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Original article

Subjects with *TNF-A-857TT* and *-1031TT* genotypes showed the highest *Helicobacter pylori* seropositive rate compared with those with other genotypes

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Abstract

Background. **A possible association between *Helicobacter pylori* seropositivity and *tumor necrosis factor* (*TNF*) *A* G-308A has been reported in Korea. The present study examined the associations of *H. pylori* with functional polymorphisms, *TNF-A* G-308A, C-857T, and T-1031C, and *TNF-B* A252G in Japanese subjects.**

Methods. **The total of 1374 study subjects included 241 outpatients who participated in an *H. pylori* eradication program (HPE), 679 first-visit outpatients (FVO) at a regional cancer hospital, and 454 local residents who received a health checkup examination (HCE).**

Results. **The frequency of the *TNF-A -308A* allele was only 1.3% of 480 chromosomes in the HPE group, so the FVO and HCE groups were not genotyped for that polymorphism. The genotype frequency of *TNF-A* C-857T was 69.2% *CC*, 27.7% *CT*, and 3.1% *TT*; that of *TNF-A* T-1031C was 69.4% *TT*, 28.1% *TC*, and 2.5% *CC*; and that of *TNF-B* A252G was 36.8% *AA*, 48.2% *AG*, and 15.0% *GG*. *TNF-A -857T* was tightly linked to *TNF-A -1031T* and *TNF-B 252A*. No significant associations between *H. pylori* seropositivity and polymorphisms of *TNF-A* C-857T and *TNF-B* A252G were observed. However, a reduced odds ratio adjusted for sex, age, and recruitment source was observed for *TNF-A -1031CC* (0.43; 95% confidence interval, 0.20–0.91) relative to *TNF-A -1031TT*. Subjects with *TNF-A -857CC* and *-1031CC* showed the lowest seropositivity (38.2% of 34 participants), while those with *TNF-A -857TT* and *-1031TT* showed the highest (66.7% of 42 participants).**

Conclusion. **This study suggests that the possibly high expression genotype of *TNF-A* may increase susceptibility to persistent *H. pylori* infection.**

Key words *Helicobacter pylori* · Tumor necrosis factor · Polymorphism

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Introduction

Susceptibility to infection with pathogenic organisms is partly determined by genetic traits. For example, a finding that the CCR5 Δ32 polymorphism is a cause of an "exposed uninfected" individuals exposed to HIV [1] highlighted the importance of genetic polymorphism studies in the field of infectious diseases. Concerning *Helicobacter pylori* infection, a well-known cause of digestive diseases including gastric cancer, polymorphism studies of the host are still limited. To date, associations of *H. pylori* seropositivity with HLA types [2] and polymorphisms of *secretor* [3], *Lewis* [3], *interleukin 1B* (*IL-1B*) [4–7], *myeloperoxidase* [8], *tumor necrosis factor A* (*TNF-A*) [9], and *TCRBV6S1* [10] have been reported.

Tumor necrosis factor (TNF)-α is a cytokine induced by *H. pylori* [11], and inhibits gastric acid secretion [12]. The *TNF-A* gene on chromosome 6p21.3 encoding TNF-α is known to have five biallelic single-nucleotide polymorphisms in the promoter region; G-238A, G-308A, C-857T, C-863A, and T-1031C [13]. Among Japanese, the *-238A* and *-308A* alleles are rare (2.0% and 1.7%, respectively), and C-863A is tightly liked with T-1031C [14]. Recently, a significant association between infection with CagA-positive *H. pylori* and the *TNF-A -308A* allele (a high expression allele [15]) was reported for Koreans [9]. In Germany, in a study by Kunstmann et al. [16], the *-308A* allele was not found among 14 *H. pylori*-positive female patients with duodenal ulcer, while 26.8% of 98 *H. pylori*-positive female patients without duodenal ulcer had at least one *-308A* allele. In their subjects, no difference in G-308A genotype distribution was observed between subjects who were *H. pylori*-positive and those who were -negative [16]. To our knowledge, there has been no study of the associa-

tion between *H. pylori* and other polymorphisms of *TNF-A*.

TNF-β is encoded by the *TNF-B* gene, which is located near the *TNF-A* gene on chromosome 6p21.3. Both TNFs bind to the TNF receptor and exert similar biological activities; TNF-α is derived from macrophages and TNF-β from helper T-lymphocytes type I (Th1). The *TNF-B* A252G (formerly designated as LTα *Nco*I) *G* allele was reportedly associated with lung cancer risk [17], and poor prognosis of sarcoidosis [18]. The combination of *TNF-A* G-308A and *TNF-B* A252G was reported to have an association with asthma [19]. These findings are consistent with the finding that the *TNF-B 252G* allele is associated with higher expression of TNF-β than the 252A allele [20].

In the present study, we examined the associations of *H. pylori* seropositivity with four *TNF* polymorphisms, *TNF-A* G-308A, *TNF-A* C-857T, *TNF-A* T-1031C, and *TNF-B* A252G, in Japan, where the great majority of elderly persons had been exposed to *H. pylori* in their childhood.

Subjects and methods

Three sets of data derived from three recruitment sources were used for the present analysis. The first group included 241 outpatients with no history of cancer who participated in an *H. pylori* eradication program (HPE group) at Aichi Cancer Center Hospital in 1999 [4], and whose genotype background was reported for 50 polymorphisms [21]. The group included 97 patients who were stated to be receiving medication (a total of 107 diseases; for gastric/duodenal ulcer, in 23; so-called gastritis, in 23; hypertension, in 16; and for other 123 miscellaneous diseases). The second group included 679 first-visit outpatients (FVO group) of the Aichi Cancer Center Hospital in 2001 [5], about 20% of whom were expected to have a cancer. The third group included 454 health checkup examinees without a history of cancer (HCE group) in Nagoya [6].

Anti-*H. pylori* antibody was tested by SRL (Tokyo, Japan) with a high-molecular-weight Campylobacter-associated protein (HM-CAP) enzyme-linked immunosorbent assay (ELISA) (Enteric Products, Westbury, NY, USA). According to the commonly used definition, 2.3 EV (ELISA value) or higher was regarded as *H. pylori* seropositive. Genotyping for the four polymorphisms was conducted by polymerase chain reaction with confronting two-pair primers (PCR-CTPP) [22, 23], using 25 μl of PCR mixture. PCR conditions are summarized in Table 1. Figure 1 shows representative gels for the genotyping.

Frequencies were compared by a χ^2 test. Odds ratios (ORs) and 95% confidence intervals (CIs) were

Table 1. List of primers and conditions for polymerase chain reaction with confronting two-pair primers

Polymorphism (GenBank accession number), common band length, polymerase, glycerol, and annealing temperature Allele-specific band lengths and primers	
***TNF-A* G-308A** (X02910), common band: 415 bp, TaKaRaTaq,[a] 10% glycerol, and 65 °C	
A allele: 266 bp	F1:5′ TCC TGA GGC CTC AAG CCT GC
G allele: 190 bp	F2:5′ GCA ATA GGT TTT GAG GGG CAT GG
***TNF-A* C-857T** (AB048818), common band: 336 bp, AmpliTaq Gold,[b] no glycerol, and 68 °C	
C allele: 160 bp	F1:5′ GGG AGC TCC TGG GAG ATA TGG
T allele: 219 bp	F2:5′ AGT ATG GGG ACC CCC CCT TAA T
***TNF-A* T-1031C** (AB048818), common band: 444 bp AmpliTaq Gold, 8% glycerol, and 66 °C	
T allele: 316 bp	F1:5′ AAG GCT CTG AAA GCC AGC TG
C allele: 174 bp	F2:5′ GAA GCA AAG GAG AAG CTG AGA AGA C
***TNF-B* A252G** (M55913), common band: 425 bp AmpliTaq Gold, no glycerol, 61 °C	
A allele: 218 bp	F1:5′ TGC TTC GTG CTT TGG ACT AC
G allele: 250 bp	F2:5′ CAT TCT CTG TTT CTG CCA TGG

R1:5′ GGA GGC TGA ACC CCG TCC T
R2:5′ TGT CTC GGT TTC TTC TCC ATC GC
R1:5′ CCT CTA CAT GGC CCT GTC TTC G
R2:5′ TCT GAC CCG GAG ACT CAT AAT GC
R1:5′ CCA GAC CCT GAC TTT TCC TTC A
R2:5′ CTT CCA TAG CCC TGG ACA TTC T
R1:5′ AGG AAG GGA ACA GAG AGG AAT
R2:5′ GAG ACG TTC AGG TGG TGT CA

Initial denaturing was done for 10 min at 95 °C for AmpliTaq Gold, and for 5 min at 94 °C for TaKaRa Taq, followed by 30 cycles of 1 min each at the denaturing temperature, the above-mentioned annealing temperature, and at 72 °C for extension, and 5 min at 72 °C for final extension
The underlined bases indicate the sites of single nucleotide polymorphisms
[a] Takara Shuzo, Otsu, Japan
[b] Applied Biosystems, Foster City, CA, USA

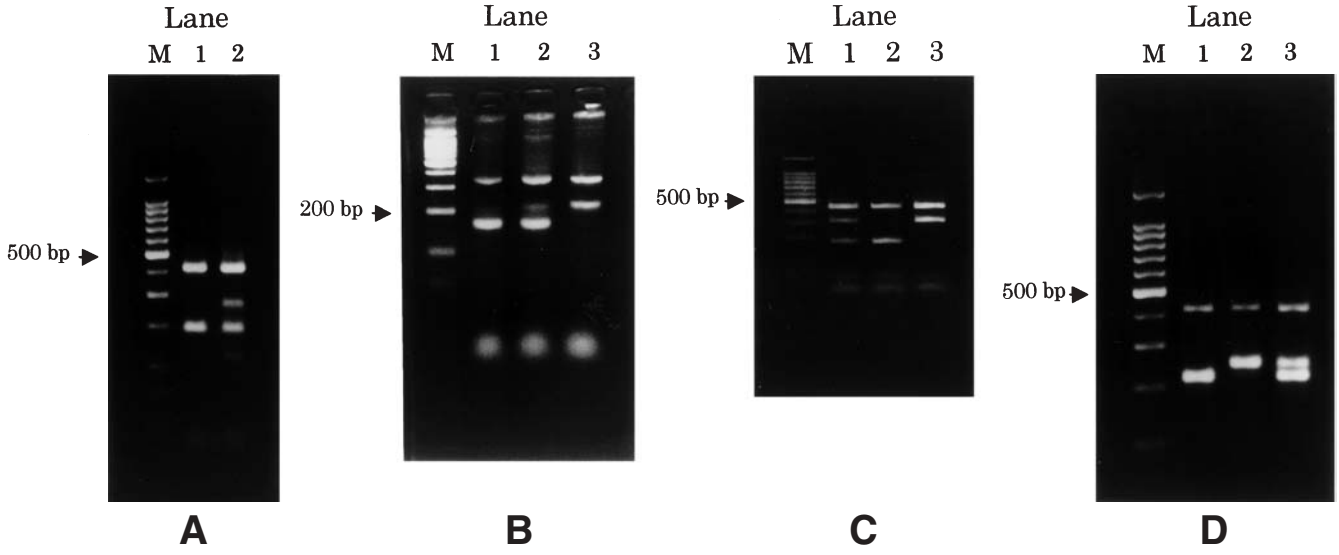

Fig. 1A–D. Representative examples of agarose gel electrophoresis. **A** *TNF-A* G-308A; *lane M*, 100-bp ladder marker; *lane 1*, *GG*; *lane 2*, *GA*. **B** *TNF-A* C-857T; *lane M*, 100-bp ladder marker; *lane 1*, *CC*; *lane 2*, *CT*; *lane 3*, *TT*. **C** *TNF-A* T-1031C; *lane M*, 100-bp ladder marker; *lane 1*, *TC*; *lane 2*, *CC*; *lane 3*, *TT*. **D** *TNF-B* A252G; *lane M*, 100-bp ladder marker; *lane 1*, *AA*; *lane 2*, *GG*; *lane 3*, *AG*. No *TNF-A* G-308A *AA* genotype was found

Table 2. Characteristics of subjects and *Helicobacter pylori* seropositive percentages (*HP*+%) according to recruitment source

	Study subjects					
	HPE		FVO		HCE	
Characteristics	*n* (%)	*HP*+%	*n* (%)	*HP*+%	*n* (%)	*HP*+%
Sex						
Males	118 (49.0)	69.5	315 (46.4)	60.6	126 (27.8)	65.9
Females	123 (51.0)	56.1	364 (53.6)	47.0	328 (72.2)	50.9
Age (years)						
≦39	2 (0.8)	0.0	132 (19.4)	22.7	48 (10.6)	14.6
40–49	44 (18.3)	45.5	101 (14.9)	49.5	58 (12.8)	39.7
50–59	90 (37.3)	57.8	210 (30.9)	58.6	102 (22.5)	59.8
60–69	105 (43.6)	75.2	151 (22.2)	68.9	176 (38.8)	64.1
≧70	0 (0.0)	—	85 (12.5)	64.7	70 (15.4)	65.7
Range	39–69 Years		18–79 Years		35–85 Years	
Smoking						
Never	140 (58.1)	58.1	351 (51.7)	49.6	376 (82.8)	55.3
Former	46 (19.1)	65.2	166 (24.4)	59.6	10 (2.2)	50.0
Current	55 (22.8)	70.9	161 (23.7)	55.3	68 (15.0)	54.4
Unknown	0 (0.0)	—	1 (0.1)	0.0	0 (0.0)	—
Total	241 (100)	62.7	679 (100)	53.3	454 (100)	54.8

HPE, participants in *Helicobacter pylori* eradication program; FVO, first-visit outpatients; HCE, health checkup examinees

estimated by an unconditional logistic model. STATA Version 7 (STATA, College Station, TX, USA) was used for these calculations. Command "genhwi" of STATA was used for examining Hardy-Weinberg equilibrium.

Results

Characteristics of study subjects by recruitment source are shown in Table 2. Although some of these summary statistics have been described previously [4–6], they are

Table 3. Genotype distributions of *TNF-A* G-308A, *TNF-A* C-857T, *TNF-A* T-1031C, and *TNF-B* A252G and *Helicobacter pylori* seropositivity according to recruitment source

	Study subjects			
Polymorphism	HPE *n* (%)	FVO *n* (%)	HCE *n* (%)	Total *n* (%)
TNF-A G-308A				
GG	234 (97.5)	NG	NG	
GA	6 (2.5)	NG	NG	
AA	0 (0.0)	NG	NG	
Total	240 (100)	NG	NG	
TNF-A C-857T				
CC	158 (65.8)	456 (69.2)	317 (70.9)	931 (69.2)
CT	74 (30.8)	177 (26.9)	122 (27.3)	373 (27.7)
TT	8 (3.3)	26 (3.9)	8 (1.8)	42 (3.1)
Total	240 (100)	659 (100)	447 (100)	1346 (100)
TNF-A T-1031C				
TT	164 (68.3)	476 (70.3)	312 (68.7)	952 (69.4)
TC	72 (30.0)	186 (27.5)	127 (28.0)	385 (28.1)
CC	4 (1.7)	15 (2.2)	15 (3.3)	34 (2.5)
Total	240 (100)	677 (100)	454 (100)	1371 (100)
TNF-B A252G				
AA	88 (36.5)	258 (38.5)	155 (34.6)	501 (36.8)
AG	116 (48.1)	315 (46.9)	225 (50.1)	656 (48.2)
GG	37 (15.4)	98 (14.6)	69 (15.4)	204 (15.0)
Total	241 (100)	671 (100)	449 (100)	1361 (100)

HPE, participants in *Helicobacter pylori* eradication program; FVO, first-visit outpatients; HCE, health checkup examinees; NG, not genotyped

Table 4. Linkages of *TNF-A* C-857T with *TNF-A* T-1031C and *TNF-B* A252G

	TNF-A T-1031C				*TNF-B* A252G			
TNF-A C-857T	*TT*	*TC*	*CC*	Total	*AA*	*AG*	*GG*	Total
CC	595	301	34	930	247	475	202	924
CT	297	76	0	373	200	171	2	373
TT	42	0	0	42	39	3	0	42
Total	934	377	34	1345	486	649	204	1339

$\chi^2 = 56.2$; degree of freedom (df), 4; $P < 0.001$ for an independent test between *TNF-A* C-857T and *TNF-A* T-1031C
$\chi^2 = 194.0$; df, 4; $P < 0.001$ for an independent test between *TNF-A* C-857T and *TNF-B* A252G

shown here again for the readers' convenience. The *H. pylori* seropositivity increased with age in each study group. Current smokers had a higher seroprevalence in the HPE group, but not in the FVO and HCE groups.

Table 3 shows the genotype distributions for the four polymorphisms. The total number of subjects shown in Table 3 is less than that in Table 2 because genotyping was not successful for some samples. In the HPE group, no participants harboring the *TNF-A -308AA* genotype were found, and only six individuals had the *TNF-A -308GA* genotype. Among these six, five had *TNF-A -857CC* and one, *TNF-A -857CT*. The linkage between *-308A* and *-857C* was not statistically significant, though the two alleles could be completely linked. Because the *TNF-A -308A* allele was so rare in HPE, the polymorphism was not genotyped for the FVO and HCE groups. The genotype distributions of *TNF-A* C-857T, *TNF-A* T-1031C, and *TNF-B* A252G were similar among the three groups of subjects. The *TNF-A -857TT* and *TNF-A -1031CC* genotypes were rare; 3.1% and 2.5%, respectively, when all subjects were combined. The distributions were in Hardy-Weinberg equilibrium; $\chi^2 = 0.378$; $P = 0.539$ for *TNF-A C-857T* with 0.830 of *C* allele; $\chi^2 = 0.448$; $P = 0.503$ for *TNF-A* T-1031C with 0.835 of *T* allele; and $\chi^2 = 0.203$; $P = 0.653$ for *TNF-B* A252G with 0.609 of *A* allele. As shown in Table 4, strong linkages were observed for *TNF-A* C-857T with *TNF-A* T-1031C and *TNF-B* A252G. The *TNF-A -857T*

Table 5. *Helicobacter pylori* seropositivity with odds ratio (ORs) and 95% confidence intervals (95% CIs) for *TNF-A* C-857T, *TNF-A* T-1031C, and *TNF-B* A252G polymorphisms

Polymorphism	Seropositive %				OR (95% CI)[a]
	HPE	FVO	HCE	Total	
TNF-A C-857T					
CC	62.0	51.1	55.5	54.5	1.00
CT	62.2	55.4	53.3	56.0	1.06 (0.82–1.37)
TT	75.0	61.5	75.0	66.7	1.69 (0.85–3.35)
TNF-A T-1031C					
TT	62.8	55.0	56.1	56.7	1.00
TC	63.9	50.5	53.5	54.0	0.92 (0.72–1.18)
CC	50.0	26.7	46.7	38.2	0.43 (0.20–0.91)
TNF-B A252G					
AA	59.1	53.5	55.5	55.1	1.00
AG	62.1	54.0	55.6	55.9	1.05 (0.82–1.34)
GG	73.0	52.0	52.2	55.9	1.05 (0.75–1.49)

HPE, participants in *Helicobacter pylori* eradication program; FVO, first-visit outpatients; HCE, health checkup examinees

[a] Adjusted for sex, age, and recruitment source

Table 6. *Helicobacter pylori* seropositivity with odds ratio (ORs) and 95% confidence intervals (95% CIs) for the combination of *TNF-A* C-857T and T-1031C

C-857T	T-1031C	*n*	Seropositive %	OR	(95% CI)[a]
CC	*CC*	34	38.2	1.00	Reference
CC	*TC*	301	51.5	1.95	(0.90–4.27)
CC	*TT*	595	57.0	2.37	(1.11–5.08)
CT	*TC*	76	61.8	2.84	(1.17–6.91)
CT	*TT*	297	54.5	2.16	(0.99–4.72)
TT	*TT*	42	66.7	3.63	(1.33–9.91)

[a] Adjusted for sex, age, and recruitment source

allele was almost completely linked to the *TNF-A -1031T* and *TNF-B 252A* alleles. All 34 participants with *TNF-A -1031CC* were found to have *TNF-A -857CC* and *TNF-B 252AA*, except for one individual not genotyped for *TNF-B A252G*.

Table 5 shows the *H. pylori* seropositivity with odds ratios (ORs) and 95% confidence intervals (CIs) for *TNF-A* C-857T, *TNF-A* T-1031C, and *TNF-B* A252G. No statistically significant differences in seropositivity were found for the subjects with the *TNF-A* C-857T and *TNF-B* A252G polymorphisms. However, those with *TNF-A* -1031CC showed the lowest seropositive rate (38.2% of 34 participants). The lowest rate was observed across the three datasets. The OR, adjusted for sex, age, and recruitment source, was 0.43 (95% CI, 0.20–0.91) for the *-1031CC* relative to the *-1031TT*.

When subjects with the combined genotype of *TNF-A* C-857T and T-1031C were classified, those with *TNF-A -857CC* and *-1031CC* showed the lowest rate (38.2%), and those with *TNF-A -857TT* and *-1031TT* the highest (66.7% of 42 participants). The OR, adjusted for sex, age, and recruitment source, was 3.63 (95% CI, 1.33–9.91) for those with *TNF-A -857TT* and *-1031TT* relative to those with *TNF-A -857CC* and *-1031CC*. The other genotype combinations had seropositivity rates between those of these two groups (Table 6).

Discussion

H. pylori is a gram-negative bacterium whose cell wall includes lipopolysaccharide. Lipopolysaccharide binds CD14 on the cell surface, and the message is transduced through toll-like receptor 4 (TLR4) to transcription factors such as nuclear factor (NF)-κB for *TNF-A* and interleukin 1-B *IL-1B* [24]. TNF-α and IL-1β induce themselves, as well as other inflammation-related cytokines. Accordingly, TNF-α and IL-1β play a key role in the early stage of inflammation in gastric mucosa [11]. For *H. pylori,* regulating inflammation to an opti-

mal level is crucial for its survival in the stomach [25]. The finding that *IL-1B C-31T* was associated with *H. pylori* seropositivity [4–7] prompted us to examine *TNF-A* and *TNF-B* polymorphisms. The documented associations between *TNF* polymorphisms and several diseases [13, 26–29] indicate that these polymorphisms are functional or that they are linked with a functional polymorphism, and this feature was also part of the rationale for the present study.

As described in the "Introduction", the *TNF-A* gene has five known polymorphisms in the promoter region, as well as G-244A, whose *A* allele has not been observed among the Japanese [18]. Of the five polymorphisms, C-857T and T-1031C (or C-863T tightly linked with T-1031C) were thought to be suitable candidates for susceptibility screening in Japanese. The less common allele, G-308A, was actually too rare in the present data sets to provide adequate statistical power for investigation. The frequency of the *-308A* allele was 1.3% (n = 240) in this study and 1.7% (n = 575) in another Japanese study [13], 3.1% (n = 113) in Koreans [9], 7.4% (n = 121) in Chinese [27], and 16.5% in Tunisia [26]. The *TNF-A* -857T allele is not rare, and its frequency seems to be similar among ethnic groups; 17.0% (457/2690) in this study, 17.3% (n = 575) in another study in Japan [13], and 23.2% (n = 235) in Northern Ireland [28]. The frequency of the *-1031C* allele observed in this study (16.5%) was also similar to that in another study in Japan (allele frequency, 16.0%) [13]. In an in vitro study, the TNF-α level and the transcriptional promoter activity produced by concanavalin A-activated peripheral blood mononuclear cells were higher for the *-857T* or *-1031C* alleles than for the *-857C* or *-1031T* alleles, respectively (n = 9). However, another research group reported that subjects with *-863A* tightly linked with *-1031C* showed a significantly lower serum TNF-α level (n = 156) [30]. The present study indicated that the combination of *-857TT* and *-1031TT* might be the most favorable condition for *H. pylori*. If the combination of *-857TT* and *-1031TT* produces the highest level of TNF-α, resulting in low gastric acid secretion, the findings of this study would make sense biologically. Further investigation of the pathophysiology involved in immune responses and *H. pylori* infection will be required to elucidate the associations between TNF-α production and *TNF-A* genotypes.

The *TNF-B 252G* allele was reported to be in complete linkage with the *Asn* allele of the *TNF-B* gene Asn26Thr polymorphism, which was associated with a higher level of TNF-β [20]. The present study demonstrated that *TNF-A -857T* was linked with *TNF-B 252A*, while *TNF-A -857C* was linked with both *TNF-B 252A* and *TNF-B 252G*. To our knowledge, the strong linkage between *TNF-A* C-857T and *TNF-B* A252G has not been examined for any ethnic group, although there has been much discussion, in published studies, of the association of diseases with either polymorphism of *TNF-A* or that of *TNF-B*. Based on the observed linkage, a haplotype of *TNF-B 26Thr*, *TNF-B 252A*, and *TNF-A -857T* is possibly related to a lower level of TNF-β than the other two major haplotypes, *TNF-B 26Thr — TNF-B 252A — TNF-A -857C* and *TNF-B 26Asn — TNF-B 252G — TNF-A -857C*. This new information should be taken into account in interpreting the published studies.

A small study in Korea showed that the *-308A* allele (a high expression allele) was significantly more frequent in patients infected with CagA+ *H. pylori* (9 out of 46) than in healthy controls with unknown *H. pylori* infection status (7 out of 113) [9]. The report is consistent with the hypothesis that possible higher expression alleles of *TNF-A -857T* and *-1031T* confer susceptibility to persistent *H. pylori* infection. Along with the documented association with a possible higher expression allele *-31T* of the *IL-1B* gene, it is plausible that the high expression *TNF-A* genotype increases the risk of persistent *H. pylori* infection.

Analysis for the genotype combinations of *TNF-A* C-858T or T-1031C with *IL-1B* C-31T seemed promising, but the sample size was too limited to further pursue potential gene-gene interactions. Although no statistically significant association was found, in 34 subjects with *TNF-A -1031CC*, the seropositive rate of *H. pylori* was 1 (16.7%) of 6 with *IL-1B -31CC*, 6 (31.6%) of 19 with *IL-1B -31CT*, and 6 (66.7%) of 9 with *IL-1B -31TT*. There was no difference in seropositivity among 42 subjects with *TNF-A -857TT* according to *IL-1B* C-31T; 6 (66.7%) of 9 with *-31CC*, 10 (76.9%) of 13 with *-31CT*, 12 (63.2%) of 19 with *IL-1B -31TT*, and none of 1 unsuccessful for the genotyping. The possible association of *H. pylori* seropositivity with these genotype combinations should be examined in future studies with a larger sample size. Similarly, analysis of interaction with smoking did not yield statistically significant results, but among the subjects with *-1031CC*, 4 (57.1%) of 7 smokers, 2 (22.2%) of 9 former smokers, and 7 (38.9%) of 18 never smokers were seropositive, while among the other subjects, seropositivity according to smoking status, as above, was 57.8%, 62.3%, and 53.8%, respectively. Among subjects with *-858TT*, 10 (90.9%) of 11 smokers, 7 (77.8%) of 9 former smokers, and 11 (50.0%) of 22 never smokers were seropositive. Gene-gene and gene-environment interactions concerning *H. pylori* infection need further investigation.

In summary, this study demonstrated: (1) a low frequency of *TNF-A -308A* in Japanese, (2) linkages of *TNF-A* C-858T with *TNF-A* T-1031C and *TNF-B* A252G, (3) no associations between *H. pylori* seropositivity and *TNF-A* C-857T and *TNF-B* A252G, and (4) the lowest *H. pylori* seropositivity for the *TNF-A -857CC* and *-1031CC* genotype, and the highest for the

TNF-A -857TT and *-1031TT* genotype. Because both TNF-α and IL-1β play an important role in inflammation, *TNF-A* functional polymorphisms could be useful to predict susceptibility to persistent *H. pylori* infection. Different ethnic groups differ in genetic makeup and exogenous risk factors. The contribution of these polymorphisms to persistent *H. pylori* infection may be worth investigating in other ethnic groups.

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