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

Nobuyuki Hamajima¹, Hidemi Ito¹², Keitaro Matsuo¹³, Kazuo Tajima¹, Suketami Tominaga⁴

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.

¹Division of Epidemiology and Prevention, ¹Aichi Cancer Center, Nagoya 464-8681 Japan, ²The Second Department of Internal Medicine, Nagoya City University School of Medicine, Nagoya 467-8601 Japan, ³Nagoya University Graduate School of Medicine, Nagoya 466-8550 Japan
Corresponding Author: Nobuyuki Hamajima, M.D., M.P.H., Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, 1-1 Kanokoden, Chikusa-ku, Nagoya 464-8681 Japan, TEL:+81-52-762-6111, FAX:+81-52-763-5233, E-mail: nhamajima@aichi-cc.jp
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IL-1β, which is induced by HP infection (Jung et al., 1997), is a pro-inflammatory 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 (Hulkonen 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 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 χ² 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 (χ²=0.83, p=0.661 for the 2 by 3 table in males, and χ²=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 χ²=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 between HP seropositivity and IL-1B C-31T genotype for Japanese
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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

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

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
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 Scotland (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-1β 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→T polymorphism 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.

Acknowledgements

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References


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.


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