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Relation between *Helicobacter pylori cagA* Status and Risk of Peptic Ulcer Disease

Abraham M. Y. Nomura,[1] Guillermo I. Pérez-Pérez,[2] James Lee,[1] Grant Stemmermann,[3] and Martin J. Blaser[2,4]

Although colonization with any *Helicobacter pylori* strain is associated with peptic ulcer, it is uncertain whether the risk is greater with *cagA*$^+$ or *cagA*$^-$ strains, which differ in their biology. A nested case-control study was done, based on a cohort of 5,443 Japanese-American men examined on the Hawaiian island of Oahu from 1967 to 1970. A total of 150 men with gastric ulcer, 65 with duodenal ulcer, and 14 with both diseases were identified. The authors matched the 229 cases with 229 population controls and tested their serum for immunoglobulin G antibodies to *H. pylori* and immunoglobulin G antibodies to the *cagA* product of *H. pylori* using enzyme-linked immunosorbent assays. Persons with *H. pylori* positivity had an odds ratio of 4.0 (95% confidence interval (CI): 1.9, 8.5) for gastric ulcer and 2.5 (95% CI: 0.8, 7.4) for duodenal ulcer. For CagA positivity, the odds ratios were 1.4 (95% CI: 0.9, 2.4) for gastric ulcer and 2.6 (95% CI: 1.1, 5.8) for duodenal ulcer. Subjects who were seropositive for both *H. pylori* and *CagA* had an odds ratio of 4.4 (95% CI: 1.8, 10.5) for gastric ulcer and 5.8 (95% CI: 1.1, 30.0) for duodenal ulcer. The results suggest that colonization with a *cag*$^+$ *H. pylori* strain elevates the risk beyond that of a *cag*$^-$ *H. pylori* strain for both gastric and duodenal ulcers. *Am J Epidemiol* 2002;155:1054–9.

duodenal ulcer; *Helicobacter pylori*; peptic ulcer; stomach ulcer

Helicobacter pylori is commonly present in the human stomach, and investigations over the past 15 years have focused on its relation to disease (1–3). The treatment of peptic ulcer disease patients with antimicrobial regimens can eliminate *H. pylori* and reduce the risk of ulcer recurrence (4, 5). A prospective study of Japanese-American men in Hawaii has shown that the presence of *H. pylori*, as detected serologically, was associated with a three- to fourfold increased risk of developing either duodenal or gastric ulcer over a subsequent 21-year period (6). However, most persons carrying *H. pylori* never develop ulcers; thus, other factors, such as cigarette smoking, may play a role in the occurrence of this disease (7–9). *H. pylori* strains are highly diverse (10), yet a fundamental distinction among strains is the cag pathogenicity island, a region of about 40 kb that is present or absent in the *H. pylori* chromosome (11, 12). One gene, *cagA*, was the first discovered gene on the island and is a marker for its presence (13, 14). The *H. pylori* strains *cag*$^+$ and *cag*$^-$ differ substantially in their biology, in that the former are much more interactive with the host (15), injecting the CagA protein into epithelial cells (16) and inducing a more profound tissue response (17, 18). *H. pylori* strains may occupy different microniches in the stomach according to *cagA* status (19), which may affect the microecology of the stomach with consequent differences in clinical outcome (20).

Carriage of *cag*$^+$ strains may be determined by detection of specific-serum immunoglobulin G (IgG) antibodies to native or recombinant CagA (21, 22). Studies in the United States and Western Europe that have compared *H. pylori*$^+$ patients with peptic ulcer disease with similar patients without ulcers have shown a significant association of CagA positivity and duodenal ulceration (17, 18, 22, 23). In contrast, among Asian populations in which *cagA*$^+$ strains predominate, no clear-cut relation with ulcer disease has been found (24–27). This different ascertainment of the significance of CagA positivity may reflect differences in the populations studied as well as the cross-sectional, rather than prospective, nature of the previous investigations.

In this report, we return to the prospective cohort of Japanese-American men we have previously examined to assess relations between *H. pylori* carriage and disease (6, 21, 28, 29). We now ask whether carriage of a *cagA*$^+$ strain affects the risk that a person will develop gastric or duodenal ulceration. By examining 229 men who developed such disease and their matched controls in a nested case-control study with 21 years of follow-up, we are able to address that question.

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Abbreviations: CI, confidence interval; IgG, immunoglobulin G.

[1] Japan-Hawaii Cancer Study, Kuakini Medical Center, Honolulu, HI.
[2] Division of Infectious Diseases, Vanderbilt University School of Medicine, Nashville, TN.
[3] Department of Pathology, University of Cincinnati Medical Center, Cincinnati, OH.
[4] Department of Veterans Affairs Medical Center, Nashville, TN.
Reprint requests to Dr. Abraham M. Y. Nomura, Japan-Hawaii Cancer Study, Kuakini Medical Center, 347 N. Kuakini Street, Honolulu, HI 96817 (e-mail: abe@kuakini.net).

MATERIALS AND METHODS

All of the participants in this study were part of the Japan-Hawaii Cancer Study cohort, as described previously (6, 28). Briefly, 8,006 Japanese-American men were examined on the Hawaiian island of Oahu from 1965 to 1968. The data collected included birthplace, marital status, history of alcohol use, history of cigarette smoking, blood pressure, and body mass index (weight (kg)/height (m^2)). Serum cholesterol values were determined by the Auto Analyzer N-24A method, and serum glucose values were determined by the Auto Analyzer N-2B method 1 hour after a 50-g glucose load was given (30).

A total of 7,498 (93.7 percent) of the 8,006 men returned for a second examination between 1967 and 1970, and a blood sample was obtained at that time. Serum specimens for a 20 percent random sample of the men were sent to the US Public Health Service Hospital in San Francisco, California, and were not available for this study, while specimens from the remaining 5,924 men were stored at –20°C at the study site. A total of 481 patients with previous gastrectomy or a previous diagnosis of peptic ulcer disease were excluded, leaving 5,443 men in the study.

During the 21-year surveillance period from 1968 to 1989, 258 men were hospitalized and diagnosed with peptic ulcer disease. Sufficient serum frozen from the examination of the men in 1967–1970 was available from 229 of these patients. In total, there were 150 men with gastric ulcer, 65 with duodenal ulcer, and 14 with both types of ulcer. Each of these patients was matched with one control from the study cohort on the basis of age at examination, date of serum collection, availability of sufficient amount of sera, and being alive at the time of hospitalization of the matched case, so that death was not a competing factor. If a potential control had a diagnosis of gastric cancer before or after the serum was obtained, he was excluded from the study because of the reported association between *H. pylori* infection and gastric cancer (28, 31, 32). As a consequence, 160 patients (3.1 percent) were removed from the control pool of 5,185 men. Of the remaining 5,025 men, 336 (6.7 percent) were excluded because they had previously had cardiovascular disease or other cancer, and 1,532 (30.5 percent) were excluded because they were diagnosed with cardiovascular disease or other cancer after serum collection. This exclusion was done because the serum specimens from these patients were to be used for other studies. A total of 3,157 subjects remained in the pool of controls, from which 229 were matched to incident cases of peptic ulcer, as previously described (6). The average age of the cases at time of examination was 56.6 years (range, 48.1–71.3 years).

Serologic methods

The presence of serum IgG antibodies to *H. pylori* was determined by an enzyme-linked immunosorbent assay with the Pyloristat kit (Wampole Laboratories, Inc., Cranbury, New Jersey), as described (6, 28). The presence of serum IgG antibodies to CagA also was determined by enzyme-linked immunosorbent assay using a recombinant CagA antigen (orv220), as described (21, 33). The laboratory technician could not distinguish the serum specimens of cases from those of controls and was blinded to their *H. pylori* IgG status as well. An optical density ratio of 0.35 or greater was considered positive, and a ratio less than 0.35 was considered negative.

Data analysis

We used the binomial probability test, which is the exact probability test counterpart of the McNemar test (34), and the paired *t* test to compare, respectively, the proportion and mean value between cases and their matched controls. The risk of ulcer associated with the presence of IgG antibody to either *H. pylori* or CagA was assessed by the odds ratios and confidence intervals estimated by age-matched conditional logistic regression (35). Each exposure variable (*H. pylori* or CagA) was categorized into discrete classes according to the frequency distribution of the matched controls to create a set of binary indicator variables, with the lowest class as reference group. These indicator variables and the confounding covariate (smoking history) were used as explanatory variables in the conditional logistic regression model. Adjustment of cigarette history was done because it was positively associated with the risk of peptic ulcer in this cohort (8, 9). The test for trend was performed using the discretized class midpoints as explanatory variables, and the score statistic (36) was used to assess statistical significance. All of the reported p values are two-sided. Statistical analyses were performed with the SAS software (37).

RESULTS

The characteristics of the 229 patients with peptic ulcer and their matched controls are presented in table 1. As expected (8, 9), more cases than controls had a history of cigarette smoking. Otherwise, the two groups of men were similar with respect to their demographic characteristics and laboratory values.

Relation of anti-*CagA* antibodies and peptic ulcer disease risk

Next, we asked whether colonization with any *H. pylori* strain or with a *cag*$^+$ *H. pylori* strain was associated with risk of developing peptic ulcer disease. As shown in our previous analysis (6), colonization with *H. pylori* was associated with a threefold increase in peptic ulcer disease risk, with significant ($p < 0.05$) increases for both gastric and duodenal ulcers (table 2). Subjects with the negative serologic response in this comparison were those who did not have *H. pylori* antibodies. Adjustment for cigarette smoking history reduced the extent of the association with duodenal ulcer (odds ratio = 2.5), but not with gastric ulcer.

Colonization by a *cag*$^+$ strain was associated with a 1.5-fold increase in risk of developing peptic ulcer disease ($p = 0.07$). There was an odds ratio of 2.1 ($p = 0.06$) for duodenal ulcer, but only 1.3 ($p = 0.40$) for gastric ulcer. Subjects with the negative serologic response in this comparison were those who did not have antibodies to the CagA protein,

TABLE 1. Characteristics of peptic ulcer patients and controls in Japanese-American men, 1967–1970

Characteristic	Patients (n = 229)	Controls (n = 229)	Two-sided p value*
Born in United States (%)	90.4	89.1	0.76
Married (%)	91.3	91.7	0.87
Alcohol user (%)	65.9	65.5	0.92
Ever smoked cigarettes (%)	86.5	61.1	<0.0001
Mean body mass index (kg/m^2)	23.7	23.9	0.56
Mean diastolic pressure (mmHg)	82.1	82.2	0.95
Mean serum cholesterol (mg/dl)	218.9	217.9	0.89
Mean serum glucose (mg/dl)	167.0	157.8	0.08

* Exact binomial probability test for matched sample was used for comparing proportions; paired t test was used for comparing means.

TABLE 2. Odds ratios for the association between peptic ulcer and colonization with *Helicobacter pylori* or with *cagA*$^+$ *Helicobacter pylori* strains in Japanese-American men, 1967–1970

Antigen and type of ulcer	Matched-pair status* (patients/controls)					OR†	95% CI‡	Adjusted odds ratio§	95% CI
	+/+	+/−	−/+	−/−	Total				
Helicobacter pylori									
All ulcers¶	161	48	16	4	229	3.0	1.7, 5.2	3.1	1.7, 5.4
Gastric ulcer	107	32	10	1	150	3.2	1.6, 6.4	4.0	1.9, 8.5
Duodenal ulcer	48	12	3	2	65	4.0	1.2, 13.2	2.5	0.8, 7.4
cagA									
All ulcers¶	63	64	44	58	229	1.5	1.0, 2.1	1.7	1.1, 2.6
Gastric ulcer	41	38	30	41	150	1.3	0.8, 2.0	1.4	0.9, 2.4
Duodenal ulcer	17	23	11	14	65	2.1	1.0, 4.2	2.6	1.1, 5.8

* +/+, both patient and matched control show positive serologic response; +/−, patient, but not matched control, shows positive serologic response; −/+, matched control, but not patient, shows positive serological response; −/−, neither patient nor matched control shows positive serologic response.
† OR, odds ratio.
‡ 95% confidence intervals (CI) were based on two-tailed analysis.
§ Odds ratios were estimated by age-matched conditional logistic regression, adjusted for cigarette smoking history.
¶ Includes 14 persons with both gastric and duodenal ulcers.

regardless of whether or not they had antibodies to *H. pylori* whole-cell antigen. With adjustment for cigarette smoking, the positive association was statistically significant for all ulcers ($p = 0.01$) and for duodenal ulcer ($p = 0.02$).

We next asked whether the height of the specific antibody responses correlated with risk of peptic ulcer disease. As shown in table 3, high-titer antibodies to the *H. pylori* whole-cell antigen were associated with a higher risk of ulcer development. Although a similar pattern was observed in relation to host responses to the CagA antigen, the trend between the level of anti-CagA antibodies and peptic ulcer disease risk was not statistically significant ($p = 0.07$).

TABLE 3. Odds ratios for all peptic ulcers according to *Helicobacter pylori* and *cagA* test results and antibody levels* in Japanese-American men, 1967–1970

	Odds ratio	95% CI†	p value for trend
H. pylori			
Negative (<0.75)‡	1.0		
Positive (≥1.00)§			
≤1.75	2.1	1.1, 4.3	
1.76–2.50	4.7	2.3, 9.5	<0.001
>2.50	4.0	1.9, 8.1	
cagA			
Negative (<0.35)	1.0		
Positive (≥0.35)§			
≤0.53	1.8	1.0, 3.1	
0.54–0.66	1.5	0.8, 2.7	0.07
>0.66	2.2	1.2, 3.9	

* Odds ratios and 95% confidence intervals were estimated by age-matched conditional logistic regression, adjusted for cigarette smoking history.
† CI, confidence interval.
‡ Two subjects with values between 0.76 and 1 were excluded.
§ The positive levels were divided into tertiles based on the cutpoint values of the controls.

Joint effect of *H. pylori* and *CagA* positivity on peptic ulcer disease risk

To evaluate the joint effect of *H. pylori* and CagA positivity on peptic ulcer disease risk, we first defined the *H. pylori*$^-$, *cagA*$^-$ population as the referent group in table 4. Analysis of case-control pairs in which either the case or the control was *H. pylori*$^-$ and *cagA*$^+$ was not done, since there were too few pairs (10) for meaningful results. With age-matched conditional logistic regression analyses in which values were adjusted for history of cigarette smoking, subjects who were seropositive for *H. pylori* but negative for CagA antibodies were at increased risk for developing peptic ulcer disease (odds ratio = 2.9) and gastric ulcer (odds ratio = 3.5). They also had an increased risk for duodenal ulcer, but it was not statistically significant. In comparison, subjects who were seropositive for both *H. pylori* and CagA

TABLE 4. Odds ratios and 95% confidence intervals* of *Helicobacter pylori* and *cagA* serology for all peptic ulcers, gastric ulcers, and duodenal ulcers in Japanese-American men, 1967–1970

Peptic ulcer	No. of case-control pairs	*H. pylori* serology/*cagA* serology				
		H. pylori$^-$/*cagA*$^-$	*H. pylori*$^+$/*cagA*$^-$		*H. pylori*$^+$/*cagA*$^+$	
			OR	95% CI	OR	95% CI
All	219	1.0	2.9	1.4, 6.0	4.2	2.1, 8.5
Gastric	145	1.0	3.5	1.4, 8.4	4.4	1.8, 10.5
Duodenal	61	1.0	2.7	0.5, 13.6	5.8	1.1, 30.0

* Odds ratios (OR) and confidence intervals (CI) were estimated by age-matched conditional logistic regression adjusted for cigarette smoking history.

had a significantly increased risk for both gastric and duodenal ulcers. The difference in odds ratio between *H. pylori*-positive persons colonized with *cagA*$^-$ or *cagA*$^+$ strains was relatively small in relation to gastric ulcer, but it more than doubled for duodenal ulcer (5.8 vs. 2.7). However, this difference was not statistically significant.

The effect of age at diagnosis and time interval from examination to diagnosis was examined by subgroup analysis for gastric ulcer in table 5. For cases diagnosed at less than age 65 years, the odds ratio was high for those who were positive for both *H. pylori* and *cagA* (odds ratio = 9.8). The same pattern was not present for cases diagnosed at age 65 years or older. For gastric ulcer cases diagnosed within 10 years of their examination, the odds ratio was high for those who were both *H. pylori* and *cagA* positive (odds ratio = 6.2) The same pattern was not present for cases diagnosed 10 or more years after examination. Similar analyses for duodenal ulcer were not statistically significant, probably because of the small numbers of subjects in each subgroup (data not shown).

DISCUSSION

This study extends the observations of our previous report, which found that colonization with any *H. pylori* strain was associated with a three- to fourfold increased risk for peptic ulcer disease (6). We now report that colonization with a *cag*$^+$ *H. pylori* strain elevated the risks beyond that of a *cag*$^-$ *H. pylori* bacterial strain, an effect present for both gastric and duodenal ulcer cases (table 4). Controlling for the effects of smoking accentuated the risk of ulcer of subjects colonized with *cagA* strains, as shown in table 2. There was weak evidence that the height of the anti-CagA antibody response was related to the risk of peptic ulcer disease.

Previous cross-sectional studies among patients who had gastroscopy in Western countries generally show a significant positive association between presence of a *cagA*$^+$ *H. pylori* strain and duodenal ulcer, with the control patients having nonulcer dyspepsia (17, 18, 22, 23). In the Far East, where *cagA*$^+$ *H. pylori* strains predominate, such cross-sectional studies have not shown an association of *cagA*$^+$ *H. pylori* strains with peptic ulcer (24–26), except in one report (27). This is probably due to the observation that more than 80 percent of the nonulcer controls in these Asian studies were *cagA* positive, compared with 47 percent of the controls in our study.

There are several advantages in this study. Most important, it has a cohort study design in which the blood samples were obtained before the diagnosis of peptic ulcer. The cohort is a relatively homogeneous population that tends to minimize undefined confounding variables, and there are appreciable numbers of gastric and duodenal ulcer cases in this investigation. Last, the control group represents persons who were not hospitalized for peptic ulcer and is not self-selected for subjects with gastrointestinal symptoms. The results from this study show that carriage of *cagA*$^+$ *H. pylori*

TABLE 5. Odds ratios and 95% confidence intervals* of *Helicobacter pylori* and *cagA* serology for gastric ulcers by age at ulcer diagnosis and time interval from exam to diagnosis in Japanese-American men, 1967–1970

Gastric ulcer	No. of case-control pairs	*H. pylori* serology/*cagA* serology				
		H. pylori$^-$/*cagA*$^-$	*H. pylori*$^+$/*cagA*$^-$		*H. pylori*$^+$/*cagA*$^+$	
			OR	95% CI	OR	95% CI
Age at diagnosis of gastric ulcer (years)						
<65	58	1.0	4.6	1.1, 10.3	9.8	2.5, 39.1
≥65	87	1.0	3.0	1.0, 8.9	2.0	0.6, 6.2
Interval from examination to diagnosis of gastric ulcer (years)						
<10	62	1.0	3.6	1.1, 11.7	6.2	1.8, 21.9
≥10	83	1.0	4.0	1.2, 13.9	3.3	1.0, 10.8

* Odds ratios (OR) and confidence intervals (CI) estimated by age-matched conditional logistic regression, adjusted for cigarette smoking history. The reference group in each analysis are the men who were both *H. pylori*$^-$ and *cagA*$^-$.

strains was strongly associated with both gastric and duodenal ulcers, especially duodenal ulcers.

The CagA protein of *H. pylori* is an immunodominant antigen with marked antigenicity despite a relatively low molar content among cellular proteins (13, 14). There is a strong correlation between the presence of serum antibodies to CagA and colonization by a *cag*$^+$ strain (21, 22, 38). Since elements of the *cag* island can be lost and persons can simultaneously carry *cagA*$^+$ and *cagA*$^-$ that are otherwise identical (39, 40), serologic testing can more accurately assess a person's *cag* status than does characterization of a single *H. pylori* isolate, as is sometimes done. Furthermore, the CagA protein is a well-conserved antigen among strains from different geographic locations. An earlier study showed that CagA proteins from strains around the world can be detected by immune antiserum raised to the protein from a single strain and thus are closely related (41). Persons carrying *cag*$^+$ strains have greater degrees of gastric inflammation and epithelial cell damage than do those from whom *cagA*$^-$ strains have been isolated (17). Both intensity of inflammation and epithelial damage may be involved in the pathogenesis of peptic ulceration (42).

Although *cagA* positivity was associated with both gastric and duodenal ulcers in this study as well as in prior cross-sectional studies (22, 27), it is uncertain why persons develop one type of ulcer instead of the other when colonized with *cagA*$^+$ *H. pylori* strains. Patients with duodenal ulcer have significantly more inflammation and epithelial degeneration in the gastric antrum than in the gastric corpus compared with gastric ulcer patients (43). Furthermore, it has been suggested that *H. pylori* increases the risk of duodenal ulceration due to the cumulative effects of antral predominant gastritis, leading to increased acid secretion and to consequent gastric metaplasia in the duodenum (44). However, there are many complexities in addressing the issue of ulcer location, as indicated by others (45).

The data in table 5 indicate that gastric ulcer cases diagnosed before age 65 years have a highly significant association with both CagA and *H. pylori* positivity, especially compared with cases diagnosed at an older age. Other studies are needed to confirm this relation to age at diagnosis. It is uncertain whether age at the time of acquisition of *cag*$^+$ *H. pylori* strains has an effect on this association, but an earlier study suggested that early age of acquisition of any *H. pylori* strain might increase risk of gastric ulcer (29). These data are consistent with the association of carriage of *cag*$^+$ strains with both atrophic gastritis (46) and gastric adenocarcinoma (21, 47), since these conditions are nosologically related to gastric ulcer.

In conclusion, the results of these studies confirm and extend our understanding of the role that carriage of *cag*$^+$ strains play in peptic ulcer disease. That carriage of *cag*$^+$ strains is associated with enhanced risk of ulcer disease must be tempered by the recent observation, using the same serologic assays, that their carriage is inversely associated with gastroesophageal reflux disease and its sequelae (48, 49). Thus, all *H. pylori* strains are not equal in their relation to disease (50), and physicians should consider *H. pylori* strain characteristics, such as *cagA* status, in attempting to optimize care for their patients.

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