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Role of *Helicobacter pylori* Plasticity Region Genes in Development of Gastroduodenal Diseases

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The plasticity region of *Helicobacter pylori* is a large chromosomal segment including isolate-specific open reading frames with characteristics of pathogenicity islands. It remains unclear whether genes in the plasticity region play a role in the pathogenesis of gastric mucosal inflammation and gastroduodenal disease. Our aim was to assess the role of selected genes in the plasticity region in relation to risk of *H. pylori*-related disease and the severity of gastric mucosal damage. We used PCR to study the relation of disease outcome and mucosal damage with four genes in the *H. pylori* plasticity region (*jhp0940*, *jhp0945*, *jhp0947*, and *jhp0949*) from isolates obtained from both Western ($n = 296$) and East Asian ($n = 217$) patients. The prevalence of *jhp0945*, *jhp0947*, and *jhp0949* differed significantly between Western and East Asian isolates. In Western isolates, the presence of *jhp0945* was significantly associated with gastric ulcer, duodenal ulcer, and gastric cancer (odds ratios [95% confidence intervals]: 2.27 [1.04 to 4.98], 1.86 [1.03 to 3.34], and 1.92 [1.03 to 3.56], respectively). *jhp0940*-positive Western isolates were significantly associated with absence of gastric ulcer or duodenal ulcer (0.21 [0.05 to 0.94] and 0.31 [0.12 to 0.78], respectively). No significant difference was observed between inflammatory cell infiltration or atrophy and the presence or absence of plasticity region genes. The outcome of *H. pylori* infections varies widely geographically. These data suggest a possible role for difference in the prevalence of plasticity region genes in the geographic variation in *H. pylori*-related diseases.

Helicobacter pylori infection of human gastric mucosa results in chronic gastritis which may eventuate in gastric ulcer or gastric cancer. Gastric cancer is generally thought to arise through a series of steps in which progressive mucosal damage ultimately results in mucosal atrophy (28). The presence of *H. pylori*-induced gastric mucosal atrophy varies among different populations, and this variation in outcome has been associated with differences in *H. pylori* virulence factors, host genetics, and/or environment factors (7, 25, 26, 29, 34).

A number of important *H. pylori* virulence factors have been identified, such as the *cag* pathogenicity island (PAI) and VacA (3). Putative *H. pylori* virulence genes have been classified into three general types: (i) isolate-specific genes (e.g., *cagA*, *dupA*, and plasticity region genes), (ii) the virulent gene with different genotypic gene activities (e.g., *vacA*, *cagA* repeat region, and *hopQ*), and (iii) the phase-variable genes (e.g., *oipA* and *babA*) (31). The presence, absence, and activity of these different virulence factors have been related to the severity of gastric mucosal injury and inflammation and thus to the risk of development of different gastroduodenal diseases (3, 4, 26, 29, 32–34).

By definition, strain-specific genes are present in only some *H. pylori* isolates. The *cag* PAI, which encodes a type IV secretion system that delivers the CagA protein into host gastric epithelial cells (5, 27), is the best-studied virulence factor. However, many strain-specific genes lie outside the *cag* PAI; up to 50% of the strain-specific genes transferred from other species are located in the plasticity region (20). For example, *H. pylori* strain J99, isolated in the United States in 1994 from patients with a duodenal ulcer, has 48 genes in the plasticity region (i.e., *jhp0914* to *jhp0961*) (1, 2). Each *H. pylori* isolate shows variability in gene content such that different components of the plasticity region may be responsible for differences in virulence potential (1, 2). The prevalence of genes *jhp0914* to *jhp0961* ranges from 13% to 100%, with only three genes being present within more than 90% of *H. pylori* (i.e., *jhp0915* [100%], *jhp0955* [94%], and *jhp0957* [94%]). Genes found in less than 20% include *jhp0914* (17%), *jhp0925* (19%), *jhp0926* (19%), and *jhp0959* (13%) (11, 21, 31). It remains unknown whether strain-specific genes or combinations of strain-specific genes influence the severity of gastric mucosal inflammation and the risk of different *H. pylori*-related diseases. In addition, the biological functions of most open reading frames (ORFs) in the plasticity region remain unknown. Recently *jhp0940*, *jhp0945*, *jhp0947*, and *jhp0949* in *H. pylori* obtained in the West have been reported to be associated with an increased risk of gastroduodenal disease and an increase in inflammatory cytokines (e.g., interleukin 8 [IL-8], IL-12, and tumor necrosis factor alpha [TNF-α]) (6, 15–17, 19, 20, 23). In previous reports, however, the role of selected genes in the plasticity region in relation to the risk of *H. pylori*-related disease and the severity of gastric mucosal damage was controversial and unclear. Moreover, there were no reports comparing *H. pylori* obtained from different geographic populations. Here, we used more than 500 Western and East Asian *H. pylori* isolates to examine the prevalence of *jhp0940*, *jhp0945*, *jhp0947*, and *jhp0949* and their relation to mucosal inflammation and *H. pylori*-related disease.

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TABLE 1 Demographic characteristics of *H. pylori*-positive Western and East Asian patients enrolled in this study

Characteristic	Results for isolates from:					*P* value[b]
	United States	Colombia	South Korea	Japan	Total	
No. of patients	202	94	105	112	513	
Age (mean ± SD)	50.8 ± 1.0	56.8 ± 1.5	47.6 ± 1.2	58.9 ± 1.3	52.5 ± 0.7	<0.01
Sex (% male)	84	59	76	61	71	<0.01
Disease[a]						
Gastritis	106 (52.5%)	32 (34.0%)	32 (30.5%)	27 (24.1%)	197 (38.4%)	<0.01
GU	32 (15.8%)	0 (0.0%)	19 (18.1%)	30 (26.8%)	81 (15.8%)	
DU	43 (21.3%)	25 (26.6%)	18 (17.1%)	26 (23.2%)	112 (21.8%)	
GC	21 (10.4%)	37 (39.4%)	36 (34.3%)	29 (25.9%)	123 (24.0%)	

[a] Abbreviations: GU, gastric ulcer; DU, duodenal ulcer; GC, gastric cancer.
[b] *P* values were from analyzed demographic data from four different countries by one-way ANOVA for age and Fisher's exact test for sex and disease.

MATERIALS AND METHODS

Patients. *H. pylori* isolates were obtained from infected patients in Western countries (United States [$n = 202$] and Colombia [$n = 94$]) and East Asian countries (South Korea [$n = 105$] and Japan [$n = 112$]) in the isolate collection period of 2002 to 2005 (Table 1). The study population consisted of patients with gastric ulcers (GU) ($n = 81$), duodenal ulcers (DU) ($n = 112$), gastric cancer ($n = 123$), or gastritis alone ($n = 197$). Gastritis was endoscopically and pathologically defined as *H. pylori* gastritis without peptic ulcers or gastric cancer. Informed consent had been obtained from all patients under protocols approved by the local hospital's ethics committee.

***jhp0940*, *jhp0945*, *jhp0947*, and *jhp0949* status by PCR.** *H. pylori* taken from each patient by endoscopy was grown at 37°C on brain heart infusion (BHI) (BD, Sparks, MD) plates containing 7% horse blood (Cocalico Biological, Inc., Reamstown, PA) in a microaerobic condition. Chromosomal DNA of *H. pylori* was isolated from confluent plate cultures expanded from a single colony using the QIAamp tissue kit (Qiagen Inc., Santa Clarita, CA) according to the manufacturer's instructions. Genotypes of *vacA* signal (s), middle (m), and intermediate (i) regions and *cagA* status were determined by PCR as described previously (3, 13, 32). We also checked the status of *H. pylori* DNA by checking *H. pylori*-specific 16S rRNA as controls by PCR. The *jhp0940*, *jhp0945*, *jhp0947*, and *jhp0949* status were determined by PCR methods using primer pairs shown in Table 2 (6, 16). The PCR conditions were 95°C for 5 min, then 35 cycles of 95°C for 30 s, 48°C for 45 s, and 72°C for 30 s, and finally 72°C for 5 min. When a PCR using two different primer pairs was positive, the status of *jhp0949* was determined as positive.

TABLE 2 Primer pairs of four kinds of plasticity regions

Gene and primer type	Sequence	Reference
jhp0940		
Forward	5′-GAA ATG TCC TAT ACC AAT GG	16
Reverse	5′-CCT AAG TAG TGC ATC AAG G	
jhp0945		
Forward	5′-ACT CCA GCC AGT ATT GTA AA-3′	6
Reverse	5′-TTC TTG CGA GTT AGG ATT GG-3′	
jhp0947		
Forward	5′-GAT AAT CCT ACG CAG AAC G-3′	16
Reverse	5′-GCT AAA GTC ATT TGG CTG TC-3′	
jhp0949		
Forward	5′-ATA GGA GTG GGT GCT TAC TT-3′	6
Reverse	5′-AGC AAC AAC AAA GGC ATG TA-3′	
Forward	5′-TTC AAA AAG TCC CCG AAA TG-3′	This study
Reverse	5′-GGA TGT CCT GGC ATG TCT CT-3′	

When the gene status was considered negative by PCR, we further confirmed the results using dot blot analyses. A total of 500 ng of total DNA was added to 100 μl of TE buffer and mixed with 100 μl of a denaturing buffer (0.5 M NaOH, 1.5 M NaCl). The denatured DNA was transferred to a Hybond-N^+ membrane (Amersham, GE Healthcare) by means of a Bio-Dot microfiltration apparatus (Bio-Rad Laboratories, Inc.). DNA of *H. pylori* J99 and human DNA were also transferred to the membrane as positive and negative controls, respectively. The membranes were hybridized at 42°C overnight in plastic bags containing ECL Gold hybridization buffer supplemented with 5% (wt/vol) blocking agent and 0.5 M NaCl. The membranes were washed three times in primary washing buffer (0.5× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate] [pH 7.0], 0.4% sodium dodecyl sulfate) at room temperature for 15 min and three times in secondary washing buffer (2× SSC) at room temperature for 15 min. Finally, the membranes were exposed to Hyperfilm ECL film (Amersham, GE Healthcare). If PCR results yielded negative results but dot blot testing showed a positive blot, we considered the samples positive.

Histology. Gastric biopsy specimens had been taken from the antrum (pyloric gland area) and the body (fundic gland area). The biopsy specimens were fixed in 10% buffered formalin and embedded in paraffin, and the paraffin was cut into sequential 4-μm sections. The *H. pylori* density, activity of gastritis (neutrophil infiltration), and atrophy were graded from 0 (absent/normal) to 5 (maximal) as previously described (8). The score is presented as the mean scores of biopsy samples from the corpus and antral areas.

Data analysis. Statistical differences in demographic characteristics, the status of four genes in the plasticity region (*jhp0940*, *jhp0945*, *jhp0947*, and *jhp0949*), and histological scoring among the different geographic groups were determined by one-way analysis of variance (ANOVA) or the chi-square test. A multiple linear regression analysis, where age, sex, bacterial factors, and clinical outcome were explanatory variables, was performed to determine which factor(s) was related to severity of histology. A *P* value of less than 0.05 was accepted as statistically significant. Calculations were carried out using statistical software StatView 5.0 (SAS Institute Inc., Cary, NC).

RESULTS

The status of *jhp0940*, *jhp0945*, *jhp0947*, and *jhp0949* was determined from 513 *H. pylori* isolates, including 296 isolates from Western countries and 217 isolates from East Asian countries (Table 1). The mean age of subjects, the sex ratio, and the population of *H. pylori*-related diseases differed significantly among different countries and between areas ($P < 0.01$) (Table 1).

TABLE 3 Prevalence of different virulence factors in different ethnic groups

Virulence factor or region	Genotype	No. (%) found							
		Western countries			East Asian countries				
		USA ($n = 202$)	Colombia ($n = 94$)	Total ($n = 296$)	South Korea ($n = 105$)	Japan ($n = 112$)	Total ($n = 217$)	Total ($n = 513$)	*P* value[a]
		35 (17.3%)	16 (17.0%)	51 (17.2%)	27 (25.7%)	24 (21.4%)	51 (23.5%)	102 (19.9%)	0.12
jhp0945	Present	96 (47.5%)	46 (48.9%)	142 (48.0%)	30 (28.6%)	31 (27.7%)	61 (28.1%)	203 (39.6%)	<0.01
jhp0947	Present	76 (37.6%)	52 (55.3%)	128 (43.2%)	9 (8.6%)	3 (2.7%)	12 (5.5%)	140 (27.3%)	<0.01
jhp0949	Present	101 (50.0%)	42 (44.7%)	143 (48.3%)	51 (48.6%)	77 (68.8%)	128 (59.0%)	271 (52.8%)	0.02
cagA	Present	169 (83.7%)	75 (79.8%)	244 (82.4%)	102 (97.1%)	112 (100%)	214 (98.6%)	458 (89.3%)	<0.01
vacA s region	s1	167 (82.7%)	72 (76.6%)	239 (80.7%)	105 (100%)	112 (100%)	217 (100%)	456 (88.9%)	<0.01
vacA m region	m1	130 (64.4%)	63 (67.0%)	193 (65.2%)	91 (86.7%)	109 (97.3%)	200 (92.2%)	393 (76.6%)	< 0.01
vacA i region	i1	153 (75.7%)	67 (71.3%)	220 (74.3%)	102 (97.1%)	110 (98.2%)	212 (97.7%)	432 (84.2%)	< 0.01

[a] *P* values were from analyzed demographic data for Western countries versus East Asian countries.

The prevalence of *jhp0940*, *jhp0945*, *jhp0947*, and *jhp0949*. The prevalence rates of *jhp0940*, *jhp0945*, *jhp0947*, and *jhp0949* in patients with *H. pylori* were 19.8% (102/513), 39.6% (203/513), 27.3% (140/513), and 52.8% (271/513), respectively. All samples considered negative by PCR were confirmed as negative by dot blot hybridization. When we combined the results from South Korea and Japan into East Asian isolates and from the United States and Colombia into Western isolates, there was no statistical difference in the prevalence of *jhp0940* between East Asian and Western isolates (17.2% and 23.5%; $P = 0.078$). In contrast, the prevalence of *jhp0945* and *jhp0947* in East Asian isolates was significantly lower in Western isolates and that of *jhp0949* was significantly higher in East Asian isolates compared to Western isolates (Table 3). The prevalence of *jhp0947* in East Asian isolates was only 5.5%.

The prevalences of *cagA* and *vacA* s1, m1 and i1 regions in *H. pylori* isolated from patients with gastritis were similar: 89.3% (458/513), 88.9% (456/513), 76.6% (393/513) and 84.2% (432/513), respectively (Table 3). The prevalence of *vacA* s1, m1 and i1 genotypes combined with *cagA*-positive status in East Asian isolates was significantly greater than that in Western isolates ($P =$ 0.001, 0.001, and 0.001, respectively) (Table 3).

The positive rates of *jhp0940* in isolates from patients with duodenal ulcer (15.2%) and gastric cancer (15.4%) were significantly lower than those from gastritis-only patients (24.9%, $P =$ 0.047 and 0.048, respectively). In contrast, the prevalence of *jhp0945* was similar in gastric ulcer and duodenal ulcer isolates (45.7% and 45.5%, respectively) and was significantly higher than that in gastritis-only patients (33.0%; $P = 0.046$ for gastric ulcer and 0.029 for duodenal ulcer, respectively).

When the results were divided into two groups, the prevalence rates of *jhp0940*, *jhp0945*, *jhp0947*, and *jhp0949* in Western isolates isolated from gastritis-only patients were 23.9%, 39.1%, 37.7%, and 45.7%, respectively (Fig. 1). The positive rates of *jhp0940* in patients with gastric ulcer and duodenal ulcer were significantly lower than that from gastritis patients (12.5% and 8.8%, respectively; $P = 0.029$ and 0.009, respectively). In contrast, the prevalence rates of *jhp0945* in gastric ulcer, duodenal ulcer, and gastric cancer patients were similar (i.e., 59.4%, 54.4%, and 55.2%, respectively) and significantly higher than in gastritis patients (39.1%, $P = 0.037$, 0.038, and 0.039, respectively). However, there were no significant differences in the prevalence of *jhp0947* and *jhp0949* between patients with gastritis and those with *H. pylori*-related diseases.

In East Asia, although the presence of *jhp0945* was significantly higher in patients with gastric ulcer (36.7%) than that in gastritis patients (18.6%, $P = 0.035$), there were no significant associations between the development of gastroduodenal diseases and the status of *jhp0940*, *jhp0945*, and *jhp0949* (Fig. 1). The prevalence of *jhp0947* in East Asian isolates was less than 10% irrespective of different diseases, with no significant differences.

Relation between plasticity region gene status and other virulence factors. The statuses of *jhp0940*, *jhp0945*, and *jhp0949* in Western isolates were significantly associated with each other ($P =$ 0.001 [*jhp0940* versus *jhp0945*], 0.006 [*jhp0940* versus *jhp0949*], and 0.006 [*jhp0945* versus *jhp0949*] (Table 4). However, the majority of East Asian *H. pylori* isolates were *jhp0947* negative and

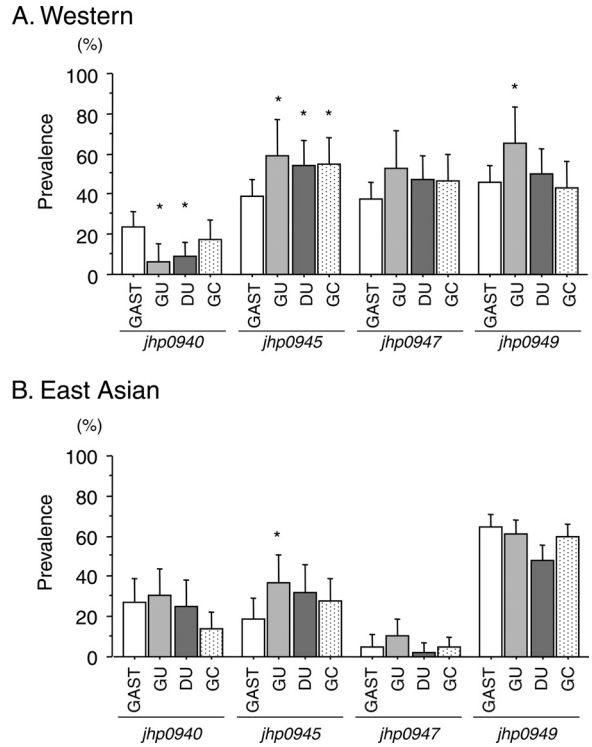

FIG 1 Prevalence of *jhp0940*, *jhp0945*, *jhp0947* and *jhp0949* status in patients with gastritis (GAST), gastric ulcer (GU), duodenal ulcer (DU), and gastric cancer (GC) in Western (A) and East Asian (B) regions.

TABLE 4 Relationship among different *H. pylori* virulence factors and plasticity regions

Region and factor	Relationship[a]			
Western	*jhp0940*	*jhp0945*	*jhp0947*	*jhp0949*
jhp0940		0.185*	0.078	0.160*
jhp0945			<0.001	0.161*
jhp0947				0.344*
jhp0949				
vacA s1	0.003	0.027	0.009	0.044
vacA m1	0.016	0.073	0.031	0.072
vacA i1	0.027	0.017	0.006	0.033
cagA	0.007	0.061	0.041	0.014
East Asian	*jhp0940*	*jhp0945*	*jhp0947*	*jhp0949*
jhp0940		0.109	0.095	0.024
jhp0945			0.115	
jhp0947				0.038
jhp0949				
vacA s1				
vacA m1	0.113	0.009	0.012	0.071
vacA i1	0.054	0.054	0.042	0.184
cagA	0.068	0.114	0.123	0.142

[a] The ϕ coefficient value as association between the two factors was analyzed by Fisher's exact test. *, $P < 0.05$.

TABLE 5 Combination of *jhp0940*, *jhp0945*, *jhp0947*, and *jhp0949* status

Genotype pattern				No. (%) of isolates with pattern	
jhp0940	*jhp0945*	*jhp0947*	*jhp0949*	East Asian	Western
−	−	−	−	47 (21.7%)	76 (25.7%)
−	−	−	+	65 (30.0%)	15 (5.1%)
−	−	+	−	0 (0%)	11 (3.7%)
−	+	−	−	18 (8.3%)	26 (8.8%)
+	−	−	−	16 (7.4%)	14 (4.7%)
−	−	+	+	4 (1.8%)	29 (9.8%)
−	+	+	−	2 (0.9%)	14 (4.7%)
+	+	−	−	4 (1.8%)	7 (2.4%)
−	+	+	+	2 (0.9%)	60 (20.3%)
+	+	+	−	1 (0.5%)	4 (1.4%)
+	+	+	+	1 (0.5%)	7 (2.4%)
+	−	−	+	22 (10.1%)	6 (2.0%)
+	−	+	−	1 (0.5%)	1 (0.3%)
+	−	+	+	1 (0.5%)	2 (0.7%)
+	+	−	+	5 (2.3%)	11 (3.7%)
−	+	−	+	28 (12.9%)	13 (4.4%)

there were no significant correlations among *jhp0940*, *jhp0945*, *jhp0947*, and *jhp0949* statuses. Moreover, the status of these four plasticity region genes had no associations with *vacA* s1, m1, and i1 genotypes and *cagA*-positive status in either Western or East Asian isolates (Table 4).

As far as combination of the four plasticity region genes was concerned, the majority genotype in East Asian isolates and Western isolates was that of the genes lacking *jhp0940-jhp0945-jhp0947-jhp0949* (the −/−/−/− genotype) ($n = 47$, 21.7% in the East Asian region and $n = 76$, 25.7% in the Western region) (Table 5). In contrast, the all-four-positive genotype (+/+/+/+ genotype) in East Asian and Western isolates was present only in 0.5% ($n = 1$) and 2.4% ($n = 7$) (Table 5); a mosaic pattern was more common, such as the −/+/−/+ and +/+/−/+ genotypes in 90 isolates ($n = 57$ [26.3%] in the East Asian region and $n = 33$ [11.1%] in the Western region) (Table 5).

Histology. Although the *jhp0947* status was associated with a significantly increased risk of corpus atrophy, most of the parameters of inflammatory cell infiltration and mucosal atrophy were not different irrespective of different gastric locations or plasticity region gene status among Western patients (Fig. 2).

The gastric mucosa in patients with gastric cancer and gastric ulcer is generally atrophic and that of those with duodenal ulcer generally shows enhanced antral inflammation such that inclusion of these patients in the histological analyses might have introduced a bias; we therefore evaluated the histological analyses using gastritis-only cases, and we found no significant differences (data not shown).

Influence of *jhp0940*, *jhp0945*, *jhp0947*, and *jhp0949* status and clinical outcomes. In East Asia, univariant analysis showed that the *jhp0945* status was associated with an increased a risk of gastric ulcer (odds ratio [OR], 2.58; 95% confidence interval [CI], 1.06 to 6.27) (Table 6). However, in other parameters, there were no significant associations between clinical outcomes and *jhp0940*, *jhp0945*, *jhp0947*, and *jhp0949* status among East Asian isolates (Table 6).

In Western isolates, the status of *jhp0940* was significantly associated with *H. pylori*-related disease and with significantly lower risks of gastric ulcer and duodenal ulcer (OR [95% CI], 0.21 [0.05 to 0.94] and 0.31 [0.12 to 0.78], respectively) (Table 6). In contrast, the *jhp0945*-positive isolates significantly increased the risk for gastric ulcer, duodenal ulcer, and gastric cancer compared with *jhp0945*-negative isolates (OR [95% CI], 2.27 [1.04 to 4.98], 1.86 [1.03 to 3.34], and 1.92 [1.03 to 3.56], respectively). The carriage of *jhp0949* was also related to an increased risk of gastric ulcer (OR, 2.28; 95% CI, 1.02 to 5.07).

Multivariant analysis, including age, sex, *jhp0940*, *jhp0945*, *jhp0947*, and *jhp0949* status, *cagA* status, and *vacA* s, m, or i region genotype, was performed to determine which factor(s) was related to clinical outcome (Table 7). The *jhp0945* and *cagA* status for gastric ulcer and the *vacA* m1 genotype for gastric cancer significantly increased the risks of a clinical outcome. In contrast, the *jhp0940* status significantly decreased the risk for duodenal ulcer (OR, 0.49; 95% CI, 0.24 to 0.99).

DISCUSSION

There has been increasing interest in strain-specific *H. pylori* genes transferred from other species that are located outside the *cag* PAI, especially genes within the plasticity regions (6, 15–17, 19, 20, 23). The plasticity regions were a large region of 45 kb in *H. pylori* J99 and 68 kb in *H. pylori* 26695, and the plasticity region is encoded by 38 genes in J99, of which 33 are absent in 26695. The gene content of *H. pylori* is variable because of different combinations of genes within plasticity regions (2). This gene variability is thought to possibly be responsible for the difference in virulence among different *H. pylori* strains that then may result in different risks of specific clinical outcomes (6, 15–17, 19, 20, 23). ORFs in the plasticity region—e.g., *jhp0917* and *jhp0918* [*dupA* (*virB4*)], *jhp0919*, *jhp0920*, and *jhp0931*; genes for DNA topoisomerase I involved in DNA replication (*jhp0921-jhp0924*); genes for DNA transformation competence ComB8 [*jhp0921* (*virB8*)], ComB9 [*jhp0923* (*virB9*)], or ComB10 [*jhp0924* (*virB10*)]; and others

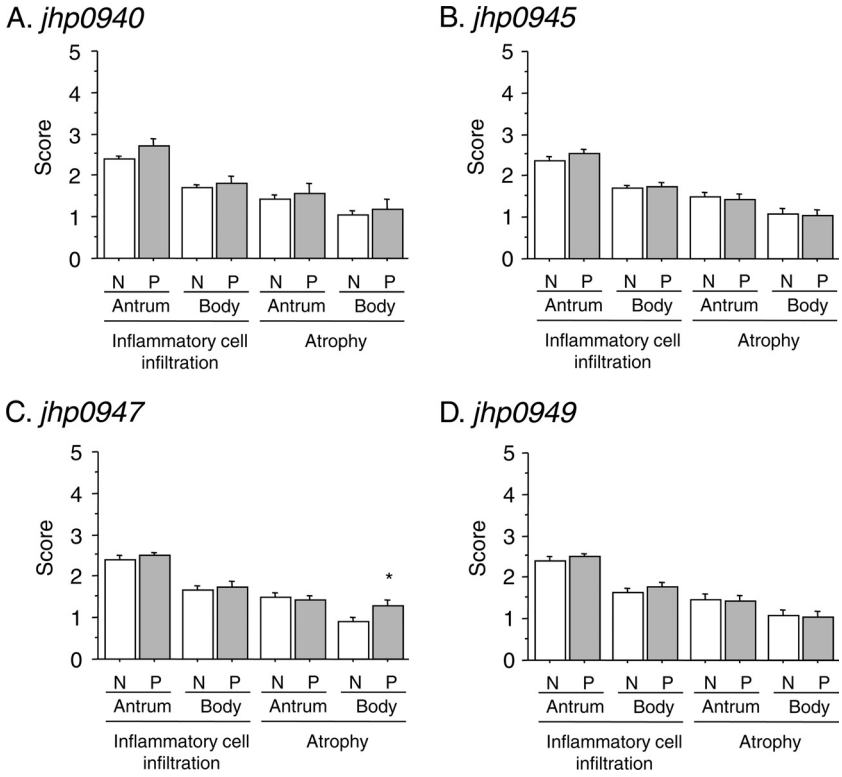

FIG 2 Scores of gastric mucosal atrophy and inflammation cell infiltration in gastric antrum and corpus between *jhp0940*, *jhp0945*, *jhp0947*, and *jhp0949* status in Western isolates. *, $P < 0.05$ compared with negative strain. Abbreviations: N, plasticity region gene-negative strain; P, plasticity region-positive strain.

(*jhp0928*, *jhp0931*, *jhp0935*, *jhp0941*, and *jhp0951*)—share similarity with genes encoding functional proteins, but overall, the biological function of most ORFs in the plasticity region remains unclear. Recently, *jhp0940*, *jhp0945*, *jhp0947*, and *jhp0949* in *H. pylori* have been reported to be associated with an increase in inflammatory cytokines, enhancement of the NF-κB signaling pathway, and gastroduodenal disease (6, 15–17, 19, 20, 23). It has been suggested that *jhp0940*, *jhp0945*, *jhp0947*, and *jhp0949* may be related to *H. pylori*-related disease pathogenesis and may be a marker for risk of peptic ulcer disease (Table 8).

The *jhp940* gene is widely prevalent geographically (India, France, Spain, Peru, Japan, South Africa, and Costa Rica), and the lowest prevalence of *jhp940* was seen in the Spanish (5%) and Costa Rican (30%) isolates, followed by the Japanese (40%) and Peruvian (60%) isolates (19). In an animal model, *jhp940* is strongly expressed in response to the interaction of *H. pylori* with the gerbil gastric mucosa (10). Although as of recently the roles of most regions remain unknown, the recombinant Jhp0940 protein has been shown to elicit tumor necrosis factor alpha (TNF-α) and interleukin-8 (IL-8) from activated human macrophages as well as enhanced translocation of NF-κB in cultured macrophages (19). Moreover, Kim et al. (14) reported that JHP940 is catalytically active as a protein kinase and translocates into cultured human cells and that the kinase activity is indispensable for indirectly

TABLE 6 Age- and sex-adjusted risk for peptic ulcer and gastric cancer in relation to *H. pylori* virulence factors

Virulence factor for region	Gastric ulcer			Duodenal ulcer			Gastric cancer		
	OR	95% CI	*P* value	OR	95% CI	*P* value	OR	95% CI	*P* value
Western[a]									
jhp0940	0.21	0.05–0.94	0.04	0.31	0.12–0.78	0.01	0.66	0.30–1.45	0.31
jhp0945	2.27	1.04–4.98	0.04	1.86	1.03–3.34	0.04	1.92	1.03–3.56	0.04
jhp0947	1.87	0.86–4.07	0.11	1.47	0.82–2.65	0.20	1.44	0.78–2.68	0.25
jhp0949	2.27	1.02–5.07	0.05	1.19	0.67–2.13	0.56	0.09	0.49–1.67	0.74
East Asian[b]									
jhp0940	1.24	0.53–2.93	0.62	0.81	0.33–2.02	0.66	0.49	0.19–1.26	0.14
jhp0945	2.58	1.06–6.27	0.04	2.06	0.82–5.20	0.12	1.67	0.70–4.02	0.25
jhp0947	2.17	0.48–9.80	0.31	0.43	0.04–4.38	0.48	0.92	0.17–4.96	0.92
jhp0949	0.85	0.38–1.91	0.90	0.55	0.24–1.25	0.15	0.72	0.33–1.56	0.40

[a] Gastric ulcer, $n = 32$; duodenal ulcer, $n = 68$; gastric cancer, $n = 58$.
[b] Gastric ulcer, $n = 49$; duodenal ulcer, $n = 44$; gastric cancer, $n = 65$.

TABLE 7 Multivariant analysis for the risk of peptic ulcer and gastric cancer in Western[a] isolates

Disease	Parameter	OR	95% CI	*P* value
Gastric ulcer	*jhp0945*	2.18	1.16–4.10	0.02
	cagA	27.69	1.79–428.7	0.02
Duodenal ulcer	*jhp0940*	0.49	0.24–0.99	0.05
Gastric cancer	*vacA* m1	5.06	1.05–24.48	0.04

[a] No significant parameters remained for East Asian strains.

upregulating phosphorylation of NF-κB p65 at Ser276. These observations might suggest that *jhp0940* may play a role enhancing the severity of gastric mucosal inflammation and inflammation-related outcomes such as gastric cancer (7, 9, 12). In support of this hypothesis, Occhialini et al. (16) reported *jhp0940* positivity in 41.2% of isolates from gastric cancer patients versus none of the isolates from patients with gastritis ($P < 0.0006$). However, other studies showed no significant relationship between the presence of *jhp0940* and any clinical outcomes (23) or even a decreased risk of gastric cancer (20). For example, we previously demonstrated that in Western isolates the presence of *jhp0940* was associated with a significant negative association with gastric ulcer or duodenal ulcer. The prevalence of *jhp0940* status was also found to be low in areas of high gastric cancer incidence, such as East Asian and Latin America, compared with low-incidence areas, such as Africa, South Asia, and Europe (19, 20, 23). These recent results might suggest that *jhp0940* has a preventive effect on gastroduodenal diseases. At best, one can surmise that no consistent effect of *jhp0940* has yet been demonstrated.

Previous studies of the prevalence of *jhp0945* status were limited (Table 8). Small studies using fewer than 20 isolates from Turkey, Costa Rica, and Netherlands found that the prevalence of *jhp0945* status was similar between *H. pylori* from patients with peptic ulcer and that from patients with gastritis (6, 16, 22). In this study, we used about 300 *H. pylori* isolates and showed a significant association between *jhp0945*-positive isolates and gastric ulcer, duodenal ulcer, and gastric cancer (OR, 1.86 to 2.27) compared with *jhp0945*-negative isolates cultured from Western patients. In East Asian isolates, there was a significant correlation with *jhp0945* status and gastric ulcer. Because *jhp940* status had no significant association with *cagA* status or with *vacA* s, m, and i region genotypes, the overall conclusion from our study is that among genes we examined, *jhp0945* was the best candidate for a disease marker, especially in Western isolates.

In previous studies, *jhp0947* was found to be homologous to *jhp0938* (*hp0990*) and *jhp253* (*hp1333*), which all encode hypothetical proteins, and was considered to be the most sensitive *H.*

TABLE 8 Literature survey of plasticity region genes

Gene	Source	Yr	Disease[a]	No. isolated	No. (%) of positive patients
jhp0940	Occhialini et al. (16)	2000	Gastritis	26	0 (0%)
			GC	17	7 (41%)
	Santos et al. (23)	2003	Gastritis	68	1 (2%)
			DU	53	1 (2%)
			GC	79	1 (1%)
	Yakoob et al. (30)	2010	Gastritis	36	14 (39%)
			GU	22	17 (77%)
			GC	29	22 (76%)
			DU	27	18 (67%)
jhp0945	de Jonge et al. (6)	2004	Gastritis	26	11 (42%)
			DU	19	7 (39%)
jhp0947	Occhialini et al. (16)	2000	Gastritis	26	9 (35%)
			GC	17	11 (65%)
	Santos et al. (23)	2003	Gastritis	68	13 (44%)
			DU	53	42 (79%)
			GC	79	68 (86%)
	de Jonge et al. (6)	2004	Gastritis	26	5 (19%)
			DU	19	10 (53%)
	Proenca Modena et al. (17)	2007	Gastritis	39	11 (38%)
			GU	24	15 (63%)
			DU	22	14 (64%)
	Yakoob et al. (30)	2010	Gastritis	36	13 (39%)
			GU	22	8 (36%)
			GC	29	22 (76%)
			DU	27	23 (85%)
	Siavoshi et al. (24)	2011	Gastritis	143	83 (58%)
jhp0949	de Jonge et al. (6)	2004	Gastritis	26	5 (19%)
			DU	19	10 (53%)
	Lehours et al. (15)	2004	Gastritis	39	14 (36%)
			ML	43	17 (40%)

[a] Abbreviations: DU, duodenal ulcer; GC, gastric cancer; GU, gastric ulcer; ML, malignant lymphoma.

pylori-related-disease plasticity region marker (16, 23). Although *jhp0917* and *jhp0918* (*dupA*) are known to be homologous with *virB4* as *cag* PAI markers in the plasticity zone, the 5′ region of *jhp0947* is also homologous to that of *jhp0477* (*hp0528*), which is part of the *cag* PAI (*virB9* homologue) and has been identified as an important structural component of the type IV secretion system (16, 23). In 2000, Occhialini et al. (16) reported that *jhp0947* was found more frequently in isolates from gastric cancer patients (64.7%) than in those from gastritis patients (34.6%) (16). Moreover, Santos et al. (23) reported that in multivariate analysis the presence of the *jhp0947* remained associated only with gastric cancer (OR, 2.94; 95% CI, 1.86 to 4.64) and with duodenal ulcer disease (4.84, 2.13 to 10.96). The *cagA* and *jhp0947* genes were independently associated with development of duodenal ulcer, and among the 140 *H. pylori* strains harboring *jhp0947*, 127 (90.7%) were also *cagA* positive (16). The presence of *cagA* and *jhp0947* in the *H. pylori* strains was associated with the severity of gastritis in the subset of patients without duodenal ulcer or gastric cancer (16). Moreover, Yakoob et al. (30) reported that *jhp0947* was significantly associated with chronic active inflammation, and we also showed that the *jhp0947* status was associated with a significantly increased risk of corpus mucosal atrophy. The presence of *jhp0947* was completely linked with that of *jhp0949* and was roughly associated with that of *jhp0945* (6). Disruption of the *jhp0945/jhp0947/jhp0949* genes, such as in *H. pylori* 26695, significantly decreased IL-12 production from mononuclear cells (THP-1 cells) *in vitro*; IL-12 directs native T cell development into inflammatory T1 cells (6), and prolonged activation of Th1 cells might result in severe tissue damage. However, this study and other reports have not confirmed that *jhp0947* and *jhp0949* status is associated with peptic ulcer and gastric cancer (17). Importantly, the prevalence of *jhp0947* in East Asian isolates was only 5.5%. Moreover, there was no association with *jhp0947* and *jhp0949* status and histological evaluation of inflammatory cell infiltration and atrophy in Brazil (23), Colombia, the United States, or East Asian countries. Overall, these data are not consistent with the notion that *jhp0947* and *jhp0949* constitute a sensitive plasticity region marker for *H. pylori*-related diseases in East Asia.

It is well known that atrophic gastritis is positively associated with both gastric ulcer and gastric cancer whereas antral predominant gastritis is associated with development of duodenal ulcer. The high-activity and high-producer genotype of *H. pylori* virulence factors and the *cagA*-positive, *vacA* s1, m1, and i1 genotypes have been associated with enhanced gastric mucosal inflammation and mucosal atrophy (18). Although the 90.7% of *H. pylori* isolates with the *jhp0947* status in Brazil were reported to be associated with *cagA* (23), this relationship was not observed in Indian (19), Dutch (6), or other Brazilian groups (17). In this study, we showed that the *jhp0940*, *jhp0945*, *jhp0947*, and *jhp0949* status had no significant associations with *vacA* s1, m1, and i1 genotypes or with *cagA* positivity in either Western or East Asian populations. We found that in multivariance analysis *jhp0940* and *jhp0945* status was related to peptic ulcer and gastric cancer and to gastric mucosal inflammation and atrophy, suggesting it may be a new virulent marker for *H. pylori*-related diseases. However, none of the associations were significant.

In conclusion, we concluded that the *jhp0940* and *jhp0945* genotype was a marker of peptic ulcer and gastric cancer development irrespective of severe inflammation and gastric mucosal atrophy, as is the current genotyping of *vacA* s, m, and i regions and *cagA* status.

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