Intrafamilial transmission of *Helicobacter pylori*: the association between a parent and an offspring with respect to the presence of anti-CagA antibody

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Abstract In this study, we examined whether the transmission of *Helicobacter pylori* occurred in married couples. We also examined whether a correlation existed between *H. pylori* seropositivity in a parent and an offspring, which may be related to *H. pylori* transmission. A cross-sectional study of residents living in a rural area in Japan was conducted in 1998. The study population consisted of 1910 volunteers, aged from 22 to 79 years, residing in this area. We serologically confirmed the presence of the antigen, CagA (cytotoxin-associated gene A), of *H. pylori*, using a recombinant CagA antigen, in addition to examining for antibodies to *H. pylori*, as determined by an IgG-specific enzyme immunoassay. The data were analyzed using logistic regression models. A significant association of *H. pylori* seropositivity was observed (*P* < 0.001) in married couples, but no significant difference with respect to the presence of anti-CagA antibodies was observed in the married couples with *H. pylori* seropositive results (*P* = 0.053). The odds ratio was 8.08 (95% confidence interval [CI], 1.39–40.05) for infection in offspring with seropositive mothers and 2.93 (95% CI, 0.25–33.85) for infection in offspring with seropositive fathers when compared with seronegative fathers or mothers as the reference. There was a concordance between the presence or absence of anti-CagA antibodies in 11 of 13 groups of mothers and offspring (84.6%) compared with concordance in 6 of 14 groups of fathers and offspring (42.9%; *P* < 0.05). Our results suggest that maternal influence is likely to be more powerful than paternal influence in the transmission of *H. pylori*.

Key words *Helicobacter pylori* · CagA · Intrafamilial transmission

Introduction

*Helicobacter pylori* is a curved, microaerophilic, gram-negative bacterium that was first isolated in 1983 from stomach biopsy specimens of patients with chronic gastritis. This discovery resulted in a revised view of the human stomach as an environment for bacterial growth. *H. pylori* infection is a major factor in the etiology of peptic ulcer diseases, is the predominant cause of chronic gastritis, and increases the incidence of gastric cancer. Once the infection is acquired, its duration seems to be very long, possibly for life. The International Agency for Research on Cancer has classified *H. pylori* as a human class I carcinogen.

Serodiagnostic tests have frequently been used to measure the prevalence of infection in various populations. *H. pylori* strains are subdivided into two types according to the presence of cagA, which encodes a high-molecular weight protein (CagA). Strains expressing CagA as a virulence factor are considered to produce more severe gastritis than CagA-negative strains.

The purpose of this cross-sectional study, carried out by examining the presence of anti-CagA antibodies, was to investigate whether the spouse of an *H. pylori*-seropositive person was more likely than the spouse of an *H. pylori*-seronegative person to be seropositive for *H. pylori*, and whether *H. pylori* infection in the offspring occurs via the paternal or the maternal route.

Subjects and methods

Study population

The Japanese Ministry of Health and Welfare provides health services for adult residents living in communities.

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Health services are conducted by the municipalities for the purpose of health maintenance within the general population. This cross-sectional study was carried out in residents living in a rural area in the western part of Fukuoka, Japan; the municipality has a population of 18000. The main sources of income are farming and forestry. Water for drinking and cooking is obtained from tube wells or supplied by the City Corporation. This study was conducted from June to August 1998. Two thousand, seven hundred and fourteen residents (938 men and 1776 women) came to the municipality to receive health services. We explained our study to them, and 1910 volunteers, aged from 22 to 79 years, in general good health participate in the study, after providing their informed consent; the response rate was 70.4%. The subjects comprised 671 men (35.1%) and 1239 women (64.9%). Response rates were not significantly different between the sexes (71.5% in men and 69.8% in women; \( P = 0.336 \)). The mean age of the men was 58.2 ± 13.7 years (range, 22–78 years) and that of the women was 58.6 ± 13.4 years (range, 23–79 years). The mean age was similar in both sexes.

Each subject completed a self-administered questionnaire, the results of which were reviewed by a trained public health nurse. In addition to sociodemographic information, information on medical conditions, including treatment of *H. pylori*, was sought through the questionnaire. None of the subjects had a history of *H. pylori* eradication therapy. The questionnaire also requested various personal details, including age, sex, height, weight, and family history. Married couples or parents and offspring were identified from the municipal population registers.

Serological methods

A blood sample was drawn from a peripheral vein into a siliconized disposable plastic tube. Blood samples for the serum analysis were collected from all subjects. Centrifugation and separation took place within 1 h after venepuncture. Sera were stored at −20°C until measurement for evaluation of the seroprevalence of *H. pylori* infection.

*H. pylori* seropositivity was determined with an IgG-specific enzyme immunoassay (EIA), using a Prika Plate G Helicobacter II assay kit (Biomerica, Newport Beach, CA, USA), which had a sensitivity of 94.9% and a specificity of 91.3%; plus two SDs was taken as the cutoff level.

Antibodies to CagA were determined with an enzyme-linked immunosorbent assay (ELISA). The antigen used in this study was a recombinant CagA antigen, which was kindly supplied by Dr. Antonello Covacci (Immunobiological Research Institute, Siena, Italy). The method of preparation and purification of the recombinant protein basically followed that described by Xiang et al., but except for the following points. Fusion was done using a His-Tagged protein instead of MS2 polymerase. The DNA fragment, digested with EcoRI, filled in and cloned in the small site of the vector pQE31 (Qiagen Inc., Valenica, CA), was expressed in *Escherichia coli* host strain M15 [prep4]. The induced protein was purified using a Ni²⁺-NTA Agarose column (Qiagen Inc.). The ELISA was performed as described by Xiang et al., and the sensitivity and specificity were 96.2% and 96.6%, respectively. All tests were carried out in duplicate.

Statistical analysis

The Mantel-Haenszel \( \chi^2 \) test was used to examine relationships between *H. pylori* seropositivity and age or sex. The \( \chi^2 \) test was employed to evaluate an association between *H. pylori*-seropositives and *H. pylori*-seronegatives in married couples. Fisher’s exact test was used when an expected value was less than 5. The logistic regression model was applied to analyze a relationship between a parent and offspring in regard to *H. pylori* seropositivity. Odds ratios (ORs) and their 95% confidence intervals (CIs) were determined to evaluate the strength of the association. Probability values less than 0.05 were considered significant. The data were analyzed with the Statistical Analysis System (SAS Institute, Cary, NC, USA).

### Results

The overall seropositivity rate for *H. pylori* was 62.3%. No significant difference was found in the prevalence of *H. pylori* infection between men (61.6%) and women (62.6%; \( P = 0.642 \)), but seropositivity rates for *H. pylori* showed a significant increase with age for both sexes (\( P < 0.01; \) Table 1).

#### Table 1. Seropositive rates of Helicobacter pylori by age and sex

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Men</th>
<th></th>
<th>Women</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of sera</td>
<td>Number of seropositive cases</td>
<td>Percentage</td>
<td>Number of sera</td>
<td>Number of seropositive cases</td>
<td>Percentage</td>
</tr>
<tr>
<td>20–29</td>
<td>28</td>
<td>8</td>
<td>28.6</td>
<td>51</td>
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<td>30–39</td>
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<td>41.7</td>
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<td>67</td>
<td>46.9</td>
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<tr>
<td>40–49</td>
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<td>66</td>
<td>56.9</td>
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<td>145</td>
<td>59.7</td>
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<td>50–59</td>
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<td>299</td>
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<td>70–79</td>
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<td>63</td>
<td>61.8</td>
<td>71</td>
<td>43</td>
<td>60.6</td>
</tr>
<tr>
<td>Total</td>
<td>671</td>
<td>413</td>
<td>61.6</td>
<td>1239</td>
<td>776</td>
<td>62.6</td>
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<tr>
<td>( P ) value for trend</td>
<td></td>
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