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Short communication

Association of the *interleukin-1 β* genetic polymorphism and gastric cancer risk in Japanese

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Background. *Helicobacter pylori* infection is associated not only with gastroduodenal ulcers but with the development of gastric cancer. *Interleukin-1 β* (*IL-1 β*) is a potent inhibitor of gastric secretion. The −31 C-to-T base transition in the intron of this gene has been reported to be involved in carcinogenic changes within the stomach, especially in *H. pylori*-infected individuals. ***Methods.*** In this study, the −511 T-to-C polymorphism in the *IL-1 β* gene was investigated in 669 patients with gastric diseases. ***Results.*** The allelic frequencies of the C allele, which indicates low acid secretion and is a component of a supposedly high-risk genotype for gastric cancer, were 0.48 in *H. pylori*-negative noncancer controls, 0.52 in *H. pylori*-positive noncancer controls, 0.57 in subjects with chronic active gastritis (CAG) with *H. pylori*, 0.58 in subjects with intestinal metaplasia (IM) or CAG without *H. pylori*, and 0.52 in gastric cancer patients. Significant differences among the groups were observed between the IM or CAG without *H. pylori* group and the gastric cancer group and between the IM or CAG without *H. pylori* group and the *H. pylori*-negative noncancer control group ($P < 0.05$). ***Conclusions.*** The *IL-1 β* −511 genetic polymorphism was not associated with gastric cancer in a multistep carcinogenesis model. However, in view of the results for the IM or CAG without *H. pylori* group, the presence of the C allele may also indicate a risk of mucosal atrophy of the stomach in the Japanese population.

Key words: *interleukin-1 β* polymorphism. gastric cancer susceptibility, *Helicobacter pylori* infection, intestinal metaplasia

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Helicobacter pylori infection is associated with many kinds of gastric diseases, including gastroduodenal ulcers and gastric cancer.[1] According to previous reports, inflammation associated with active gastritis can be caused by *H. pylori* infection. Over a period of decades, active gastritis can lead to gastric mucosal atrophy, which is thought to be a high-risk factor for the development of gastric cancer.[1–3] Recently, genetic polymorphisms of *interleukin-1* (*IL-1*) *β*, which is a 100-fold more potent inhibitor of gastric secretion than a proton pump inhibitor, have been found to be associated with functional differences in the effect of *IL-1 β* on gastric secretion among individuals.[4] In addition, genetic polymorphisms involving a C-to-T base transition (−31) and a T-to-C base transition (−511) in the intron of the *IL-1 β* gene have been associated with a risk of gastric cancer in *H. pylori*-infected individuals in a study involving a Western population.[5]

Subjects and methods

In this study, the −511 T-to-C genetic polymorphism was analyzed, using polymerase chain reaction (PCR) amplification with *Ava*I enzyme digestion to investigate its association with gastric diseases in a Japanese population. Briefly, genomic DNA was amplified using primers 5′-GCCTGAACCCTGCATACCGT and 5′-GCCAATAGCCCTCCCTGTCT under the following PCR conditions: an initial melting temperature of 94°C (10 min), followed by 5 cycles of melting (94°C, 30 s), annealing (65°C, 30 s), and extension (72°C, 30 s), followed by 30 cycles of melting (94°C, 30 s), annealing (60°C, 30 s), and extension (72°C, 30 s), and, finally, 5 cycles of melting (94°C, 30 s), annealing (55°C, 30 s), and extension (72°C, 30 s).[5–7] The PCR product was subjected to *Ava*I restriction enzyme digestion (5 units; Takara, Tokyo, Japan) in a buffer at 37°C for 18 h, and analyzed by electrophoresis in an agarose gel (3%). The digestion

1) DNA extraction from peripheral blood
2) DNA amplification by PCR with
F-Primer: 5'-GCCTGAACCCTGCATACCGT
R-Primer: 5'-GCCAATAGCCCTCCCTGTCT
3) Restriction enzyme digestion using *Ava* I
4) Electrophoresis

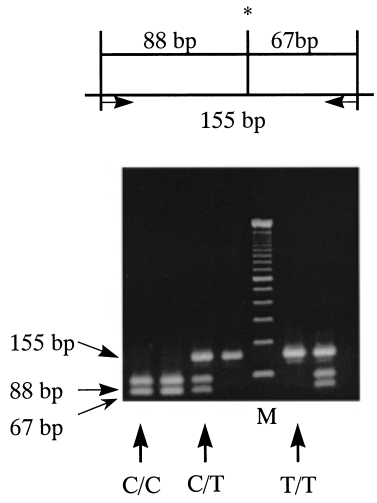

Fig. 1. Detection of interleukin 1 (*IL-1*) β T → C transition at −511 by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP). *C/C* (homozygons C allele) and *C/T* (heterozygote) are expected to be the low-acid (high pH) genotypes in *Helicobacter pylori* infected gastric diseases

Table 1. Interleukin-1 (*IL-1*) β genotypes and the status of gastric mucosa

Cases	genotype TT	CT	CC	C allele (%)	
(1) HP negative non-cancer controls (Superficial Gastritis or Erosive Gastritis without HP infection)	29	61	24	48	
(2) HP positive non-cancer controls	50	110	61	52	*
(3) Chronic atrophic gastritis (CAG) with HP	9	21	15	57	
(4) Intestinal metaplasia or CAG without HP	25	51	43	58	
(5) Gastric cancer with HP	25	73	29	52	*

* $P < 0.05$

produced two fragments, of 88 and 67 base pairs, representing the homozygous C allele (CC), the heterozygote (CT), and the homozygous T allele (TT) (Fig. 1).

Six hundred and sixty-nine patients with gastric diseases were classified into five subgroups according to the presence or absence of *H. pylori* infection: (1) *H. pylori*-negative noncancer controls, including those with superficial gastritis (SG) or erosive gastritis (EG) (n = 114; men, 36; women, 78; average age, 43.1 ± 16.7 years); (2) *H. pylori*-positive noncancer controls, including those with SG, EG, and chronic active gastritis (CAG) without atrophy (n = 210; men, 111; women, 99; average age, 48.7 ± 14.6 years), (3) CAG with *H. pylori* infection (n = 45; men, 21; women, 24; average age, 59.8 ± 14.0 years), (4) intestinal metaplasia (IM) or CAG without *H. pylori* infection (n = 19; men, 78; women, 41; average age, 58.5 ± 12.0 years), and (5) gastric cancer patients (GCA) (n = 127; men, 81; women, 46; average age, 59.8 ± 10.8 years). In all patients, diagnosis was done by gastrointestinal endoscopy and by histological findings of biopsy specimens, or by detection of serum anti-*H. pylori* IgG antibody positivity (Silenus Laboratories, Sydney, Australia), and all those with chronic atrophic gastritis had type B gastritis.[8] In CAG patients without *H. pylori* infection, the extensive mucosal atrophy of the stomach prevented *H. pylori* from colonizing the gastric mucosa.[9,10] All patients were seen at our hospital (in Tokyo, Japan) between 1993 and 1999; oral informed consent was obtained from each patient. The data were analyzed using a χ^2 test, and the SPSS statistical analysis program for the PC (SPSS, Tokyo, Japan).

Results

The genotypes of the −511 genetic polymorphism are summarized in Table 1. The allelic frequencies of the C allele, which indicates low acidic secretion and is a component of a supposedly high-risk genotype for gastric cancer compared with the homozygous T or heterzygote genotype, were 0.48, 0.52, 0.57, 0.58, and 0.52 for the five subgroups (1)–(5) above, respectively. In combination analysis, significant differences were observed between the IM or CAG without *H. pylori*

infection and the GCA group ($P < 0.05$), and between the IM or CAG without *H. pylori* group and the *H. pylori*-negative noncancer control group ($P < 0.05$), but, in the study overall, the specific genotypes of the *IL-1 β* −511 polymorphism were no associated with gastric cancer.

In this study, we could not find any associations between the *IL-1 β* specific genotypes of the patients with *H. pylori*-positive gastric cancer and the *H. pylori*-negative noncancer controls in our Japanese population. The *H. pylori* infection rate in Japan is higher than that in other populations, especially those in Western countries.[1–3] According to previous reports, 60% to 70% of the population over 60 years of age in Japan are infected with *H. pylori*. This positivity rate is close to the *H. pylori* positivity rate of gastric cancer cases in Japan. According to epidemiological studies, high-risk factors for gastric cancer have been reported to be sex differences, *H. pylori* infection, high salt intake and low ascorbic acid intake, exposure to exogenous or endogenously induced nitroso compounds, and intramucosal changes from a precancer-cancer sequence in the stomach.[11–13]

Discussion

Recently, *IL-1 β* has been reported to be an important proinflammatory cytokine that is highly expressed in the gastric mucosa of *H. pylori*-positive hosts; gastric acid secretion is low in this population.[4,5] El-Omar et al.[5] examined the association of particular genotypes with a low acid state in Scottish and Polish gastric cancer patients and controls. They suggested that individuals with the −31 TT or CT genotypes overexpress gastric *IL-1 β* in response to *H. pylori* infection, which leads to an increase in inflammation and a lower stomach acidity. This hypothetical scheme is suitable for the precancer-cancer sequence of gastric carcinogenesis. Indeed, the pH of gastric juice depends on the *IL-1 β* −511 polymorphism genotype in Japanese gastric cancer patients; the pH of gastric juice is significantly higher in these with the TT genotype than in those with either the CT or the CC genotype (T. Furuta and H. Sugimura, personal communication), but the most recent publication revealed that the −31 C-to-T base transition was inverted, in association with the −511 T-to-C base transition this means −31C equal to −511-T similar to the −511 T-to-C base transition in Japanese;[14] the El-Omar group also confirmed this inversion in their study subjects (E.M. El-Omar, personal communication). According to the these studies, the C allele could be the risk factor for low acid secretion in the stomach. Neutralization of acid in the stomach is one of several important factors in gastric carcinogenesis.[13] Pepsinogen I (≤50 to 70 ng/ml) and the pepsinogen I/pepsinogen II ratio (<3.0) are good markers for the degree of mucosal atrophy in the stomach of Japanese patients,[15] and mucosal atrophy is believed to be an important cause of acid neutralization in the stomach, especially in the presence of an *H. pylori* infection.[10] Moreover, the possibility of inter-individual differences in gastric cavity pH, depending on the *IL-1 β* polymorphism, should be emphasized. In this study, we could not find any association between the existence of the C allele and a risk of gastric cancer in the precancer-cancer sequence, although the sample numbers for the disease categories were limited. A rigorous case-control study is needed to obtain definitive results, but our study suggests that this polymorphism may not contribute to the risk of gastric cancer in the Japanese population. However, the difference in C allele frequencies between the IM or CAG without *H. pylori* group (58%) and the gastric cancer group (52%), and the difference between the IM or CAG without *H. pylori* group (58%) and the *H. pylori*-negative noncancer control group (48%) were significant. *H. pylori* is known to be unable to survive in the mucosa of an IM region. In patients with extensive mucosal atrophy, the spontaneous disappearance of *H. pylori* was also observed, indicating that the mucosa of IM or CAG patients without *H. pylori* infection had undergone precancerous mucosal changes.[10,16] In this study, the frequency of the C allele in the IM or CAG without *H. pylori* infection group was significantly higher than that in the *H. pylori*-negative noncancer controls. This result indicated that this −511 T-to-C base transition may be important for the mucosal atrophy of the stomach. IM and CAG represent a potent carcinogenic state for gastric cancer, but, according to a cohort follow-up study, only 2% of patients with IM mucosa develop gastric cancer.[17] In this population, i.e., those with 1 m or CAG, long-term acid neutralization in the stomach of individuals with the C allele may induce the growth of certain intestinal bacteria that produce carcinogenic nitroso-compounds in the stomach,[18] and other factors, including activation enzymes for nitrosamines or detoxification enzymes for specific carcinogenic compounds, may be important for stomach carcinogensis.[19]

In conclusion, the C allele of the *IL-1 β* −511 genetic polymorphism was not associated with gastric cancer in our Japanese population. However, a possibility does exist that populations who possess the C allele of the *IL-1 β* polymorphism may be more likely to progress to mucosal atrophy with IM.

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References

1. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Infection with *Helicobacter pylori*. Schistosomes, liver flukes and *Helicobacter pylori*. IARC monographs on the evaluation of carcinogenic risks to humans. IARC 1994;61:177–240.
2. Nomura A, Stemmermann GN, Chyou PH, Kato I, Perez-Perez GI, Blaser MJ. *Helicobacter pylori* infection and gastric carcinoma among Japanese Americans in Hawaii. N Engl J Med 1991;325:1132–6.
3. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al. *Helicobacter pylori* infection and the risk of gastric carcinoma. N Engl J Med 1991;325:1127–31.
4. Wolfe MM, Nompleggi DJ, Cytokine inhibition of gastric acid secretion—a little goes a long way. Gastroenterology 1992;102: 2177–8.
5. El-Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, et al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature 2000;404:398–402.
6. Santtila S, Savinainen K, Hurme M. Presence of the IL-1RA allele 2 (IL1RN*2) is associated with enhanced IL-1beta production in vitro. Scand J Immunol 1998;47:195–8.
7. di Giovine FS, Takhsh E, Blakemore AI, Duff GW, Single base polymorphism at −511 in the human *interleukin-1 β* gene (*IL-1 β*). Hum Mol Genet 1992;1:450.
8. Strickland RG, Mackay IR. A reappraisal of the nature and significance of chronic atrophic gastritis. Am J Dig Dis 1973;18: 426–40.
9. Fukuda H, Saito D, Hayashi S, Hisai H, Ono H, Yoshida S, et al. *Helicobacter pylori* infection, serum pepsinogen level and gastric cancer: a case-control study in Japan. Jpn J Cancer Res 1995;86: 64–71.
10. Karnes WE Jr, Samloff IM, Siurala M, Kekki M, Sipponen P, Kim SW, et al. Positive serum antibody and negative tissue staining for *Helicobacter pylori* in subjects with atrophic body gastritis. Gastroenterology 1991;101:167–74.
11. Correa P. Human gastric carcinogenesis: a multistep and multifactorial process. First American Cancer Society award lecture on cancer epidemiology and prevention. Cancer Res 1992;52:6735–40.
12. Sato S, Fukuyama S, Susuki T, Takanagi J. The relationship between gastric cancer mortality and salted food intake in several places in Japan. Bull Inst Public Health 1959;8:187–98.
13. Tsugane S, Tei Y, Takahashi T, Watanabe S, Sugano K. Salty food intake and risk of *Helicobacter pylori* infection. Jpn J Cancer Res 1994;85:474–8.
14. Hamajima N, Matsuo K, Saito T, Tajima K, Okuma K, Yamao K, et al. Interleukin 1 polymorphisms, lifestyle factors, and *Helicobacter pylori* infection. Jpn J Cancer Res 2001;92:383–9.
15. Miki K, Ichinose M, Kawamura N, Matsushima M, Ahmad HB, Kimura M, et al. The significance of low serum pepsinogen levels to detect stomach cancer associated with extensive chronic gastritis in Japanese subjects. Jpn J Cancer Res 1989;80:111–4.
16. Kitahara F, Shimazaki R, Sato T, Kojima Y, Morozumi A, Fujino M. Severe atrophic gastritis with *Helicobacter pylori* infection and gastric cancer. Gastric Cancer 1998;1:118–24.
17. Correa P, Haenszel W, Cuello C, Zavala D, Fontham E, Zarama G, et al. Gastric precancerous process in a high risk population: cohort follow-up. Cancer Res 1990;50:4737–40.
18. Fox JG, The role of *Helicobacter pylori* species in newly recognized gastrointestinal tract diseases of animals. Lab Anim Sci 1997;47:222–55.
19. Kato S, Onda M, Matsukura N, Tokunaga A, Matsuda N, Yamashita K, et al. Genetic polymorphisms of the cancer related gene and *Helicobacter pylori* infection in Japanese gastric cancer patients. An age and gender matched case-control study. Cancer 1996;77:1654–61.