**Helicobacter pylori** CagA Seropositivity and Gastric Carcinoma Risk in a Japanese American Population

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*Helicobacter pylori* colonization is associated with gastric cancer, but whether and to what extent the risk is greater for strains with the cagA gene than for those without needs to be determined. Between 1967 and 1977, 9963 Japanese American men were recruited and examined. By 1996, incident cases of gastric carcinoma of the distal stomach had been diagnosed in 261 men. Stored serum samples from these case patients and 261 age-matched control subjects were tested for immunoglobulin G antibodies to *H. pylori* and to the CagA product of *H. pylori*, using antibody-specific enzyme-linked immunosorbent assays. Compared with *H. pylori*-negative, CagA-negative men, *H. pylori*-positive, CagA-negative men had an odds ratio (OR) of 2.7 (95% confidence interval [CI], 1.3–5.6) for intestinal gastric carcinoma. Men seropositive for both *H. pylori* and CagA had an OR of 4.1 (95% CI, 2.2–7.7). This suggests that colonization by an *H. pylori* strain with the cagA gene is associated with a greater risk of intestinal gastric carcinoma.

*Helicobacter pylori*, a spiral, gram-negative microaerophilic bacterium, has been labeled a group I carcinogen by the International Agency for Research on Cancer [1]. We and others have shown that prior acquisition of *H. pylori* (detected by the presence of specific IgG antibodies in serum) increases the risk for both the intestinal and the diffuse histologic types of gastric carcinoma [2–4]. A recent study also found that *H. pylori*-positive patients with gastric ulcers, gastric hyperplastic polyps, or nonulcer dyspepsia were at far greater risk for gastric cancer than were *H. pylori*-negative patients [5]. However, only a small percentage of *H. pylori* carriers actually develop gastric cancer [6]. This has led to a search to identify additional factors in the host, the bacterium, or the environment that can be used to better characterize persons at high risk for this disease.

One target of interest is the cag island of *H. pylori* [7]. This genomic island of ~40 kb contains cagA and type IV secretion genes, the products of which inject the CagA protein into epithelial cells [8]. CagA is an immunodominant, high–molecular-weight protein of variable size (120–140 kDa) that is present in ~60%–70% of *H. pylori* strains [9, 10]. Persons carrying strains with the cagA gene (cagA+) have a more marked gastric inflammatory response, which results in greater injury to epithelial cells than occurs in persons carrying strains without the gene (cagA−) [11, 12]. Both intensity of inflammation and epithelial damage may be involved in the pathogenesis of gastric cancer [13]. In addition, multifocal atrophic gastritis of the antrum, a precursor lesion of gastric cancer, is more likely to develop in persons with cagA+ strains than in those with cagA− strains [14, 15].

There have been 9 case-control studies that reported on the association between CagA and gastric cancer and also tested for the presence of serum IgG antibodies to *H. pylori*. We and other researchers in the United States, Germany, Sweden, and Japan noted that cagA+ *H. pylori* strains increase the risk of gastric cancer beyond that associated with other *H. pylori* strains [16–21]. However, this finding was not universal; some investigators in Asia reported either no accentuation of the risk associated with cagA+ strains or no relationship between the presence of cagA+ strains and an increased risk for gastric carcinoma [22–24]. In China [24] and Japan [25, 26], the predominant types of *H. pylori* circulating in the population produce the CagA protein. This predominance of cagA+ strains in Asian countries may obscure interstrain differences.

The number of gastric cancer cases in the earlier studies, except for the 2001 study in Sweden [21], ranged from 48 to 110. Furthermore, only 2 [16, 17] of the 9 investigations used serum samples obtained many years before the diagnosis of gastric cancer. The other studies [18–24], which were cross-
Subjects and Methods

**Study population.** The study population consisted of 7498 Japanese American men born between 1900 and 1919 who were interviewed and examined between 1967 and 1970 as part of the Honolulu Heart Program on the Hawaiian island of Oahu [29]. The study was done at the Japan-Hawaii Cancer Study site, Kukanini Medical Center, Honolulu. Nonfasting blood samples were obtained from these men, and a 20% random sample of the specimens was sent to the US Public Health Service Hospital in San Francisco. Serum specimens from the remaining 5924 men were obtained from these men, and a 20% random sample of the specimens was sent to the US Public Health Service Hospital in San Francisco. Serum specimens from the remaining 5924 men were stored at −20°C at the study site. Between 1971 and 1975, 6860 of the 7498 men returned for a second examination; serum samples were available after phlebotomy for 6813 of these men. Serum samples collected at the 1967–1970 examination were available for 616 men, serum samples collected at both the 1967–1970 and the 1971–1975 examinations were available for 5308 men, and serum samples from only the 1971–1975 examination were available for 1505 men. In total, serum samples were available for 7429 men.

At the time of the second examination, the 6860 men who returned for reexamination were asked to name their brothers. As a result, 3843 additional men were identified, of whom 2553 (66% of the total) who were born between 1889 and 1938 were subsequently recruited and examined between 1975 and 1977. Nonfasting venous blood samples were obtained from 2534 of the examined brothers and stored at −75°C.

The data collected on these men included birthplace, religion, education, marital status, alcohol-use history, cigarette smoking history, blood pressure, and body mass index (weight in kilograms divided by the square of the height in meters). Serum cholesterol values were obtained using the AutoAnalyzer N-24A method. Of the 9963 men for whom blood samples were available, 28 had received a diagnosis of gastric cancer before the first examination and were excluded from the study.

**Serologic methods.** Serum specimens were diluted 1:800 for the *H. pylori* assay and 1:100 for the CagA assay. Sonicates of 5 *H. pylori* strains were used for the *H. pylori* antigen in an ELISA to detect *H. pylori*-specific serum IgG, as described elsewhere [2]. The CagA antigen was a recombinant fragment cloned in *E. coli* on pORV220 and was used in the CagA ELISA [16]. The sensitivity

**Selection of control subjects.** Each case patient was matched with a control subject from the study cohort by age at examination and date of serum collection. If a control subject had undergone gastrectomy before the serum sample was obtained or had received a diagnosis of peptic ulcer disease, according to hospital records, before or after the serum sample was obtained, he was excluded from the study. As a result, 774 potential control subjects (8%) were removed from the pool of 9656 men without gastric cancer. This was done because of the reported association between *H. pylori* and peptic ulcer disease [35–37]. Of the 261 case-control pairs identified, the members of each pair had been born within 1 year of each other, except for 3 pairs (median age difference, 2.0 years), and were examined within 1 month of each other, except for 35 pairs (median difference in examination times, 3 months). A total of 104 case-control pairs (39.8%) of the 261 in this study were included in our earlier report, which examined the association between *H. pylori*–specific IgG antibodies and gastric carcinoma [2]. Each control subject was alive and did not have any cancer diagnosis at the time of diagnosis of cancer in the matched case patient. Therefore, death was not a competing risk in this study. The frozen serum samples were shipped in dry ice to Vanderbilt University in Nashville, Tennessee, for analysis. The laboratory technician could not distinguish the serum of case patients from the serum of control subjects and treated them identically in the analysis.
Table 1. Characteristics of 261 case patients with gastric carcinoma and 261 matched control subjects.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Case patients</th>
<th>Control subjects</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Born in United States, %</td>
<td>83.9</td>
<td>83.5</td>
<td>.100</td>
</tr>
<tr>
<td>Buddhist/Shinto religion, %</td>
<td>69.7</td>
<td>69.0</td>
<td>.84</td>
</tr>
<tr>
<td>High school education, %</td>
<td>46.4</td>
<td>48.7</td>
<td>.60</td>
</tr>
<tr>
<td>Married once, %</td>
<td>97.3</td>
<td>95.8</td>
<td>.59</td>
</tr>
<tr>
<td>History of alcohol use, %</td>
<td>73.2</td>
<td>69.4</td>
<td>.69</td>
</tr>
<tr>
<td>Mean systolic blood pressure, mm Hg</td>
<td>136.6</td>
<td>136.2</td>
<td>.83</td>
</tr>
<tr>
<td>Mean serum cholesterol level, mg/dL</td>
<td>215.8</td>
<td>217.0</td>
<td>.71</td>
</tr>
<tr>
<td>Mean body mass index, kg/m²</td>
<td>23.7</td>
<td>23.5</td>
<td>.67</td>
</tr>
<tr>
<td>Ever smoked cigarettes, %</td>
<td>75.5</td>
<td>63.2</td>
<td>.003</td>
</tr>
</tbody>
</table>

* The exact binomial probability test for matched samples was used for comparing proportions; the paired Student’s t test was used for comparing mean values.

and specificity of the H. pylori and CagA assays were described in earlier reports [16, 38]. All serum samples were examined at least in duplicate on at least 2 separate days. Coding procedures were such that the laboratory technician was blinded to the status of the samples as case or control.

Statistical analysis. We used the binomial probability test, which is the exact test counterpart of the McNemar test [39], and the paired Student’s t test to compare the proportions and mean values, respectively, for case patients and matched control subjects (table 1). The risk of gastric cancer associated with serum H. pylori and CagA (tables 2-5) was assessed by the odds ratio (OR), estimated by the generalized linear model, of which the response variable (gastric cancer) is binomial and the link function is logit [40]. Because some of the men in our study sample are brothers, for whom a correlation in the risk for gastric cancer is likely, we used the generalized estimating equation approach, specifying an exchangeable “working” correlation matrix, to correct for possible intracluster correlation [41, 42].

The exposure variables serum H. pylori and CagA were assigned to categories according to the frequency distribution among the matched control subjects. The categorized exposure variables were used to create a set of binary indicator variables, with the lowest category (negative) of H. pylori or CagA as the reference group [16, 38]. These indicator variables were used as explanatory variables in the generalized linear model for the estimation of ORs. The test for trend was performed using the 4 class midpoints as explanatory variables, and the score statistic was used to determine statistical significance. All P values and confidence intervals (CIs) are based on a 2-sided test. P < .05 was considered to be significant.

Results

For the 261 patients with gastric adenocarcinoma distal to the cardia, the average age at the time of diagnosis was 72.5 years (range, 50.2–90.3 years). Table 1 presents some of the characteristics of the case patients and the 261 age-matched control subjects. Although more case patients than control subjects had a history of smoking cigarettes, the 2 groups of men were otherwise similar with regard to demographic characteristics and laboratory values.

H. pylori and CagA test results. Table 2 shows the ORs for the association of gastric carcinoma with H. pylori and CagA seropositivity. Eighty-nine percent of the men with gastric cancer (231 of 261 men) and 74% of the control subjects (193 of 261 men) had positive results of testing for H. pylori IgG antibody, which resulted in an OR of 3.0 (95% CI, 1.8–5.0). When the case patients were separated according to the histologic carcinoma type, the OR was 3.2 (statistically significant) for intestinal gastric cancer and 3.0 (not significant) for diffuse gastric cancer. Adjustment for cigarette smoking had little effect on the ORs.

Colonization by a cagA+ H. pylori strain was associated with a 1.9-fold increase in the risk of developing gastric cancer (table 2). The OR was 2.1 (95% CI, 1.3–3.2) for intestinal gastric cancer and 1.5 (95% CI, 0.6–4.0) for diffuse gastric cancer. There was little effect on the association when the analysis was adjusted for cigarette smoking history.

Patients with intestinal and patients with diffuse gastric cancer were separated into tertile groupings, according to the distribution of H. pylori IgG and CagA antibody levels among control subjects, to determine whether the height of the specific antibody responses correlated with cancer risk (table 3). There was no positive monotonic linear trend in the ORs.

Next, we examined the association between antibody positivity and intestinal gastric adenocarcinoma in relation to the time interval from phlebotomy to diagnosis. The mean interval from examination to diagnosis was 12.7 years (range, 0.1–26.7 years). Because the results up to this point were more notable for intestinal than for diffuse cancer and because there were only 49 patients with diffuse gastric cancer, the data in table 4 include only the patients with intestinal cancer. The adjusted OR of H. pylori seropositivity was 3.5 (95% CI, 1.8–6.8) for cases diagnosed ≥10 years after phlebotomy and only 1.7 (95% CI, 0.6–4.8) for cases of intestinal cancer diagnosed ≤9 years of examination of the patients. The association with CagA antibodies was positive for cases diagnosed ≤9 years (adjusted OR, 2.9 (95% CI, 1.7–5.1)).
The association was strongest in cases of intestinal cancer, all beyond that associated with colonization by any \textit{H. pylori} strain. In addition, colonization with \textit{cagA} + \textit{H. pylori} strains increased the risk of distal gastric carcinoma beyond that associated with colonization by any \textit{H. pylori} strain. The association was strongest in cases of intestinal cancer, although there also was a positive relationship to diffuse gastric cancer. Persons who were \textit{H. pylori} positive and \textit{CagA} positive had an OR of 4.1 for intestinal cancer, compared with those who were seronegative for both \textit{H. pylori} IgG and \textit{CagA} antibodies. This result supports our earlier observations of the same associations with \textit{H. pylori} IgG and \textit{CagA} positivity, in which only 75 patients with intestinal cancer were compared with control subjects who were positive for \textit{H. pylori} IgG antibodies [16]. It also strengthens the finding from an earlier cohort-based study that reported that the \textit{CagA} phenotype was positively related to malignancy in 77 patients with intestinal cancer [17]. In that investigation, persons who were \textit{H. pylori} and \textit{CagA} positive had an OR for intestinal cancer (5.1) that was 3 times that of persons who were \textit{H. pylori} positive and \textit{CagA} negative (OR, 1.4). These earlier studies demonstrated that \textit{CagA} seropositivity increases the risk of intestinal carcinoma of the an-

### Discussion

This study confirms the observation that \textit{H. pylori} seropositivity increases the risk for gastric adenocarcinoma of the corpus or antrum [2–4]. In addition, colonization with \textit{cagA} + \textit{H. pylori} strains increased the risk of distal gastric carcinoma beyond that associated with colonization by any \textit{H. pylori} strain. The association was strongest in cases of intestinal cancer, although there also was a positive relationship to diffuse gastric cancer. Persons who were \textit{H. pylori} positive and \textit{CagA} positive had an OR of 4.1 for intestinal cancer, compared with those who were seronegative for both \textit{H. pylori} IgG and \textit{CagA} antibodies. This result supports our earlier observations of the same associations with \textit{H. pylori} IgG and \textit{CagA} positivity, in which only 75 patients with intestinal cancer were compared with control subjects who were positive for \textit{H. pylori} IgG antibodies [16]. It also strengthens the finding from an earlier cohort-based study that reported that the \textit{CagA} phenotype was positively related to malignancy in 77 patients with intestinal cancer [17]. In that investigation, persons who were \textit{H. pylori} and \textit{CagA} positive had an OR for intestinal cancer (5.1) that was 3 times that of persons who were \textit{H. pylori} positive and \textit{CagA} negative (OR, 1.4). These earlier studies demonstrated that \textit{CagA} seropositivity increases the risk of intestinal carcinoma of the an-

### Table 3. Adjusted odds ratios (ORs) for gastric carcinoma, by histologic type of carcinoma, according to results of serologic testing for \textit{Helicobacter pylori} and \textit{CagA} and antibody levels, among 254 case patients with intestinal or diffuse gastric cancer and 254 matched control subjects.

<table>
<thead>
<tr>
<th>Serologic test, results</th>
<th>Intestinal carcinoma ((n = 205))</th>
<th>Diffuse carcinoma ((n = 49))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of case patients/control subjects</td>
<td>Adjusted OR (95% CI)</td>
</tr>
<tr>
<td>\textit{H. pylori}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>23/54</td>
<td>1.0</td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low antibody levels</td>
<td>64/50</td>
<td>3.1 (1.7–5.8)</td>
</tr>
<tr>
<td>Intermediate antibody levels</td>
<td>54/47</td>
<td>2.8 (1.5–5.4)</td>
</tr>
<tr>
<td>High antibody levels</td>
<td>64/54</td>
<td>2.9 (1.5–5.3)</td>
</tr>
<tr>
<td>\textit{CagA}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>57/89</td>
<td>1.0</td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low antibody levels</td>
<td>70/42</td>
<td>2.7 (1.6–4.4)</td>
</tr>
<tr>
<td>Intermediate antibody levels</td>
<td>45/33</td>
<td>2.3 (1.3–4.0)</td>
</tr>
<tr>
<td>High antibody levels</td>
<td>33/41</td>
<td>1.5 (0.9–2.7)</td>
</tr>
</tbody>
</table>

NOTE: Adjusted ORs and 95% confidence intervals (CIs) were estimated using the generalized estimating equations approach, to correct for intracluster correlation. The ORs were statistically adjusted for cigarette smoking history and age (by matching).

### Table 4. Odds ratios (ORs) for the association of intestinal gastric carcinoma with \textit{Helicobacter pylori} and \textit{CagA} seropositivity, according to time interval from phlebotomy to diagnosis, among 205 case patients with gastric cancer and 205 matched control subjects.

<table>
<thead>
<tr>
<th>Serologic test, time interval</th>
<th>Matched-pair status</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{H. pylori}</td>
<td></td>
</tr>
<tr>
<td>0–9 years</td>
<td>+/+ 44 (1.5 1.5)</td>
</tr>
<tr>
<td>10 years</td>
<td>+/+ 44 (1.5 1.5)</td>
</tr>
<tr>
<td>\textit{CagA}</td>
<td></td>
</tr>
<tr>
<td>0–9 years</td>
<td>22 (1.5 1.5)</td>
</tr>
<tr>
<td>10 years</td>
<td>22 (1.5 1.5)</td>
</tr>
</tbody>
</table>

NOTE: CI, confidence interval; +/+ , positive test results for case patient and control subject; +/− , positive test result for case patient, negative result for control subject; −/+ , negative test result for case patient, positive result for control subject; −/− , negative test results for case patient and control subject.

\(^a\) Adjusted ORs and 95% CIs were estimated using the generalized estimating equations approach, to correct for intracluster correlation. The ORs were statistically adjusted for cigarette smoking history and age (by matching).
Other researchers have also investigated the link between CagA and gastric carcinoma. Four cross-sectional studies found that the risk of gastric cancer associated with cagA+ H. pylori strains was greater than that associated with other H. pylori strains [18–21], but they did not find a difference between intestinal and diffuse cancer in this association. Whether differences in control selection and study design affected the findings for diffuse cancer of these 4 cross-sectional studies and 2 cohort-based investigations is uncertain.

In contrast to the above, 3 cross-sectional studies in Asia did not find an increased risk of gastric cancer or any association with CagA seropositivity [22–24]. The percentage of control subjects who were CagA positive had no bearing on the difference in the results of the various studies. For example, CagA positivity was found in 31% and 66% of the control subjects in the negative studies by Kikuchi et al. [22] and Yamaoka et al. [23], respectively, versus 37% and 75% in the positive studies by Parsonnet et al. [17] and Rudi et al. [20]. Laboratory methods to detect CagA antibodies may have had an effect. Although all but 3 studies [20, 21, 24] used ELISA, the CagA antigens used were not uniform.

Statistically, the generalized estimating equations approach was used to correct for intracluster correlation, because 2534 men were brothers of the original group of 6860 men recruited into the study. In actuality, there were only 11 pairs of brothers in which both were case patients, no pairs of brothers in which both were control subjects, and 1 pair of brothers in which one was a case patient and the other was a control subject. There were no instances in which ≥3 brothers were included in the study. As a result, ORs and CIs estimated using conditional logistic regression analysis (which does not correct for intracluster correlation) produced results virtually identical to those shown in tables 2–5 (data not shown).

The present study included a total of 8 case patients and 12 control subjects (3.9% of 508 subjects with gastric cancer of a known histologic type) who had anti-CagA antibodies in serum but had negative results of testing for H. pylori IgG antibodies. We have recently confirmed that some persons who are culture positive for H. pylori do not produce sufficiently high antibody levels to the group antigens present in our whole-cell ELISA (designated "H. pylori positive") but meet the threshold only for anti-CagA antibodies [43].

In the report from Sweden [21], 69 (13.3%) of 517 study subjects were H. pylori negative and CagA positive. When these persons were excluded from the analysis of data from case patients and control subjects who were H. pylori negative, the positive association of gastric cancer with H. pylori and CagA increased. The same occurred among the case patients with intestinal gastric cancer in our study. This suggests that identifying H. pylori–negative persons on the basis of both the H. pylori IgG antibody test and the anti-CagA antibody test is more useful than the H. pylori IgG antibody test alone.

There are a number of advantages in the present study. First, it is a cohort study in which the blood samples were obtained before the diagnosis of gastric carcinoma. On average, ∼13 years elapsed from the time of examination to diagnosis. Therefore, the analysis of the stored serum samples provides a more definitive determination of the subjects’ CagA and H. pylori status during the period in which cancers were developing and is less likely to be affected by the presence of gastrointestinal responses to H. pylori. Responses to long-term colonization, especially by cagA− strains [14], include extensive intestinal metaplasia, which subsequently reduces the bacterial load to H. pylori [27, 28]. The presence of cancer and its associated secondary events (including antibiotic treatment of infectious complications) also may contribute to false-negative serological results for H. pylori IgG antibodies. By obtaining the serum samples years before the detection of cancer, the occurrence of these events is minimized. Second, the cohort studied is a relatively homogeneous population, which tends to lessen the effects of undefined confounding variables. Third, the control subjects, appropriately, are persons without gastric carcinoma and are not self-selected for individuals with gastrointestinal symptoms, as occurs in cross-sectional studies that include control subjects undergoing upper gastrointestinal endoscopy. Lastly, to our knowledge, this is the largest prospec-
tive study to date of the association between \textit{H. pylori} and gastric cancer.

CagA positivity was associated with risk regardless of the interval from examination to diagnosis. Because the development of atrophic gastritis, a precursor to the development of intestinal gastric cancer, reduces \textit{H. pylori} populations [27] and leads to their elimination [28], antibodies to CagA may be preserved until a later stage in this process. Alternatively, anti-CagA seropositivity may be indicative of severe gastric inflammation [22, 23], which enhances the risk of gastric carcinoma. The increased risk of intestinal cancer associated with colonization by a cagA\textsuperscript{+} strain could reflect a strong association of this histologic type of gastric cancer with atrophic gastritis and intestinal metaplasia [44] and may indicate that long-term colonization with cagA\textsuperscript{+} strains results in more-intense gastritis [14, 15] and the development of both atrophy and intestinal metaplasia [15]. This phenomenon is likely related to the triggering of epithelial cell chemokine responses, which is dependent on multiple genes within the \textit{cag} island [8, 45–47]. Reactive oxygen and nitrogen species released as a consequence of the inflammation contribute to epithelial cell injury and DNA damage. These laboratory observations support a link between gastric carriage of cagA\textsuperscript{+} \textit{H. pylori} strains and the risk of noncardia intestinal adenocarcinoma of the stomach.

Acknowledgments

We thank the Honolulu Heart Program for use of its data and the following institutions for their cooperation: Kaiser Medical Center, Queen's Medical Center, St. Francis Medical Center, Straub Clinic and Hospital, Tripler Medical Center, and the Hawaii Tumor Registry (Honolulu); Castle Medical Center (Kaneohe, HI); Pali Momi Medical Center (Aiea, HI); St. Francis West Medical Center (Ewa, HI); and Wahiawa General Hospital (Wahiawa, HI).

References


