Original Contribution

Decreased Risk of Celiac Disease in Patients With *Helicobacter pylori* Colonization

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The prevalence of celiac disease (CD) has increased in recent decades without a clear explanation. The "hygiene hypothesis" theorizes that decreased exposure to bacterial antigens may trigger autoimmunity. We aimed to determine whether *Helicobacter pylori* infection and CD were associated among patients undergoing upper gastrointestinal endoscopy. We performed a cross-sectional study of patients who underwent esophagogastroduodenoscopy with submission of gastric and duodenal biopsies to Miraca Life Sciences, Inc. (Irving, Texas), a US commercial pathology laboratory, during a 4.5-year period (January 2008–June 2012). We compared the prevalence of *H. pylori* in CD patients with that in persons without CD. We performed multiple logistic regression analysis, adjusting odds ratios for patient age, gender, and racial, ethnic, and socioeconomic factors. Among 136,179 patients, a total of 2,689 (2.0%) had CD. *H. pylori* prevalence was significantly lower in patients with CD (4.4%) than in those without CD (8.8%; $P < 0.0001$). After adjustment for the above covariates, this inverse relationship remained strong (adjusted odds ratio (OR) = 0.48, 95% confidence interval (CI): 0.40, 0.58). The relationships were similar in men (unadjusted OR = 0.51, 95% CI: 0.38, 0.69) and women (unadjusted OR = 0.46, 95% CI: 0.36, 0.58) and in all age groups. We conclude that *H. pylori* presence and CD are inversely associated, a relationship that persists after adjustment for socioeconomic factors. Future studies should address whether *H. pylori* modulates immune responses to ingested gluten.

celiac disease; gluten; *Helicobacter pylori*

Abbreviations: aOR, adjusted odds ratio; CD, celiac disease; CI, confidence interval; IEL, intraepithelial lymphocytosis.

Celiac disease (CD) is a common autoimmune condition, affecting 0.8%–1.0% of Western populations. The prevalence of CD in the United States has increased sharply in recent decades, with up to a 4-fold increase in the past 50 years (1). Studies of stored serum have shown that this is a true rise in disease incidence, rather than a by-product of increased detection (1–3). Although the cause of this rise is not known, given its rapidity, a number of environmental risk factors have been proposed. The timing of gluten introduction and other infant feeding practices may play a role (4), as may rotavirus infection (5). One recent population-based study linked CD to elective, but not emergent, cesarian section, suggesting that differential perinatal exposure to bacterial microbiota could modulate CD risk (6).

Considering a role for microbial exposure in affecting CD risk, we sought to test for an association between *Helicobacter pylori* infection and CD. Several lines of evidence suggest that an inverse relationship between *H. pylori* and CD risk may exist. CD is triggered by the ingestion of gluten, digestion of which may be based on the pH and status of the gastric mucosa. Gastric *H. pylori* colonization appears to confer protection against asthma and other atopic diseases (7–10), and increased CD prevalence in the United States coincides temporally with declining *H. pylori* prevalence (11).

We sought to examine whether there is an association between histologically confirmed *H. pylori* and CD. Using a large pathology database, we hypothesized that the presence

of *H. pylori* is independently associated with decreased risk of CD.

MATERIALS AND METHODS

Miraca Life Sciences, Inc. (Irving, Texas) is a commercial pathology laboratory that receives specimens submitted by approximately 1,500 gastroenterologists from 43 states, the District of Columbia, and Puerto Rico. A prospectively maintained database of pathology specimens contains more than 2,000 patients with CD spanning the years 2008–2012; this database was the source for a recent report on the epidemiology of *H. pylori* in the United States (12).

We performed a cross-sectional study of all patients undergoing upper gastrointestinal endoscopy with submission of gastric and duodenal biopsies in this database during a 4.5-year period from January 1, 2008, to June 30, 2012. Patients with only gastric or only duodenal biopsies submitted were excluded from this analysis. Among patients with multiple endoscopies, only the first chronological examination with both gastric and duodenal biopsies in the database was included. Patients with a histopathological diagnosis of any upper gastrointestinal malignancy (carcinoma or lymphoma) or gastric or duodenal ulcers were excluded.

In the primary analysis, we studied patients who had concurrent gastric and duodenal biopsies in order to compare *H. pylori* prevalences in patients with CD and those without CD. In this analysis, we considered any patient with villous atrophy to have CD, and we compared the *H. pylori* prevalence with that in a reference group comprised of patients with a normal concentration of intraepithelial lymphocytes (i.e., ≤25 lymphocytes per 100 enterocytes) (13) and normal duodenal villous architecture. This group included patients with focal foveolar metaplasia ("peptic duodenitis") or foci of active inflammation ("duodenitis") (14). As a secondary outcome, we also compared the prevalence of *H. pylori* in patients who had intraepithelial lymphocytosis (IEL) but normal villous architecture (Marsh stage 1) with that in the reference group.

Histological definitions

As in prior studies, CD was defined as duodenal histology featuring blunt villi (equivalent to Marsh stage 3A) or flat villi (Marsh stage 3B or 3C) (15). Patients with a documented history of CD in the "clinical indication" field but with normal villous architecture (*n* = 263) were excluded from this analysis.

Gastric biopsy specimens were evaluated according to the updated Sydney System (16). Specifically, *H. pylori* gastritis was diagnosed when *H. pylori* organisms were demonstrated by a specific polyclonal immunochemical stain (Cell Marque Corporation, Rocklin, California), routinely performed on all gastric biopsy specimens. *H. pylori*-negative chronic active gastritis was defined by focal or diffuse chronic inflammation (lymphocytes and plasma cells) with neutrophils infiltrating surface or foveolar epithelium in the absence of *H. pylori* organisms. Chronic inactive gastritis was characterized by focal or diffuse chronic inflammation without neutrophilic granulocytes or *H. pylori* organisms. Criteria for reactive

gastropathy were based on the 2005 definition, which includes various combinations of foveolar hyperplasia, regenerative changes in the surface epithelium, edema or hyperemia of the lamina propria, erosions, and smooth muscle proliferation (17). Lymphocytic gastritis was diagnosed when the gastric surface or foveolar epithelium contained more than 25 intra-epithelial lymphocytes per 100 epithelial cells; in equivocal cases, an immunohistochemical stain for CD3 lymphocytes (Cell Marque Corporation) was performed (18).

Duodenitis was diagnosed when the duodenal mucosa showed active (neutrophilic) inflammation in the epithelium, irrespective of erosion or the magnitude of the lymphoplasmacytic infiltrates, and often was accompanied by foci of gastric foveolar metaplasia (the putative "peptic duodenitis") (12).

Prior procedures

Because some patients undergoing duodenal biopsy may have had prior evidence of gastric *H. pylori*, we repeated the analysis, now including all patients with a duodenal biopsy and any prior gastric biopsy dating back 5 years to January 1, 2003. For such patients, if any prior gastric biopsy showed *H. pylori*, for this analysis they were categorized as having *H. pylori*.

Accounting for socioeconomic status, race, and ethnicity

Rates of CD and *H. pylori* differ by socioeconomic status, race, and ethnicity. Information on medical insurance was available for all subjects in this analysis, and 3% of these subjects were enrolled in the Medicaid program at the time of specimen submission. Medicaid enrollment status was included as a dichotomous variable in the multivariate model.

For a subset of specimens (comprising 35% of the patients in this study), the residential zip code of the patient was available. We performed an additional sensitivity analysis, incorporating zip-code-level data on area racial and ethnic composition and household income. For each zip code, we obtained data on median household income, percentage of black residents, and percentage of Hispanic residents from the US Census Bureau's American Community Survey, using data collected during the years spanning 2007–2011 (19). Based on their zip codes of residence, patients were then divided into quartiles of zip-code-level household income, percentage of black residents, and percentage of Hispanic residents. We then compared the associations between CD and *H. pylori* among persons with and without residential zip code information available in the database, so as to measure possible selection bias in this sample. Sociodemographic predictors of the availability of residential zip code data were also assessed. Within the subset of patients for whom residential zip codes were available, the main logistic regression models were refitted with adjustment for percentage of black residents, percentage of Hispanic residents, and median household income in the residential zip code, with the variables coded into quartiles. In additional sensitivity analyses, inverse probability weights for the presence of a residential zip code were calculated; patient age was the only variable that predicted the availability of zip code data (20–22). These sensitivity analyses including inverse

probability weights assessed potential bias due to the exclusion of subjects for whom zip code data were not available.

Statistical analyses

The association between CD and *H. pylori* infection was measured using odds ratios and 95% confidence intervals. We also performed multiple logistic regression analysis adjusting for age, gender, and Medicaid enrollment. To search for an association between the presence of *H. pylori* and the degree of villous architectural disturbance, we subsequently calculated crude and adjusted odds ratios relating *H. pylori* infection to 1) blunt villi (Marsh stage 3A) and 2) flat villi (Marsh stages 3B and 3C). We also performed age-stratified analyses using predetermined age groups ($\leq$19, 20–39, 40–59, and $\geq$60 years) and analyses stratified by gender and Medicaid enrollment status (versus non-Medicaid status). In a subsequent multivariate model limited to those patients whose residential zip codes were available, we employed generalized estimating equations with robust standard error estimation to account for nonindependence of observations arising from clustering of subjects within zip codes (23). These models adjusted for percentage of black residents, percentage of Hispanic residents, and median household income in the residential zip code. The generalized estimating equations models were then refitted incorporating inverse probability weights for the availability of residential zip code data.

All statistical calculations were performed using SAS, version 9.2 (SAS Institute Inc., Cary, North Carolina). This analysis was submitted to the Institutional Review Board of Columbia University and was deemed non-human-subjects research, since all data were deidentified prior to being provided to the investigators.

RESULTS

After the exclusions detailed above, we identified 142,729 patients with duodenal biopsies during the 4.5 years spanning January 1, 2008–June 30, 2012, and any gastric biopsy going back to January 1, 2003. We excluded 263 patients who had a history of CD in the clinical indication field of the endoscopy report but had a normal duodenal biopsy, which left 142,466 patients. When the data set was restricted to those patients whose gastric and duodenal biopsies were performed during the same procedure, 136,179 persons remained.

The characteristics of patients are shown in Table 1. The mean age was 51 (standard deviation, 18) years, and 89,940 patients (66%) were female. The prevalence of CD was stable during the time period of this analysis (1.9% and 2.1% in the years 2008–2010 and 2011–2012, respectively), as was the prevalence of *H. pylori* (8.8% and 9.0% in the years 2008–2010 and 2011–2012, respectively). Of the 2,689 patients with histological evidence of CD, gastric *H. pylori* was detected in 117 (4.4%) patients. *H. pylori* was significantly less common in CD patients than among patients without CD (8.8%; $P < 0.0001$ (Table 2)). After adjustment for age, gender, and Medicaid enrollment status, the inverse relationship between CD and *H. pylori* changed little (adjusted odds ratio (aOR) = 0.48, 95% confidence interval (CI): 0.40, 0.58) (see Table 2). In contrast, the

Table 1. Characteristics of 136,179 Patients Who Underwent Gastric and Duodenal Biopsy in the Same Procedure and Whose Biopsy Specimens Were Submitted to Miraca Life Sciences (Irving, Texas) During the Period January 1, 2008–June 30, 2012

Characteristic	Duodenal Histology					
	Normal[a] (*n* = 127,619)		Celiac Disease (*n* = 2,689)		IEL (*n* = 5,871)	
	No.	%	No.	%	No.	%
Mean age, years	51 (18)[b]		49 (18)		44 (17)	
0–19	6,009	5	160	6	382	7
20–39	26,327	21	657	24	1,979	34
40–59	51,313	40	1,046	39	2,411	41
≥60	43,970	34	826	31	1,099	19
Gender						
Male	43,989	34	846	31	1,404	24
Female	83,630	66	1,843	69	4,467	76
Insurance						
Medicaid	3,846	3	51	2	268	5
Non-Medicaid	123,773	97	2,638	98	5,603	95
Gastric histology						
Normal	32,240	25	473	18	1,273	22
Active *Helicobacter pylori* gastritis	11,207	9	117	4	780	13
Chronic active (*H. pylori*-negative) gastritis	2,081	2	85	3	162	3
Chronic inactive (*H. pylori*-negative) gastritis	11,819	9	553	21	742	13
Reactive gastropathy	17,858	14	215	8	538	9
Lymphocytic gastritis	172	0.1	197	7	55	1
Other[c]	52,242	41	1,049	39	2,321	40

Abbreviation: IEL, intraepithelial lymphocytosis.
[a] Includes normal duodenal mucosa and duodenitis.
[b] Numbers in parentheses, standard deviation.
[c] Other diagnoses included mild chronic inactive gastritis, mild reactive gastropathy, atrophic gastritis, collagenous gastritis, granulomatous gastritis, focally enhanced gastritis, and polyps (fundic gland, hyperplastic, and adenomatous).

prevalence of *H. pylori* was higher among persons who had IEL and normal villous architecture (13.3%), an association that remained significant after adjustment for the same covariates.

Results of analyses stratified by age group, gender, and Medicaid enrollment are shown in Table 3. The prevalence of *H. pylori* was greatest in older subjects, men, and Medicaid enrollees. The inverse relationship between *H. pylori* and CD was similar in most age strata. The relationships were similar in men and women. The association was diminished among patients enrolled in Medicaid, though the confidence intervals

Table 2. Association Between Celiac Disease and *Helicobacter pylori* Infection, by Degree of Villous Atrophy, Among Patients With Biopsy Specimens Submitted to Miraca Life Sciences (Irving, Texas) During the Period January 1, 2008–June 30, 2012

Characteristic	Total No.	H. pylori Prevalence		Unadjusted OR	95% CI	Adjusted[a] OR	95% CI
		No.	%				
Normal duodenal biopsy	127,619	11,207	8.8	1.0		1.0	
IEL with normal villi	5,871	780	13.3	1.61	1.49, 1.74	1.72	1.59, 1.86
All celiac disease patients	2,689	117	4.4	0.46	0.38, 0.56	0.48	0.40, 0.58
Blunt villi (Marsh stage 3A)	1,334	56	4.2	0.45	0.34, 0.58	0.46	0.35, 0.60
Flat villi (Marsh stage 3B/C)	1,355	61	4.5	0.48	0.37, 0.62	0.50	0.39, 0.65

Abbreviations: CI, confidence interval; IEL, intraepithelial lymphocytosis; OR, odds ratio.
[a] Adjusted for age, gender, and Medicaid enrollment status.

were wide. The prevalences of CD and *H. pylori* by age group are shown in Figure 1. *H. pylori* prevalence increased with age, while CD prevalence was highest in the younger age strata, although this trend was not apparent among men. Despite the positive correlation between *H. pylori* and IEL, the prevalence of the latter declined with age (Figure 2). Results of analysis stratified by the most common clinical indications for endoscopy are shown in Table 4. While the prevalences of CD and *H. pylori* varied by indication, the inverse relationships were present and similar in magnitude regardless of the indication for the procedure.

Repeating the multivariate analysis, now including all patients with a duodenal biopsy and concurrent *or previous* gastric biopsy (*n* = 142,466), the inverse relationship between CD and *H. pylori* remained essentially unchanged (aOR = 0.48, 95% CI: 0.40, 0.58). The positive relationship between *H. pylori* and IEL with normal villous architecture was similarly stable in this sensitivity analysis (aOR = 1.71, 95% CI: 1.58, 1.85).

Adjustment for residential zip-code racial, ethnic, and socioeconomic data

Residential zip code was available for 47,440 patients residing in 6,232 zip codes (35% of the cohort). In the subsets for whom zip code data were available, the multivariate analysis was rerun without adjustment for zip-code-level characteristics. The association between CD and *H. pylori* remained similar in magnitude and direction (aOR = 0.55, 95% CI: 0.41, 0.74) to that observed in the full data set.

Table 3. Association Between Celiac Disease and *Helicobacter pylori* Infection, by Age, Gender, and Medicaid Status, Among Patients With Biopsy Specimens Submitted to Miraca Life Sciences (Irving, Texas) During the Period January 1, 2008–June 30, 2012

| Characteristic	No. of Patients With CD	H. pylori Prevalence in CD Patients		H. pylori Prevalence in Non-CD Patients		Unadjusted Odds Ratio	95% Confidence Interval
		No.	%	No.	%		
Age, years							
0–19	160	2	1.3	161	2.7	0.46	0.11, 1.87
20–39	657	21	3.2	1,975	7.5	0.41	0.26, 0.63
40–59	1,046	44	4.2	4,906	9.6	0.42	0.31, 0.56
≥60	826	50	6.1	4,165	9.5	0.62	0.46, 0.82
Gender							
Male	846	45	5.3	4,379	10.0	0.51	0.38, 0.69
Female	1,843	72	3.9	6,828	8.2	0.46	0.36, 0.58
Insurance							
Medicaid	51	7	13.7	716	18.6	0.70	0.31, 1.56
Non-Medicaid	2,638	110	4.2	10,491	8.5	0.47	0.39, 0.57

Abbreviation: CD, celiac disease.
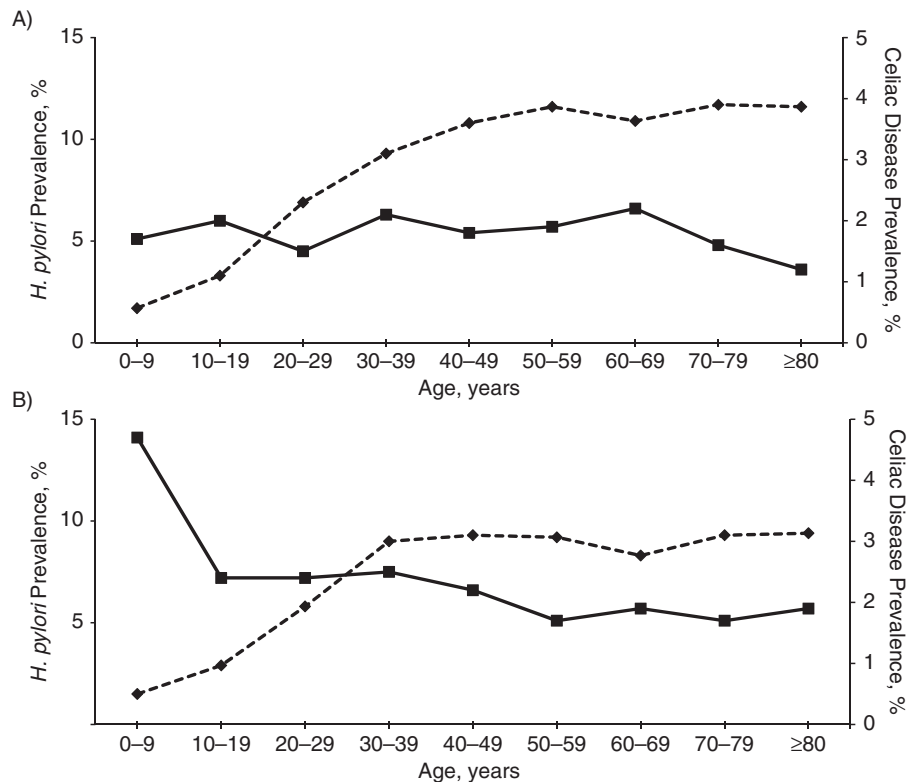
Figure 1. Prevalences of *Helicobacter pylori* infection and celiac disease, by age and gender, among patients with biopsy specimens submitted to Miraca Life Sciences (Irving, Texas) during the period January 1, 2008–June 30, 2012. A) men; B) women. Solid line, celiac disease; dashed line, *H. pylori*.

Results for quartiles of median household income, percentage of black residents, and percentage of Hispanic residents are shown in Table 5. There was a modest positive association between median income quartile and CD prevalence and an inverse relationship between median income quartile and *H. pylori* prevalence. Both percentage of black residents and percentage of Hispanic residents were positively associated with *H. pylori* prevalence and negatively associated with CD prevalence (see Table 5). The correlation between median income and percentage of black residents was modest ($r = -0.27$), and the correlation between median income and percentage of Hispanic residents was similar ($r = -0.24$). However, the correlation between percentage of black residents and percentage of Hispanic residents was negligible ($r = -0.03$). Therefore, all 3 variables (median income quartile, percentage of black residents, and percentage of Hispanic residents) were included in the adjusted model.

Analyses using generalized estimating equations and adjusting for zip-code-level data showed that the inverse relationship between CD and *H. pylori* remained strong (aOR = 0.59, 95% CI: 0.44, 0.78). Additional analyses that further incorporated inverse probability weights for availability of the zip code data found similar results (aOR = 0.56, 95% CI: 0.47, 0.67).

DISCUSSION

In this analysis of a nationwide pathology database, we found a strong inverse relationship between the presence of *H. pylori* and CD among patients undergoing gastrointestinal endoscopy for a variety of symptoms. After adjustment for potentially confounding variables, such as age, gender, and socioeconomic status, the inverse relationship between *H. pylori* and CD remained strong. This association was present in all age groups and both genders, and the relationships were similar regardless of the degree of villous atrophy found in the duodenal biopsy. Because this was a cross-sectional study, the temporality of exposure and outcome could not be ascertained for each individual. However, previous studies have established that *H. pylori* is usually acquired during the first few years of life (24), while studies of serial serum sampling have shown that CD can develop at any age (2, 25). Therefore, these results raise the possibility that the presence of *H. pylori* is protective against the development of CD. The epidemiology of these two conditions argues against the reverse—namely, that CD is somehow protective against the acquisition of *H. pylori*.

The few studies that have investigated the relationship between these two entities have yielded conflicting results. Four studies noted a decreased prevalence of *H. pylori*
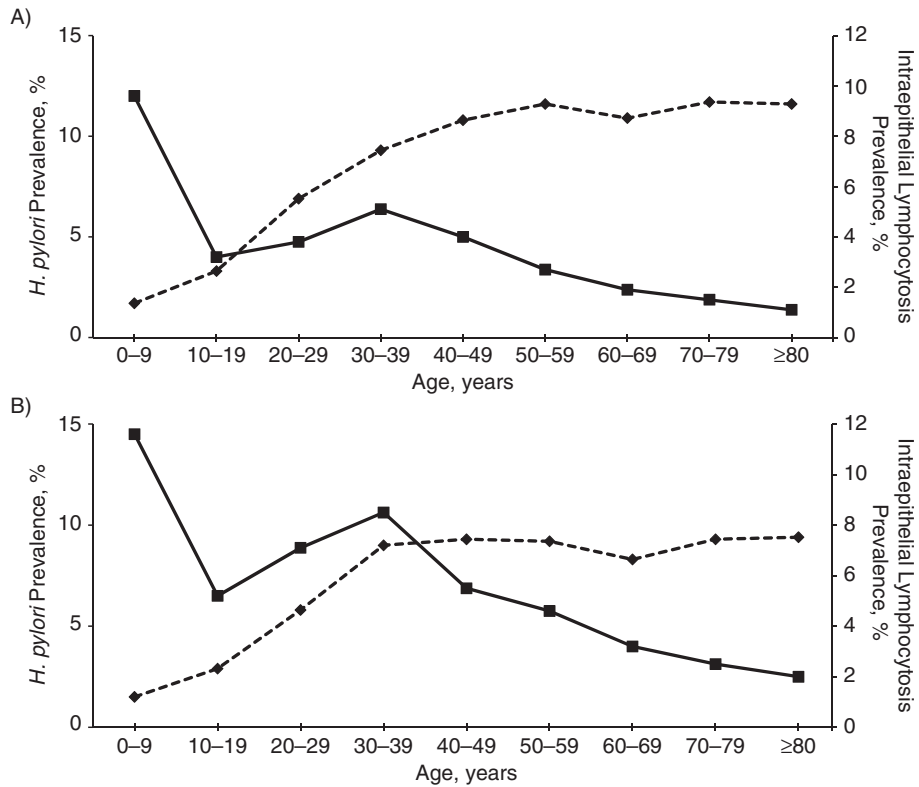
Figure 2.   Prevalences of *Helicobacter pylori* infection and intraepithelial lymphocytosis, by age and gender, among patients with biopsy specimens submitted to Miraca Life Sciences (Irving, Texas) during the period January 1, 2008–June 30, 2012. A) men; B) women. Solid line, intraepithelial lymphocytosis; dashed line, *H. pylori*.

among CD patients compared with controls, with the absolute difference in prevalence ranging between 4% and 35% (26–29). Three studies showed no difference in *H. pylori* prevalence (30–32), while 1 study showed a slightly increased prevalence of *H. pylori* in CD patients (26% vs. 20%) (33). One other study found that among patients with CD, *H. pylori* presence is associated with less severe degrees of villous atrophy (34). Variability in these results is probably due to large differences in *H. pylori* prevalence, small sample

sizes (with most studies including fewer than 200 CD patients), lack of histological confirmation of *H. pylori* in some studies, and lack of adjustment for sociodemographic characteristics. In contrast, the present study comprised a large sample (including 2,689 persons with CD) and included patients from multiple sites throughout the United States. This analysis also adjusted for gender (since *H. pylori* is more common in men (12) and CD is more often diagnosed in women (35)), age (since *H. pylori* prevalence increases

Table 4.   Association Between Celiac Disease and *Helicobacter pylori* Infection, by Clinical Indication for Endoscopy, Among Patients With Biopsy Specimens Submitted to Miraca Life Sciences (Irving, Texas) During the Period January 1, 2008–June 30, 2012

Indication	Total No.	Celiac Disease Prevalence, %	*H. pylori* Prevalence, %	Adjusted[a] Odds Ratio	95% Confidence Interval	P Value
Dyspepsia	27,719	1.4	9.8	0.47	0.30, 0.75	0.0016
Abdominal pain	62,846	1.5	8.4	0.44	0.32, 0.62	<0.0001
GERD	47,518	1.3	8.7	0.37	0.24, 0.58	<0.0001
Anemia	17,698	2.2	12.6	0.41	0.26, 0.63	<0.0001
Diarrhea	21,297	2.5	6.4	0.59	0.38, 0.92	0.0191

Abbreviation: GERD, gastroesophageal reflux disease.
[a] Adjusted for age, gender, and Medicaid enrollment status.

Table 5. Association of Zip-Code-level Participant Characteristics With Celiac Disease and *Helicobacter pylori* Infection Among Patients With Biopsy Specimens Submitted to Miraca Life Sciences (Irving, Texas) During the Period January 1, 2008–June 30, 2012

Zip-Code-level Characteristic and Quartile	Prevalence of Celiac Disease, %	Adjusted[a] OR	95% CI	Prevalence of *H. pylori*, %	Adjusted[a] OR	95% CI
Median household income[b]						
1	1.7	1.0		13.4	1.0	
2	2.0	1.17	0.94, 1.47	8.8	0.75	0.67, 0.84
3	2.2	1.25	1.00, 1.56	7.6	0.70	0.63, 0.78
4	2.3	1.25	1.00, 1.58	6.4	0.66	0.59, 0.75
% of black residents[c]						
1	2.3	1.0		7.5	1.0	
2	2.3	1.00	0.81, 1.22	7.5	0.98	0.86, 1.12
3	1.9	0.83	0.67, 1.03	8.8	1.07	0.94, 1.21
4	1.8	0.83	0.66, 1.03	11.2	1.42	1.25, 1.60
% of Hispanic residents[d]						
1	2.3	1.0		6.8	1.0	
2	2.3	1.01	0.83, 1.25	6.7	1.00	0.87, 1.14
3	1.9	0.85	0.67, 1.03	7.9	1.18	1.04, 1.35
4	1.9	0.91	0.73, 1.13	13.4	1.86	1.65, 2.10

Abbreviations: CI, confidence interval; OR, odds ratio.
[a] Adjusted for age, gender, and Medicaid enrollment status.
[b] Quartile 1, <$42,104; quartile 2, $42,104–$54,642; quartile 3, $54,643–$74,034; quartile 4, >$74,034.
[c] Quartile 1, <1%; quartile 2, 1%–3.7%; quartile 3, 3.8%–12.5%; quartile 4, >12.5%.
[d] Quartile 1, <2.5%; quartile 2, 2.5%–6.4%; quartile 3, 6.5%–16.8%; quartile 4, >16.8%.

with age (12)), and socioeconomic status (since *H. pylori* is more common among patients with lower socioeconomic status (11, 12), whereas CD may be less common among patients with lower socioeconomic status (36)). The fact that a strong inverse relationship between *H. pylori* and CD persisted after adjustment for these covariates provides evidence that the relationship is robust and independent of confounding influences. We also found a positive association between *H. pylori* and duodenal IEL, a finding consistent with that of prior investigations (37, 38), providing external validation for the present study. The presence of IEL in the absence of villous atrophy is a finding with a wide differential diagnosis, including *H. pylori* (39).

The mechanism by which *H. pylori* might be protective against CD is uncertain. *H. pylori* is associated with decreased risks of allergic and atopic disease and other inflammatory conditions (7, 9, 40). This may reflect the additional recruitment of both local and systemic regulatory T-regulatory lymphocytes to the gastric mucosa in patients with *H. pylori*, with both local and systemic effects (41, 42). Experimental murine infections provide strong evidence for the hypothesis that the T-regulatory lymphocytes recruited by *H. pylori* have systemic effects and specifically protect against allergen-induced asthma (9, 10, 43). T-regulatory lymphocytes also may play a role in the pathogenesis of CD, since the down-regulation of cellular responses mediated by T-regulatory cells in the bowel wall is diminished or lost in

CD patients (44). Thus, persons without *H. pylori* and without the recruited gastric T-regulatory cells may be less likely to down-regulate immune responses to gluten. Alternatively, *H. pylori* may affect ingested gluten through its modification by gastric pH or through its proteases (24), reducing its immunogenicity.

Our study had a number of strengths, including its large sample size, geographic diversity, and uniformity of histological interpretation. The limitations include the fact that the study was restricted to patients who underwent concurrent gastric and duodenal biopsies. Such patients may differ from patients undergoing only gastric biopsy or only duodenal biopsy. To address this issue, we repeated the analysis after including patients with duodenal biopsy and any prior gastric biopsy on record, and found an unchanged inverse relationship. The definition of CD was limited to histological diagnosis, and there are other conditions that can mimic CD histologically (45, 46). However, CD is the most common form of villous atrophy, and we performed separate analyses for Marsh 3A and Marsh 3B/C lesions, the latter being highly specific for CD (47). The gold standard for CD diagnosis is small-bowel biopsy; thus, identification of patients with histological evidence of villous atrophy is likewise a highly sensitive method. Since most patients with CD in the United States are undiagnosed (48, 49), identifying a risk or protective factor for diagnosed disease may not result in the same effect magnitude when considering undiagnosed CD patients

as well. As such, the direction of the association between *H. pylori* and undiagnosed CD cannot be deduced from this analysis. We found an association between *H. pylori* and increased IEL; because CD3 immunohistochemical staining was not done routinely on small-intestine biopsies in these patients, subtle or borderline cases of IEL may have been missed, yielding an underestimate of the calculated association. We did not have access to information on medication use in this database, and proton pump inhibitors may decrease the sensitivity of gastric biopsy for the diagnosis of *H. pylori*; however, the fact that the inverse association between CD and *H. pylori* was present regardless of clinical indication (Table 4) illustrates that such differential use of acid suppression medication is unlikely to have been a confounding variable.

H. pylori may be a marker for another exposure, such as crowded living conditions, dietary habits, or not receiving antibiotics. Adjusting for Medicaid status partially addressed this issue, since Medicaid status has been used previously in analyses of socioeconomic disparities in health-care outcomes (50). To further account for racial, ethnic, and socioeconomic factors that may affect the risk of CD and *H. pylori*, in a subset of the study population we were able to perform additional analyses taking into account zip-code-level data on these characteristics. This additional analysis uncovered associations between household income, zip-code racial and ethnic composition, and the outcomes of CD and *H. pylori* in our data set. However, adjustment for these variables did not alter the inverse relationship between CD and *H. pylori* infection. Our analyses, including incorporation of inverse probability weights, suggest that the subset of patients from whom zip code data were available was representative of the overall studied patient population. Estimates of the association between CD and *H. pylori* may vary based on the population prevalence of *H. pylori*, and testing of this association in nations with a higher prevalence of *H. pylori* may be warranted.

Because this was a cross-sectional study, it is unknown whether gastric colonization with *H. pylori* preceded the onset of CD or developed subsequent to this condition. While it was impossible to ascertain this information for each individual patient in this analysis, on the whole *H. pylori* precedes CD development given the known epidemiology of *H. pylori* (which is usually acquired in childhood) and CD, which can develop de novo at any age.

In conclusion, we found that patients with CD have significantly lower rates of gastric *H. pylori* infection than patients with normal duodenal mucosa. This association remained unaffected by the confounding influences of age, gender, and socioeconomic status. These results raise the hypothesis that *H. pylori* confers protection against the development of CD. Additional studies may be warranted for confirmation and to examine whether *H. pylori* could modulate gluten immunogenicity among genetically susceptible hosts.

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