RESEARCH COMMUNICATION

Helicobacter Pylori Seropositivity, the *Interleukin 1B* Polymorphism, and Smoking among First-visit Outpatients

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Abstract

Our previous studies of 241 re-visit outpatients in the *Helicobacter pylori* (*HP*) eradication program (HPE) of Aichi Cancer Center Hospital (Jpn J Cancer Res 2001;92:383-389) and of 462 health checkup examinees (HCE) in Nagoya (Jpn J Public Health 2001;48:604-612) found a significant association between *HP* seropositivity and the *Interleukin 1B* (*IL-1B*) C-31T genotype, especially among current smokers. This study aimed to confirm the association for 547 first-visit outpatients (277 males and 270 females) of Aichi Cancer Center Hospital aged 40 to 79 years. Samples were genotyped by polymerase chain reaction with confronting two-pair primers (PCR-CTPP), the same method as that used in the previous studies. Sex-age-adjusted odds ratio (aOR) was 1.32 (95% confidence interval, 0.84-2.08) for *CT* genotype and 1.35 (0.84-2.08) for *TT* genotype. The aOR was higher in never smokers (aOR=1.69, 0.86-3.32 for *TT* genotype) than in current smokers (aOR=1.01, 0.34-2.98 for *TT* genotype). The obtained aORs for *TT* genotype were inconsistent to those in our previous studies; aOR=2.46 (1.06-5.74) for 241 HPE, aOR=1.74 (1.05-2.89) for 462 HCE, aOR=22.9 (1.97-266) for 55 HPE current smokers, and aOR=4.62 (0.94-22.7) for 67 HCE current smokers. Since the 95% confidence intervals of aORs for *TT* genotype from the three study subjects overlapped, the inconsistent findings could be due to random errors. Alternatively, there might be other effect modifiers for the association with the polymorphism. Further studies will be required to elucidate the causes of the observed inconsistent findings.

Key Words: *Helicobacter pylori – IL-1B* C-31T – smoking – PCR-CTPP

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Introduction

It is well documented that *Helicobacter pylori* (*HP*) infection causes gastric diseases including gastric cancer (Munoz, 1994; Asaka et al., 1997). The *HP* infection is prevalent in many developing countries (Bardhan, 1997), and poses health problems. The mode of infection is considered to be oral-oral and/or fecal-oral routes, and the infection chance depends largely on sanitary conditions, especially in childhood (Brown, 2000). The virulence or strains of the bacterium may also influence the infection rate, as well as disease risk of the infected (Montecucco and Rappuoli, 2001; Blaser and Berg, 2001).

There is no doubt that the chance of *HP* exposure is deterministic for the infection, however, genetic factors of the host could also affect the susceptibility to *HP* infection and the persistence. A twin study showed that the concordance of anti-*HP* antibody status was higher in monozygotic twin pairs than in dizygotic twin pairs (Malaty et al., 1994), strongly indicating the genetic roles in persistent *HP* infection. To date, the associations with HLA types (Go, 1997) and polymorphisms of *secretor* (Ikehara et al., 2001), Lewis (Ikehara et al., 2001), *interleukin 1B* (*IL-1B*) (Hamajima et al., 2001a; Katsuda et al., 2001), *myeloperoxidase* (Hamajima et al., 2001b) and *tumor necrosis factor A* (Yea et al., 2001) have been reported.

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IL-1β, which is induced by *HP* infection (Jung et al., 1997), is a proinflammatory cytokine with multiple biological effects (Dianarello, 1996). It is a strong inhibitor of gastric acid secretion (Beales and Calam, 1998), possibly leading to *HP* spread from the pylorus to the corpus. The spread increases the risk of gastric atrophy and gastric cancer (El-Omar, 2001). *IL-1B* encoding IL-1β have been reported to have polymorphisms, among which tightly linked C-31T and C-511T polymorphisms (*-31C* with *-511T* and *-31T* with *-511C*) (Hamajima et al., 2001a) are considered to be functional (Hulkkonen et al., 2000). The *-511T* allele carriers were reported to have a higher risk of stomach cancer (El-Omar et al., 2000, corrections 2001; Machado et al., 2001).

We reported the significant association with *IL-1B* C-31T polymorphism for 241 non-cancer outpatients who participated in *HP* eradication program (Hamajima et al., 2001a) and for 462 health checkup examinees (Katsuda et al., 2001). Among current smokers, anti-*HP* IgG antibody seropositivity was higher for individuals with *TT* genotype than for those with *CC* genotype. This study was conducted to confirm the association for another Japanese population.

Materials and Methods

Subjects

Subjects were first-visit outpatients of all clinics in Aichi Cancer Center Hospital who donated a 7ml of peripheral blood for the Hospital-based Epidemiological Research Program at Aichi Cancer Center II (HERPACC-II) during February to June 2001 (Hamajima et al., 2001c). They included roughly 20% of cancer patients diagnosed at other hospitals or before diagnosis. The participation rate for the blood donation was about 60% of the first-visit outpatients. Those aged 40 to 79 years were sampled from the HERPACC-II participants for this study.

All the participants provided written informed consent for genotyping after the explanation by staff of Division of Epidemiology and Prevention. This study was approved in October 2000 (Approval No. 41-2), by the Ethical Committee for Genetic Research of Aichi Cancer Center organized according to the Guideline for Genetic Research issued by Ministry of Health and Welfare on May 30, 2000.

Laboratory Methods

An anti-*HP* IgG antibody test ("HM-CAP", Enteric Products Inc., Westbury, NY) was used for the identification of *HP* seropositive participants. According to the usual definition, 2.3 EV (ELISA Value) or over was regarded as *HP* infection positive. The sensitivity of HM-CAP was reported to be 98.7% and specificity 100% in the United States (Evans et al., 1989), though the sensitivity was not so high for Japanese (Matsuo et al., 2000). The antibody test was conducted by SRL Co., Ltd, Tokyo, Japan.

DNA was extracted from buffy coat fraction by QIAamp DNA Blood Mini Kit (Qiagen Inc., Valencia, CA). *IL-1B* C-31T polymorphism was genotyped by a PCR-CTPP (polymerase chain reaction with confronting two-pair primers) method described in the previous papers (Hamajima et al., 2000; Hamajima et al., 2001a)

Statistical Analysis

Statistical analysis was conducted by a computer program STATA Version 7 (STATA Corp., College Station, TX). A $\chi 2$ test was applied for examining the Hardy-Weinberg equilibrium and independence between two categorical variables. An unconditional logistic model was used for estimating odds ratios (ORs) and 95% confidence intervals (95% CIs).

Age-adjustment was conducted by a variable with a continuous value in years. Smoking habits were obtained by a self-administered questionnaire. Current smokers included ex-smokers within one year after smoking cessation. Never smokes included individuals who smoked less than 100 cigarettes in their lifetime.

Results

For the present analysis, 547 participants (277 males and 270 females) were available. The seropositive rate was the highest for 60-69 year age group in each sex, as shown in Table 1. The rate was higher in males than in females, and the difference was the largest in 50-59 year age group (66.0% vs 52.2%). Current smokers were 35.4% in males and 10.7% in females. There was no significant association between the seropositivity and smoking habits ($\chi 2=0.83$, $p=0.661$ for the 2 by 3 table in males, and $\chi 2=3.14$, $p=0.208$ for the 2 by 3 table in females).

The genotyping succeeded for 531 participants (271 males and 260 females). The genotype frequency among them was 21.8% for *CC* genotype, 44.6% for *CT* genotype, and 33.5% for *TT* genotype, and the allele frequency was 44.2% for *C* allele and 55.8% for *T* allele. A Hardy-Weinberg equilibrium test produced $p=0.029$ with $\chi 2=4.79$, indicating a genotype distribution significantly discrepant from the allele frequency. However, the difference was not substantially large; the expected was 103.6 for 116 individuals with *CC* genotype, 261.9 for 237 individuals with *CT* genotype, and 165.6 for 178 individuals with *TT* genotype. The maximum difference was 12%, (116-103.6)/ 103.6 x100, for the *CC* genotype.

Table 2 shows the crude and sex-age-adjusted ORs. Individuals with *CT* or *TT* genotype had a higher OR of being seropositive relative to *CC* genotype among all participants (adjusted OR=1.32, 95%CI, 0.84-2.08 for *CT* and 1.35, 0.83-2.18 for *TT*), though it was not significant. The ORs for *CT* and *TT* genotypes were the highest among never smokers, and about unity among current smokers. The 95% CIs for *CT* or *TT* genotype overlapped each other, and there was no interaction with smoking status.

Discussion

The first study on the association betwee*HP* seropositivity and *IL-1B* C-31T genotype for Japannese

Table 1. Anti-*HP* Antibody Status According to Sex, Age and Smoking Habit

Characteristic	Males			Females			Total	
	HP- [a]	*HP*+ [b]	*HP*+ % [c]	*HP*-	*HP*+	*HP*+ %	n	(%)
Age								
40 - 49	16	19	54.3	35	31	47.0	101	(18.5)
50 - 59	33	64	66.0	54	59	52.2	210	(38.4)
60 - 69	28	63	69.2	19	41	68.3	151	(27.6)
70 - 79	18	36	66.7	12	19	61.3	85	(15.5)
Smoking								
Never	17	38	69.1	103	117	53.2	275	(50.3)
Former	46	78	62.9	6	15	71.4	145	(26.5)
Current	32	66	67.3	11	18	62.1	127	(23.2)
Total	95	182	65.7	120	150	55.6	547	(100)

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

revealed a remarkable role of the functional polymorphism for 241 re-visit outpatients of Aichi Cancer Center Hospital who participated in *HP* eradication program (HPE) (Hamajima et al., 2001a). Since it is not rare that the association with polymorphisms is not reproduced for other subjects, we conducted the second study. It provided a weaker, but significant association for 462 health checkup examinees in Nagoya (HCE) (Katsuda et al., 2001), as shown in Figure 1. Both studies showed that the association was marked for current smokers, indicating that smoking was

Table 2. Crude and Sex-age-adjusted Odds Ratios (ORs) for *Helicobacter pylori* IgG Antibody Seropositivity with Respect to the *Interleukin 1B* (*IL-1B*) C-31T Genotype in Japanese First-visit Outpatients

Genotype	N	*HP*- [a]	*HP*+ [b]	*HP*+ %[c]	cOR	(95% CI [d])	aOR	(95% CI)
All participants								
CC	116	51	65	56.0	1	(Reference)	1	(Reference)
CT	237	90	147	62.0	1.28	(0.82-2.01)	1.32	(0.84-2.08)
TT	178	66	112	62.9	1.33	(0.83-2.14)	1.35	(0.83-2.18)
NG[e]	16	8	8	50.0				
Total	547	215	332	60.7				
Never smokers								
CC	57	30	27	47.4	1	(Reference)	1	(Reference)
CT	118	47	67	58.8	1.58	(0.84-3.00)	1.70	(0.88-3.27)
TT	96	39	57	59.4	1.62	(0.84-3.14)	1.69	(0.86-3.32)
NG	8	4	4	50.0				
Former smokers								
CC	36	13	23	63.9	1	(Reference)	1	(Reference)
CT	63	23	40	63.5	0.98	(0.42-2.30)	1.04	(0.44-2.48)
TT	41	13	28	68.3	1.21	(0.47-3.14)	1.25	(0.48-3.27)
NG	5	3	2	40.0				
Current smokers								
CC	23	8	15	65.2	1	(Reference)	1	(Reference)
CT	60	20	40	66.7	1.07	(0.39-2.93)	1.12	(0.40-3.11)
TT	41	14	27	65.9	1.03	(0.35-3.01)	1.01	(0.34-2.98)
NG	3	1	2	66.7				

[a] Anti-*HP* antibody test negative.
[b] Anti-*HP* antibody test positive.
[c] Percentage of anti-*HP* antibody test positive.
[d] Confidence interval
[e] Not genotyped

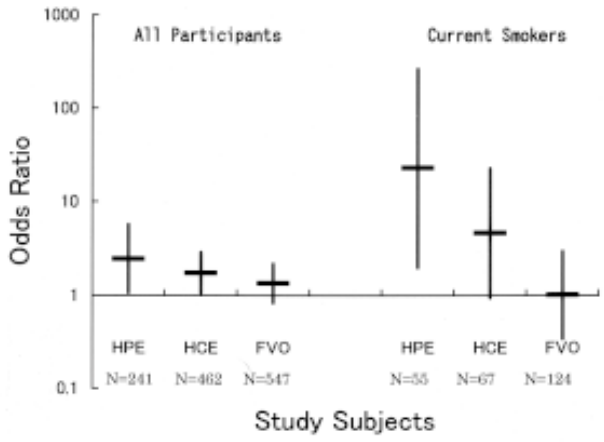

Figure 1. Sex-age-adjusted Odds Ratios (Horizontal Bars) and 95% Confidence Intervals (Vertical Bars) for the *TT* Genotype of *Interleukin 1B* C-31T Polymorphism Relative to the *CC* Genotype for Three Groups of Study Subjects; HPE for *Helicobacter pylori* Eradication Program Participants (Hamajima et al., 2001a), HCE for Health Checkup Examinees (Katsuda et al., 2001), and FVO for First-visit Outpatients of the present study.

an effect modifier for the association with the *IL-1B* polymorphism. The present study found a weaker association for first-visit outpatients (FVO) of Aichi Cancer Center Hospital, and no association for current smokers. The reasons were unknown.

All samples of the three studies were tested for anti-*HP* IgG antibody by the same method at the same laboratory. The genotyping method was also the same, PCR-CTPP in our laboratory. Since the tests were conducted independently, the results of the seropositivity and genotype were not influenced each other. Information bias concerning smoking status was limited, because the participants and interviewers did not know the antibody status and genotype.

The genotype frequency for the present subjects was not in Hardy-Weinberg equilibrium. It seemed partly due to the relative large number of study subjects enabling to detect a small size of discrepancy, and may partly due to the inclusion of more cancer patients in the participants than in general population. The frequency for the *T* allele was 55.8%, which was similar to that in our previous studies (55.0% in HPE and 55.1% in HCE). The proportion was smaller than that observed in Poland (70.2%, n=429, El-Omar et al., 2000)

As Figure 1 demonstrates, the 95% confidence intervals of the *TT* genotype ORs for all participants and current smokers of the three studies overlapped. It was not denied that the inconsistent results were due to the random errors. The inconsistency was larger for current smokers among the three studies, which could be partly due to unknown modifiers on the association with the polymorphism in current smokers.

The association between *HP* infection and smoking is very controversial. Majority of the cross-sectional studies for inhabitants showed no association with smoking habits (The EUROGAST Study Group, 1993; Tsugane et al., 1994; Katsuda et al., 2001). The exceptions were the studies in Northern Ireland (Murray et al., 1997), in Scottland (Woodward et al.,2000) and for Japanese Americans in Seattle (Namekata et al., 2000). Meanwhile, a significant association was documented in cross-sectional studies for patients (Fontham et al., 1995; Hamajima et al., 1997). A lower eradication rate for smokers has been reported in many clinical studies on *HP* eradication treatments (Labenz et al., 1995; Bertoni et al., 1996; Goddard and Spiller, 1996; Moayyedi et al., 1997; Kamada et al., 1999; Maconi et al., 2001; Perri et al., 2001), though no association between the eradication rate and smoking was also reported (Cutler and Schubert, 1993; Moshkowitz et al., 1996).

The modification by smoking on the association between *HP* seropositivity and *IL-1B* functional polymorphism seems biologically plausible. Although cigarette smoke extracts suppress IL-1β production of human peripheral blood mononuclear cells (Ouyang et al., 2000), IL-1β concentration in bronchoalveolar lavage is higher among smokers than among non-smokers (Kuschner et al., 1996). Since there are no experimental studies according to the *IL-1B* genotype, explanation is very hypothetical. However, the elevation of IL-1β concentration could occur in gastric mucosa, like in the lung. If individuals with *TT* genotype are high responders to cigarette smoke and have a higher level of IL-1β, inhibited gastric acid secretion by IL-1β, makes a favorable condition for *HP* to survive in the stomach, which may prevent spontaneous elimination (Xia and Talley, 1997).

In conclusion, the association between *HP* seropositivity and *IL-1B* C-31T *TT* genotype was weak in the present study, and no association was found among current smokers. The inconsistent result could be due to random errors. Alternative explanation might be due to the unknown effect modifiers through gene-gene interaction and/or gene-environment interaction, especially among current smokers. Further studies will be required to elucidate the causes of the observed inconsistent findings.

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