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Interleukin 1 Polymorphisms, Lifestyle Factors, and *Helicobacter pylori* Infection

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Associations between *Helicobacter pylori* (*HP*) infection and lifestyle factors have been reported by several authors, but little is known about the host factors associated with the infection. This study aims to examine the infection rate of *HP* according to gene polymorphisms of interleukin (IL)-1A, IL-1B, and IL-1RN, and to investigate the interactions with lifestyle factors. Subjects were 241 non-cancer outpatients who had participated in a *HP* eradication program. Polymorphisms at −889 (T to C) of IL-1A, at −31 (C to T; T allele makes a TATA box) and −511 (C to T) of IL-1B, and at intron 2 (86-bp VNTR (variable number of tandem repeats)) of IL-1RN were genotyped by PCR (polymerase chain reaction), PCR-RFLP (restriction fragment length polymorphism) and PCR-CTPP (PCR with confronting two-pair primers). It was found that IL-1B polymorphisms at −31 and −511 were near-completely linked, but in the opposite way to that in Caucasians; −31C/−511T and −31T/−511C alleles were dominant in the present subjects. The *HP* infection rate was substantially different among the genotypes of IL-1B C−31T; 45.2% (19/42) for the C/C, 67.7% (90/133) for the C/T, and 63.6% (42/66) for the T/T. The age-sex adjusted odds ratio (OR) relative to the C/C genotype was 2.32 (95%CI (confidence interval), 1.10–4.92) for the T/C genotype and 2.46 (1.06–5.74) for the T/T genotype. The OR for the T/T genotype was significantly modified by smoking status; interaction term=14.6 (1.12–190). The polymorphisms of IL-1A and IL-1RN were not associated with the infection rate. The results suggested that the T allele of IL-1B C−31T is associated with vulnerability to persistent *HP* infection, and that the vulnerability is modified by smoking.

Key words: *Helicobacter pylori* infection — Interleukin 1 — Lifestyle factors

Helicobacter pylori (*HP*) infection, a well-known risk factor for stomach cancer, depends largely on sanitary conditions, especially in childhood.[1–3] There are still substantial differences in childhood prevalence between developed and developing countries, ranging from 4% to 82% reportedly.[2] An increase in infected individuals with age is observed in many developed countries, which is considered to be due to a cohort effect.[1] Although there is no doubt that exposure to the bacterium increases the infection rate in the population, host susceptibility is also an important factor. Even after an ordinary level of *HP* exposure, uninfected persons still seem to exist in any population. In addition, spontaneous eradication after childhood infection may be influenced by host factors.

Polymorphisms of interleukin-1β gene (IL-1B) and interleukin 1 receptor antagonist gene (IL-1RN) were reportedly associated with stomach cancer risk for a population from Poland.[4] IL-1β is a pro-inflammatory cytokine with multiple biological effects,[5] and is induced by *HP* infection.[6] IL-1B has three diallelic polymorphisms at −511, −31, and 3954 base pairs (bp) from the transcriptional start site. Though the differences were not statistically significant, persistent *HP* infection was observed for 60% of individuals harboring the C/C genotype (n=58) at −31 of IL-1B, for 73% of those with the C/T genotype (n=67), and for 79% of those with the T/T genotype (n=24) in a population from Scotland.[4] The T allele at −31 forms a TATA box, suspected of enhancing gene expression. The study reported that the polymorphism at −511 linked tightly with the polymorphism at −31, and therefore quite similar findings on the risks of stomach cancer and *HP* infection were obtained for IL-1B C−511T. The polymorphism at 3954 was not associated with the risks of stomach cancer and *HP* infection in the study.[4] IL-1RN has a penta-allelic 86-bp tandem repeat polymorphism in intron 2, which was found to affect IL-1β production *in vitro*,[7] but it was not associated with *HP* infection.[4] IL-1α is another member of IL-1, which is encoded by IL-1A. It is primarily a cytosolic cytokine. About 10% or 15% is myristoylated and transported to the cell surface (membrane IL-1), but it is not detected in the serum under normal conditions.[5] IL-1A was reported to have three polymorphisms; C−889T, 46-bp variable number of tandem

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repeats (VNTR) at intron 6, and G4845T. A study showed that the combination of T/T of IL-1A C−889T and T/− of IL-1B C−511T was related to high plasma levels of IL-1β.[8] To date, no studies have been reported on the association of IL-1A polymorphisms with stomach cancer risk or *HP* infection.

The present case-control study examines the associations between persistent *HP* infection and four polymorphisms of the non-coding regions of IL-1; at −889 (T to C) of IL-1A, at −31 (C to T) and −511 (C to T) of IL-1B, and at intron 2 (86-bp VNTR) of IL-RN. To our knowledge, this is the first report on their genotype frequencies in Japanese. The interactions with lifestyle factors are also considered in this paper.

MATERIALS AND METHODS

Study subjects Subjects were participants in an *HP* eradication program at Aichi Cancer Center Hospital, who were scheduled for gastroscopy. Written informed consent was obtained for a lifestyle questionnaire and gene polymorphism tests. The lifestyle questionnaire included smoking, alcohol, food intake frequency, physical exercise, and past history of cancer. In total, 283 outpatients (138 males and 145 females) participated in the study up to December 1999. We excluded 42 participants (38 with a history of cancer, 3 hepatitis virus carriers whose blood was not stored, and 1 who refused blood sampling after entry), and the remaining 241 outpatients were analyzed.

***HP* infection** An anti-*HP* IgG antibody test, High-molecular-weight *Campylobacter*-associated protein (HM-CAP) ELISA ("Detaminor *H. pylori* antibody," Enteric Products Inc., Westbury, NY) was used for detecting *HP*-infected participants. An ELISA value of 2.3 or over was regarded as indicating *HP* infection-positive status. The sensitivity of HM-CAP was reported to be 98.7% and specificity 100% in the United States,[9] though the sensitivity was not so high for Japanese.[10]

Genotyping DNA was extracted from 200 μl of buffy coat preserved at −40°C by the use of a QIAamp DNA Blood Mini Kit (QIAGEN Inc., Valencia, CA). The polymerase chain reaction (PCR) amplification was conducted using the primers listed in Table I. The primers of IL-1RN were the same as described by Mansfield *et al.*,[11] and the others were chosen for this study. Genomic DNA (30 to 100 ng) was used in a volume of 25 μl with 0.1 m*M* dNTPs, 25 pmol of each primer, 0.5 units of "TaKaRa Taq" (TaKaRa Shuzo Co., Ltd., Otsu) or "AmpliTaq Gold" (Perkin-Elmer Corp., Foster City, CA), and 2.5 μl of 10× PCR buffer including 15 m*M* $MgCl_2$. The PCR-RFLP (restriction fragment length polymorphism) method was used for the C-to-T single nucleotide polymorphism of IL-1B C−511T. The PCR products for tandem repeat polymorphisms of IL-1RN were used directly for electrophoresis. For the polymorphisms at −889 of IL-1A and at −31 of IL-1B, a new method named PCR-CTPP (PCR with confronting two-pair primers) was applied, which does not require a step to digest DNA products for single nucleotide polymorphism genotyping.[12] The primer setting for IL-1B C−31T is a revised version providing clearer bands than the former version.[12] Fig. 1 shows the results of PCR-CTPP for IL-1A C−889T and IL-1B C−31T, PCR-RFLP for IL-1B C−511T, and PCR for IL-1RN. All PCR products were visualized on a 2% agarose gel with ethidium bromide staining. To confirm genotyping for IL-1B C−31T, PCR-RFLP was conducted using a restriction enzyme

Table I. PCR Conditions for IL-1A, IL-1B, and IL-1RN

Polymorphism	Primers	Temperature, time, and cycles for PCR; polymerase; PCR method (restriction enzyme); and definitions of the allele
IL-1A C to T at −889	F1 5′-ATC ACA CCT AGT TCA TTT CC-3′ R1 5′-TAC ATA TGA GCC TTC AAT G<u>A</u>-3′ F2 5′-TAA TAG TAA CCA GGC AAC A<u>C</u>-3′ R2 5′-CAA CAC ATT TCC TAT AGA GG-3′	10 min at 95°C, 30 cycles of 1 min at 95°C, 48°C, and 72°C, and 5 min at 72°C; AmpliTaq Gold; PCR-CTPP; C allele: 279 bp and 428 bp, T allele: 186 bp and 428 bp
IL-1B C to T at −31	F1 5′-AAT GTG GAC ATC AAC TGC A-3′ R1 5′-CTC CCT CGC TGT TTT TAT <u>A</u>-3′ F2 5′-ACT TCT GCT TTT GAA AGC <u>C</u>-3′ R2 5′-TCA GCT GTT AGA TAA GCA G-3′	5 min at 94°C, 30 cycles of 1 min at 94°C, 54°C, and 72°C, and 5 min at 72°C; TaKaRa Taq; PCR-CTPP; C allele: 574 bp and 345 bp, and T allele: 574 bp and 266 bp
IL-1B C to T at −511	F 5′-CTG CAT ACC GTA TGT TCT CTG CC-3′ R 5′-GGA ATC TTC CCA CTT ACA GAT GG-3′	5 min at 94°C, 30 cycles of 30 s at 94°C, 59°C, and 72°C and 5 min at 72°C; AmpliTaq Gold; PCR-RFLP (*Eco*81I); C allele: 194 bp, T allele: 109 bp and 85 bp
IL-1RN 86 bp VNTR at intron 2	F 5′-CTC AGC AAC ACT CCT AT-3′ R 5′-TCC TGG TCT GCA GGT AA-3′	5 min at 94°C, 30 cycles of 1 min at 94°C, 60°C, and 72°C, and 5 min at 72°C; TaKaRa Taq; PCR; 2 repeat allele: 240 bp, 3 repeat allele: 326 bp, 4 repeat allele: 412 bp, 5 repeat allele: 498 bp

Underlined is the allele-specific base for PCR-CTPP.

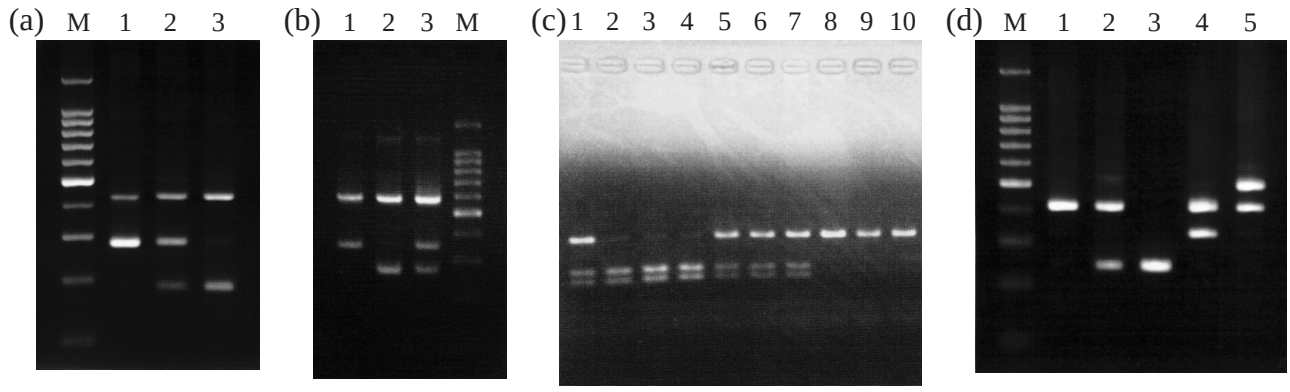

Fig. 1. Gel showing the genotypes for polymorphisms at −889 of IL-1A, at −31 and −511 of IL-1B, and at intron 2 of IL-1RN. Lane M contains a 100-bp DNA ladder. (a) IL-1A T−889C: lane 1 for C/C (279 and 428 bp), lane 2 for C/T (186, 279 and 428 bp), and lane 3 for T/T (186 and 428 bp), (b) IL-1B C−31T: lane 1 for C/C (345 and 574 bp), lane 2 for T/T (266 and 574 bp), and lane 3 for C/T (266, 345 and 574 bp), (c) IL-1B C−511T: lanes 8, 9 and 10 for C/C (194 bp), lanes 1, 5, 6 and 7 for C/T (85, 109 and194 bp), lanes 2, 3 and 4 for T/T (85 and 109 bp), and (d) IL-1RN: lane 1 for 4/4 (412 bp), lane 2 for 2/4 (240 and 412 bp), lane 3 for 2/2 (240 bp), lane 4 for 3/4 (326 and 412 bp), and lane 5 for 4/5 (412 and 498 bp).

(*Alu*I) recognizing 5′-AGCT-3′, and DNA sequencing around −31 and −511 of IL-1B was performed for 4 samples.

Statistical analysis An unconditional logistic model was applied for estimating odds ratios (ORs) and interaction terms by a computer program, STATA Version 6 (STATA Corp., College Station, TX).

Approval by Ethical Committee This study had been approved by the Ethical Committee at Aichi Cancer Center in 1999 before the study started (Ethical Committee Approval Number 12-23).

RESULTS

The genotype frequencies are listed in Table II. Forty subjects (16.6%) were found to harbor at least one T allele of IL-1A C−889T. A near-complete linkage disequilibrium opposite to that for Caucasians[4] was observed between polymorphisms at −31 and −511 of IL-1B; 17.2% (41/239) for C/C at −31 and T/T at −511, 54.0% (129/239) for C/T and C/T, 27.3% (65/239) for T/T and C/C, 1.3% (3/239) for C/T and C/C, 0.4% (1/239) for C/C and C/C, and none for the other combinations. When all C/T at −31 and C/T at −511 were C-T and T-C alleles, only 5 out of 478 alleles (1.0%) were C-C allele and there was no T-T allele in the subjects. All genotypes of IL-1B C−31T determined by PCR-CTPP were the same as those by PCR-RFLP. DNA sequencing conducted for four samples (one T/T and C/C, one C/C and C/C, and two T/C and C/C) confirmed that the genotyping was correct. The participants harboring the 4/4 genotype of IL-1RN represented 90.0% of the total.

As shown in Table II, the *HP* infection rate increased with age; this was especially clear for males. The rate was not different between the C/C genotype (61.7%) and C/T genotype (66.7%) of IL-1A C−889T (not significant), when males and females were combined. Those with the C/C genotype of IL-1B C−31T showed a lower infection rate (45.2%) than those with the C/T or T/T genotype (66.3%, χ^2=5.62 with *d.f.*=1, *P*=0.018). Since the alleles of IL-1B C−511T are strongly linked with those of IL-1B C−31T, a corresponding finding was observed for the IL-1B C−511T polymorphism. No significant difference in the infection rate between the 4/4 genotype (62.2%, 135/217) and the others (66.7%, 16/24) of IL-1RN was observed.

Based on the above results, the ORs were calculated for the IL-1B C−31T genotype. The age-sex adjusted OR relative to the C/C genotype was 2.32 (95%CI (confidence interval), 1.10−4.92) for the C/T and 2.46 (1.06−5.74) for the T/T. Table III shows the ORs according to lifestyle factor. The largest OR was observed for the T/T genotype among current smokers; OR=22.9 (1.97−266). The odds of antibody negative to positive were 10 to 6 for smokers with C/C, and 1 to 11 for smokers with T/T. The adjusted ORs of current smokers relative to non-current smokers were estimated for *HP* infection; 0.69 (0.14−3.27) for C/C, 2.68 (0.86−8.32) for C/T, and 9.06 (0.97−84.1) for T/T, indicating that smoking habit is a potent factor for persistent *HP* infection among individuals with the T/T genotype. When sex, age (a continuous variable), the C/T and T/T genotypes (two dummy variables), smoking (current vs. non-current), and interaction terms of smoking with the C/T and T/T genotypes (two variables) were included in a

Table II. Distribution of Age and Genotypes of IL-1A, IL-1B, and IL-RN According to Sex and Anti-*HP* Antibody Status

	Males			Females			Total
	HP−[a]	*HP*+[b]	*HP*+%[c]	*HP*−	*HP*+	*HP*+%	*n* (%)
Age							
39–49	13	10	43.5	13	10	43.5	46 (19.1)
50–59	11	23	67.7	27	29	51.8	90 (37.3)
60–69	12	49	80.3	14	30	66.2	105 (43.6)
IL-1A: T−889C							
C/C	29	69	70.4	48	55	53.4	201 (83.4)
C/T	7	13	65.0	6	13	68.4	39 (16.2)
T/T	0	0	—	0	1	100.0	1 (0.4)
IL-1B: C−31T							
C/C	12	12	50.0	11	7	38.9	42 (17.4)
C/T	18	52	74.3	25	38	60.3	133 (55.2)
T/T	6	18	75.0	18	24	57.1	66 (27.4)
IL-1B: C−511T (2 cases were not genotyped)							
C/C	8	18	69.2	19	24	55.8	69 (28.9)
C/T	17	52	75.4	24	36	60.0	129 (54.0)
T/T	11	12	52.2	11	7	38.9	41 (17.2)
IL-1RN: 86 bp VNTR at intron 2							
2/2	0	1	100.0	0	0	—	1 (0.4)
2/4	2	7	77.8	3	5	62.5	17 (7.1)
2/5	0	0	—	1	0	0.0	1 (0.4)
3/4	0	1	100.0	0	0	—	1 (0.4)
4/4	32	72	79.2	50	63	55.8	217 (90.0)
4/5	2	1	33.3	0	1	100.0	4 (1.7)
Total	36	82	69.5	54	69	56.1	241 (100)

a) Anti-*HP* antibody test negative.
b) Anti-*HP* antibody test positive.
c) Percentage of anti-*HP* antibody test positive.

logistic model, the estimated interaction term between smoking and the T/T genotype was statistically significant; OR=14.6 (1.12–190). This means that the effect of the T/T genotype for the current smokers was 14.6 times higher than that for the non-current smokers, or that the effect of current smoking for the T/T genotype was 14.6 times higher than that for the C/C genotype. Differences in OR were observed for milk intake (everyday vs. occasionally), fruits intake (≥4 times/week vs. less frequent), and physical exercise (≥3 times/week vs. less frequent), but the interaction terms with the genotype were not statistically significant. The subgroup analysis showed similar ORs for alcohol drinking (≥5 days/week and ≥1 gou or equivalent alcohol vs. other), salty seasoning (salty and average vs. not salty), intake frequencies of meat (≥4 times/week vs. less frequent), fish (≥4 times/week vs. less frequent), tofu (≥4 times/week vs. less frequent), fresh vegetables (≥4 times/week vs. less frequent), Japanese tea (≥6 cups/day vs. less), and coffee (everyday vs. occasionally).

DISCUSSION

Since anti-*HP* antibody tests have been most frequently used for epidemiologic studies on *HP* infection,[13–16] almost all epidemiologic findings on *HP* infection have been based on antibody tests. We also used an anti-*HP* antibody to determine persistent *HP* infection, although the test does not have 100% sensitivity for the existence of *HP* in the stomach.[10] Elevated antibody may reflect the response of the human body to *HP*, which is appropriate for the aim of investigating the association between disease risk of *HP* infection and genetic factors.

IL-1 is a multifunctional cytokine with high inflammatory activity. It has three members, IL-1α, IL-1β, and IL-1Ra, and there are two types of receptors, type I (IL-1RI) and type II (IL-1RII). Both receptors have a membrane-bound form and a soluble form. Only membrane-bound IL-1RI transduces a signal when combined with IL-1. IL-1RII is called a decoy receptor, because the membrane-bound form does not transduce a signal, nor does the solu-

Table III. Age-sex Adjusted Odds Ratios and 95% Confidence Intervals According to Lifestyle Factors

Lifestyle factor	IL-1B−31 genotype				
	n	*HP*+%[a]	C/C	C/T	T/T
All subjects	241	62.7	1.00	2.32 (1.10–4.92)	2.46 (1.06–5.74)
Smoking					
Current	55	70.9	1.00	6.18 (1.34–28.6)	22.9 (1.97–266)
Former	46	65.2	1.00	2.87 (0.54–15.3)	1.30 (0.18–9.20)
Never	140	58.6	1.00	1.30 (0.44–3.84)	1.50 (0.47–4.80)
Drinking					
Drinker[b]	45	68.9	1.00	3.57 (0.60–21.1)	1.79 (0.23–13.8)
Others	196	61.2	1.00	2.19 (0.94–5.12)	2.64 (1.02–6.83)
Milk					
Everyday	121	65.3	1.00	1.84 (0.61–5.55)	1.45 (0.44–4.76)
Occasionally	120	60.0	1.00	3.72 (1.00–9.45)	4.72 (1.27–12.6)
Fruits					
≥4 times/week	127	57.5	1.00	1.62 (0.52–5.08)	1.50 (0.43–5.23)
<4 times/week	114	68.4	1.00	2.85 (1.02–7.95)	4.39 (1.23–15.8)
Seasoning (no answer for 2 subjects)					
Salty	160	64.4	1.00	2.71 (1.09–6.74)	2.03 (0.74–5.53)
Not salty	79	59.5	1.00	1.97 (0.50–7.81)	3.46 (0.69–17.4)
Physical exercise:					
≥3 times/week	68	69.1	1.00	0.93 (0.15–5.56)	0.57 (0.09–3.67)
<3 times/week	173	60.1	1.00	2.86 (1.19–6.87)	3.94 (1.42–10.9)

a) Percentage of anti-*HP* antibody positive.
b) ≥5 days/week and ≥1 gou (equivalent to alcohol 25 ml).

ble form. The five players (three IL-1 members and two receptors) regulate complex biologic reactions, in concert with other cytokines and small mediator molecules.[5] The polymorphisms of each player, whether known[17, 18] or unknown, may affect IL-1 functions, independently and/or in combination. In this study, four polymorphisms were examined. Although the whole picture of their roles in the infection remains to be elucidated, the observed significant associations provide useful information to approach the host factors.

For IL-1B, three polymorphisms, C−511T, C−31T, and C3954T, have been reported. A near-complete linkage disequilibrium between polymorphisms at −31 and −511 was found for the present participants. El-Omar *et al.* reported that the alleles at −31 were tightly linked with the alleles at −511; 0.610 for C-C combination, 0.010 for C-T, 0.005 for T-C, and 0.375 for T-T among the controls in Scotland (n=100), and 0.699 for C-C, 0.002 for C-T, 0.000 for T-C, and 0.298 for T-T among the controls in Poland (n=429).[4] In the present study, the corresponding values were 0.010, 0.438, 0.552, and 0.000, assuming that those with C/T at −31 and C/T at −511 did not harbor C-C or T-T allele. The sequence of IL-1B registered in GenBank (accession X04500) is the T-C type, very rare among Caucasians and common among our subjects. This complete discrepancy in the linkage within such a short distance as 480-bp between Caucasians and Japanese seems remarkable and should be further investigated. Since both studies showed that the T allele at −31 was related to a higher infection rate, though the effect was not significant in the El-Omar *et al.* study, the polymorphism at −511 seemed to be unrelated to the *HP* infection rate.

The T/T genotype at −31, a higher risk genotype for *HP* infection, was more frequent among our Japanese subjects (27.8%: 67/241) than among Caucasians (10.7%: 46/429[4]). This suggests that Japanese are more vulnerable to persistent *HP* infection than Caucasians. The T allele of IL-1A was less frequent for our Japanese subjects (8.5%: 41/482) than for Caucasians (33.1%: 265/800 in Finland,[8] and 28.3%: 137/484 in England[11]). The alleles other than 4 repeats of IL-1RN were also rarer in the study participants (5.4%: 26/482) than in Caucasians (27.9%: 239/858,[4] 29.9%: 239/800 in Finland,[8] and 26.6%: 139/522 in England[11]). Although IL-1A, IL-1B, and IL-1RN are mapped within a 430 kilobase region on chromosome 2q, no clear linkages were observed among the polymorphisms except C−31T and C−511T of IL-1B in this study.

It was reported that 18 out of 59 patients with reflux esophagitis treated with omeprazole developed corpus atrophic gastritis, but none of 31 patients treated with fun-

doplication did so,[19] suggesting that acid secretion inhibition leads to *HP* re-distribution to the corpus and gastric atrophy, and possibly a condition favoring persistent infection. Since IL-1β is a strong inhibitor of gastric acid secretion,[20] individuals with the T/T genotype at −31, making a TATA box, may have a disadvantage in acquisition of *HP* infection and/or spontaneous eradication.

In the present study, lifestyle factors were found to modify the effect of IL-1B C−31T polymorphism on the *HP* infection. Some may be by chance due to multiple comparisons, while others may actually affect the association of the IL-1B polymorphism. Although further studies are needed to confirm the modification observed in this study, the findings are quite interesting in a biological sense. Smoking was reported to be associated with *HP* infection for inhabitants,[21, 22] outpatients,[23, 24] and participants in an *HP* eradication program,[25] while there are many studies reporting no association.[13, 15] Smoking interacted with the IL-1B C−31T polymorphism in this study. The infection rate was especially high among smokers harboring the T/T genotype, which is relatively rare for Caucasians. This difference in the genotype frequency may explain in part the above inconsistent results among the epidemiologic studies. Smoking is a well-known risk factor for gastric ulcer, which may suggest a synergistic effect with IL-1B activity for inflammation caused by *HP* infection, although there is no biological evidence for this.

Subgroup analysis for milk, fruits, and physical exercise showed different ORs, but the interaction was not significant. Accordingly, the difference could be caused by chance, while a small statistical power might fail to detect a significant interaction. Frequent fruits intake was reported to reduce the infection prevalence.[24] This study demonstrated that the effect of IL-1B C−31T polymorphism was strong among those eating fruits 3 times or less per week. Salty food was reported to increase the *HP* infection rate,[15] but it did not interact with this polymorphism. The other lifestyle factors, including alcohol drinking, also showed no interaction with the IL-1B C−31T polymorphism.

The interactions found in this study, significant or not, may provide a clue to resolve the biological mechanism of persistent *HP* infection. To confirm these interactions with lifestyle factors, as well as the association between this IL-1B polymorphism and *HP* infection, further studies will be required with different subjects.

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