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PERSISTENT *HELICOBACTER PYLORI* INFECTION AND GENETIC POLYMORPHISMS OF THE HOST

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ABSTRACT

Helicobacter pylori (*HP*) infection elevates the risk of gastric diseases including peptic ulcer and gastric cancer. The infection induces inflammatory cytokines, which could work both for and against lifetime infection in the human stomach. Genetic polymorphisms of the cytokines and other related ligands, receptors, and enzymes may influence persistent *HP* infection. This paper summarizes studies done on the associations between anti-*HP* antibody seropositivity and polymorphism genotypes. To date, the associations with the polymorphisms of *fucosyl transferase 2* (*FUT2 or secretor gene*), *FUT3* (*Lewis gene*), *interleukin 1A* (*IL-1A*), *IL-1B, IL-1RN, IL-8, IL-10, myeloperoxidase* (*MPO*), and *tumor necrosis factor A* (*TNF-A*) and *TNF-B* have been reported. Polymorphisms of other related genes, *CD14*, *CXC chemokine receptor 2* (*CXCR2*), *IL-1RI*, *nuclear factor KB2* (*NF-KB2*), and *Toll-like receptor 4* (*TLR4*), have the potential to influence persistent infection. Unpublished results from our datasets are reported here for all these polymorphisms except *TLR4*. Gene-environment interactions between these genotypes and smoking are reviewed. An effect on OR due to the involvement of unexposed subjects is demonstrated to elucidate a disadvantage in the studies done in areas where the majority of the population is not exposed to *HP*.

Key Words: *Helicobacter pylori*, Polymorphisms, Interleukins, Odds ratio dilution

INTRODUCTION

Helicobacter pylori (*HP*) is a gram-negative bacterium which, upon infecting in the gastric mucosa, increases the risk of gastric diseases including peptic ulcers and stomach cancer.[1-3] Person-to-person infection largely depends on sanitary conditions, especially in childhood.[4-7] In addition, lifestyle factors such as salty food intake,[8] fruit intake,[9] and smoking[9-13] are considered to influence the infection rate. Meanwhile, a twin study reported that the concordance of anti-*HP* antibody status was higher in monozygotic twin pairs than in dizygotic twin pairs,[14] strongly underscoring the genetic role in persistent *HP* infection.

This paper reviews the reports on the associations between anti-*HP* antibody seropositivity and polymorphism genotypes. The associations between HLA genotypes and that seropositivity have also been reported,[15] but they are not covered here. First, the mechanism of signal transduction from *HP* is described to provide biological background on the selected gene polymorphisms. After the polymorphism studies are introduced, the limitations of association studies are discussed.

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BIOLOGICAL MECHANISMS

Gram-negative bacteria such as *HP* have a cell wall that contains lipopolysaccharide (LPS). The innate immune response, a preprogrammed nonspecific first line of defense responsible for eliminating pathogens at the site of entrance into the host, recognizes LPS with a pattern recognition receptor, CD14, on the cell surface. CD14 is a 55-kd glucosylphospatidylinositol-anchored receptor lacking an intracellular domain, which binds LPS with high affinitiy. The LPS-CD14 complex then activates Toll-like receptor 4 (TLR4) with an intracellular domain for signal transduction. TLR4 is stabilized in the form of a homodimer by MD-2. The signal from LPS is transduced through MyD88, IRAK (IL-1 receptor-associated kinase), TRAF6 (TNF receptor-associated factor 6), NIK/MKK (KF-κB-inducing kinase/mitogen-activated protein kinase kinase), and IKK (inhibitory κB kinase) to NF-κB.[16] (Fig. 1)

NF-κB is a group of proteins (NF-κB/REL proteins) that bind a common sequence motif known as the κB site.[17] They transcript inflammation-related genes such as *IL-1A, IL-1B, IL-2, IL-6, IL-8, TNF-A, TNF-B,* and *GM-CSF*.[18] Other pathways of LPS signaling may also exist for *IL-1B*,[19] and for *TNF-A* through extracellular signal-regulated kinase (ERK).[20] LPS-induced IL-1β and TNF-α induce other cytokines and enzymes for inflammation as well as IL-1β and TNF-α themselves through the NF-κB pathway.[21] IL-1 receptor antagonist coded by *IL-1RN* disturbs IL-1β binding to IL-1 receptor I (IL-1RI), resulting in the inhibition of the IL-1β function.

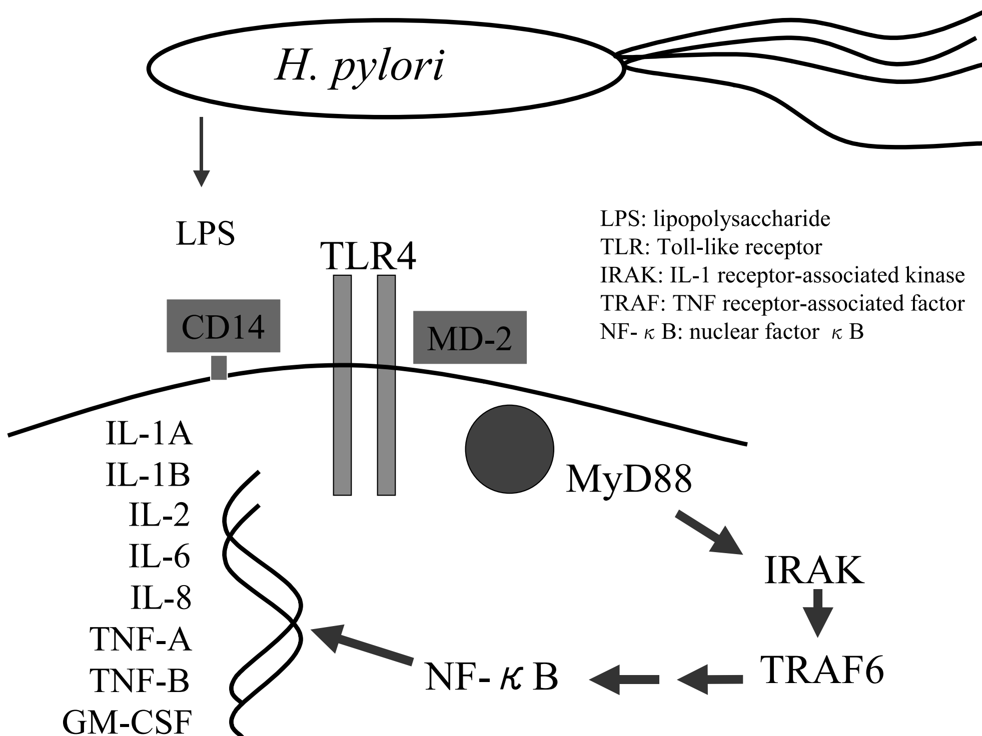

Fig. 1 Signal pathway from *Helicobacter pylori* to cytokine gene expression.

IL-1β and TNF-α inhibit gastric acid secretion.[22] The inhibited acid secretion causes *HP* distribution to the corpus, resulting in gastric atrophy.[23] Accordingly, the level of cytokines could influence persistent *HP* infection.[24] IL-8 is a CXC chemokine that mediates the activation and migration of neutrophils into tissue from peripheral blood. As is the case with IL-1β and TNF-α, IL-8 induced in gastric epithelial cells[25] and in neutrophils[26] by *HP* serves to trigger the inflammation. It combines CXCR-1 (previously called IL-8RA) and CXCR-2 (IL-8RB) with similar affinity. IL-10, a cytokine produced by type 2 T-helper cells (Th2 cells), inhibits the production of IL-1β and IL-8.[27,28] In mice, cytokine expressions by *Helicobacter felis* are modified by a concurrent infection of the enteric helminth, *Heligmosomoides polygyrus*, which drives the immune response through type 2 T-helper cells (Th2 cells). The co-infection increases the mRNA of interleukin 10 (IL-10) in comparison with *Helicobacter felis* infection alone, resulting in reduced *Helicobacter*-associated gastric atrophy and high *Helicobacter* colonization.[29] These findings suggest that a high level of IL-10 and a lower level of IL-8 create favorable conditions for prolonging the *HP* infection in human gastric mucosa. MPO is a lysosomal enzyme in polymorphonuclear leukocytes and monocytes. Hypochlorous acid produced by MPO shows microbicidal activity against a wide range of organisms[30] producing tissue inflammation. It was reported that HP water extract can activate neutrophils[31] and enhance the secretion of MPO.[26]

Another line of genetic traits involved in the susceptibility to *HP* concerns *HP* binding to gastric epithelium. *HP* with *babA2* gene is attached to gastric mucosa with blood group antigen-binding adhesion (BabA). [32,33] BabA binds both *Lewis b* and H type I blood group carbohydrate structure on the foveolar epithelium of human gastric mucosa. Type I precursor is converted to H type I antigen by fucosyltransferase 2 (FUT2, secretor enzyme), then to *Lewis b* antigen by fucosyltransferase 3 (FUT3, *Lewis* enzyme). FUT3 also converts type I precursor to *Lewis a* antigen. Although other binding mechanisms exist in *HP*, individuals lacking H type I and/or *Lewis b* may have a lower susceptibility to persistent *HP* infection.

POLYMORPHISMS

The molecules on the pathways originating from LPS seem to have a potential to enhance the susceptibility to *HP* infection and prolong the infection. The candidate molecules and their polymorphisms are discussed below in alphabetical order. Table 1 summarizes the findings of reported studies on the associations with these polymorphisms. "Unpublished data" in Table 1 are derived from our own unpublished studies on the same subjects as those in published reports.

CD14

CD14, located in chromosome 5q31.1, has a single nucleotide polymorphism (SNP) C-159T. Serum soluble CD14 was reported to be significantly higher in those with -159TT (n=42, median=4.5μg/ml) than in those with -159CC (n=67, median=4.1μg/ml).[34] Our unpublished data indicated no association with this polymorphism (Table 1).

CXCR2

CXCR2 in 2q35 was reported to have three SNPs; C785T causing a silent codon change in leucine, and T1208C and G1440A in the 3' untranslated region of exon 3.[35] These SNPs are tightly linked, forming a haplotype with 785C, 1208T, and 1440G. Accordingly, any SNP may be used for a pilot association study on disease risk. Our pilot study showed no substantial difference in the seropositive rate among the three genotypes of C785T.

Table 1 Sex-age-adjusted odds ratios (OR) and 95% confidence interval (95%CI) of polymorphism genotypes for *Helicobacter pylori* seropositivity

Authors and year Enrolled subjects	Polymorphism		N[*1]	OR	95% CI or *HP*+[*2]
Unpublished data	*CD14* C-159T	*TT*	413	1	reference
1,374 participants[*3]		*TC*	678	0.94	0.73–1.22
		CC	413	1.16	0.84–1.60
Unpublished data[41)]	*CXCR2* C785T	*CC*	110	*HP*+[*3]	64.5%
241 non-cancer		*CT*	100	*HP*+	63.0%
outpatients		*TT*	25	*HP*+	56.0%
Ikehara *et al.* 2001[37)]	*FUT2*	*SeSe*	61	1	reference
241 non-cancer		*Sese*	127	0.79	0.39–1.58
outpatients		*sese*	51	0.35	0.15–0.80
Hamajima *et al.* 2002[38)]	*FUT2*	*SeSe*	170	1	reference
679 first-visit		*Sese*	328	1.51	1.02–2.22
outpatients		*sese*	181	1.50	0.97–2.33
		SeSe	139	1	reference
465 health-checkup		*Sese*	218	1.57	1.00–2.46
examinees		*sese*	107	1.29	0.76–2.19
Ikehara *et al.* 2001[37)]	*FUT3*	*LeLe*	124	1	reference
241 non-cancer		*Lele*	98	1.95	1.08–3.50
outpatients		*lele*	17	2.80	0.81–9.74
Hamajima *et al.* 2002[38)]	*FUT3*	*LeLe*	353	1	reference
679 first-visit		*Lele*	251	0.98	0.70–1.37
outpatients		*lele*	59	1.31	0.73–2.37
		LeLe	235	1	reference
465 health-checkup		*Lele*	155	1.06	0.69–1.63
examinees		*lele*	33	1.40	0.63–3.07
Hamajima *et al.* 2001[45)]	*IL-1A* T-889C	*CC*	201	*HP*+	61.7%
241 non-cancer		*CT*	39	*HP*+	33.3%
outpatients		*TT*	1	*HP*+	100%
Hamajima *et al.* 2001[45)]	*IL-1B* C-31T	*CC*	42	1	reference
241 non-cancer		*CT*	133	2.32	1.10–4.92
outpatients		*TT*	66	2.46	1.06–5.74
Katsuda *et al.* 2001[46)]	*IL-1B* C-31T	*CC*	116	1	reference
465 health checkup		*CT*	183	0.97[*4]	0.59-1.57
examinees		*TT*	163	1.73[*4]	1.04–2.87
Hamajima *et al.* 2002[47)]	*IL-1B* C-31T	*CC*	116	1	reference
547 first visit		*CT*	237	1.32	0.84–2.08
outpatients[*5]		*TT*	178	1.35	0.83–2.18
Uno *et al.* 2002[48)]	*IL-1B* C-31T	*CC*	226	1	reference
963 Japanese		*CT*	432	1.30	0.94–1.81
Brazilians		*TT*	289	1.45	1.02–2.06

Kato *et al.* 2001[49]	*IL-1B* C-511T	*CC*	143[*6]	*HP*+	53.1%[*6]
499 patients with		*CT*	243[*6]	*HP*+	53.9%[*6]
gastric diseases other		*TT*	113[*6]	*HP*+	52.2%[*6]
than gastric cancer					
Hamajima *et al.* 2001[45]	*IL-1RN* VNTR	*4/4*	217	*HP*+	62.2%
241 non-cancer		others	24	*HP*+	66.7%
outpatients					
Unpublished data[41]	*IL-1RI* C-116T	*CC*	93	*HP*+	64.5%
241 non-cancer		*CT*	114	*HP*+	57.9%
outpatients		*TT*	32	*HP*+	71.9%
Hamajima *et al.* 2003[53]	*IL-8* T-251A	*TT*	234	1	reference
454 health-checkup		*TA*	177	0.86	0.57–1.29
examinees[*7]		*AA*	37	0.70	0.34–1.45
Hamajima *et al.* 2003[53]	*IL-10* T-819C	*TT*	220	1	reference
454 health-checkup		*TC*	177	0.67	0.44–1.02
examinees[*7]		*CC*	47	0.82	0.42–1.60
Hamajima *et al.* 2003[53]	*IL-8* & *IL-10*	*TT&TT*	115	1	reference
454 health-checkup		Others	327	0.62	0.39–0.98
examinees[*7]					
Unpublished data[41]	*IL-8* & *IL-10*	*TT&TT*	57	1	reference
241 non-cancer		Others	178	1.04.	0.54–1.99
outpatients					
Unpublished data	*IL-8* & *IL-10*	*TT&TT*	164	1	reference
679 first-visit		Others	507	1.49	1.03–2.17
outpatients					
Hamajima *et al.* 2001[60]	*MPO* G-463A	*GG*	192	1	reference
241 non-cancer		*GA/AA*	49	0.69	0.35–1.35
outpatients					
Katsuda *et al.* 2001[61]	*MPO* G-463A	*GG*	354	1	reference
454 health checkup		*GA/AA*	83	0.84	0.51–1.37
examinees[*7]					
Unpublished data	*NF-KB2* -10G	*Ins/Ins*	513	1	reference
1,374 participants[*2]		*Ins/De*	648	1.03	0.81–1.32
		Del/Del	199	1.15	0.81–1.62
Hamajima *et al.* 2003[72]	*TNF-A* T-1031C	*TT*	952	1	reference
1,374 participants[*2]		*TC*	385	0.92	0.72–1.18
		CC	34	0.43	0.20–0.91
Hamajima *et al.* 2003[72]	*TNF-A* C-857T	*CC*	931	1	reference
1,374 participants[*2]		*CT*	373	1.06	0.82–1.37
		TT	42	1.69	0.85–3.35
Hamajima *et al.* 2003[72]	-1031 & -857	*CC&CC*	34	1	reference
1,374 participants[*2]		*TC&CC*	301	1.95	0.90–4.27

		TT&CC	595	2.37	1.11–5.08
		TC&CT	76	2.84	1.17–6.91
		TT&CT	297	2.16	0.99–4.72
		TT&TT	42	3.63	1.33–9.91
Kunstman *et al.* 1999[71) 393 patients with abdominal or chest pain	*TNF-A* G-308A	*GG*	277[*8]	*HP*+	52.3%[*8]
		GA	89[*8]	*HP*+	56.2%[*8]
		AA	18[*8]	*HP*+	55.6%[*8]
Hamajima *et al.* 2003[72) 1,374 participants[*2]	*TNF-B* A252G	*AA*	501	1	reference
		AG	656	1.05	0.82–1.34
		GG	204	1.05	0.75–1.49

[*1] Subjects successfully genotyped. Difference between enrolled subjects and a total of the successfully genotyped subjects yields the subjects not genotyped.
[*2] *HP* seropositive rate.
[*3] Subjects consisted of 241 non-cancer outpatients, 679 first-visit outpatients, and 454 health-checkup examinees without a history of cancer. ORs are adjusted for sex, age, and the three data sources.
[*4] Age-sex-smoking adjusted OR.
[*5] Those aged 40–69 years out of the 679 first-visit outpatients.
[*6] Numbers were constructed from Table 1 by *Kato et al.*[49)]
[*7] Those without a history of cancer out of 465 health-checkup examinees.
[*8] Numbers were constructed from Table 3 by Kunstman *et al.*[71)]

FUT2 (*secretor gene*) and *FUT3* (*Lewis gene*)

Six alleles have been identified for *FUT2* located in 19q13.3. They are *Se1* (*357C, 385A, 571C, 628C*), *Se2* (*357T, 385A, 571C, 628C*), *sej* (*357T, 385T, 571C, 628C*), *se3* (*357C, 385A, 571T, 628C*), *se4* (*357C, 385A, 571C, 628T*), and *se5* (combined with a pseudogene). *Se1* and *Se2* exhibit full enzyme activity, *sej* shows very low activity, and the others reveal no activity. Accordingly, *Se1* and *Se2* are denoted by *Se*, and the others by *se*. Among Japanese, *se3* and *se4* are very rare.[36)]

FUT3 has three SNPs; T59G, G508A, and T1067A. An *Le* allele is defined as one with 59T, 508G, and 1067T, an *le1* allele one with 59G, 508A, and 1067T, an *le2* allele one with 59G, 508G, and 1067A, and an *le3* allele one with 59G, 508G, and 1067T. The *le1* and *le2,* denoted by *le,* lack enzyme activity. Since *le3* shows almost full enzyme activity, it is grouped into *Le*.[36)]

Our first report successfully demonstrated that those with *sese* genotype who cannot synthesize H type I nor *Lewis b* had the lowest seropositive rate.[37)] In addition, *LeLe* genotype, which may disturb the synthesis of H type I by FUT2 through sharing the same substrate (type I precursor), showed the lowest rate.[37)] The odds ratio for study subjects with *sese* and *LeLe* was about one tenth of those with *SeSe/lele*, *SeSe/Lele*, or *Sese/lele*.[37)] In order to confirm that association, two datasets were analyzed, but the association was not reproduced.[38)] Unknown effect modification may exist among the three datasets.

IL-1A

IL-1A making a cluster in chromosome 2q14 with *IL-1B* and *IL-1RN* has two SNPs of C-889T and G4845T, as well as a 46-bp VNTR (variable number of tandem repeats) polymorphism.[39)] It was reported that the combination *IL-1A -889TT* and *IL-1B -511T* (*TT* or *TC*) was related to high plasma levels of IL-1β.[40)] Since among Japanese -889T allele represented only

8.5%,[41] a large study is required to evaluate the association with *HP* seropositivity in that population. The 46-bp VNTR polymorphism was not polymorphic among Japanese in our study.[41] Polymorphism G4845T was reported to be concordant with C-889T.[42]

IL-1B

Three polymorphisms of *IL-1B*, C-511T, C-31T, and C3954T, have been studied for dozens of diseases.[43] An electrophoretic mobility-shift assay demonstrated that C-31T was a functional polymorphism,[44] while C3954T is unlikely to be functional. Since -511T and -511C are tightly linked with -31C and -31T, respectively,[45] either one is enough for genotyping *IL-1B*.

Our team conducted four studies,[45-48] all of which showed a similar result, i.e., study sujbects with -31TT ran a higher risk of *HP* seropositivity. No studies other than ours focused on the associations between *HP* infection and *IL-1B* polymorphisms. The *HP* seropositivity constructed from a study by Kato *et al.* showed no association with C-511T.[49] Meanwhile, there was a study reporting that *-511T/-31C* allele increased the risk for atrophic gastritis, intestinal metaplasia, and severe inflammation among the infected.[50]

IL-1RN

IL-1RN has an 86-bp VNTR polymorphism. Among 241 Japanese, the allele frequency was 4.1% for *2 repeat* allele, 0.2% for *3 repeat* allele, 94.6% for *4 repeat* allele, and 1.0% for *5 repeat* allele.[41] No difference in *HP* soropositivity was observed between *4/4* and other genotypes (Table 1).[45] Since *4 repeat* allele is dominant, studies on this polymorphism among Japanese pose difficulies. The *2 repeat* allele (*IL-1RN*2* allele) was reportedly associated with atrophic gastritis, intestinal metaplasia, and severe inflammation among the infected,[50] as well as stomach cancer risk.[44]

IL-1RI

There are two receptors for IL-1β; IL-1RI and IL-1RII. The former transduces the signal, but the latter does not. *IL-1RI* in 2q12 has reportedly four SNPs; C-116T (RFLP-A), C-90T, T49C, and RFLP-B at an unknown site.[51] There are no reports on the association between these polymorphisms and *HP* seropositivity. As shown in Table 1, our unpublished pilot study for C-116T indicated no association.

IL-8 and *IL-10*

IL-8 located in chromosome 4q12-21 was reported to have nine polymorphisms (four in 5' upstream regions, four at introns, and one in 3' downstream region),[52] and more are listed in GenBank Accession No. AF385628 including some rare allele polymorphisms. Among those polymorphisms, A-251T from the transcription start site (-353 from the translation start site) in Accession No. M28130 is considered to influence *IL-8* expression; *A* allele has a greater expression than *T* allele.[52] Among Europeans, two haplotypes, one with *-1722delT, -251A, 396G, 781T, 1633T*, and *2767T* termed *delTAGTTT* in the order of those polymorphisms, and the other with *delTTTCCA*, are dominant with frequencies of 0.41 and 0.52, respectively. The haplotypes are more diverse among Africans; *delTAGCCA* (frequency 0.36), *delTATCCA* (0.19), *insTATCCA* (0.18), and *delTTTCCA* (0.10), respectively,[52] but the genotyping of T-251A and T396G is sufficient for classifying the haplotypes. Our preliminary genotyping for 227 outpatients identified a strong linkage between the two polymorphisms; *396TT* was found in 90.0% of 110 individuals with *-251TT*, *396TG* in 90.5% of 95 individuals with *-251TA,* and *396GG* in 100% of 22 individuals with *-251AA*.[53] *IL-8 -251AA* was found to be more frequent among patients with tuberculosis than among the controls (OR=3.41, 95%CI, 1.52-7.64 for Caucasians and OR=3.46,

95%CI, 1.48-8.08 for African Americans).[54)]

IL-10 G-1082A and T-819C polymorphisms are considered to influence the expression of *IL-10* mapped on 1q31-32; *-1082A* and *-819T* are reportedly higher expression alleles.[55,56)] Among Japanese, the *-1082G* allele frequency was found to be 0.04,[57)] and there were no Japanese studies on the T-819C allele frequency except ours.[53)]

Our first study found that high seropositivity was associated with the combination of *IL-8 -251TT* and *IL-10 -819TT.*[53)] That association was significant among smokers, as will be described later (Table 2).[53)] However, the other datasets did not reproduce such an association. On the contrary, for reasons that remain unclear, one dataset of 679 first-visit outpatients showed an opposite association.

MPO

While *MPO* Arg569Trp, Val173Cys, or Met251Thr causes fatal diseases such as chronic granulomatous disease due to severe enzyme activity deficiency,[58)] *MPO* G-463A exhibits a different level of expression; the *G* allele has a 25-fold higher transcription level than the *A* allele.[59)] This gene polymorphism located in 17q23.1 was found to show a similar association by univariate analysis in two datasets, though not a significant one (Table 1).[60,61)]

NF-KB2

NF-KB2 coding NF-κB2 (p100) has Ins/Del -10G (or 1867GG/G[62)]), as well as two SNPs with a rare minor allele.[62)] The function of Ins/Del -10G was not demonstrated, but our unpublished data suggested no association with *HP* seropositivity (Table 1).

TLR4

In *TLR4*, two co-segregating polymorphisms have been reported, Asp299Gly and Thr399Ile. The *299Gly/399Ile* allele is less sensitive to LSP than *299Asp/399Thr* allele, resulting in lower NF-κB activity.[63)] The LPS-hyposensitive allele was found to be 5.9% among 879 blood donors in England[64)] and not found at all among 275 Japanese.[65)]

TNF-A and *TNF-B*

TNF-A and *TNF-B* genes are located between HLA-B and HLA-DR on 6p21.3. In the promoter area of *TNF-A*, G-238A, G-244A, G-308A, C-857T, C-863A, and T-1031C were reported.[66,67)] Among Japanese, *-244A* was not found,[67)] *-238A* and *-308A* alleles were rare (2.0% and 1.7%, respectively),[66)] and C-863A was tightly linked with T-1031C.[68)] Recently, a significant association between infection with CagA-positive *H. pylori* and *TNF-A -308A* allele (a high-expression allele[69)]) was reported among Koreans.[70)] In Germany, the *-308A* allele was not found among 14 *HP*-positive female patients with duodenal ulcer, while 26.8% of 98 *HP*-positive female patients without duodenal ulcer had at least one *-308A* allele. In those subjects, no difference in G-308A genotype distribution was observed between the *H. pylori* positive and negative patients (Table 1).[71)] Our study of 1,374 participants from three datasets showed that those with *TNF-A -857TT* and *-1031TT* ran the highest risk of being *HP* seropositive, and those with *TNF-A -857CC* and *-1031CC* the lowest.[72)]

TNF-B A252G, whose *G* allele is strongly linked with *TNF-A -857C* allele in Japanese,[72)] was not associated with seropositivity, as shown in Table 1. Another SNP, *TNF-B* Thr26Asn, was found in our dataset to link completely with A252G.

Table 2 Age-sex-adjusted odds ratios (OR) and 95% confidence interval (95%CI) of polymorphism genotypes for *Helicobacter pylori* seropositivity, taking account of smoking habit

Authors and year / Enrolled subjects	Polymorphism		N[*1]	OR	95% CI
Hamajima *et al.* 2001[45]	*IL-1B* C-31T	Non-cancer outpatients			
55 current smokers		*CC*	16	1	reference
		CT	27	6.18	1.34–28.6
		TT	12	22.9	1.97–266
Katsuda *et al.* 2001[46]	*IL-1B* C-31T	Health checkup examinees			
80 ever smokers		*CC*	23	1	reference
		CT	34	1.68	0.50–5.71
		TT	22	5.29	1.11–25.1
Hamajima *et al.* 2002[47]	*IL-1B* C-31T	First visit outpatients[*2]			
127 current smokers		*CC*	23	1	reference
		CT	60	1.12	0.40–3.11
		TT	41	1.01	0.34–2.98
Uno *et al.* 2002[48]	*IL-1B* C-31T	Japanese Brazilians			
124 Current smokers		*CC*	NA	1	reference
		CT	NA	2.45	0.91–6.55
		TT	NA	3.49	1.17–10.4
Hamajima *et al.* 2003[53]	*IL-8* & *IL-10*	Health checkup examinees			
65 current smokers		*TT&TT*	17	1	reference
		Others	48	0.13	0.03–0.61
Unpublished data	*IL-8* & *IL-10*	Non-cancer patient			
55 current smokers		*TT&TT*	14	1	reference
		Others	41	0.45	0.09–2.23
Unpublished data	*IL-8* & *IL-10*	First-visit outpatients			
158 current smokers		*TT&TT*	38	1	reference
		Others	120	0.89	0.40–1.98
Hamajima *et al.* 2001[60]	*MPO* G-463A	Non-cancer outpatients			
55 currrent smokers		*GG*	45	1	reference
		GA/AA	10	0.21	0.04–0.98
186 non-current smokers		*GG*	147	0.39	0.16–0.92
		GA/AA	39	0.37	0.13–1.03
Katsuda *et al.* 2001[61]	*MPO* G-463A	Health checkup examinees			
64 current smokers		*GG*	49	1	reference
		GA/AA	15	0.83	0.22–3.05
363 never smokers		*GG*	297	1	reference
		GA/AA	66	0.87	0.50–1.51

[*1] Subjects successfully genotyped. Difference between enrolled subjects and a total of the successfully genotyped subjects yields subjects not genotyped.
[*2] Those aged 40–69 years.
NA: data not available.

GENE-ENVIRONMENTAL INTERACTION

Inconsistent results observed in polymorphism studies are not rare. Such discrepancies are explained partly by gene-environment or gene-gene interaction. We have examined the interaction between genotypes and several lifestyle factors, among which smoking was found to be a major candidate for modifing the effect of genotypes.

Table 2 shows the odds ratios among smokers or for smoking in combination with genotypes. Concerning *IL-1B* C-31T,[45-48] the odds ratios tended to be higher among smokers with one exception.[47] The first dataset for the combination of *IL-8 -251TT* and *IL-10 -819TT* showed a marked association among current smokers.[53] Subsequent datasets produced insignificant ORs with the same direction as the first report. A marginal interaction was observed for *MPO* (*GG* vs. *GA/AA*) and smoking (current vs. non-current); OR=4.57 and p=0.08.[60] No difference in OR was observed between current smokers and never smokers in another dataset.[61]

LIMITATIONS OF ASSOCIATION STUDIES

For the purpose of measuring the associations between *HP* infection and genotypes, the optimal study population is one in which all subjects were exposed to *HP*. The true strength of the association is diluted with each increase in the proportion of the unexposed. If 50% of study subjects were not exposed to *HP*, OR = 4 among the exposed consisting of half with a high-risk allele and the rest without that allele, becomes 2 (Table 3). Accordingly, any study on the association with *HP* infection is difficult in areas where the majority has not been exposed to *HP*.[73]

We learned that the frequency of minor alleles varies widely among different ethnic groups. When the frequency is too low to generate sufficient statistical power for detecting a significant OR, such a study would be meaningless for that ethic group. In this case, comparisons across different ethnic groups are impossible.

Gene-gene or gene-environment interactions are quite attractive themes in the field of polymorphism studies. However, studies on such interactions require a larger sample size, e.g., in the thousands. Only major research groups accustomed to routinely enrolling many participants could successfully conduct such a study.

Table 3 An example of odds ratio dilution

		Exposure to *Helicobacter pylori*					
		Yes		No		Total	
		Pos.[*1]	Neg.[*2]	Pos.	Neg.	Pos.	Neg.
High risk allele	Yes	40	10	0	50	40	60
	No	25	25	0	50	25	75
	Total	65	35	0	100	65	135

The subjects consist of 100 exposed and 100 unexposed, a total of 200 individuals. The OR of high risk allele is 4 among the exposed, but 2 among all subjects.

[*1] Seropositive, [*2] Seronegative

CONCLUSIONS

The great majority of reports on the association between *HP* seropositivity and polymorphisms have been produced by our teams. The datasets were derived from 241 non-cancer outpatients who participated in an *HP*-eradication program, 465 health checkup examinees in Nagoya (454 of which were without a history of cancer), 679 first-visit outpatients to the Aichi Cancer Center (547 outpatients aged 40 to 69 years), and 963 Japanese Brazilians in Sao Paulo. We enjoyed some advantages, i.e., 1) our research team at the Aichi Cancer Center was fully cooperative, 2) a hospital-based epidemiologic research program (HERPACC) was ongoing,[74] 3) a newly developed PCR method, PCR-CTPP (polymerase chain reaction with confronting two-pair primers) was available[75], and 4) *HP* exposure is very common in Japan, especially among the elderly.

Our findings were rather consistent for *IL-1B* and *TNF-A*, both of which encode cytokines that inhibit gastric acid secretion. The finding that acid inhibition from medication established favorable conditions for *HP* to form colonies provides biological plausibility for the polymorphisms of both cytokines. Another pathway to influence persistent *HP* infection may exist in relation to immunological response, but this remains to be investigated.

To date, strong and specific associations with polymorphisms have not been identified for *HP* infection. In the case of HIV, it is well known that the β chemokine receptor gene (*CCR5*) *Δ32* homozygous genotype completely blocks the infection through inhibiting the successful cell surface transportation of the CCR5 molecule, the essential molecule for HIV to enter CD4-positive T cells.[76] On the contrary, since *HP* remains restricted to the mucus layer of the stomach, similarly strong mechanisms specific to *HP* may not exist.

There are several studies reporting the associations between genotypes and stomach cancer risk.[44,49,77-81] Such studies are essential in measuring the net contribution of genotypes to stomach carcinogenesis, which consists of three steps in areas where *HP* infection is prevalent.; 1) persistent *HP* infection among the exposed, 2) gastric atrophy among the infected, and 3) stomach cancer among those with gastric atrophy. In view of stomach cancer prevention, analytical studies on each step are required. The present paper reviewed progress to date in the first step to stomach carcinogenesis through *Helicobacter pylori*.

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