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Duodenal Ulcer Promoting Gene of *Helicobacter pylori*

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Abstract

Background & Aims—Identification of a disease-specific *H pylori* virulence factors predictive of the outcome of infection remains unachieved.

Methods—We used the polymerase chain reaction and Southern blot to compare the presence of 14 *vir* homologue genes with clinical presentation of *H pylori* infection, mucosal histology, and mucosal interleukin (IL)-8 levels.

Results—We examined 500 *H pylori* strains from East Asia and South America, including 120 with gastritis, 140 with duodenal ulcer (DU), 110 with gastric ulcer (GU), and 130 with gastric cancer. Only 1 gene that encompassed both *jhp0917* and *jhp0918* called *dupA* (duodenal ulcer promoting gene) was associated with a specific clinical outcome. *dupA* was present in 42% of DU vs. 21% of gastritis (adjusted odds ratio [OR] = 3.1, 95% confidence interval [CI]: 1.7–5.7). Its presence was also associated with more intense antral neutrophil infiltration and IL-8 levels and was a marker for protection against gastric atrophy, intestinal metaplasia, and gastric cancer (OR for gastric cancer = 0.42, 95% CI: 0.2–0.9 compared with gastritis). In vitro studies in gastric epithelial cells using *dupA*-deleted and -complemented mutants showed that the *dupA* plays roles in IL-8 production, in activation of transcription factors responsible for IL-8 promoter activity, and in increased survivability at low pH.

Conclusions—*dupA* is a novel marker associated with an increased risk for DU and reduced risk for gastric atrophy and cancer. Its association with DU-promoting and -protective effects against atrophy/cancer was evident in both Asian and Western countries.

The outcome of a *Helicobacter pylori* infection is thought to reflect an interplay between the virulence of the infecting strain, host genetics, and environmental factors. Although a number of putative *H pylori* virulence genes such as the *cag* pathogenicity island (PAI), *VacA*, *BabA*, *OipA*, and *HrgA* have been associated with increased risks of a clinical outcome such as peptic ulcer or gastric cancer, none can be clearly linked to 1 specific *H pylori*-related disease (eg, duodenal ulcer).^{1–12} It is known that different patterns of gastritis are associated with different clinical outcomes.^{13,14} For example, antral-predominant gastritis is associated with hyperchlorhydria and duodenal ulcer (DU), whereas corpus gastritis leads to hypochlorhydria and gastric atrophy and an increased risk of distal gastric cancer.^{15,16}

Comparison of the sequences of the whole genomes of 2 *H pylori* strains (26695 and J99) revealed several regions whose G + C content differed from that of *H pylori*, suggesting the presence of acquired DNA within the *H pylori* genome.^{17–19} One such region is the *cag*

PAI. The other has been termed the “plasticity region,” based on the variability in gene content among different isolates (approximately 3% to 4% of the chromosome).^{17–19} Genome sequence comparisons indicated that nearly half of *H pylori*’s strain-specific genes are located in the plasticity region. There has been considerable recent interest in this plasticity region.^{20–23} For example, Occhialini et al found that the *jhp0940* and *jhp0947* genes in the plasticity region were more likely to be found in *H pylori* from gastric cancer patients from a small group of 17 gastric cancer and 26 gastritis patients from Costa Rica.²⁰ A follow-up to test the hypothetical relationship was done in 200 strains of *H pylori* from Brazil, including stains from DU, gastric cancer, and *H pylori* gastritis patients. The original hypothesis was not confirmed, but the new study suggested a relation between *jhp0947* (not *jhp0940*) and both DU and gastric cancer.²¹ More recently, de Jonge et al reported that the presence of the *jhp0947* and *jhp0949* genes (which are linked) was significantly associated with DU disease when compared with gastritis from a small group of 26 duodenal ulcer and 19 gastritis patients from Dutch population.²² Kersulyte et al recently reported a novel 16.3-kb segment (*tfs3*) in the plasticity region, 7 of whose 16 open reading frames were homologues of the type IV secretion genes.²³ Full-length and partial *tfs3* elements were each found in about one fifth of *H pylori* strains. Overall, these data suggest that some genes or combination of genes in the plasticity region may play critical roles in the pathogenesis of *H pylori*-associated gastroduodenal diseases.

H pylori contains a number of *vir* genes homologues.^{17,18} For example, the *cag* PAI contains 7 *vir* genes homologues: *virB4*, *virB7*, *virB8*, *virB9*, *virB10*, *virB11*, and *virD4* of the so-called VirB/D complex of type IV secretion systems known from diverse bacteria such as *Agrobacterium tumefaciens* and *Bordetella pertussis*.^{24–28} Mutation studies suggest that VirB homologues in the *cag* PAI are involved in the induction of proinflammatory cytokine interleukin (IL)-8 and activation of transcription factors such as nuclear factor (NF)- κ B in gastric epithelial cells.^{24,26,29,30} In this study, we examined the relationship between the presence of *vir* homologues and clinical outcomes and histologic changes in patients with different clinical presentations of *H pylori* infection. We show that the *jhp0917* and *jhp0918* genes (VirB4 homologue genes), which are present in the plasticity region, form 1 continuous gene. The gene is a marker for development of DU diseases and for protection against gastric atrophy, intestinal metaplasia, and gastric cancer. We have tentatively designated *jhp0917-jhp0918* as the DU-promoting (*dupA*) gene of *H pylori*.

Materials and Methods

H Pylori Studied

For the study of the possible relation between *vir* homologues and clinical outcome in *H pylori* infection, we examined a large number of *H pylori* from different geographic regions to reduce the likelihood of spurious associations based on examining small numbers or restricted regional circulation of a particular genotype. We examined *H pylori* obtained from patients in East Asia (Kyoto Prefectural University of Medicine, Kyoto, Japan, and Guro Hospital, Korea University College of Medicine, Seoul, Korea) and from South America (Universidad Nacional de Colombia Bogotá, Colombia). We chose 4 different clinical presentations: simple *H pylori* gastritis, DU disease, gastric ulcer disease (GU), and noncardiac gastric adenocarcinoma. DU and GU were identified endoscopically; patients with ulcer scars were included. We excluded GU cases with DU. Gastritis was defined as histologic gastritis with no peptic ulcers or gastric cancer. No subjects had received treatment for *H pylori* infection.

H pylori isolates were chosen from those collected between 1997 and 2002. Thirty-seven percent (187 of 500) of *H pylori* had been used in our previous study for genotyping *iceA*, *cagA*, *vacA*, and/or *hrgA* genes.^{12,31} In addition, to assess the relation to gastric mucosal

IL-8 levels, we used 98 strains from Japanese patients whose mucosal IL-8 levels in gastric biopsy specimens had been measured previously.³² None of these 98 patients were included among the 500 patients in the disease-association study. Informed consent had been obtained from all patients, and samples were taken under protocols approved by each local ethics committee.

Histology

Gastric mucosal biopsy specimens were fixed in 10% buffered formalin, embedded in paraffin, cut in sequential 4- μ m sections, and stained with Genta stain³³ or El-Zimaity triple stain³⁴ (Korea and Colombia) or modified Giemsa stain (Japan). We examined sections unaware of the patient's clinical diagnosis or the characteristics of the *H. pylori* strain. Each specimen was scored for *H. pylori* density, neutrophil infiltration, intestinal metaplasia, and atrophy. All the variables were graded using the visual analogue scale graded from 0 (absent/normal) to 5 (maximal intensity), as described previously.³⁵

Analysis of *vir* Homologues

Chromosomal DNA 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. We examined all 14 *vir* homologue genes from strain 26695 and/or J99: 7 genes in the *cag* PAI (*jhp0473/hp0524*: *virD4* homologue, *jhp0474/hp0525*: *virB11* homologue, *jhp0476/hp0527*: *virB10* homologue, *jhp0477/hp0528*: *virB9* homologue, *jhp0479/hp0530*: *virB8* homologue, *jhp0481/hp0532* [*cagT*]: *virB7* homologue and *jhp0492/hp0544* [*cagE*]: *virB4* homologue), 4 in the plasticity region (*hp0441*, *hp0459*, *jhp0917*, and *jhp0918*: *virB4* homologues), and 3 genes outside of the *cag* PAI and the plasticity region (*jhp0015/hp0017*: *virB4* homologue [*comB4*], *hp1006*: *virD4* homologue, and *jhp1316/hp1421*: *virB11* homologue). Primers for *jhp0473/hp0524*, *jhp0474/hp0525*, *jhp0476/hp0527*, *jhp0477/hp0528*, *cagT*, and *cagE* had been reported previously.^{36,37} Primers for other genes are presented in Table 1. When the polymerase chain reaction (PCR) yielded negative results, Southern blot hybridization was performed to confirm the accuracy of PCR methods as previously described.³⁶ In the case of a given region detected only by hybridization and not by PCR, the isolate was considered positive for this region.

Recently, Kersulyte et al reported a novel 16.3-kb segment (*tfs3*) in the plasticity region, 7 of whose 16 ORFs were *vir* homologues (*virB4*, *virB7* to *virB11*, and *virD4*).²³ Therefore, we also examined the presence of *tfs3* by PCR as previously described.²³ *H. pylori* 26695 (ATCC700392: American Type Culture Collection, Rockville, MD) and J99 (ATCC700824) were used as reference *H. pylori* strains.

Construction of Gene Deleted Strains and Complemented Strains of *H. pylori*

For construction of the whole *jhp0917-jhp0918*-deleted mutants, we used gene replacement mutagenesis techniques. PCR fragments containing the most downstream fragment of the *jhp0916* and the most upstream of the *jhp0919* were cloned into the plasmid (pT7Blue; Novagen) resulting in pTJHP916 and pTJHP919, respectively. A chloramphenicol (*cat*) cassette (a gift from D. E. Taylor, University of Alberta, Edmonton, Canada) was inserted at the *Bam*HI site of pTJHP916 resulting in pTJHP916::*cat*. Finally, the blunt fragment of *jhp0916-cat* (*Hinc*II/*Sma*I) was inserted at the *Hinc*II site of pTJHP919, resulting in pTJHP916/919::*cat*. The purified plasmids were used to inactivate chromosomal *H. pylori* genes by natural transformation as previously described.³⁸ Inactivation of the genes was confirmed by PCR amplification and/or Southern blot analyses (data not shown).

For construction of the *jhp0917-jhp0918*-complemented mutants, PCR fragments containing *jhp0917-jhp0918* with the promoter of *jhp0917* were cloned into the plasmid (pBlueScript;

Stratagene, La Jolla, CA). The *Bam*HI/*Hinc*II fragment was cut from the plasmid and cloned into the *Bam*HI/*Nru*I sites of *H. pylori*/*Escherichia coli* shuttle vector (pHel3)³⁹ (a gift from R. Haas, Max von Pettenkofer Institut, München, Germany) to generate the complementation construct pHel3-JHP917-918. The purified plasmid was used to introduce the gene into the *jhp0917-jhp0918*-deleted mutants with selection onto kanamycin and chloramphenicol. The plasmids were extracted from the complemented strains and subjected to restriction analyses to ensure their integrity. Transformants harboring intact plasmids were selected for further studies. The *jhp0917-jhp0918*-deleted mutants introduced by the empty shuttle vector (pHel3) were used as control.

IL-8 Levels From Gastric Cancer Cells Cocultured With *H. pylori*

In vitro IL-8 secretion from gastric epithelial cells was examined as previously described.³² Briefly, gastric epithelial cell line, MKN45 cells (Japanese Cancer Research Resource Bank, Tsukuba, Japan) (approximately 5×10^5 /mL) were plated into 24-well plates and cultured for 2 days (approximately 1×10^6 /mL for each). Experiments were performed in duplicate for clinical samples and were performed at least 3 times for wild type and its isogenic mutants. *H. pylori* (multiplicity of infection [MOI] of 100) or brain heart infusion (BHI) broth (control) were added to the cultured cells for 20 hours, and IL-8 in the supernatant was assayed by an enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN) in duplicate.

Luciferase Reporter Gene Assay

The PathDetect cis-reporting plasmids pISREluc, pNF- κ B_{luc}, pAP-1_{luc}, and pCRE_{luc}, which contain the luciferase reporter gene driven by the TATA box plus tandem repeats of the consensus-binding sequence for the corresponding transcription factor (interferon-stimulated responsive element [ISRE], NF- κ B, activator protein [AP]-1, and cAMP responsive element [CRE]), respectively, were obtained from Stratagene. Luciferase reporter gene assay was performed as previously described⁴⁰ in duplicate for clinical samples and was performed at least 4 times for wild type and its isogenic mutants. Luciferase activity was normalized to control renilla luciferase activity and expressed as firefly luciferase activity/renilla luciferase activity (normalized luciferase activity).

Protein/DNA Array for Different Components of the Signal Transduction System

Recently, a protein/DNA array that provides a profile to the DNA-binding activity of multiple transcription factors in a single array experiments has been introduced (TranSignal Arrays, Panomics, Inc., Redwood City, CA). The basic steps involved include the following: (1) A set of biotin-labeled DNA-binding oligonucleotides (TranSignal Probe Mix) are preincubated with a nuclear extract of interest to allow the formation of DNA/protein complexes; (2) the protein/DNA complexes are separated from the free probes; and (3) the probes in the complexes are then extracted and hybridized to the TranSignal Array. Each kit includes the reagents for HRP-based chemiluminescence detection. This array allows 54 transcription factors to be identified simultaneously. MKN45 cells (approximately 5×10^5 /mL) were plated into 10-cm²-well plates and cultured for 2 days (approximately 1×10^6 /mL for each). *H. pylori* (MOI of 100) or BHI broth (control) was added to the cultured cells for 90 minutes, and nuclear extracts of gastric cells were prepared using hypotonic/nonionic detergent lysis. An equal amount of protein was used for protein/DNA array according to the manufacturer's instruction. Assays were performed in triplicate. Quantitation of the activity of the transcription factors was determined using Scion Image beta 4.02 (Scion Corp., Frederick, MA).

Survival of *H Pylori* After Exposure to Low pH

Susceptibility of *H pylori* to low pH was measured as described previously in duplicate for clinical samples and at least 4 times for wild type and its isogenic mutants.⁴¹ Briefly, *H pylori* (approximately 10⁸ CFU/mL) were grown in BHI broth adjusted to pH 6 with citrate-phosphate buffer (CPB) and supplemented with 5% horse serum on a rotating shaker for 24 hours. We preexposed *H pylori* to pH 6 based on previous studies showing that *H pylori* has an acid-tolerance response, and *H pylori* adapted at pH 6 survived exposure to pH 3 approximately 100-fold better than did unadapted cells (exposed to pH 7).⁴² *H pylori* were then centrifuged at 2000g, resuspended in CPB at low pH (3 to 5), and incubated at 37°C for 20 minutes. All buffer solutions were urea free to ensure that any effect was independent of urease enzymatic activity. Viable counts before and after acid stress was assessed by serial 10-fold dilution in BHI broth onto BHI agar plates for 3–5 days. Difference in viable counts before and after exposure to acid stress was presented as log10 kill. Intraassay variation in our laboratory was within 10%.

Data Analysis

For univariate analysis, Fisher exact test, χ^2 test, and Mann-Whitney rank sum test were used, depending on the data set of concern. A multiple logistic regression analysis was performed to determine which *vir* homologue factor(s) was the most discriminating for clinical outcome, where *vir* homologues, age, and sex were explanatory variables. Multiple linear regression analyses were used for the histologic data because the data showed normal distributions. In the analyses, *vir* homologue factors, sex, age, and clinical outcomes were included as explanatory variables, and mutually adjusted associations with the criterion variables were calculated. Selections of variables were by backward stepwise deletion in the logistic regression and by F-out and F-in stepwise method in the linear regression, where F values were both 2.0. A *P* value of less than .05 was accepted as statistically significant. Calculations were carried out using statistical software “HALBAU” (Gendai-sugaku-sha, Kyoto, Japan).

Results

Prevalence of the *vir* Homologue Genes and Clinical Outcomes

We examined 500 *H pylori* isolates for the 14 known *vir* homologue genes previously detected within the *H pylori* genome. Specimens were obtained from 294 men and 206 women with a mean age of 52.4 years. There were 160 subjects from Japan (presentations included 50 gastritis, 30 DU, 50 GU, and 30 gastric cancer), 175 from Korea (presentations included 30 gastritis, 65 DU, 30 GU, and 50 gastric cancer), and 165 from Colombia (presentations included 40 gastritis, 45 DU, 30 GU, and 50 gastric cancer).

Tables 2 and 3 show the association among the prevalence of the 14 *vir* homologue genes in relation to clinical presentation. Independent univariate analysis showed that the prevalence of the *jhp0917* and *jhp0918* genes (*virB4* homologue genes) in the plasticity region was significantly greater among strains isolated from patients with DU (42%) than from patients with non-DU *H pylori* gastritis (21%), with GU (27%) or with gastric cancer (9%) (*P* < .001 for DU vs. gastritis or cancer (*P* < .05 for GU vs. cancer). The prevalence of the *jhp0917* and *jhp0918* genes was closely linked, with only 10 of 500 (2%) strains being *jhp0917* negative/*jhp0918* positive (5 from DU, 3 from gastritis, and 2 from gastric cancer), and the status of the 2 genes in the remaining strains was identical (both positive or both negative). We therefore categorized strains as either genotype positive or negative based on the combination of the *jhp0917* and *jhp0918* genes (*jhp0917-0918*). Ten cases were *jhp0917* negative and *jhp0918* positive and were excluded for further analyses.

None of the other genes examined were related to clinical outcome (Table 3). However, there was a trend for the prevalence of an intact *cag* PAI with all 7 *vir* homologue genes in the island being positive to be greater among strains from gastric cancer patients (94%) compared with those from gastritis patients (80%) ($P = .06$). Only 7 of 500 strains (1%) possessed a partially deleted *cag* PAI, and they were excluded for further analyses. The *cag* PAI status was then categorized into 2 types: positive for all 7 *cag* PAI genes (intact *cag* PAI) or *cag* PAI negative. The *hp0441* gene was very rarely present (3%). All strains studied possessed the *jhp1316/hp1421* gene.

Multiple logistic regression analysis was performed for 14 *vir* homologue genes, adjusting for age, sex, and country of origin. As shown in Table 4, only the presence of the *jhp0917-0918* genes was related to the clinical outcomes. The presence of the *jhp0917-0918* genes was an independent determinant predictor of DU and was protective against gastric cancer (DU vs. gastric cancer; OR = 8.3; 95% CI: 4.0–17.4). Importantly, this pattern was similar in all 3 geographic regions examined, confirming that the observation was not a reflection of restricted regional circulation of a particular genotype. In Colombia, the presence of the *jhp0917-0918* genes was also an independent determinant protective (or linked to another protective factor) against gastric cancer vs. gastritis and GU (Table 4).

We also examine the presence of *tfs3* in 24 strains (12 *jhp0917-0918* positive and 12 *jhp0917-0918*-negative) for each country. Complete *tfs3* was detected in 8 (33%) of Colombian strains, 2 (8%) of Japanese strains, and 3 (13%) of Korean strains. The presence of complete *tfs3* was not linked to other *vir* homologue genes (eg, 7 [19%] of the *jhp0917-0918*-positive strains and 6 [17%] of the *jhp0917-0918*-negative strains possessed complete *tfs3*). The presence of partial *tfs3* was also not linked to other *vir* homologue genes, except for that of *hp0459* (*hp0459* is a member of *tfs3*) (data not shown).

Prevalence of the *vir* Homologue Genes and Gastric Mucosal Histology

Gastric mucosal biopsy specimens were analyzed histologically and scored for *H. pylori* density, neutrophil infiltration, intestinal metaplasia, and atrophy. All the variables were graded using the visual analogue scale graded from 0 (absent/normal) to 5 (maximal intensity), as described previously.³⁵ Histologic analyses were made using biopsy specimens from normal-appearing gastric mucosa of Japanese and Colombian patients. Twenty-one Colombian patients (11 from gastric cancer, 6 from GU, 2 from DU, and 2 from gastritis) were excluded from these analyses because we had only 1 biopsy sample in either antrum or corpus because gastric cancer was widely spread in the antrum or corpus.

Independent univariate analysis showed that the presence of the *jhp0917-0918* genes was positively related to neutrophil infiltration and inversely related to intestinal metaplasia and atrophy (Table 5). When we examined each disease separately, similar tendency was observed in each disease in both countries (Figure 1). The effect of the *cag* PAI was restricted to biopsy specimens from Colombia because *cag* PAI-negative strains are rarely found in East Asia. Scores for gastric mucosal atrophy were significantly higher in patients with infection with intact *cag* PAI compared with PAI-negative cases (Table 5). No other genes examined were related to changes in gastric histology (data not shown).

A backward stepwise multiple linear regression analysis showed the similar results for determining the *vir* factors related to the severity of histology (Table 6). For these analyses, we used the *vir* factors as well as age, sex, and clinical outcomes as explanatory variables. The presence of the *jhp0917-0918* genes was significantly associated with severe neutrophil infiltration and with reduced intestinal metaplasia and atrophy, confirming that the *jhp0917-0918* genes were markers for DU, which typically is associated with severe antral inflammation and high acid secretion. Importantly, these patterns were confirmed in both

Japan and Colombia (Table 6). For example, patients infected with the *jhp0917-0918*-positive strains would be expected to have 1.21 ± 0.26 (= partial regression coefficient $\pm$ SE) higher scores for the neutrophil infiltration than those with the *jhp0917-0918*-negative strains. In contrast to the *jhp0917-0918* status, the *cag* PAI-positive status was associated with intestinal metaplasia and atrophy in agreement with previous studies.¹⁰ In fact, no intestinal metaplasia and atrophy were observed in patients infected with the *jhp0917-0918*-positive/*cag* PAI-negative strains ($n = 10$).

In Vivo Mucosal IL-8 Production and the *jhp0917-0918* Status

In a previous study, we measured in vivo gastric mucosal IL-8 levels and *H pylori* density in gastric mucosal biopsy specimens from 98 Japanese patients infected with the *cag* PAI-positive strains (50 from DU and 48 from gastritis patients).³² Antral IL-8 levels were measured from all patients, and corpus IL-8 levels were measured in 35 patients for each disease. We therefore used those data to examine the relationship between mucosal IL-8 production and the *jhp0917-0918* status determined using PCR. The results confirmed that the presence of the *jhp0917-0918* genes was strongly related to the presence of DU because 23 of 50 (46%) strains from DU were *jhp0917-0918*-positive compared with 15% (7 of 48) with simple gastritis ($P < .001$). The presence of the *jhp0917-0918* genes was also strongly related to the cellular inflammation and negatively related to intestinal metaplasia and atrophy (data not shown).

Antral mucosal IL-8 levels were significantly higher in biopsy specimens obtained from DU patients infected with *jhp0917-0918*-positive strains compared with those with *jhp0917-0918*-negative strains (160.9 ± 19 vs. 101.2 ± 14 pg/mg protein, respectively) ($P < .05$) (Figure 2). Adjustment of antral IL-8 levels for *H pylori* density (IL-8 per *H pylori*) also showed a similar tendency (347 ± 10 vs. 258 ± 7 pg/CFU $\times 10^3$, respectively) ($P < .05$). Antral IL-8 levels were also higher in gastritis patients with *jhp0917-0918*-positive infections (IL-8 per *H pylori* = 302 ± 7 vs. 150 ± 19 pg/CFU $\times 10^3$, respectively) ($P < .01$). In contrast, we did not find a relation between IL-8 levels in the corpus and the presence of the *jhp0917-0918* genes in either DU or gastritis patients (Figure 2). These close relationships between the presences of the *jhp0917-0918* genes and high antral IL-8 production are consistent with prior studies showing that DU is typically associated with severe antral inflammation with high antral IL-8 production.³²

Structural Analyses of the *jhp0917-0918* Genes

Sequence analyses (BLAST: <http://www.ddbj.nig.ac.jp/search/blast-e.html>, GTOP: <http://spock.genes.nig.ac.jp/%7Egenome/gtop.html> and Pfam: <http://www.sanger.ac.uk/Software/Pfam/>) shows that neither *jhp0917* nor *jhp0918* genes possess a complete functional *virB4* homologue gene in genome sequence strain J99. The *jhp0917* gene consists of 475 amino acids and lacks most of the 3' end of the *virB4* gene. The *jhp0918* gene consists of 140 amino acids and contains a fragment of the *virB4* gene from the 3' end. We therefore sequenced the *jhp0917-0918* genes in 24 *jhp0917-0918*-positive strains (8 randomly selected from each country). The total size from the 5' end of the *jhp0917* gene to 3' end of the *jhp0918* gene in all clinical isolates was the same as that of strain J99 by PCR; however, all the clinical isolates contains 1 base pair insertion (C or T) in the 3' region of the *jhp0917* gene (after position 1385), resulting in a frameshift leading to a continuous open reading frame (Figure 3). In contrast, J99 strain in our stock showed exactly the same sequences as those deposited in GenBank, forming separate 2 genes: *jhp0917* and *jhp0918* genes. Therefore, we conclude that *jhp0917-0918* is 1 gene homologous to *virB4* gene, with strain J99 being an exception.

The above in vivo data clearly showed that the presence of the *jhp0917-0918* genes were strongly related to the existence of DU and that the prevalence of the *jhp0917* gene and *jhp0918* gene was closely linked to each other. We therefore designated the *jhp0917-0918* as the DU-promoting (*dupA*) gene of *H. pylori*. Full sequences of the *dupA* gene has been deposited in GenBank (accession number: AB196363).

In Vitro IL-8 Production From the Epithelial Cells and *dupA* Status

We examined the relationship between in vitro IL-8 induction from the epithelial cells and *dupA* status. We used a wild-type *dupA*-positive strain, its *dupA*-deleted mutant, and its complemented *dupA* mutant. We selected strain C142 as the *dupA*-positive parental strain, because it contains 13 of the 14 *vir* homologue genes (only *jhp0441* gene is absent), for use to investigate the specific effect of the *dupA* gene among the *vir* homologue genes. Strain C142 was originally isolated from Colombian DU patients, and sequence analyses showed that this strain contained intact *dupA* gene (continuous from the 5' end of the *jhp0917* to 3' end of the *jhp0918*) (GenBank accession number: AB196363). IL-8 levels were significantly higher in supernatants cocultured with C142 wild type than in those with its *dupA*-deleted mutant (mean $\pm$ SE = 3421 ± 175 vs. 2480 ± 124 pg/mL, respectively) ($P < .005$). IL-8 induction was restored using the complemented *dupA* mutant (3354 ± 216 pg/mL), confirming that the *dupA* gene is involved in IL-8 induction (Figure 4). The *dupA*-deleted mutants introduced by empty shuttle vector (control) failed to restore IL-8 production (data not shown).

To confirm the relationship between in vitro IL-8 induction and the *dupA* status, we also examined clinical isolates. Because both the *cag* PAI and *OipA* affect IL-8 induction in vitro,^{38,40} we limited the analysis to the *cag* PAI-positive/*oipA* “on” strains. We selected 26 *dupA*-positive strains and 28 *dupA*-negative strains from among the 500 strains (6 strains with each disease from each country). IL-8 levels from MKN45 cells were significantly higher in supernatants cocultured with *dupA*-positive strains than in those with *dupA*-negative strains (mean $\pm$ SE = 3207 ± 123 vs. 2681 ± 118 pg/mL, respectively) ($P < .001$) (Figure 4).

Relationship Between Gene Promoter Activation and *dupA* Status

Transcription of genes is regulated by transcription factors that bind to their promoter. For example, we previously reported that full activation of the IL-8 promoter in *H. pylori* infection required binding sites for NF- κ B, AP-1, and ISRE-like elements.⁴⁰ Full activation of IL-6 and RANTES promoters, which are also important inflammatory cytokine and chemokine, in *H. pylori* infection required binding sites for CRE-binding protein (CREB) (unpublished observation). The luciferase reporter gene assay was performed to investigate the relationship between the *dupA* status and gene promoter activation by the ISRE, NF- κ B, AP-1, and CREB. When we used the wild-type strain (C142) and its *dupA*-deleted mutants, luciferase activity for ISRE, AP-1, and CRE was significantly higher in cases cocultured with the wild-type *H. pylori* compared with those with the *dupA*-deleted mutants (fold induction: 3.6 ± 0.2 vs. 1.8 ± 0.1 , respectively [$P < .001$], for ISRE; 4.6 ± 0.2 vs. 2.8 ± 0.2 , respectively [$P < .001$], for AP-1; and 2.1 ± 0.1 vs. 1.4 ± 0.1 , respectively [$P < .05$] for CREB) (Figure 5A). The *dupA*-complemented mutants restored the activity for these 3 factors (fold induction: 3.8 ± 0.2 for ISRE, 4.9 ± 0.2 for AP, and 2.4 ± 0.1 for CREB). The *dupA*-deleted mutants introduced by empty shuttle vector (control) did not show restoration (data not shown). The *dupA*-positive wild-type *H. pylori* also induced higher luciferase activity for NF- κ B compared with the *dupA*-deleted mutants, although the differences were not statistically significant (fold induction: 6.4 ± 0.3 vs. 5.5 ± 0.2 , respectively). Luciferase activity for all 4 factors was similar between strain J99 and its *jhp0917-0918*-deleted mutant

(data not shown), indicating that *jhp0917* region of the *dupA* gene is not involved in activation of these transcription factors.

We next used the clinical isolates that are the same strains as used for in vitro IL-8 levels as shown above. The *dupA*-positive *H pylori* induced luciferase activity for all 4 factors significantly higher compared with the *dupA*-negative strains (Figure 5B). These data confirmed that the *dupA* genes are involved in the activation of transcription factors responsible for the IL-8 promoter.

Protein/DNA Array for Different Components of the Signal Transduction System

Using the novel protein/DNA array, we assessed relationship between levels of activated transcription factors and *dupA* status. Wild-type *H pylori* C142 produced more than a 2-fold induction of 34 of 54 transcription factors evaluated compared with uninfected control (Table 7). Twelve transcription factors were activated in both wild-type strains and *dupA*-deleted mutants (eg, YY1, Ets-1/PEA3), whereas 22 transcription factors were activated only by wild-type strains (eg, AP-1, CREB, p53). Results for ISRE, AP-1, and CREB were in agreement with the above results using luciferase reporter gene assay. The accuracy of the protein/DNA array was further confirmed by using electrophoretic mobility shift assay for NF- κ B, AP-1, CREB, and ISRE (unpublished observation).

Susceptibility of *H pylori* to Low pH

The above in vivo experiments clearly show that the *dupA* status was associated with intestinal metaplasia and atrophy. Because intestinal metaplasia and atrophy were related to acid secretion, we evaluated the acid tolerance. We used wild-type strain (C142) and its *dupA*-deleted mutant and *dupA*-complemented mutant. Viable counts of *H pylori* grown at pH 6 for 24 hours were approximately 10^{10} CFU/mL, irrespective of the strains. We exposed *H pylori* to pH 3 BHI broth for 20 minutes after preexposure of pH 6 BHI broth for 24 hours. The *dupA* deleted mutant was more susceptible to pH 3 BHI broth than wild-type strains in 5 independent experiments ($\log_{10}$ killing = 6.6 ± 0.1 vs. 5.8 ± 0.1 , respectively) ($P < .001$), and the *dupA* complemented mutant had susceptibility similar to the wild type (6.8 ± 0.1) ($P < .001$ compared with *dupA*-deleted mutant) (Figure 6). When *H pylori* was exposed to pH 4 BHI broth for 20 minutes, similar results were observed (Figure 6). In contrast, most strains survived exposure of pH 5 BHI broth, irrespective of the strain used (data not shown). The *cag* PAI-deleted knockout mutants were not related to acid tolerance (data not shown).

We next examined 54 clinical isolates that were also used for in vitro IL-8 induction. We exposed *H pylori* to pH 3 BHI broth for 20 minutes after pre-exposure of pH 6 BHI broth for 24 hours. The *dupA*-negative strains were significantly more susceptible to pH 3 BHI broth than the *dupA*-positive strains ($\log_{10}$ killing = 6.4 ± 0.9 vs. 6.0 ± 0.9 , respectively; $P < .005$). These findings suggest that the *dupA*-positive strains were resistant to high acid conditions (eg, DU) and were in agreement with the above in vivo data.

Discussion

Although a number of putative virulence factors of *H pylori* had been reported (eg, the *cag* PAI, *vacA*, *babA*, and *oipA*), their presence has typically been associated with an increased risk of both gastric cancer and peptic ulcers.^{1–10} We report an *H pylori* gene (*jhp0917-0918*: *dupA*) whose presence was related to increased risk of DU and with neutrophil infiltration as well as protection against atrophy, intestinal metaplasia, and gastric cancer. This pattern mirrors the clinical observation that the presence of a DU is known to be protective against gastric cancer and may eventually prove to solve that conundrum.^{43,44} To reduce known

causes of bias associated with nonrandom selections of disease or by restricted circulation of a particular strain or strains that may produce spurious associations, we used both a large number of isolates as well as isolates from different geographic regions and clinical presentations.⁴⁵ With the exception of strain J99, the *jhp0917-jhp0918* gene forms 1 continuous reading frame and has been designated as the duodenal ulcer promoting (*dupA*) gene of *H pylori*. The results of the current study were consistent with the *dupA* gene being a marker for duodenal DU disease and also for a protective effect for atrophy, intestinal metaplasia, and gastric cancer. It is unknown whether either or both of these effects are directly related to the gene or whether the gene is a marker for other disease-modifying virulence factors. However, we believe that the results from complementation experiments strengthen our hypothesis that the *dupA* gene plays important roles in development of DU. The effects were consistent in both Asian and Western countries. We propose that, basically, the presence of the *dupA* gene is related to development of a pattern of nonatrophic gastritis. The presence of the *dupA* gene was also associated with increased IL-8 production from the antral gastric mucosa in vivo as well as from the gastric epithelial cells in vitro. We further confirmed that the *dupA* gene is involved in the activation of transcription factors responsible for IL-8 promoter. These results are in agreement with in vivo results showing higher neutrophil infiltration and IL-8 production in the antral gastric mucosa of DU patients compared with other diseases.^{32,46}

However, more than half of all isolates from DU patients did not possess the *dupA* gene. In addition, the prevalence of the *dupA* gene was higher in Colombia compared with East Asia, irrespective of the disease outcomes, consistent with the notion that outcome results from a combination of factors including *H pylori* virulence factors, host genetics (eg, genetic polymorphisms affecting the immune response), and environmental factors (eg, diet or smoking) as well as combinations and interactions among various factors.

Of interest, preliminary in vitro experiments showed that the absence of the *dupA* gene was associated with increased susceptibility to low pH, and this observation was confirmed using the *dupA*-deleted and -complemented mutants. Changes in acid secretion, whether caused by drugs or by surgery, are associated with a change in the ability of *H pylori* to interact with the gastric mucosa.^{47,48} For example, in humans, it is known that antral predominant gastritis in DU patients spreads into the corpus after surgery to reduce acid (eg, selective vagotomy),^{47,49,50} suggesting that the ability of the organism to interact with acid may be an important outcome determinant. Subsequent experiments will be done to identify and characterize the pH-regulated genes that are responsible for the difference between the *dupA*-positive and the *dupA*-deleted mutants and whether there is a requirement for coinducers required in addition to pH stress.⁵¹

The *dupA* gene encodes homologues of VirB4 ATPase. Pfam search shows that *jhp0917* region (position 3–201 of *dupA*) contains CagE_TrbE_VirB domain (Pfam accession number: PF03135) and FtsK/SpoIIIE family (PF01580). The region from the 3' region *jhp0917* to *jhp0918* region (position 203–610) is homologue to TraG/TraD family (PF02534). The FtsK/SpoIIIE domain contains a putative ATP-binding P-loop motif and is involved in cell division and peptidoglycan synthesis or modification and is implicated in intercellular chromosomal DNA transfer.^{52,53} Members of the TraG/TraD family are potential NTP hydrolases that are essential for DNA transfer in bacterial conjugation and are thought to mediate interactions between the DNA processing and the mating pair formation systems.^{54,55} Many *H pylori* contain at least 2 functional, independent type IV transport systems: the secretion system for effector proteins such as CagA (*cag* PAI system) and the DNA import system via natural transformation (ComB system).^{28,56–58} VirB4-homologous ATPases are contained within both systems (*jhp0492/hp0544* [*cagE*] and *jhp0015/hp0017* [*comB4*], respectively). Such ATPases typically provide energy for the correct assembly of

the secretion apparatus or the delivery of the effector molecules. However, in *A. tumefaciens*, it has been suggested that the oligomeric structure of VirB4, but not its capacity to bind ATP, is important for the assembly of VirB proteins as a DNA uptake system.⁵⁹ It remains to be shown whether ATPase activity is necessary for the function of the *dupA* VirB4-homologues in type IV secretion system. The details of the relation between the *dupA* and type IV secretion remain to be elucidated, particularly because a search for genes in the plasticity region in strain J99 did not find partner genes with the *dupA* genes as components of a type IV secretion system. Kersulyte et al recently reported a new 16.3-kb segment (*tfs3*) located in the plasticity region in which 7 of the 16 ORFs were homologues of type IV secretion genes (*virB4*, *virB7* to *virB11*, and *virD4*), the third putative type IV secretion system found in *H. pylori*.²³ However, we did not find any linkage between the presence of the *dupA* gene and full-length or partial *tfs3* elements, which is in agreement with their report that the presence of the *tfs3* elements was independent of clinical diseases.²³ The function of the *dupA* gene is unknown; however, it might be possible that the *dupA* gene in combination with other novel *vir* homologue genes in the plasticity region might form a type IV secretion system similar to the *cag* PAI, which is also related to IL-8 secretion.

Preliminary data examining the effect of the presence of the *dupA* gene on transcription factors showed that activation of transcription factors for tumor suppressor gene p53 was decreased and activation of the YY1 was increased using the *dupA*-deleted mutants (Table 7). This pattern, shown in the *dupA*-deleted mutants, has been reported to be related to cancer development, with YY1 inhibiting activation of the p53 via binding competitively to the p53-binding site that contains the ACAT sequence.^{60–62} The activation of the Ets-1/PEA3 was induced by the absence of the *dupA* gene (Table 7). Ets-1/PEA3 is an oncogene and has been used as a marker of malignant potential in gastric cancer⁶³ and also plays an important role in angiogenesis in the early stage of ulcer healing.⁶⁴ Additional studies in vitro and confirmatory studies in vivo are needed to put these in vitro observations into a clinical context.

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Abbreviations used in this paper

AP-1	activator protein-1
BHI	brain heart infusion
CRE	cAMP responsive element
CREB	CRE binding protein
CPB	citrate-phosphate buffer
DU	duodenal ulcer
ELISA	enzyme-linked immunosorbent assay

GU	gastric ulcer
IL	interleukin
ISRE	interferon-stimulated responsive element
MOI	multiplicity of infection
NF	nuclear factor
PAI	pathogenicity island
PCR	polymerase chain reaction

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Figure 1.

Univariate analysis showing the relationship between the *jhp0917-0918* gene (*dupA*) and the gastric mucosal histology. The scores (0: absent/normal to 5: maximal intensity) for neutrophil infiltration, intestinal metaplasia, and atrophy scores are presented separately in relation to clinical presentation in 1 East Asian and 1 Western country (Japan and Colombia, respectively). The strains from Japanese patients included 34 *dupA*-positive strains (7 from gastritis, 11 from DU, 13 from GU, and 3 from gastric cancer) and 124 *dupA*-negative strains (42 from gastritis, 19 from DU, 37 from GU, and 26 from gastric cancer). Strains from Colombia included 50 *dupA*-positive strains (14 from gastritis, 21 from DU, 9 from GU, and 6 from gastric cancer) and 87 *dupA*-negative strains (22 from gastritis, 17 from DU, 15 from GU, and 33 from gastric cancer). The data are expressed as mean $\pm$ standard error.

Figure 2.

In vivo gastric mucosal IL-8 levels and the *jhp0917-0918* status. Mucosal IL-8 levels from biopsy specimens measured by ELISA are presented on the *left panel*, and adjusted IL-8 levels for *H. pylori* density (IL-8 per *H. pylori*) are presented on the *right panel*. IL-8 in the supernatant was assayed in duplicate. Data are expressed as mean $\pm$ standard error.

Figure 3. Schematic structure of the *jhp0917* gene and *jhp0918* gene in strain J99 and that of the *dupA* gene in the clinical isolates. All 24 clinical isolates examined contains “C or T” insertion after position 1385 to produce 1 continuous open-reading frame homologue to the *virB4* gene. We denote the gene containing the sequences of the *jhp0917* gene and *jhp0918* as the duodenal ulcer-promoting (*dupA*) gene.

Figure 4.

In vitro IL-8 production from MKN45 cells cocultured with wild-type strain C142 and its *dupA*-deleted mutant and *dupA*-complemented mutant and clinical isolates is shown. *H. pylori* (MOI of 100) was cocultured with MKN45 cells for 20 hours. Experiments were performed at least 4 times for the wild type and its isogenic mutants and were performed in duplicate for clinical samples. IL-8 in the supernatant was assayed by an ELISA in duplicate. Data are expressed as mean $\pm$ standard error.

Figure 5.

Shows the luciferase reporter activity of ISRE, NF-κB, AP-1, and CRE associated with *H. pylori* infection. Luciferase reporter gene assay was performed as previously described³⁵ at least 4 times for (A) wild type and its isogenic mutant and was performed in duplicate for (B) clinical samples. Luciferase activity was normalized by renilla luciferase vector DNA (Promega), and normalized luciferase activity (fold induction) is presented. Data are expressed as mean ± standard error of normalized luciferase activity.

Figure 6. Susceptibility of *H. pylori* to exposure to low pH. Log10 kill represents the difference in viable *H. pylori* before and after exposure to low pH. Assays were performed at least 4 times. Data are expressed as mean $\pm$ standard error.

Table 1PCR Primers for Amplification of the *vir* Homologue Genes

Gene	Primer	Primer sequence (5'→3')	Size (bp) of PCR product
<i>jhp0479/hp0530 (virB8)</i>	ORF16 (+)	ATGTCAAACACTTTGAAGCC	574
	ORF16 (–)	CCAATAGGACTTTCAAAAGGGC	
<i>hp0441 (virB4)</i>	HP441 (+)	AGGCGCTCTTAAATTAGAGG	573
	HP441 (–)	TCATGCAAACGACCTCTAGC	
<i>hp0459 (virB4)</i>	HP459 (+)	CTACGCAGAATACATCAATGGC	363
	HP459 (–)	CTCAAACGATTGGCTTGAC	
<i>jhp0917 (virB4)</i>	JHP917 (+)	TGGTTTCTACTGACAGAGCGC	307
	JHP917 (–)	AACACGCTGACAGGACAATCTCCC	
<i>jhp0918 (virB4)</i>	JHP918 (+)	CCTATATCGCTAACGCGCGCTC	276
	JHP918 (–)	AAGCTGAAGCGTTTGTAACG	
<i>jhp0015/hp0017 (comB4, virB4)</i>	JHP15 (+)	AAACTTAGTGGGCATCCTCCG	276
	JHP15 (–)	ACGCCATGCAAAGAGTGAGTG	
<i>hp1006 (virD4)</i>	HP1006 (+)	AAGTAGGAGCTATGGTGTGG	336
	HP1006 (–)	GGTTTAGCCTTATGACCGCTTAC	
<i>jhp1316/hp1421 (virB11)</i>	JHP1316 (+)	ACCCATAGAGTTTACAAGCC	267
	JHP1316 (–)	ATTCGCGCTCACCTATAGC	

Association Between the *vir* Homologue Genes Outside of the *cag* PAI and Disease Outcomes

Table 2

	No.	<i>hp0441 (virB4)</i> %	<i>hp0459 (virB4)</i> %	<i>jhp0917 (virB4)</i> %	<i>jhp0918 (virB4)</i> %	<i>jhp0917-0918 (dupA)</i> %	<i>jhp0015/hp0017 (virB4)</i> %	<i>hp1006 (virD4)</i> %	<i>jhp1316/hp1421 (virB11)</i> %
Total									
Gastritis	n = 120	3	35	20 ^a	23 ^a	21 ^a	82	38	100
DU	n = 140	1	38	41 ^{b,c,d}	44 ^{b,c,d}	42 ^{b,c,d}	86	44	100
GU	n = 110	2	37	27 ^c	27 ^c	27 ^c	83	41	100
Gastric cancer	n = 130	2	37	9	11	9	82	37	100
Japan									
Gastritis	n = 50	2	40	14	16	14	82	34	100
DU	n = 30	0	47	37 ^{a,e}	37	37 ^{a,e}	83	43	100
GU	n = 50	2	40	26	26	26	78	40	100
Gastric cancer	n = 30	7	43	10	13	10	83	37	100
Korea									
Gastritis	n = 30	3	30	7	7	7	67	33	100
DU	n = 65	2	37	37 ^{b,c}	37 ^{b,c}	37 ^{b,c}	80	51	100
GU	n = 30	3	37	17	17	17	83	43	100
Gastric cancer	n = 50	0	32	6	8	6	78	36	100
Colombia									
Gastritis	n = 40	5	33	38 ^c	43 ^c	39 ^c	93	45	100
DU	n = 45	2	33	49 ^c	60 ^c	55 ^c	96	36	100
GU	n = 30	0	33	40 ^a	40 ^a	40 ^a	90	40	100
Gastric cancer	n = 50	2	38	12	12	12	84	38	100

NOTE. *P* value was determined by Fisher exact test or χ^2 test.
For *jhp0917-0918*, 10 samples with *jhp0917*-negative/*jhp0918*-positive samples were excluded.

^a*P* < .05 compared with gastric cancer.

^b*P* < .01 compared with gastritis.

^c*P* < .01 compared with gastric cancer.

^d*P* < .05 compared with gastric ulcer.

$eP < .05$ compared with gastritis.

Table 3

Association Between the *vir* Homologue Genes in the *cag* PAI and Disease Outcomes

	No.	<i>jhp0473/hp0524 (virD4)</i> %	<i>jhp0474/hp0525 (virB11)</i> %	<i>jhp0476/hp0527 (virB10)</i> %	<i>jhp0477/hp0528 (virB9)</i> %	<i>jhp0478/hp0529 (virB8)</i> %	<i>cagT (virB7)</i> %	<i>cagE (virB4)</i> %	<i>cag</i> PAI (intact) %
Total									
Gastritis	n = 120	93	93	93	93	93	93	94	95
DU	n = 140	94	94	95	95	95	95	96	96
GU	n = 110	96	96	96	96	96	95	95	97
Gastric cancer	n = 130	98	98	98	98	98	98	98	98
Japan									
Gastritis	n = 50	100	100	100	100	100	100	100	100
DU	n = 30	97	97	97	97	97	97	100	100
GU	n = 50	100	100	100	100	100	100	100	100
Gastric cancer	n = 30	100	100	100	100	100	100	100	100
Korea									
Gastritis	n = 30	97	97	97	97	97	97	97	100
DU	n = 65	97	97	98	98	98	98	98	98
GU	n = 30	100	100	100	100	100	97	97	100
Gastric cancer	n = 50	100	100	100	100	100	100	100	100
Colombia									
Gastritis	n = 40	80	80	80	80	80	83	85	84
DU	n = 45	89	89	89	89	89	89	89	89
GU	n = 30	87	87	87	87	87	87	87	87
Gastric cancer	n = 50	94	94	94	94	94	94	94	94

NOTE. For the *cag* PAI, 7 samples with partial deleted *cag* PAI were excluded from analyses.

Table 4Multiple Logistic Regression Analysis: *H pylori* Factors Associated With Clinical Presentation

Factors (positive vs. negative)		P value	Adjusted OR	95% CI
Duodenal ulcer vs. gastric cancer				
Total	<i>jhp0917-jhp0918</i>	<.001	8.3	4.0–17.4
Japan	<i>jhp0917-jhp0918</i>	.023	5.4	1.3–23.0
Korea	<i>jhp0917-jhp0918</i>	<.001	10.9	1.8–66.9
Colombia	<i>jhp0917-jhp0918</i>	<.001	9.0	3.0–26.6
Duodenal ulcer vs. gastritis				
Total	<i>jhp0917-jhp0918</i>	<.001	3.1	1.7–5.7
Japan	<i>jhp0917-jhp0918</i>	.027	3.7	1.2–11.5
Korea	<i>jhp0917-jhp0918</i>	.005	9.2	2.0–43.0
Gastric cancer vs. gastritis				
Total	<i>jhp0917-jhp0918</i>	.042	0.42	0.2–0.9
Colombia	<i>jhp0917-jhp0918</i>	.012	0.22	0.1–0.7
Gastric cancer vs. gastric ulcer				
Colombia	<i>jhp0917-jhp0918</i>	.034	0.29	0.1–0.8

NOTE. The data with statistically significant are presented. When we analyzed data in combinations of 3 counties (total), countries were also included in explanatory variables.

Table 5
Univariate Analysis of Relationship Between the *jhp0917-0918* and *cag* PAI Status and Histology

	No.	Antrum				Corpus			
		<i>H. pylori</i> density	Neutrophil infiltration	Intestinal metaplasia	Atrophy	<i>H. pylori</i> density	Neutrophil infiltration	Intestinal metaplasia	Atrophy
Japan									
<i>jhp0917-0918</i>									
Positive	34	3.0 (3)	3.5 (3)	0.4 (0)	0.6 (0)	2.6 (3)	2.3 (3)	0.3 (0)	0.3 (0)
Negative	124	2.7 (3)	2.1 (2)	1.1 (1)	2.1 (2)	2.1 (2)	1.8 (2)	1.0 (1)	1.8 (1)
<i>P</i> value		.07	<.001	.006	<.001	NS	<.001	.043	<.001
Colombia									
<i>jhp0917-0918</i>									
Positive	50	2.5 (2)	2.8 (3)	0.2 (0)	0.6 (0)	2.4 (2)	2.3 (2)	0.1 (0)	0.3 (0)
Negative	87	2.2 (2)	1.7 (2)	1.1 (0)	1.7 (1)	2.2 (2)	1.7 (2)	1.0 (0)	1.1 (0.5)
<i>P</i> value		NS	.002	.004	<.001	NS	.023	.003	<.001
<i>cag</i> PAI									
Positive	125	2.4 (2)	2.5 (2)	0.9 (0)	1.4 (1)	2.3 (2)	2.0 (2)	0.7 (0)	0.9 (0)
Negative	17	2.3 (2.5)	1.6 (2)	0.4 (0)	0.5 (0)	2.2 (2)	1.3 (1)	0.1 (0)	0 (0)
<i>P</i> value		NS	.057	NS	.037	NS	.063	NS	.009

NOTE. For histologic scores (minimum 0 to maximum 5), mean (median) scores are presented. The *cag* PAI data in Japan did not show because there were no *cag* PAI negative strains in Japan. Other *vir* factors were not related to changes in gastric histology and were not shown.

NS, not significant; *P* > .10.

Table 6

Final Model Using Multiple Linear Regression Analysis: *H pylori vir* Homologue Genes Associated with Histology

Histology	Site	Factors (positive vs. negative)	Partial regression coefficient $\pm$ SE	P value
Japan				
Neutrophil infiltration	Antrum	<i>jhp0917-jhp0918</i>	1.21 ± 0.26	<.001
	Corpus	<i>jhp0917-jhp0918</i>	0.49 ± 0.25	.021
Intestinal metaplasia	Antrum	<i>jhp0917-jhp0918</i>	-0.52 ± 0.21	.023
	Corpus	<i>jhp0917-jhp0918</i>	-0.47 ± 0.23	.034
Atrophy	Antrum	<i>jhp0917-jhp0918</i>	-1.31 ± 0.25	<.001
	Corpus	<i>jhp0917-jhp0918</i>	-1.03 ± 0.24	<.001
Colombia				
Neutrophil infiltration	Antrum	<i>jhp0917-jhp0918</i>	0.84 ± 0.26	.002
		<i>cag</i> PAI	0.74 ± 0.36	.068
	Corpus	<i>cag</i> PAI	0.70 ± 0.32	.049
		<i>jhp0917-jhp0918</i>	0.41 ± 0.24	.076
Intestinal metaplasia	Antrum	<i>jhp0917-jhp0918</i>	-0.62 ± 0.23	.009
	Corpus	<i>jhp0917-jhp0918</i>	-0.53 ± 0.23	.018
		<i>cag</i> PAI	0.54 ± 0.32	.079
Atrophy	Antrum	<i>jhp0917-jhp0918</i>	-0.81 ± 0.27	.004
		<i>cag</i> PAI	0.74 ± 0.39	.077
	Corpus	<i>cag</i> PAI	0.96 ± 0.35	.004
		<i>jhp0917-jhp0918</i>	-0.63 ± 0.25	.009

NOTE. Factors that remained in final model are presented. The partial regression coefficient provides an estimate of the expected changes of the histologic scores (minimum 0 to maximum 5) between the different diseases.

Table 7

Differential Activation of Transcription Factors Induced by *H pylori*: Comparison Between Wild Type Strain (C142) and its *dupA*-Deleted Mutant

Name	Wild type (C142)	<i>dupA</i> -deleted mutants	Ratio for wild type/ <i>dupA</i> deleted
Transcription factors activated in both wild type strain C142 and <i>dupA</i> deleted mutant			
YY1	2.1	2.9	0.8
Ets	2.5	2.2	1.1
NFκB	2.5	2.0	1.3
c-Myb	2.6	2.2	1.2
GATA	2.7	2.1	1.5
Brn-3	2.8	2.2	1.3
Ets-1/PEA3	4.5	6.6	0.8
Interferon regulatory factor 1 (IRF-1)	4.6	4.5	1.2
Pre-B-cell leukemia transcription factor 1 (Pbx1)	4.7	2.3	2.2
Signal transducer and activator of transcription 1 (Stat1)	5.2	2.2	2.7
Estrogen receptor (ERE)	5.9	2.4	2.6
Hepatocyte nuclear factor 4 (HNF-4)	9.4	2.2	4.6
Transcription factors activated only in wild type strain C142			
E2F transcription factor 1	2.1	1.4	1.6
Retinoic acid receptor (RAR)	2.2	1.1	1.9
Heat shock element (HSE)	2.2	1.6	1.4
Upstream transcription factor 1 (USF-1)	2.2	1.7	1.4
AP-1	2.2	1.2	2.4
Sp1	2.4	1.4	1.7
Stat4	2.4	1.9	1.3
Nuclear factor of activated T-cells, cytoplasmic (NFATc)	2.5	1.5	1.7
Nuclear factor (erythroid-derived 2) (NF-E2)	2.7	1.9	1.5
CREB	2.7	1.3	2.1
Stat5/Stat6	2.9	1.5	1.9
p53	3.0	1.3	2.4
Glucocorticoid receptor element (GRE)	3.1	1.9	1.8
Thyroid hormone receptor (TR)	3.2	1.3	2.4
CAAT box General (CEF)	4.0	1.9	2.3
Nuclear factor I (NF-1)	4.0	1.7	2.3
Stat3	4.4	1.8	3.0
Progesterone receptor element (PRE)	4.9	1.6	3.5
Serum response element (SRE)	7.4	0.9	9.0
Myocyte enhancing factor 2 (MEF2)	8.9	1.0	9.3
FAST-1	9.9	ND	
ISRE	17.4	ND	

NOTE. Fold induction compared with control is presented for wild type strains and the *dupA*-deleted mutants. Assays were performed in triplicate, and mean value are presented.

ND, Not detected.