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H pylori seropositivity and cytokine gene polymorphisms

Yasuaki Saijo, Eiji Yoshioka, Tomonori Fukui, Mariko Kawaharada, Fumihiro Sata, Hirokazu Sato, Reiko Kishi

Yasuaki Saijo, Department of Health Science, Asahikawa Medical College, Midorigaoka, E2-1-1-1, Asahikawa, Hokkaido 078-8510, Japan
Eiji Yoshioka, Tomonori Fukui, Mariko Kawaharada, Fumihiro Sata, Reiko Kishi, Department of Public Health, Hokkaido University Graduate School of Medicine, Kita 15, Nishi 7, Kita-ku, Sapporo 060-8638, Japan
Hirokazu Sato, Health Administration Department, Sapporo Railway Hospital, Kita 3, Higashi 1, Cyuo-ku, Sapporo 060-0033, Japan
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Correspondence to: Dr. Yasuaki Saijo, Department of Health Science, Asahikawa Medical College, Midorigaoka, E2-1-1-1, Asahikawa, Hokkaido 078-8510,
Japan. y-saijo@asahikawa-med.ac.jp
Telephone: +81-166-682402 Fax: +81-166-682409
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Abstract

AIM: To investigate whether the pro- and anti-inflammatory cytokine gene polymorphisms, *IL1B*-511C/T, *IL1B*-31C/T, *IL6*-634C/G, *TNF*-1031T/C, *TNF*-857C/T, and *IL10*-1082A/G, interact with smoking and drinking habits to influence infection with *H pylori*.

METHODS: The subjects were 410 Japanese transit company employees. C-reactive protein and conventional cardiovascular risk factors were evaluated. Serum anti-*H pylori* antibodies were measured. The genotypes of *IL1B*-511C/T, *IL1B*-31C/T, *IL6*-634C/G, *TNF*-1031T/C, *TNF*-857C/T, and *IL10*-1082A/G polymorphisms were determined by allelic discrimination using fluorogenic probes and a 5´nuclease assay.

RESULTS: In gender- and age-adjusted logistic analyses, the subjects with *TNF*-857T/T had a significantly lower odds ratio (OR) for *H pylori* seropositivity (reference -857C/C; OR = 0.15, 95% CI: 0.03-0.59, P = 0.007). After stratification according to smoking and drinking status, among never-smokers, the subjects with *IL1B*-511C/T had a significantly lower OR (reference -511C/C; OR = 0.30, 95% CI: 0.10-0.90, P = 0.032). Among drinkers in the 1-5 times/wk category, the subjects with *IL1B*-511T/T had a significantly lower OR (reference C/C; OR = 0.38, 95% CI: 0.16-0.95, P = 0.039), and the subjects with *IL1B*-31C/T and T/T had a significantly higher OR (reference C/C; C/T: OR = 2.59, 95% CI, P = 0.042: 1.04-6.47; C/C: OR = 3.17, 95% CI: 1.23-8.14, P = 0.017). Among current smokers, the subjects with *IL6*-634C/G had a significantly higher OR (reference C/C; OR = 2.28, 95% CI: 1.13-4.58, P = 0.021). However, the interactions terms between the aforementioned genotypes and lifestyles were not statistically significant.

CONCLUSION: Contrary to previous findings, the results herein suggest that the *TNF*-857T/T genotype may be protective against chronic infection with *H pylori*. Drinking and smoking habits may influence the effect of cytokine gene polymorphisms. Further studies are required to clarify the effects of the pro- and anti-inflammatory cytokine polymorphisms and gene-environmental interactions on *H pylori* infection.

Key words: *H pylori* seropositivity; Cytokines; Polymorphisms

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INTRODUCTION

The prevalence of *H pylori* infection is generally higher in developing countries than in developed countries[1]; however, the Japanese population has a high prevalence of *H pylori* seropositivity[2]. Infection with *H pylori* represents a key factor in the etiology of various gastrointestinal diseases, including asymptomatic chronic active gastritis, peptic ulceration, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue lymphoma[3]. *H pylori* has also been implicated in a number of extra-gastrointestinal disorders, such as atherosclerosis[4], cerebral vascular disease[5], idiopathic thrombocytopenic purpura[6], and rosacea[7]. Because of the greater prevalence and various pathogenic activities of *H pylori* in Japanese, it is important to understand the basis for genetic susceptibility and identify the environmental factors that maintain chronic infection.

The mucosal production of pro-inflammatory cytokines, such as interleukin (IL)-1β, IL6, and tumor necrosis factor (*TNF*)-α, appears to be enhanced by infection with *H pylori*[8,11]. Interleukin-1β and TNF-α inhibit gastric acid secretion, providing a favorable condition for *H pylori* to survive in the stomach[12]. Although one study failed to show

inhibition of gastric acid secretion by IL-6[13], several studies have shown that gastric colonization with *H pylori* leads to elevated IL-6 levels in the gastric mucosa[14,15]. Thus, IL-6 may be one of the factors that maintain chronic infection with *H pylori*. Furthermore, IL-10, an anti-inflammatory cytokine, may reduce the inflammation associated with *H pylori* infection[16].

The host's ability to regulate cytokine production has been shown to be influenced by the presence of cytokine gene polymorphisms. Therefore, *H pylori*-susceptible cytokine gene backgrounds have recently been investigated. Regarding the *IL1B* gene, Japanese subjects with the -31T/T genotype have a significantly higher odds ratio (OR) for *H pylori* seropositivity as compared to subjects with the -31C/C or C/T genotypes[17]. A strong relationship involving *ILIB* -31T/T has been demonstrated in Japanese Brazilians[18]; however, such an association has not been shown to exist in Italians[19] or Jamaicans[20]. Regarding the *TNF* gene, Japanese subjects with the -1031C/C genotype have a significantly lower OR compared to those with the -1031T/T genotype[21]; however, an association was not found in Italians[19], Jamaicans[20], or Japanese Brazilians[22]. Thus, the effect *IL1B* and *TNF* polymorphisms on infection with *H pylori* remains controversial. Furthermore, among Jamaicans, the *IL6*-634C/G polymorphism (denoted -572G/C) was not associated with *H pylori* seropositivity[20], and the *IL10*-1082C/G polymorphism was not associated with *H pylori* infection among Jamaicans[20] or Italians[19]. Little is known regarding the effects of the *IL6* and *IL10* promoter polymorphisms on infection with *H pylori*.

Smoking cigarettes and drinking alcohol may have an effect on chronic infection with *H pylori*[23,26]. Therefore, interactions between the genome and lifestyle factors should be elucidated. An interaction between the *IL1B* genotype and one's cigarette smoking status on the eradication of *H pylori* has been reported[27]. It has also been reported that the effect of the *IL1B*-31T/T genotype on *H pylori* infection is modified by smoking cigarettes and drinking alcohol[18,28], but the interactions between other cytokine gene polymorphisms and lifestyle factors on *H pylori* infection have not been fully investigated.

The aim of this study was to investigate whether the pro- and anti-inflammatory cytokine gene polymorphisms, *IL1B*-511C/T, *IL1B*-31C/T, *IL6*-634C/G, *TNF*-1031T/C, *TNF*-857C/T, and *IL10*-1082A/G, interact with smoking cigarettes and drinking alcohol to influence infection with *H pylori* in Japanese.

MATERIALS AND METHODS

Subjects

The subjects were transit company employees (1255 men and 94 women, 35-60 years of age), who had their annual health checkup between April 2003 and March 2004. We used a self-administered questionnaire that included items regarding clinical history, smoking cigarettes, and consumption of alcohol. The questionnaire was distributed to the subjects prior to their annual health checkup, and was collected at the time of the checkup. Answers to the questionnaire and written informed consent to view pertinent health checkup data were obtained from 413 men and 5 women, for a response rate of 32.9% and 5.3%, respectively. Eight subjects were excluded due to inadequate blood samples. Ultimately, we analyzed a total of 410 employees (405 men and 5 women). No subject had a history of an internal malignancy or gastric surgery.

This study was conducted with written informed consent from all the subjects and approved by the institutional ethical board for epidemiological studies and human gene and genome studies of the Hokkaido University Graduate School of Medicine.

Data collection

Subjects were classified as current, never- or ex-smokers. Alcohol consumption habits were categorized as never/rarely, 1-5 times/wk, or 6-7 times/wk.

Blood samples were drawn from the antecubital vein of the subject after a 12 h fast while in a seated position and with minimal tourniquet use. The anti-*H pylori* antibody titer was measured using an enzyme immunoassay (E plate; Eiken Chemical, Tokyo, Japan)[29]; an assay value < 10 U/mL was considered negative and a value > 10 U/mL was considered positive.

Genomic DNA was extracted from each subject's peripheral blood lymphocytes using an EZ1 DNA blood kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. We genotyped the *IL1B*-511C/T (dbSNP: rs16944), *IL1B*-31C/T (dbSNP: rs1143627), *IL6*-634C/G (rs1800796), *TNF*-1031T/C (dbSNP: rs1799964), *TNF*-857C/T (dbSNP: rs1799724), and *IL10*-1082A/G (dbSNP: rs1800896) polymorphisms by allelic discrimination using fluorogenic probes and the 5´nuclease (TaqMan) assay, as previously described[30,31]. To detect a polymorphism in IL-6-634C/G, the following MGB probes were prepared: a C allele-specific probe, 5'-FAM-CAACAGCCCCTCACAG-MGB-3', and a G allele-specific probe, 5'-VIC-CAACAGCCGCTCACAG-MGB-3'. Each of the reporters was quenched with MGB, which was typically located at the 3' end. The primers for the PCR involving the promoter region, including the -634C/G polymorphism of IL-6, were as follows: forward, 5'-GGATGGCCAGGCAGTTCTA-3', and reverse, 5'-CCAGTCATCTGAGTTCTTCTGTGTT-3'. The reaction mixture contained approximately 40 ng of template DNA, 5.0 μL of TaqMan Universal PCR master mixture, and 0.3 μL of 40 × assay mixture, in a volume of 10 μL. The *IL1B*-511C/T, *IL1B*-31C/T, *TNF*-1031T/C, *TNF*-857C/T, and *IL10*-1082A/G polymorphisms were similarly genotyped using the TaqMan® SNP genotyping products: C_1839943_10, C_1839944_10, C_7514871_10, C_11918223_10, and C_1747360_10, respectively (Applied Biosystems, Foster City, CA, USA). Real-time PCR was performed on a 7500 Real-time PCR System (Applied Biosystems) using a protocol consisting of incubation at 50℃ for 2 min and 95℃ for 10 min, followed by 50 cycles for *IL6* or 40 cycles for the other genotypes, denaturation at 92℃ for 15 s, and annealing/extension at 60℃ for 1 min. The FAM and VIC fluorescence levels of the PCR products were measured at 60℃ for 1 min, resulting in the

Table 1 Characteristics of *H pylori*-seropositive and -seronegative subjects

	H pylori-seropositive (*n* = 237)		*H pylori*-seronegative (*n* = 173)		*P*-value
	n	(%)	*n*	(%)	
Gender					0.645
Male	234	98.7	171	98.8	
Female	3	1.3	2	1.2	
Age (yr)					< 0.001
< 45	30	12.7	63	36.4	
45-49	62	26.2	53	30.6	
50-54	85	35.9	43	24.9	
⩾ 55	60	25.3	14	8.1	
Smoking					0.281
Never	59	24.9	32	18.5	
Former	76	32.1	57	32.9	
Current	102	43.0	84	48.6	
Drinking					0.023
Never or rarely	35	14.8	43	24.9	
1-5 times/wk	131	55.3	91	52.6	
6-7 times/wk	71	30.0	39	22.5	

clear identification of all six genotypes of *IL1B*, *IL6*, *TNF*, or *IL10* on a two-dimensional graph.

Statistical analysis

The differences in the frequency of each characteristic between the *H pylori*-seropositive and -seronegative groups were examined by the chi-square test. Hardy-Weinberg equilibrium analyses were performed to compare the observed and expected genotype frequencies using the chi-square test. A logistic regression analysis was used to evaluate the associations between each cytokine genotype and *H pylori* seropositivity, with adjustment for age and gender, to obtain the OR and 95% confidence intervals (CI). After stratification according to cigarette smoking and alcohol consumption status, the adjusted OR for each genotype of *H pylori* seropositivity was calculated. The interaction term for the genotype/lifestyle factors was included in the logistic model with the main effect.

The haplotype was analyzed using Haploview, version 3.32[32], and linkage disequilibrium between loci was measured using Lewontin's D'[33]. The adjusted OR for the *IL1B* and *TNF* haplotypes was analyzed by logistic regression models. Statistical analyses were conducted with SPSS software for Windows, version 14.0 (SPSS; Chicago, IL, USA).

RESULTS

The characteristics of the groups according to *H pylori* seropositivity are shown in Table 1. Two hundred thirty-seven subjects (57.8%) were *H pylori*-seropositive. The *H pylori*-seropositive group was older and drank alcohol more frequently than the *H pylori*-seronegative group.

H pylori seropositivity, according to the genotypes of *IL1B*, *IL-6*, *TNF*, and IL-10, are shown in Table 2. The distribution of genotypes in each group was in the Hardy-Weinberg equilibrium. *TNF*-857C/T genotypes were significantly different between the *H pylori*-seropositive and -seronegative subjects.

Table 2 *H pylori* seropositivity according to cytokine genotypes

	H pylori-seropositive (*n* = 237)		*H pylori*-seronegative (*n* = 173)		*P*-value
	n	(%)	*n*	(%)	
IL1B-511C/T					0.243
CC	93	39.2	54	31.2	
CT	109	46.0	89	51.4	
TT	35	14.8	30	17.3	
IL1B-31C/T					0.434
CC	33	13.9	29	16.8	
CT	112	47.3	87	50.3	
TT	92	38.8	57	32.9	
IL6-634C/G					0.753
CC	138	58.2	104	60.1	
CG	88	37.1	59	34.1	
GG	11	4.6	10	5.8	
TNF-1031T/C		0.0			0.170
TT	152	64.1	115	66.5	
CT	80	33.8	51	29.5	
CC	5	2.1	7	4.0	
TNF-857C/T					0.018
CC	170	71.7	115	66.5	
CT	64	27.0	47	27.2	
TT	3	1.3	11	6.4	
IL10-1082A/G					0.852
AA	211	89.0	153	88.4	
AG/GG[1]	26	11.0	20	11.6	

[1]Only one subject had the *IL10*-1082 GG genotype.

Table 3 Age, gender-adjusted ORs for *H pylori* seropositivity according to cytokine genotypes

	n	*Hp* (+)%[1]	Adjusted OR (95% CI)	*P*-value
IL1B-511C/T				
CC	147	63.3	1.00	
CT	198	55.1	0.69 (0.43-1.10)	0.121
TT	65	53.8	0.70 (0.37-1.32)	0.270
IL1B-31C/T				
CC	62	53.2	1.00	
CT	199	56.3	1.11 (0.61-2.05)	0.726
TT	149	61.7	1.47 (0.78-2.78)	0.234
IL6-634C/G				
CC	242	57.0	1.00	
CG	147	59.9	1.06 (0.68-1.66)	0.785
GG	21	52.4	0.63 (0.24-1.62)	0.335
TNF-1031T/C				
TT	267	56.9	1.00	
CT	131	61.1	1.24 (0.78-1.95)	0.361
CC	12	41.7	0.48 (0.13-1.70)	0.253
TNF-857C/T				
CC	285	59.6	1.00	
CT	111	57.7	0.93 (0.58-1.49)	0.760
TT	14	21.4	0.15 (0.03-0.59)	0.007
IL10-1082A/G				
AA	364	58.0	1.00	
AG/GG[2]	46	56.5	1.08 (0.56-2.09)	0.811

[1]*H pylori* seropositivity (%); [2]Only one subject had the *IL10*-1082 GG genotype.

The age- and gender-adjusted ORs of the genotypes for *H pylori* seropositivity are shown in Table 3. The subjects with *TNF*-857T/T had a significantly lower OR for *H pylori* seropositivity (reference -857C/C; OR = 0.15, 95% CI 0.03-0.59).

After stratification according to cigarette smoking and

Table 4 Age, gender-adjusted ORs for *H pylori* seropositivity according to cytokine genotypes and lifestyle factors

			IL1B–511C/T			
	n	*Hp* (+)%[1]	C/C	C/T	*P*-value	T/T	*P*-value
All subjects	410	57.8	1.00	0.69 (0.43-1.10)	0.121	0.70 (0.37-1.32)	0.270
Smoking							
Never	91	64.8	1.00	0.30 (0.10-0.90)	0.032	0.40 (0.10-1.62)	0.200
Former	133	57.1	1.00	0.90 (0.39-2.10)	0.819	0.74 (0.26-2.15)	0.583
Current	186	54.8	1.00	0.83 (0.42-1.63)	0.582	0.80 (0.30-2.16)	0.658
Drinking							
Never or rarely	78	44.9	1.00	0.58 (0.20-1.66)	0.310	1.38 (0.31-6.23)	0.676
1-5 times/wk	222	59.0	1.00	0.81 (0.43-1.55)	0.531	0.38 (0.16-0.95)	0.039
6-7 times/wk	110	64.5	1.00	0.66 (0.25-1.69)	0.383	1.11 (0.33-3.77)	0.862
			IL1B–31C/T			
	n	*Hp* (+)%[1]	C/C	C/T	*P*-value	T/T	*P*-value
All subjects	410	57.8	1.00	1.11 (0.61-2.05)	0.726	1.47 (0.78-2.78)	0.234
Smoking							
Never	91	64.8	1.00	0.79 (0.22-2.29)	0.723	2.25 (0.56-9.08)	0.257
Former	133	57.1	1.00	1.34 (0.50-3.62)	0.560	1.55 (0.55-4.42)	0.409
Current	186	54.8	1.00	1.20 (0.44-3.24)	0.722	1.22 (0.44-3.40)	0.705
Drinking							
Never or rarely	78	44.9	1.00	0.44 (0.11-1.82)	0.258	0.68 (0.15-3.12)	0.624
1-5 times/wk	222	59.0	1.00	2.59 (1.04-6.47)	0.042	3.17 (1.23-8.14)	0.017
6-7 times/wk	110	64.5	1.00	0.72 (0.23-2.29)	0.579	0.81 (0.24-2.73)	0.735
			IL6–634C/G			
	n	*Hp* (+)%[1]	C/C	C/G	*P*-value	G/G	*P*-value
All subjects	410	57.8	1.00	1.06 (0.68-1.66)	0.785	0.63 (0.24-1.62)	0.335
Smoking							
Never	91	64.8	1.00	0.54 (0.22-1.38)	0.200	2.79 (0.28-27.73)	0.382
Former	133	57.1	1.00	0.63 (0.28-1.41)	0.259	0.00 (0.00)	0.999
Current	186	54.8	1.00	2.28 (1.13-4.58)	0.021	0.84 (0.22-3.15)	0.796
Drinking							
Never or rarely	78	44.9	1.00	1.07 (0.41-2.82)	0.886	0.00 (0.00)	1.000
1-5 times/wk	222	59.0	1.00	1.02 (0.55-1.91)	0.940	0.40 (0.11-0.41)	0.153
6-7 times/wk	110	64.5	1.00	1.61 (0.63-4.12)	0.325	1.60 (0.28-9.28)	0.599

[1]*H pylori* seropositivity (%).

alcohol consumption status, the age- and gender-adjusted ORs of *IL1B* and *IL-6* genotypes for *H pylori* seropositivity are shown in Table 4. Among never-smokers, subjects with *IL1B*-511C/T had a significantly lower OR for *H pylori* seropositivity (reference -511C/C; OR = 0.30, 95% CI: 0.10-0.90). Among the 1-5 times/wk drinkers, *IL1B* -511T/T had a significantly lower OR (reference C/C; OR = 0.38, 95% CI: 0.16-0.95), and *IL1B*-31C/T and -T/T had significantly higher ORs (reference C/C; C/T: OR = 2.59, 95% CI: 1.04-6.47; C/C: OR = 3.17, 95% CI: 1.23-8.14). Among current smokers, *IL6*-634C/G had a significantly higher OR (reference C/C; OR = 2.28, 95% CI: 1.13-4.58); however, the interaction terms between the aforementioned genotypes and lifestyles were not statistically significant. The remaining genotypes revealed no statistically significant ORs after stratification (data not shown).

Complete linkage disequilibrium existed between the two *IL1B* promoter lesions (D' = 1, r^2 = 0.048) and strong linkage disequilibrium existed between the two *TNF* promoter lesions (D' = 0.953). The estimated haplotype frequency of *TNF* (-1031T/C and -857C/T) was as follows: TC = 64.1%, CC = 18.9%, TT = 17.0%, and CT = 0%. The estimated haplotype frequency of *IL1B* (-511C/T and -31C/T) was as follows: CT = 58.9%, TC = 38.3%, TT = 1.7%, and CC = 1.1%.

The adjusted ORs of the combination of the two *IL1B* promoter genotypes and the *TNF* genotypes for *H pylori* seropositivity are shown in Table 5. The subjects with *TNF*-1031T/T and -857T/T had significantly lower ORs for *H pylori* seropositivity (reference, all the remaining combinations of genotypes; OR = 0.15, 95% CI: 0.04-0.60); however, subjects with *TNF*-1031T/T and -857T/T were similar to the subjects with -857T/T.

Table 5 Age, gender-adjusted ORs for *H pylori* seropositivity according to the combination of the two promoter genotypes of *TNF* and *IL1B*

	n	*Hp* (+)%[1]	Adjusted OR (95% CI)	*P*-value
TNF-1031/-857				
TT/CC[2]	169	58.6	1.07 (0.70-1.64)	0.743
TC/CC[2]	104	63.5	1.38 (0.85-2.25)	0.195
CC/CC[2]	12	41.7	0.44 (0.13-1.57)	0.207
TT/CT[2]	84	59.5	1.06 (0.63-1.78)	0.830
TC/CT[2]	27	51.9	0.89 (0.39-2.02)	0.781
TT/TT[2]	14	21.4	0.15 (0.04-0.60)	0.007
IL1B-511/-31[3]				
CC/TT[2]	141	61.7	1.29 (0.83-2.01)	0.259
CT/CT[2]	191	54.5	0.74 (0.48-1.13)	0.159
TT/CC[2]	59	52.5	0.77 (0.43-1.39)	0.384

[1]*H pylori* seropositivity (%). [2]Each reference group represented all the other combinations of genotypes. [3]ORs of the groups with < 7 subjects were not analyzed: CT/TT = 4, TT/TT = 4, CC/CT = 6, TT/CC = 2, and CT/CC = 3.

DISCUSSION

In the current study, the *TNF*-857T/T genotype had a significantly reduced OR for *H pylori* seropositivity. Because the subjects with both *TNF*-1031T/T and -857T/T genotypes were similar to the subject who was classified with the *TNF* -857T/T genotype only, the combination of genotypes also had a reduced OR.

It has been reported that Japanese subjects with the -1031C/C genotype have a significantly lower OR for *H pylori* infection when compared to those with the -1031T/T genotype, and that subjects with -857T/T and -1013T/T have significantly higher ORs for *H pylori* infection when compared to those with -1031C/C and -857C/C[21]. However, neither -1031T/C nor -857C/T polymorphisms were associated with *H pylori* infection in Italians[19] or Jamaicans[20], and neither the genotypes nor the combination of genotypes were associated with Japanese Brazilians[22]. The genotype distributions of -1031T/C and -857C/T among the aforementioned Japanese subjects were quite similar to the distributions in the subjects enrolled in our study. However, the subjects between the studies differed as follows: (1) our subjects were younger than in the previously published study, (2) nearly all of our subjects were male, while approximately one-half of the previous study subjects were female, and (3) our study subjects were healthy workers, unlike the subjects in the previous study that included outpatients participating in a *H pylori* eradication program, outpatients with chronic diseases, as well as health checkup examinees; these differences may have been the basis for the discrepant results. Further studies are needed to elucidate age and sex specific effects of *TNF*-857C/T polymorphism on *H pylori* infection.

In the current study, the subjects with the *TNF*-857T/T genotype had the highest level of *TNF*-α secretion, resulting in low gastric acid secretion, and they were resistant to chronic *H pylori* infection. Higuchi *et al*[34] reported that the level of TNF-α and the transcription promoter activity produced by concanavalin A-activated peripheral blood mononuclear cells in subjects with -1031C or -857T alleles were higher than in those subjects with the -1031T or -857C alleles. Skoog *et al*[35] reported that subjects with -863A tightly linked with -1031C had a significantly lower serum TNF-α level. Moreover, in another study it was shown that *ex vivo* lipopolysaccharide-stimulated whole-blood TNF production was higher in healthy *TNF*-857C homozygotes[34]. Thus, further studies will be needed to clarify the effect of the TNF genotype on susceptibility to infection with *H pylori* and production of TNF-α.

The *TNF* gene has more than three relatively frequent bi-allelic single-nucleotide polymorphisms in the promoter region: -863C/A, -308G/A, and -238G/A[35]. It has been reported that the *TNF*-308A allele is highly associated with *H pylori* infection in Italy[19], but the -308A allele is rare in Japan, and the other major allele, -238A, is also rare in Japan (1.7% and 2.0%, respectively)[35]. Moreover, the -863C/A allele is tightly linked with -1031C/T[36]. Therefore, we investigated the two promoter region polymorphisms of *TNF*. However, since the *TNF*-857 T/T genotype is not frequent in the population, simple and easy methods for genotyping are required for practical use.

The two *IL1B* promoter genotypes were not associated with *H pylori* infection in our entire group of subjects. In like manner, no association was found in Italians[19] or Jamaicans[20]. However, a previous Japanese study showed that subjects with the -31T/T genotype had a significantly higher OR (1.74, 95% CI: 1.15-5.63) for *H pylori* infection as compared to those subjects with the -31C/T or -31CC genotypes[17]. Furthermore, a study of Japanese Brazilian subjects found an association (OR of T/T = 1.45, 95% CI: 1.02-2.07)[28]. The subjects in the two previous studies involved an adequate number of female subjects and the Japanese study subjects were older than our study subjects. These differences may have accounted for our inability to obtain statistically significant results. In addition, the sample size of the previous Japanese study was nearly the same as that of our study (n = 437), but the sample size of the Japanese Brazilian study was almost twice as large as that of our study (n = 963). If a real OR of the T/T genotype was approximately 1.5, a smaller sample size as in our study may have failed to reach statistical significance.

Smoking cigarettes and drinking alcohol augment the T/T genotype effect on *H pylori* infection[18,28]. In our study, 1-5 times/week drinkers with T/C and T/T genotypes had significant ORs. In previous studies, the subjects were divided into drinkers or non-drinkers[18,28] and the pattern of drinking enhanced the T/T genotype effect on chronic *H pylori* infection. The drinkers in the previous study involved moderate and heavy consumption of alcohol, but the difference in T/T genotype augmentation between moderate and heavy consumption of alcohol was not analyzed. In our study, the results suggested that moderate drinking enhanced the T/T genotype effect on chronic *H pylori* infection. Therefore, further studies are needed to elucidate the interactions betweens the volume of alcohol consumption and genotypes on *H pylori* infection.

In our study, 1-5times/wk drinkers with the -511T/T genotype had a significantly lower OR since -31C and -511T were tightly linked (-511T/C and -31C/T combinations: 59.8% for T-C, 1.7% for T-T, 38.3% for C-T, and 1.1% for C-C). Non-smokers with -511C/T had a significantly lower OR. Chance may have influenced the significance of the result. Moreover, because this study was cross-sectional, changes in cigarette smoking and alcohol consumption habits were not involved in the analyses. Thus, the changes from previous habits may have affected the ORs.

Lipopolysaccharide (LPS)-stimulated IL-1β expression by whole blood leukocytes *in vitro* was lower in subjects with -31T and -511C[37,38]. Since IL-1β inhibits gastric acid secretion, thereby providing a favorable environment for *H pylori* to survive in the stomach[12], the results of the previous Japanese study and the moderate drinkers of our study were compatible to the *in vitro* IL-1β expression studies.

The *IL6*-634C/G (denoted -572G/C in reference 20) polymorphism was not associated with *H pylori* seropositivity among Jamaicans[20]. In our study, the polymorphism was also not associated with *H pylori* seropositivity among our entire group of subjects. However, current smokers with

the -634C/G genotype had a significantly higher OR for *H pylori* infection.

Persons with the C allele of the *IL6*-174G/C polymorphism are common among Caucasians, but extremely rare among East Asians[39,40]. However, persons with the G allele of the *IL6*-634C/G polymorphism are common among East Asians, and this genotype significantly relates to recurrent pregnancy loss[39], bone mineral density[41], and diabetic nephropathy[42]. Additionally, the -634G allele is associated with an elevated production and secretion of IL-6 by peripheral blood mononuclear cells *in vitro*[42].

In a study of young and healthy Caucasians, the IL-6 polymorphism was not associated with the *IL6*-174 genotypes in non-smokers, but in smokers where the -174C allele was associated with a higher number of leukocytes, lymphocytes, and monocytes[43]. In our study, the smokers with the -634G/G genotype had no significant results, perhaps because of a smaller sample size. In contrast, we found that the impact of the -634G allele on CRP elevation was greater in non-smokers than in current smokers (in press at Hypertens Res). Thus, the effect of *IL6* gene polymorphisms and the gene-environment interactions on *H pylori* infection should also be further elucidated.

In our study, the *IL10*-1082C/G polymorphism was not associated with *H pylori* infection. As previously mentioned, negative results were reported for Jamaicans[20] and Italians[19]. Other *IL10* promoter polymorphisms, such as -819C/T and -592C/A, have been reported[19] and a Japanese study showed that the combination of the *IL8*-251T/A and *IL10*-819C/T polymorphisms was significantly associated with *H pylori* infection, but the *IL10*-819C/T polymorphism alone did not have a statistically significant effect[44]. Furthermore, associations between *IL10*-1013A/A[45] and -819C/T[16] genotypes on non-cardia gastric cancer were reported. An experiment in mice showed that increased IL-10 levels may reduce the inflammation of *H pylori* infection[16]. Unfortunately, we did not evaluate other *IL10* promoter polymorphisms or the *IL8*-251T/A polymorphism; further studies are required to clarify how these polymorphisms effect *H pylori* infection.

Because this study examined IgG antibodies to *H pylori*, which can reflect a previous infection, IgG seropositivity to *H pylori* may not reflect active infection. However, the relative sensitivity, specificity, and rates of agreement between the results obtained using the enzyme immunoassay employed in the present study (i.e., the E plate) and those obtained by the culture/rapid urease test have been reported to be 100%, 80.0%, and 97.1%, respectively[29]. Strains isolated from Japanese gastric ulcer patients were used as antigens to prepare the E plate. Thus, this serological method to detect *H pylori* infection in Japanese is a suitable method for this type of genotype-associated study.

In summary, we observed the *TNF*-857T/T genotype significantly reduced the OR for *H pylori* seropositivity. Because the ORs for the subjects with both *TNF*-1031T/T and -857T/T genotypes were the same as the subject who was classified with the TN-857T/T genotype alone, the combination of genotypes also revealed a reduction in the OR. In the entire group of subjects analyzed, the promoter region polymorphisms of *IL1B*, *IL6*, and *IL-10* had no association with *H pylori* infection. After stratification according to cigarette smoking and alcohol consumption, never-smokers with the *IL1B*-511C/T genotype and 1-5 times/week drinkers with the *IL1B*-511T/T, *IL1B*-31C/T, and -31T/T genotypes, had a significant association with *H pylori* infection. Among current smokers, the *IL6*-634C/G genotype also had a significant association. However, interactions terms between the aforementioned genotypes and lifestyles were not statistically significant. Further studies are required to clarify the effects of the pro- and anti-inflammatory cytokine polymorphisms and the gene-environmental interactions on *H pylori* infection.

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