1B-511/1RN	0.430	0.016	0.516	0.024	0.013	0.001	0.19	2.738	0.2543
Gastric carcinoma	IL-1B-31/1B-511	0.008	0.519	0.468	0.005			-0.97	350.2	0.0001
	IL-1B-31/1RN	0.439	0.034	0.494	0.019		0.013	0.08	7.016	0.0714
	IL-1B-511/1RN	0.491	0.019	0.442	0.034	0.014		0.75	6.985	0.0724
Colorectal carcinoma	IL-1B-31/1B-511	0.007	0.515	0.471	0.007			-0.97	128.1	0.0001
	IL-1B-31/1RN	0.407	0.064	0.505	0.017	0.006	0.001	0.62	4.537	0.0336
	IL-1B-511/1RN	0.504	0.017	0.407	0.064	0.001	0.007	0.50	6.783	0.0636

^aC is denoted by allele 1 and T is denoted by allele 2.



	Genotype											
	Control (%)	EC (%)	OR (95% CI)	P	GC (%)	OR (95% CI)	P	CC (%)	OR (95% CI)	P	UC (%)	OR (95%CI)
No.	136	75			186			69			18	
C/C	31 (23)	20 (27)	1.0		45 (24)	1.0		20 (29)	1.0		4 (22)	1.0
C/T	80 (59)	44 (59)	0.85 (0.4-1.7)	0.641	86 (46)	0.74 (0.4-1.3)	0.283	26 (38)	0.50 (0.2-1.0)	0.058	11 (61)	1.07 (0.3-3.6)
T/T	25 (18)	11 (14)	0.68 (0.3-1.7)	0.406	55 (30)	1.52 (0.8-2.9)	0.214	23 (33)	1.43 (0.6-3.1)	0.383	3 (17)	0.93 (0.2-4.5)
T carriers	105 (77)	55 (73)	0.81 (0.4-1.5)	0.529	141 (76)	0.93 (0.5-1.6)	0.770	49 (71)	0.72 (0.4-1.4)	0.332	14 (78)	1.03 (0.3-3.4)

No., number of patients; OR, odds ratio; 95% CI, 95% confidence interval; EC, esophageal carcinoma; GC, gastric carcinoma; CC, colon carcinoma; T carriers, both IL-1B-31T homozygotes and IL-1B-31 heterozygotes; UC, ulcerative colitis.

Table IV. Comparison of IL-1B-511 genotype frequencies between controls and patients with gastrointestinal cancers.

	Genotype											
	Control (%)	EC (%)	OR (95% CI)	P	GC (%)	OR (95% CI)	P	CC (%)	OR (95% CI)	P	UC (%)	OR (95%CI)
No.	136	75			186			69			18	
C/C	24 (18)	13 (17)	1.0		54 (29)	1.0		23 (33)	1.0		3 (17)	1.0
C/T	80 (59)	44 (59)	1.02 (0.5-2.2)	0.969	87 (47)	0.48 (0.3-0.9)	0.015	26 (38)	0.34 (0.2-0.7)	0.003	11 (61)	1.10 (0.3-4.3)
T/T	32 (23)	18 (24)	1.04 (0.5-2.5)	0.934	45 (24)	0.63 (0.3-1.2)	0.162	20 (29)	0.65 (0.3-1.5)	0.293	4 (22)	1.0
T carriers	112 (82)	62 (83)	1.02 (0.5-2.1)	0.954	132 (71)	0.52 (0.3-0.9)	0.019	46 (67)	0.45 (0.2-0.9)	0.012	15 (83)	1.07 (0.3-3.9)

No., number of patients; OR, odds ratio; 95% CI, 95% confidence interval; EC, esophageal carcinoma; GC, gastric carcinoma; CC, colon carcinoma; T carriers, both IL-1B-511T homozygotes and IL-1B-511 heterozygotes; UC, ulcerative colitis.

IL-1B-511C/T. There was no significant difference in genotype frequency between patients with esophageal carcinoma and control subjects ($\chi^2=0.007$, $p=0.996$, Table IV). Genotype frequencies in patients with esophageal carcinoma were similar to control subjects and genotypic specific risks were not high. IL-1B-511C/T genotypes were not significantly different between patients with esophageal carcinoma and control subjects.

A significant difference in genotype frequency was seen between patients with gastric carcinoma and control subjects ($\chi^2=6.417$, $p=0.040$). The risk of gastric carcinoma was significantly low in IL-1B-511 heterozygotes (OR, 0.48; 95% CI, 0.3-0.9; $p=0.015$), and in IL-1B-511 T carriers (both IL-1B-

511T homozygotes and IL-1B-511 heterozygotes, OR, 0.52; 95% CI, 0.3-0.9; $p=0.019$).

A significant difference in genotype frequency was also present between patients with colorectal carcinoma and control subjects ($\chi^2=9.407$, $p=0.009$). The risk of colorectal carcinoma was significantly low in IL-1B-511 heterozygotes (OR, 0.34; 95% CI, 0.2-0.7; $p=0.003$), and in IL-1B-511 T carriers (OR, 0.45; 95% CI, 0.2-0.9; $p=0.012$). Genotype frequencies in patients with UC were similar to control subjects ($\chi^2=0.034$, $p=0.983$) and genotypic specific risks were not high (Table IV).

IL-1RN VNTR. As shown in Table V, there was no significant difference in genotype frequency between control subjects

Table V. IL-1RN VNTR genotypic frequencies in controls and patients with gastrointestinal cancers.

	Control subjects (%)	Patients with			
		Esophageal carcinoma (%)	Gastric carcinoma (%)	Colon carcinoma (%)	Ulcerative colitis (%)
No.	136	75	186	69	18
1/1	126 (93)	69 (92)	164 (88)	60 (87)	16 (98)
1/2	7 (5)	3 (4)	14 (7)	7 (11)	2 (12)
1/3	2 (1)	2 (3)	3 (2)	1 (1)	0
1/4	0	0	2 (1)	0	0
2/2	1 (1)	1 (1)	3 (2)	1 (1)	0

No., number of patients.

and patients with esophageal carcinoma ($\chi^2=0.684$, $p=0.953$, Table V), gastric carcinoma ($\chi^2=2.817$, $p=0.589$), colorectal carcinoma ($\chi^2=2.077$, $p=0.722$), and UC ($\chi^2=0.408$, $p=0.982$). After reclassifying the alleles into long and short, there was no significant difference in genotype frequency between control subjects and patients with esophageal, gastric, colorectal carcinoma, and UC (Table VI). There were no significant associations between IL-1RN genotype and the risk of development of these malignancies (Table VI).

Daily consumption of alcohol and/or cigarettes. As shown in Table I, the frequency of alcohol consumption differed significantly between the esophageal carcinoma group (64 of 75; 85%) and the control group ($p<0.0001$). However, the frequency of alcohol consumption in the gastric carcinoma group (69 of 186; 37%) and the colorectal carcinoma group

(30 of 69; 43%) did not differ significantly from that in the control group ($p=0.5692$, $p=0.7050$). The frequency of daily cigarette smoking in esophageal carcinoma group (64 of 75; 85%) differed significantly from that in the control group ($p<0.0001$). In contrast, the frequency of cigarette smoking in the gastric carcinoma group (72 of 186; 39%) and the colorectal carcinoma group (27 of 69; 39%) did not differ significantly from that in the control group ($p=0.3033$, $p=0.3580$). Daily amount of alcohol intake (63 g/day) and a total amount of alcohol intake (715 kg) in patients with esophageal carcinoma differed significantly from those (15 g/day, 160 kg) in control subjects ($p<0.0001$). In contrast, daily amount of alcohol intake (g/day) and a total amount of alcohol intake (kg) in patients with gastric carcinoma (17 g/day, 230 kg/total) or colorectal carcinoma (17 g/day, 221 kg/total) did not differ significantly from control subjects (gastric carcinoma: $p=0.6530$, $p=0.2104$; colorectal carcinoma: $p=0.7077$, $p=0.3779$). Daily amount of cigarette smoking (20 pieces/day) and Brinkman index (B.I., 756) in patients with esophageal carcinoma differed significantly from those (6 pieces/day; B.I., 154) in control subjects ($p<0.0001$). In contrast, daily amount of cigarette smoking (pieces/day) and Brinkman index (B.I.) in patients with gastric carcinoma (9 pieces/day; B.I., 286) or colorectal carcinoma (7 pieces/day; B.I., 259) did not differ significantly from those in control subjects (gastric carcinoma: $p=0.0538$, $p=0.3181$; colorectal carcinoma: $p=0.3010$, $p=0.1229$).

Degree of inflammation and atrophy in the stomach. Degree of inflammation and atrophy was estimated in all subjects according to Sydney system. The mean scores of inflammation in the corpus of the stomach in patients with esophageal carcinoma, gastric carcinoma, colorectal carcinoma and control subjects were 1.8, 2.5, 2.3 and 1.9, respectively. The mean score of atrophy in the corpus body of the stomach in patients with esophageal carcinoma, gastric carcinoma, colorectal carcinoma and control subjects were 1.8, 2.6, 2.3 and 2.0, respectively. The mean scores of inflammation and atrophy

Table VI. Comparison of IL-1RN VNTR genotypic frequencies in controls and patients with gastrointestinal carcinoma and UC.

	Genotype												
	Control (%)	EC (%)	OR (95% CI)	P	GC (%)	OR (95% CI)	P	CC (%)	OR (95% CI)	P	UC (%)	OR (95%CI)	P
No.	136	75			186			69			18		
L/L	128 (94)	71 (95)	1.0		169 (91)	1.0		61 (88)	1.0		16 (89)	1.0	
L/2	7 (5)	3 (4)	0.71 (0.2-3.1)	0.714	14 (8)	1.51 (0.6-3.9)	0.381	7 (10)	2.10 (0.7-6.1)	0.175	2 (11)	1.08 (0.1-9.3)	0.890
2.2	1 (1)	1 (1)	1.80 (0.1-28)	0.674	3 (1)	2.27 (0.2-20)	0.468	1 (2)	2.10 (0.1-32)	0.594	0		

No., number of patients; OR, odds ratio; 95% CI, 95% confidence interval; EC, esophageal carcinoma; GC, gastric carcinoma; CC, colon carcinoma; UC, ulcerative colitis; L, the long allele (alleles 1, 3, 4 and 5).



SPANDIDOS PUBLICATIONS is with esophageal carcinoma, gastric carcinoma, colorectal carcinoma did not differ significantly from those in control subjects.

Discussion

IL-1 is a multifunctional cytokine with high inflammatory activity. It has three members, IL-1 α , IL-1 β and IL-1Ra, and there are two types of receptors; a membrane bound and soluble form. For IL-1B, three polymorphisms, C-511T, C-31T and C+3954T, have been reported (16). A near-complete linkage disequilibrium between polymorphisms at -31 and -511 was found for the participants. In the present study, the corresponding values in Japanese were 0.000 for C-C combination, 0.553 for C-T, 0.429 for T-C, and 0.018 for T-T among the controls. El-Omar *et al* corrected their results (24), and they confirmed the more common (IL-1B-31T/IL-1B-511C) and less common (IL-1B-31C/IL-1B-511T) haplotypes. However, our data showed that C-T type is common in Japanese subjects. It is likely that the difference in frequencies of IL-1B polymorphisms is due to difference of ethnic background between Caucasians and Japanese. Furthermore, the histologic characteristics of control subjects were estimated according to the criteria of Updated Sydney system. Since the mean scores of inflammation and atrophy in the corpus of controls tended to be low rather than those of patients with gastric carcinoma, almost all of controls did not have histologically precancerous lesions. Furuta *et al* reported that IL-1B-31T/IL-1B-511C was predominant in gastritis-only patients and *H. pylori*-negative control subjects (25). In contrast, other studies in Japanese populations reported IL-1B-31C/IL-1B-511T was dominant in *H. pylori*-negative control subjects (26,27). These results were similar to those in our study. We do not believe that the difference of IL-1B gene polymorphisms regulates infection of *H. pylori*, although the difference of IL-1B gene polymorphisms can regulate the degree of atrophy in the corpus. Thus, the allele identities in our study were not reversed.

Recent studies have demonstrated that IL-1 β is an important proinflammatory cytokine highly expressed in the gastric mucosa of *H. pylori*-positive hosts; who are also known to have low gastric acid secretion (11,16). El-Omar *et al* examined the association of particular genotypes with low acid state in Scottish and Polish gastric carcinoma patients and controls (16). They suggested that individuals with the -31T/T or C/T genotypes overexpressed gastric IL-1 β in response to inflammation and low gastric pH. This hypothetical scheme is suitable for the precancer-cancer sequence of the gastric carcinogenesis (16). Indeed, the pH of gastric juice depends on the IL-1B-511 polymorphism genotype in Japanese gastric carcinoma patients. The pH of gastric juice is significantly higher in the patients with the T/T genotype than in those with either the C/T or C/C genotype (26) but the most recent study revealed that the -31C to T base transition was inverted, in association with the -511T to C base transition; this means -31C equals to -511T, similar to the -511T to C base transition in Japanese (27).

In the present study, we investigated the association between the IL-1B -31C/T, -511C/T or IL-1RN VNTR polymorphisms and the risk of esophageal, gastric and

colorectal carcinomas in Japanese patients with *H. pylori* infection. El-Omar *et al* reported that carriers of IL-1B-31T and IL-1RN2 homozygotes (IL-1RN*2) were at higher risk for development of gastric carcinoma at ORs of 1.9 (95% CI, 1.5-2.6) and 3.7 (95% CI, 2.4-5.7), respectively (16). In the present study, the frequencies of IL-1B-31 and -511 genotype in patients with esophageal carcinoma were similar to the control. Furthermore, the risk of esophageal carcinoma was not related to IL-1B and IL-1RN VNTR polymorphisms. In Japan, 95% of esophageal carcinomas are of the squamous cell type (28). The risk factors of esophageal carcinoma include daily consumption of alcohol and/or cigarette smoking (29). In our study, the frequency of daily consumption of alcohol or cigarettes in esophageal carcinoma group differed significantly from that in the control group. However, such frequency in gastric carcinoma group or in colorectal carcinoma group did not differ significantly from that in control group. Daily consumption of alcohol and/or cigarettes is associated with the risk of esophageal carcinoma. Irritation by daily consumption of alcohol and/or cigarettes may cause carcinoma of the esophagus. We believe that host genetic factors affecting cytokines such as IL-1 β are unlikely to be associated with risk of esophageal carcinoma in Japanese population.

In contrast, *H. pylori* infection increases the risk of gastric carcinoma both in Japanese and Europeans (6,30). Gastritis involving the acid-secreting corpus region leads to hypochlorhydria and progressive gastric atrophy, which increase the risk of gastric carcinoma (8,31). In the present study, although *H. pylori* infection was found in both patients with gastric carcinoma and control subjects, a significant difference in IL-1B polymorphisms was noted between the two groups. Unlike esophageal carcinoma, we believe that genetic factors affecting cytokines such as IL-1 β are likely to be associated with the risk of gastric carcinoma in Japanese population. In this regard, Takashima *et al* (32) reported that gastric acid secretion was low and serum gastrin levels were high in Mongolian gerbils infected with *H. pylori*. They concluded that this change in gastric acid secretion appears to be mediated by IL-1 β induced by *H. pylori* infection. Based on these studies, we hypothesize that differences in IL-1B polymorphisms in hosts infected with *H. pylori* may be associated with differences in gastric acid secretion caused by IL-1 β , consequently leading to progressive gastritis and increasing the risk for gastric carcinogenesis. However, the frequencies of IL-1B polymorphisms in our study were quite different from those in the study by El-Omar *et al* (16). It is likely that the difference in frequencies of IL-1B polymorphisms is due to differences in ethnic background. Further studies are necessary to investigate the relationships between gastric carcinoma and IL-1B polymorphisms.

Our results showed significant differences in IL-1B polymorphisms between patients with colorectal carcinoma and control subjects. We believe that genetic factors affecting cytokines such as IL-1 β could be associated with increased risk of colorectal carcinoma in Japanese population. The IL-1 gene family has been extensively studied in patients with inflammatory bowel diseases (IBD) (33-39), including the relationship between gene polymorphisms and disease susceptibility or disease progression. Mansfield *et al*

investigated the relationship between UC and IL-1RN polymorphism, and IL-1RN*2 was initially reported to be associated with increased risk of UC (33). In the present study, the frequencies of IL-1B-31, -511 and IL-1RN genotype in patients with UC were similar to control subjects, and the risk of UC was not related to IL-1B and IL-1RN VNTR polymorphisms. Conversely, IL-1B-511 heterozygotes and T carriers contributed to the low risk of colorectal carcinoma despite sporadic cases. To our knowledge, there is no report of relationships between IL-1 polymorphisms and risk of colorectal carcinoma. In contrast, IL-1B-511 heterozygotes and T carriers also showed low risk of gastric carcinoma in our study. IL-1 β is a potent inhibitor of gastric acid secretion (40,41), and association of IL-1B polymorphism with gastric acid secretion is supported (16). Thus, gastric acid secretion may affect the pH of the intestinal tract. If pH in the large intestine is regulated by IL-1B polymorphisms, direct mucosal injury or abnormality of intestinal bacterial flora may depend on pH. We hypothesize that pH in the intestinal tract could be associated with the risk of carcinogenesis of the large intestine. Furthermore, polymorphism combination of IL-1B-31/IL-1B-511 and IL-1B-31/IL-1RN would be associated with the risk of colorectal carcinoma development.

In summary, our results suggest that IL-1B polymorphisms are associated with low risk of gastric and colorectal carcinogenesis. The putative underlying mechanism involves amplification of the host inflammatory response to *H. pylori* infection. While pro-inflammatory genotypes may be advantageous for the host response against certain bacterial infections in the large intestine, IL-1 β overproduction associated with such infections may damage the colorectal mucosa, eventually leading to carcinoma development. In contrast, host genetic factors involving IL-1B are unlikely to be associated with increased risk of esophageal carcinoma in Japanese population.

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