Published in final edited form as:

Nat Rev Gastroenterol Hepatol. 2010 November ; 7(11): 629–641. doi:10.1038/nrgastro.2010.154.

Mechanisms of disease: *Helicobacter pylori* virulence factors

Yoshio Yamaoka

Department of Environmental and Preventive Medicine, Oita University Faculty of Medicine, Iidaigaoka, Hasamamachi, Yufucity, Oita 879-5593, Japan

Yoshio Yamaoka: yyamaoka@oita-u.ac.jp

Abstract

Helicobacter pylori plays an essential role in the development of various gastroduodenal diseases; however, only a small proportion of people infected with *H. pylori* develop these diseases. Some populations that have a high prevalence of *H. pylori* infection also have a high incidence of gastric cancer (for example, in East Asia), whereas others do not (for example, in Africa and South Asia). Even within East Asia, the incidence of gastric cancer varies (decreasing in the south). *H. pylori* is a highly heterogeneous bacterium and its virulence varies geographically. Geographic differences in the incidence of gastric cancer can be explained, at least in part, by the presence of different types of *H. pylori* virulence factor, especially CagA, VacA and OipA. However, it is still unclear why the pathogenicity of *H. pylori* increased as it migrated from Africa to East Asia during the course of evolution. *H. pylori* infection is also thought to be involved in the development of duodenal ulcer, which is at the opposite end of the disease spectrum to gastric cancer. This discrepancy can be explained in part by the presence of *H. pylori* virulence factor DupA. Despite advances in our understanding of the development of *H. pylori*-related diseases, further work is required to clarify the roles of *H. pylori* virulence factors.

Introduction

Helicobacter pylori is a Gram-negative spiral bacterium whose ecological niche is the human stomach. *H. pylori* gastritis is etiologically associated with peptic ulcer, primary gastric B-cell lymphoma and gastric carcinoma. Despite a general decline in the incidence of gastric cancer, it remains the fourth most common cancer and second leading cause of cancer-related deaths worldwide.¹ In 2010, it is estimated that there will be 21,000 new gastric cancer cases and 10,570 deaths from gastric cancer in the USA.² In Africa and South Asia, the incidence of gastric cancer is much lower than in East Asian countries, although the prevalence of *H. pylori* infection is high in each of these regions (Table 1).³ Even within East Asia, the incidence of gastric cancer varies, decreasing at increasingly southerly latitudes. What is the cause of these geographic differences in disease pattern? In addition, *H. pylori* infection is thought to be involved in the development of both gastric cancer and duodenal ulcer, which are at opposite ends of the disease spectrum. What makes it possible for *H. pylori* infection to be involved in both of these gastroduodenal diseases?

The questions above may be answered by considering various host-related factors, the duration of infection and/or environmental factors. Host genetics play an important role in interactions between the host and *H. pylori*. For example, polymorphisms in those genes that control the host's inflammatory response can either accentuate or attenuate the inflammatory

response and thus the incidence of adverse outcomes associated with an infection.⁴ The duration of infection and the age at acquisition of an *H. pylori* infection are also important, as is poor nutrition in childhood (or childhood infections), which lead to reduced acid secretion and increased susceptibility to the development of *H. pylori*-induced atrophic gastritis and gastric cancer.⁵ Environmental factors known to protect against gastric cancer include storage of food in a refrigerator (that is, a food preservation surrogate and an indication of reduced consumption of a seasonal diet) and the increased consumption of fresh fruit and vegetables; correlates with gastric cancer include a high salt intake and nitrate consumption.⁶ In addition to host-related factors, duration of infection and/or environmental factors, considering the bacterial factors present should provide one of the most important clues needed to answer the questions.

H. pylori has co-evolved with humans at least since they migrated out of Africa approximately 58,000 years ago and probably throughout their evolution (Figure 1); *H. pylori* is, therefore, a highly heterogeneous bacterium,^{7–10} the virulence of which has also changed geographically. In this Review, current knowledge of *H. pylori* virulence factors and how they contribute to the differing geographic gastric cancer disease patterns and to the development of both gastric cancer and duodenal ulcer is discussed, with a focus on cytotoxin-associated gene A product (CagA), vacuolating cytotoxin (VacA), outer inflammatory protein (OipA) and duodenal ulcer promoting (DupA).

CagA

CagA is the most extensively studied *H. pylori* virulence factor. There are two types of clinical *H. pylori* isolate: CagA-producing (*cagA* positive) strains and CagA-nonproducing (*cagA* negative) strains. Studies of Mongolian gerbils showed that gastric cancer developed in those animals infected with wild-type *H. pylori*, but not in animals infected with isogenic *cagA* mutants.^{11,12} Since then, another study reported that gastric cancer and other malignant neoplasms occurred in some transgenic mice that had CagA protein artificially introduced.¹³ These data have led to increasing knowledge of CagA as a bacteria-derived carcinogen. In Western countries, it has been reported that individuals infected with *cagA*-positive strains of *H. pylori* are at a higher risk of peptic ulcer or gastric cancer than those infected with *cagA*-negative strains.^{14,15} In East Asia, however, most strains of *H. pylori* have the *cagA* gene irrespective of the disease; the pathogenic difference in East Asia is, therefore, difficult to explain simply in terms of the presence or absence of the *cagA* gene alone.¹⁶

Caga type: Western versus East Asian

cagA is a polymorphic gene. In particular, there are different numbers of repeat sequences located in the 3' region of the *cagA* gene of different *H. pylori* strains.^{17–19} The repeat regions were initially classified into two types—the first repeat and the second repeat—and the sequence of the second repeat region was found to differ considerably between East Asian strains and Western strains of *H. pylori*.^{17–20}

Each repeat region of the CagA protein contains Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs, including a tyrosine phosphorylation site. It has now become more common to name the first repeat region as EPIYA-A and EPIYA-B segments and to name the second repeat region as EPIYA-C or EPIYA-D segments for Western and East Asian strains, respectively.²¹ As such, each CagA is assigned a sequence type consisting of the names of the EPIYA segments in its sequence (that is, ABC, ABCC or ABCCC for Western-type CagA and ABD for East-Asian-type CagA) (Figure 2). Analyses of the repeat regions of CagA has demonstrated that, although rare, some East Asian strains have a Western-type CagA sequence, especially strains isolated from Okinawa, the south islands of Japan (Table

1).^{20,22–26} By contrast, none of the Western strains studied have an East-Asian-type CagA sequence.^{20,26} A study published in 2009 reported that the Western-type CagA detected in strains from Okinawa formed a different cluster compared with the ordinary Western-type CagA and it was named as the J-Western-type CagA subtype.²⁷ As *H. pylori*, together with humans, is thought to have migrated out of Africa approximately 58,000 years ago, and reached parts of Europe and finally East Asia via Central Asia (Figure 1), the original *H. pylori* strain is thought to have a Western-type CagA sequence that possibly evolved to the J-Western-type CagA and then to the East-Asian-type CagA. Further studies are needed to clarify the role of the different Western-type CagA sequences in the pathogenesis of disease.

Pathogenicity of multiple EPIYA segments

The first reports to suggest that the number of EPIYA segments in the second repeat region is associated with gastric cancer were published in the late 1990s.^{17,18} Subsequent studies confirmed that, in Western countries, the incidence of gastric cancer is notably higher in patients infected with strains containing multiple EPIYA-C segments than in patients infected with strains containing a single EPIYA-C segment (that is, ABCCC versus ABC).^{17,18,28,29} However, as almost all East Asian strains contain a single EPIYA-D segment,²⁶ it is difficult to differentiate between simple gastritis and gastric cancer simply by considering the number of repeated sequences in East Asia.

With regard to the function of the repeat regions, initial demonstrations suggest that *H. pylori* strains that have a larger number of EPIYA segments in their repeat regions are less resistant to gastric acid.¹⁸ This finding seems to indicate that *H. pylori* strains containing many EPIYA segments can survive only in the presence of advanced atrophic gastritis (such as gastric cancer), in which gastric acid secretion is low. If this is the case, multiple EPIYA segments may have developed after the onset of atrophy and are not the cause, but rather the effect of atrophy.

Tyrosine phosphorylation and pathogenicity

There have been many reports that the site of tyrosine phosphorylation in the EPIYA motif of CagA plays a direct role in the pathogenicity of *H. pylori*. Following injection of CagA into epithelial cells, the EPIYA motifs are tyrosine phosphorylated by Src and Abl family kinases,^{30–34} which results in the impairment of a variety of intracellular signaling systems (Figure 3). Backert *et al.* reported that there are at least 20 known cellular binding partners of CagA, which is the highest number currently known for any virulence-associated effector protein in the microbial world.³⁵ Of these binding partners, 10 host cell proteins bind CagA in a phosphorylation-dependent manner.^{35,36} The number of EPIYA motifs present is related to the level of CagA phosphorylation that occurs in epithelial cells infected by either East Asian³⁷ or Western strains.²⁸

The best studied of the host signaling factors that interact with phosphorylated CagA is the Src homology-2 domain-containing phosphatase 2 (SHP2).³⁸ SHP2 is able to bind EPIYA-B, EPIYA-C and EPIYA-D segments. Importantly, CagA with an EPIYA-D segment has a higher binding affinity for SHP2 than CagA with an EPIYA-C segment.²¹ The sequence flanking the tyrosine phosphorylation site of the EPIYA-D segment (EPIYATIDF), but not the EPIYA-C segment (EPIYATIDD), matches perfectly the consensus high-affinity binding sequence for the SH2 domains of SHP2 (Figure 2).

A study that used stable isotope labeling with amino acids in cell culture combined with mass spectrometry of bound protein (SILAC/MS) demonstrated that C-terminal Src tyrosine kinase (Csk) prefers to bind EPIYA-A and EPIYA-B segments, whereas Ras-GAP and Grb7 prefer to bind EPIYA-C segments.³⁶ Malignant neoplasms have been shown to occur with

greater frequency in transgenic mice in which East-Asian-type CagA had been introduced compared with transgenic mice in which Western-type CagA had been introduced.³⁹ The discovery that, *in vivo*, East-Asian-type CagA is more carcinogenic than Western-type CagA is important; however, in this transgenic mouse model, the gastric cancer developed without inflammation and malignancy also occurred in nongastric organs; which raises questions about the applicability of this model to human disease.

Many previous *in vitro* studies of CagA involved transfection of a CagA expression vector instead of natural injection of CagA via the *cag* pathogenicity island (PAI) type IV secretion system (T4SS). However, transfection experiments have potentially confounded our understanding of the role of CagA. For example, Grb2 reportedly interacts with phosphorylated CagA during *H. pylori* infection,³⁶ but such an interaction was not found in studies using transfected CagA.^{34,40} Importantly, transfection of CagA does not closely mimic natural injection of CagA by the T4SS. T4SS-injected CagA primarily localizes to focal adhesions,⁴¹ whereas vector-expressed CagA localizes to the entire host membrane.³⁸ Most importantly, transfection prevents assessment of critical bacterial–host cell interactions, such as the activation of cellular signaling associated with VacA and OipA. Therefore, information gained from transfection experiments should be interpreted with caution and the findings confirmed by experiments using natural injection.

Phosphorylation-independent signaling

Nonphosphorylated CagA also impairs intracellular signaling systems. Currently, there are at least 10 known phosphorylation-independent CagA host interaction partners (Figure 3).³⁵ In addition, CagA itself forms dimers in cells in a phosphorylation-independent manner, and the CagA multimerization (CM) sequence (FPLxRxxxVxDLSKVG) was identified by Ren *et al.* as the site responsible for dimerization.⁴² The CM sequence is located within the EPIYA-C segment, but is just downstream of the EPIYA-D segment (Figure 2).

The CM sequence is also essential for the formation of the CagA–PAR1 (MARK) complex—bound nonphosphorylated CagA inhibits the kinase activity of PAR1 to promote the loss of cell polarity^{42,43} and a loss of the elongation phenotype of host cells.⁴⁴ The CM sequence has also been named by Nesic *et al.* as the MARK2/PAR1b kinase inhibitor (MKI).⁴⁵ In addition, the same region was reported to be responsible for the interaction of CagA with activated c-Met and named as the conserved repeat responsible for phosphorylation-independent activity (CRPIA) by Suzuki and colleagues.⁴⁶ The interaction of CRPIA with c-Met subsequently led to the upregulation of β -catenin and nuclear factor κ B (NF κ B) transcriptional activities, which promoted proliferation and inflammation, respectively. Therefore, there are currently three acronyms that essentially correspond to the same sequence in CagA. Functional differences between the CM/MKI/CRPI sequence in Western-type CagA (FPLKRHDKVDDLKVG) and East-Asian-type CagA (FPLRRSAAVNDLSKVG) have not been detected^{42,43} and have yet to be identified.⁴⁵

Interleukin 8 production

Infection of the gastric mucosa by *H. pylori* is characterized by the production of proinflammatory cytokines, especially interleukin (IL)-8, a potent neutrophil-activating chemokine.^{47–50} The *cag* PAI, in which the *cagA* gene is localized at one end, is involved in the induction of gastric IL-8 production; whereas most reports have demonstrated that the CagA protein is not involved in IL-8 induction.^{51–53} However, one study has demonstrated that CagA is involved in IL-8 induction in a strain-dependent and time-dependent manner.⁵⁴ IL-8 induction has typically been assessed 12–24 h after infection *in vitro* using gastric epithelial cells; however, the potentiating effects of CagA only become evident 36–48 h after infection.⁵⁴ Interestingly, findings from transfection experiments suggest that the IL-8

levels induced by East-Asian-type CagA are higher than those induced by Western-type CagA.⁵⁵

Clinical importance of CagA phenotype

As described above, there has been an increasing tendency in the past 10 years to explain the higher incidence of gastric cancer in East Asia using the concept of East-Asian-type CagA and Western-type CagA. The incidence of gastric cancer is clearly higher in East Asian countries than in any other countries when age-standardized rates (ASRs) are considered (Table 1). However, it should be noted that the incidence of gastric cancer is also high in some regions where Western-type CagA strains are reported to account for the majority of *H. pylori* strains (for example, in Peru and Columbia [ASR per 100,000 population 21.2 and 17.4, respectively]).^{20,56} In addition, in Africa the rate of *H. pylori* infection is high (for example, 70–97% of patients with dyspepsia are infected with *H. pylori*, as are 80% of asymptomatic volunteers⁵⁷) but gastric cancer is generally uncommon; this seemingly contradictory situation is known as the “African Enigma”.⁵⁷ The incidence of gastric cancer is, however, extremely high in Mali, West Africa (ASR per 100,000 population 20.3) and the frequency of gastric cancer among women in this country is higher than it is among women in Japan (ASR per 100,000 population 19.3 versus 18.2) (Table 1). These facts cannot be explained by the presence of East-Asian-type CagA versus Western-type CagA alone.

In an attempt to explain the geographic difference in the incidence of gastric cancer, my group compared the *cagA* gene repeat sequences found in Columbia (ASR per 100,000 population 17.4) with those found in the USA (ASR per 100,000 population 4.1). 100 *H. pylori* isolates from patients with simple gastritis (30 from Columbia and 70 from the USA) were analyzed—57% of the isolates from Columbia had two EPIYA-C segments, whereas only 4% of the isolates from the USA had two EPIYA-C segments (Y. Yamaoka, unpublished data). Overall, the number of EPIYA-C segments may explain, to some extent, the geographic difference in the incidence of gastric cancer in Western countries.

In Thailand, the incidence of gastric cancer is extremely low compared with that in the surrounding countries (ASR per 100,000 population 3.5) (Table 1). A previous study demonstrated that gastric cancer was common among ethnic Chinese and rare among the Thai indigenous population.²⁵ All ethnic Chinese patients who had gastric cancer or peptic ulcers were infected with *H. pylori* with East-Asian-type CagA; whereas East-Asian-type CagA was found in only 40% of ethnic Chinese patients who had gastritis. These data suggest that East-Asian-type CagA may have increased virulence compared with Western-type CagA. However, interpretation of the relationship between the CagA type and outcome in Thailand is complicated by the fact that the majority of gastric cancers (82%) occurred among ethnic Chinese or Thai–Chinese individuals.²⁵ Taken together, although there is increasing evidence from *in vitro* studies and animal models that East-Asian-type CagA is more virulent than Western-type CagA, the clinical concept that East-Asian-type CagA versus Western-type CagA explains the geographic difference in the incidence of gastric cancer is still not accepted.

In East Asia, the incidence of gastric cancer decreases at increasingly southerly latitudes. Indeed, the incidence of gastric cancer in Vietnam is half that in South Korea, although most Vietnamese strains of *H. pylori* (97%) have been reported to contain East-Asian-type CagA (Table 1). In addition, most of the East Asian strains of *H. pylori* have only one EPIYA-D segment.²⁶ Therefore, in areas where infection with East-Asian-type CagA *H. pylori* strains is predominant, it is not possible to explain the difference in the incidence of gastric cancer by CagA alone. The structure of the East-Asian-type *cagA* gene in Vietnamese strains of *H. pylori* is, however, slightly different to that of strains from other East Asian countries.²⁴ An

18 bp deletion unique to Vietnamese strains was identified in the region located slightly upstream of the EPIYA-A segment, whereas a 39 bp deletion was identified in strains common in East Asian countries such as Japan, and no deletion was identified in Western strains.²⁴ Further research is required to determine whether these subtypes are involved in the pathogenesis of gastric cancer.

VacA

VacA is the second most extensively studied *H. pylori* virulence factor. In addition to inducing vacuolation, VacA can induce multiple cellular activities, including membrane-channel formation, cytochrome *c* release from mitochondria leading to apoptosis, and binding to cell-membrane receptors followed by initiation of a proinflammatory response.^{58–60} VacA can also specifically inhibit T-cell activation and proliferation.^{61–63} Studies indicate that VacA and CagA can even inhibit at least some of each other's signaling pathways.^{64–67} For example, CagA has been shown to promote the expression of the apoptotic suppressor Mcl1, and inhibit epithelial cell apoptosis caused by VacA.^{64,65} These data again emphasize the importance of *in vitro* infection experiments in which interaction among *H. pylori* virulence factors can be taken into account.

Clinical importance of *vacA* genotype

Virtually all *H. pylori* strains have a functional *vacA* gene. There is variation in the vacuolating activity of different *H. pylori* strains,^{68,69} primarily due to differences in the *vacA* gene structure at the signal (s) region (s1 and s2) and the middle (m) region (m1 and m2).⁷⁰ *In vitro* experiments demonstrated that s1/m1 strains are the most cytotoxic, followed by s1/m2 strains, whereas s2/m2 strains have no cytotoxic activity and s2/m1 strains are rare.^{70,71} In agreement with *in vitro* data, in Western countries, including Latin America, the Middle East and Africa, there have been many reports that individuals infected with s1 or m1 *H. pylori* strains have an increased risk of peptic ulcer or gastric cancer compared with individuals infected with s2 or m2 strains.^{14,70,72,73} In East Asia, however, most *H. pylori* strains have an s1-type s region; therefore the pathogenic difference cannot be explained by the type of s region present.^{16,20} Moreover, it is interesting to note that almost all *cagA*-positive strains are classified as an s1 strain, whereas almost all *cagA*-negative strains are classified as an s2/m2 strain.⁷⁰

With respect to the m region, however, there is variation within East Asia. Although m1 strains are common in parts of north East Asia, such as Japan and South Korea, m2 strains are predominant in parts of south East Asia, such as Taiwan and Vietnam (Table 2).^{20,24} As the incidence of gastric cancer is higher in the north than in the south of East Asia, the m region may play a role in the regional difference in disease pattern. Even within Vietnam, the incidence of gastric cancer is approximately 1.5 times higher in Hanoi in the north than in Ho Chi Minh in the south of the country (Table 2). Research has shown that almost all *H. pylori* strains are classified as *cagA*-positive and *vacA* s1 strains in these two cities, but that the frequency of the *vacA* m1 strain is considerably higher in Hanoi.²⁴

Novel genotypes

In 2007, a third disease-related region of *vacA* was identified between the s region and the m region; it was named the intermediate (i) region.⁷⁴ According to the authors of the study, all s1/m1 strains were classified as type i1, and all s2/m2 strains were classified as type i2, but s1/m2 strains were classified as either type i1 or i2, and i1 strains were shown to be more pathogenic. In the same study, typing of the i region was also reported to be more effective for determining the risk of gastric cancer in Iranian patients than typing of the s region or m region.⁷⁴ An additional study conducted by the same group showed that the i region plays a

role in the development of peptic ulcer in people in Iraq and Italy.^{75,76} However, in a study of patients from East and Southeast Asia, there was no association between the i region and disease.⁷⁷ More recently, a fourth disease-related region—the deletion (d) region—was identified between the i region and the m region.⁷⁸ The d region is divided into d1 (no deletion) and d2 (a 69–81 bp deletion). The study of Western strains demonstrated that d1 was a risk factor for gastric mucosal atrophy; however, almost all East Asian strains are classified as s1/i1/d1.⁷⁸ At present, therefore, typing of the m region may be the best overall marker for gastric cancer, especially in East Asia.

OipA

Approximately 4% of the *H. pylori* genome is predicted to encode outer membrane proteins (OMPs), some of which may function as adhesins.^{79–85} One such OMP is OipA, which was identified in 2000.⁸⁶ The functional status of OipA is regulated by slipped strand mispairing that is determined by the number of CT dinucleotide repeats in the 5' region of the gene (switch 'on' and OipA is functional; switch 'off' and OipA is nonfunctional).⁸⁶

Interleukin 8 production

OipA was initially identified as a proinflammatory-response-inducing protein based, in part, on the fact that *oipA* isogenic mutants reduced the production of IL-8 from gastric epithelial cell lines.⁸⁶ *In vivo* experiments using human gastric biopsy specimens also confirmed that a functional *oipA* was independently and significantly associated with high mucosal IL-8 levels.¹⁵ Some subsequent conflicting reports regarding IL-8 induction *in vitro*^{87–90} might be at least partially explained by the fact that multiple *in vitro* passages can allow factors other than OipA to enhance IL-8 induction.⁹¹ Binding sites for the transcription factors NFκB, activator protein 1 (AP1), and interferon-stimulated responsive element (ISRE)-like element within the IL-8 promoter are involved in regulating IL-8 gene transcription in *H. pylori*-infected gastric epithelial cells in both an OipA-dependent and *cag* PAI-dependent manner.^{92–94} However, data from my group demonstrated that the activation of NFκB is strain dependent and this might also be a reason for the conflicting reports (Figure 4).⁸³

Gradually it has become clear that one of the functions of OipA is to induce inflammation and actin dynamics via phosphorylation of multiple signaling pathways (Figure 4).^{92,95–99} However, most of these signaling pathways, with the exception of p38-related pathways, are also involved in *cag* PAI-related pathways. Therefore, there might have been some interaction between OipA and *cag* PAI and/or CagA; however, evidence of such an interaction has not been reported and further studies will be necessary to clarify the specific roles of OipA on these *cag* PAI-related pathways. One study has demonstrated that OipA in addition to CagA is involved in β-catenin signaling, leading to the opening of cell-cell junctions and proliferation.¹²

Gastric colonization

OipA functions as an adhesin and is reported to be involved in the attachment of *H. pylori* to gastric epithelial cells *in vitro*.^{89,92,100} In addition, animal studies have revealed that OipA plays a role in the bacterial colonization of the gastric mucosa. In one study of mice infected with either wild-type *H. pylori* strain CPY2052 or its isogenic *oipA* mutant, the bacterial density was considerably decreased in the mice infected with the mutant strain compared with the mice infected with the wild-type strain.¹⁰⁰ In a study of Mongolian gerbils, the isogenic *oipA* mutant of the TN2 wild-type *H. pylori* strain did not cause infection.⁹⁰ By contrast, the isogenic *oipA* mutant of wild-type *H. pylori* strain 7.13 has been shown to infect animals; however, no animals infected with the *oipA* mutant developed gastric cancer, whereas 27% of those infected with the wild-type strain developed gastric cancer.¹² These

animal studies demonstrate that OipA alone plays a role in the development of gastric cancer.

Clinical importance of OipA

Interestingly, the *oipA* 'on' status and *cagA* positivity are closely linked with each other (correlation coefficient = 0.82).¹⁵ As described above, the *cagA* status is also linked to the *vacA* s region type and it is further closely linked to the presence of the *babA* gene; the BabA protein is another virulence factor that is also an OMP.⁸⁰ The links among these factors should have a certain biological significance and they may somehow interact with each other. It might be more relevant to hypothesize that these factors interact synergistically with each other and induce serious diseases, rather than to discuss which factor is the most virulent. According to one investigation so far, it is interesting to note that all East Asian strains are classified as *oipA* status 'on' and as functional OipA-protein-producing strains.⁸⁶ Half-collapsed CT repeat sequences in the s region of the *oipA* gene (for example, Japanese-derived strain JK51 contains a CTGCCTTTCT repeat sequence and its status is 'on') suggest that this sequence may result from an intentional change in the status in the course of the evolution of the bacteria to prevent the switch from being turned 'off' easily.

DupA

The production of CagA, VacA and OipA is linked and the majority of *H. pylori* strains produce either all of these proteins or none of them. Almost all East Asian strains of *H. pylori* are classified as CagA-producing, VacA-producing (*vacA* s1), and OipA-producing (*oipA* status 'on') strains and are highly pathogenic. In addition, CagA, VacA and OipA are all thought to be involved in the development of both gastric cancer and duodenal ulcer, which are at the opposite ends of the disease spectrum. In 2005, the first disease-specific *H. pylori* virulence factor that induced duodenal ulcer and had a suppressive action on gastric cancer was identified, and was named duodenal ulcer promoting gene A (DupA).¹⁰¹

Cytokine induction

Initial *in vitro* experiments using *dupA*-deleted and *dupA*-complemented mutants showed that the presence of *dupA* was associated with increased IL-8 production from both the antral gastric mucosa *in vivo* and gastric epithelial cells.¹⁰¹ The relationship between *dupA* and IL-8 induction from gastric epithelial cells *in vitro* is still controversial;^{102,103} however, one study supports the original data that the presence of the *dupA* gene is associated with increased IL-8 production from gastric mucosa *in vivo*.¹⁰² Importantly, *in vitro* experiments using *dupA* mutants showed that *dupA* substantially increased *H. pylori*-induced IL-12p40 and IL-12p70 production by CD14⁺ mononuclear cells.¹⁰² The production of other T-helper-1-associated cytokines and IL-8 were also modestly induced by the cells. These data suggest that virulent *H. pylori* strains cause inflammation by stimulating epithelial cells via CagA, OipA and/or VacA-related proteins and mononuclear inflammatory cells through DupA.

Clinical outcomes

In an initial study of a total of 500 *H. pylori* isolates, including 160 from Japan, 175 from South Korea and 165 from Colombia, the positive rate for the *dupA* gene was high in patients with duodenal ulcer and low in patients with gastric cancer, regardless of a patients' nationality (42% versus 9% on average).¹⁰¹ Since this report was published, studies have been conducted all over the world.¹⁰⁴ Reports from India,¹⁰⁵ China¹⁰⁶ and Iraq⁷⁵ support the initial data, whereas reports from Iran,^{75,107} Brazil,^{108,109} Singapore,¹⁰³ Malaysia¹⁰³ and

Japan¹¹⁰ failed to demonstrate a correlation between the presence of the *dupA* gene and disease.

A pooled analysis of data from three Western countries (the USA, Belgium and South Africa) showed that the presence of the *dupA* gene was correlated with peptic ulcer as well as with gastric cancer.¹¹¹ One report from Iran, which was not supportive of the hypothesis that *dupA* contributes to the development of duodenal ulcer but not gastric cancer, showed that the incidence of dysplasia—a premalignant condition—was notably lower in *dupA*-positive patients,¹⁰⁷ partially supporting the hypothesis. One report from Brazil¹⁰⁸ was also unsupportive of the hypothesis, but showed that the rate of infection with *dupA*-positive strains in pediatric patients was 100% and higher than that in adult patients. This finding indicates that the number of *dupA*-positive strains decreases as gastric mucosal atrophy gradually progresses with aging, which also partially supports the hypothesis.¹⁰⁸ In a review of previous reports on *dupA* that included a total of 2,358 cases, the positive rate for *dupA* was 48% and confirmed that *dupA* was a risk factor for duodenal ulcer.¹¹² However, a large regional difference was also reported: *dupA* might, therefore, be a risk factor for both duodenal ulcer and gastric cancer in some regions.

Of note, an academic report on Brazilian strains that was presented at the Annual Meeting of the American Gastroenterological Association in 2008, showed that a *dupA* gene mutation (deletion or insertion) was found in 50% of patients with gastric cancer, whereas it was found in only approximately 20% of patients with duodenal ulcer.¹¹³ As a result, the positive rate for the functional *dupA* gene was considerably higher in patients with duodenal ulcer than in patients with gastric cancer, and this finding supported the original hypothesis. That such mutations occur at an unexpectedly high frequency should not be ignored. However, it is impossible to detect *dupA* mutations by using a simple PCR method; therefore, detection of functional *dupA* by measuring intact DupA protein using immunoblotting techniques, which has not been reported, should be attempted.

DupA as part of a novel T4SS

DupA is found in an area called the plasticity zone, in which many genes thought to be associated with pathogenicity can be found (Box 1).^{101,104} DupA has a high homology with the VirB4 ATPase, but there are *vir* genes before and after the region of the *dupA* locus. In the strain Shi470, for example, *virB2*, *virB3*, *virB4* (*dupA*), *virB8*, *virB9*, *virB10*, *virB11*, *virD4* and *virD2* can all be found (Figure 5). These *vir* genes before and after the region of the *dupA* locus are structurally similar to the T4SS called *cag* PAI and ComB, and are thought to be the third T4SS. Kersulyte *et al.* named the third T4SS in the plasticity zone *tfs3* (type IV secretion 3).¹¹⁴ The T4SS containing *dupA* has since been named as *tfs3a*, and T4SS containing the *virB4* sequence but not *dupA* was named as *tfs3b*.¹¹⁵ The strain G27 that contains *tfs3a* is unlikely to be functional because *dupA* has a frame-shift mutation. In addition, the strain J99 has a mutation in *dupA* and contains only a part of *tfs3a*. These observations suggest that only strains with functional *dupA* and complete *tfs3a* may be considered as pathogenic strains that have the same action as T4SS, such as *cag* PAI and ComB. The study of the plasticity zone is only at its beginning and may be the most attractive area for future investigation.

Conclusions

So, can *H. pylori* factors explain both the geographic differences in gastric cancer disease pattern and the role of *H. pylori* infection in gastric cancer and duodenal ulcer? CagA, VacA and probably OipA (that is, different CT repeat sequence patterns) are key factors needed to answer the first question and DupA is a key factor needed to answer the second question. However, although these four virulence factors are obviously important they can provide

only partial answers to the questions. First, since *H. pylori* consist of approximately 1,600 genes, there remains the possibility that additional important pathogenic genes will be identified. Second, it is also true that gastric cancer incidence has changed remarkably with environmental factors such as diet (for example, salt intake) or with migration. Host factors (for example, IL-1 polymorphisms) and duration of infection (for example, early infection with duodenal ulcer and late infection with gastric cancer) should also be taken into account. These various factors are thought to interact with each other in a complex manner in the actual development of disease.

In the past 10 years, tremendous progress has been made in understanding the mechanisms by which *H. pylori* virulence factors induce gastroduodenal damage. However, controversial data are also increasing as research moves forward. Transfection of CagA versus natural infection via T4SS gives us different results. The relationship between induction of IL-8 and CagA or OipA is also controversial, and 'time-dependent' and 'strain-dependent' phenomena have proved confusing. The terminology used in the field is also confusing, with CM, MKI and CRPIA all being used to describe the same sequence. In addition, *tfs3a* and *tfs3b* in strain P12 were both named *tfs4*.¹¹⁶ We should, therefore, take the time to understand and interpret each report carefully, with methodology and terminology used taken into account.

There also remains a considerable knowledge gap between the detailed dissection of events observed *in vitro* or in animal models and the clinical and/or epidemiological confirmation of their role in the pathogenesis of human disease. One reason for this discrepancy is that *H. pylori* is surprisingly heterogenic and we use only limited *H. pylori* strains *in vitro* or in animal models. By detailed typing of *H. pylori* in addition to searching for interactions between bacterial factors and other factors such as environmental factors and host factors, we will gradually understand the mechanisms by which *H. pylori* induces gastric injury, leading to gastric cancer.

Acknowledgments

This report is based on work supported in part by grants from the NIH (DK62813), and grants-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan (22390085 and 22659087).

References

1. International Agency for Research on Cancer. The Globocan Project. 2010. [online], <http://globocan.iarc.fr/>
2. Jemal A, Siegel R, Xu J, Ward E. Cancer Statistics. CA Cancer J Clin. 2010;10:3322/caac.20073
3. Malaty HM. Epidemiology of *Helicobacter pylori* infection. Best Pract Res Clin Gastroenterol. 2007; 21:205–214. [PubMed: 17382273]
4. El-Omar EM. Role of host genes in sporadic gastric cancer. Best Pract Res Clin Gastroenterol. 2006; 20:675–686. [PubMed: 16997152]
5. Graham DY. *Helicobacter pylori* infection in the pathogenesis of duodenal ulcer and gastric cancer: a model. Gastroenterology. 1997; 113:1983–1991. [PubMed: 9394739]
6. Graham DY, Lu H, Yamaoka Y. African, Asian or Indian enigma, the East Asian *Helicobacter pylori*: facts or medical myths. J Dig Dis. 2009; 10:77–84. [PubMed: 19426388]
7. Falush D, et al. Traces of human migrations in *Helicobacter pylori* populations. Science. 2003; 299:1582–1585. [PubMed: 12624269]
8. Linz B, et al. An African origin for the intimate association between humans and *Helicobacter pylori*. Nature. 2007; 445:915–918. [PubMed: 17287725]
9. Moodley Y, et al. The peopling of the Pacific from a bacterial perspective. Science. 2009; 323:527–530. [PubMed: 19164753]

10. Yamaoka Y. *Helicobacter pylori* typing as a tool for tracking human migration. Clin Microbiol Infect. 2009; 15:829–834. [PubMed: 19702588]
11. Franco AT, et al. Activation of beta-catenin by carcinogenic *Helicobacter pylori*. Proc Natl Acad Sci USA. 2005; 102:10646–10651. [PubMed: 16027366]
12. Franco AT, et al. Regulation of gastric carcinogenesis by *Helicobacter pylori* virulence factors. Cancer Res. 2008; 68:379–387. [PubMed: 18199531]
13. Ohnishi N, et al. Transgenic expression of *Helicobacter pylori* CagA induces gastrointestinal and hematopoietic neoplasms in mouse. Proc Natl Acad Sci USA. 2008; 105:1003–1008. [PubMed: 18192401]
14. van Doorn LJ, et al. Clinical relevance of the *cagA*, *vacA*, and *iceA* status of *Helicobacter pylori*. Gastroenterology. 1998; 115:58–66. [PubMed: 9649459]
15. Yamaoka Y, et al. Importance of *Helicobacter pylori oipA* in clinical presentation, gastric inflammation, and mucosal interleukin 8 production. Gastroenterology. 2002; 123:414–424. [PubMed: 12145793]
16. Yamaoka Y, et al. Relationship between *Helicobacter pylori iceA*, *cagA*, and *vacA* status and clinical outcome: studies in four different countries. J Clin Microbiol. 1999; 37:2274–2279. [PubMed: 10364597]
17. Yamaoka Y, Kodama T, Kashima K, Graham DY, Sepulveda AR. Variants of the 3' region of the *cagA* gene in *Helicobacter pylori* isolates from patients with different *H. pylori*-associated diseases. J Clin Microbiol. 1998; 36:2258–2263. [PubMed: 9666002]
18. Yamaoka Y, et al. Relationship between the *cagA* 3' repeat region of *Helicobacter pylori*, gastric histology, and susceptibility to low pH. Gastroenterology. 1999; 117:342–349. [PubMed: 10419915]
19. Yamaoka Y, et al. Molecular epidemiology of *Helicobacter pylori*: separation of *H. pylori* from East Asian and non-Asian countries. Epidemiol Infect. 2000; 124:91–96. [PubMed: 10722135]
20. Yamaoka Y, et al. *Helicobacter pylori* in North and South America before Columbus. FEBS Lett. 2002; 517:180–184. [PubMed: 12062433]
21. Hatakeyama M. Oncogenic mechanisms of the *Helicobacter pylori* CagA protein. Nat Rev Cancer. 2004; 4:688–694. [PubMed: 15343275]
22. Saribasak H, Salih BA, Yamaoka Y, Sander E. Analysis of *Helicobacter pylori* genotypes and correlation with clinical outcome in Turkey. J Clin Microbiol. 2004; 42:1648–1651. [PubMed: 15071020]
23. Shokrzadeh L, et al. Analysis of 3'-end variable region of the *cagA* gene in *Helicobacter pylori* isolated from Iranian population. J Gastroenterol Hepatol. 2010; 25:172–177. [PubMed: 19793167]
24. Uchida T, et al. Analysis of virulence factors of *Helicobacter pylori* isolated from a Vietnamese population. BMC Microbiol. 2009; 9:175. [PubMed: 19698173]
25. Vilaichone RK, et al. Molecular epidemiology and outcome of *Helicobacter pylori* infection in Thailand: a cultural cross roads. Helicobacter. 2004; 9:453–459. [PubMed: 15361085]
26. Xia Y, Yamaoka Y, Zhu Q, Matha I, Gao X. A comprehensive sequence and disease correlation analyses for the C-terminal region of CagA protein of *Helicobacter pylori*. PLoS ONE. 2009; 4:e7736. [PubMed: 19893742]
27. Truong BX, et al. Diverse characteristics of the CagA gene of *Helicobacter pylori* strains collected from patients from southern Vietnam with gastric cancer and peptic ulcer. J Clin Microbiol. 2009; 47:4021–4028. [PubMed: 19846630]
28. Argent RH, et al. Determinants and consequences of different levels of CagA phosphorylation for clinical isolates of *Helicobacter pylori*. Gastroenterology. 2004; 127:514–523. [PubMed: 15300584]
29. Azuma T, et al. Correlation between variation of the 3' region of the *cagA* gene in *Helicobacter pylori* and disease outcome in Japan. J Infect Dis. 2002; 186:1621–1630. [PubMed: 12447739]
30. Poppe M, Feller SM, Romer G, Wessler S. Phosphorylation of *Helicobacter pylori* CagA by c-Abl leads to cell motility. Oncogene. 2007; 26:3462–3472. [PubMed: 17160020]
31. Selbach M, Moese S, Hauck CR, Meyer TF, Backert S. Src is the kinase of the *Helicobacter pylori* CagA protein *in vitro* and *in vivo*. J Biol Chem. 2002; 277:6775–6778. [PubMed: 11788577]

32. Stein M, et al. c-Src/Lyn kinases activate *Helicobacter pylori* CagA through tyrosine phosphorylation of the EPIYA motifs. *Mol Microbiol.* 2002; 43:971–980. [PubMed: 11929545]
33. Tammer I, Brandt S, Hartig R, Konig W, Backert S. Activation of Abl by *Helicobacter pylori*: a novel kinase for CagA and crucial mediator of host cell scattering. *Gastroenterology.* 2007; 132:1309–1319. [PubMed: 17408661]
34. Tsutsumi R, Higashi H, Higuchi M, Okada M, Hatakeyama M. Attenuation of *Helicobacter pylori* CagA x SHP-2 signaling by interaction between CagA and C-terminal Src kinase. *J Biol Chem.* 2003; 278:3664–3670. [PubMed: 12446738]
35. Backert S, Tegtmeyer N, Selbach M. The versatility of *Helicobacter pylori* CagA effector protein functions: The master key hypothesis. *Helicobacter.* 2010; 15:163–176. [PubMed: 20557357]
36. Selbach M, et al. Host cell interactome of tyrosine-phosphorylated bacterial proteins. *Cell Host Microbe.* 2009; 5:397–403. [PubMed: 19380118]
37. Hirata Y, et al. Functional variability of *cagA* gene in Japanese isolates of *Helicobacter pylori*. *Gene.* 2004; 343:165–172. [PubMed: 15563842]
38. Higashi H, et al. SHP-2 tyrosine phosphatase as an intracellular target of *Helicobacter pylori* CagA protein. *Science.* 2002; 295:683–686. [PubMed: 11743164]
39. Miura M, Ohnishi N, Tanaka S, Yanagiya K, Hatakeyama M. Differential oncogenic potential of geographically distinct *Helicobacter pylori* CagA isoforms in mice. *Int J Cancer.* 2009; 125:2497–2504. [PubMed: 19588494]
40. Churin Y, et al. *Helicobacter pylori* CagA protein targets the c-Met receptor and enhances the motogenic response. *J Cell Biol.* 2003; 161:249–255. [PubMed: 12719469]
41. Kwok T, et al. *Helicobacter* exploits integrin for type IV secretion and kinase activation. *Nature.* 2007; 449:862–866. [PubMed: 17943123]
42. Ren S, Higashi H, Lu H, Azuma T, Hatakeyama M. Structural basis and functional consequence of *Helicobacter pylori* CagA multimerization in cells. *J Biol Chem.* 2006; 281:32344–32352. [PubMed: 16954210]
43. Saadat I, et al. *Helicobacter pylori* CagA targets PAR1/MARK kinase to disrupt epithelial cell polarity. *Nature.* 2007; 447:330–333. [PubMed: 17507984]
44. Umeda M, et al. *Helicobacter pylori* CagA causes mitotic impairment and induces chromosomal instability. *J Biol Chem.* 2009; 284:22166–22172. [PubMed: 19546211]
45. Nesić D, et al. *Helicobacter pylori* CagA inhibits PAR1-MARK family kinases by mimicking host substrates. *Nat Struct Mol Biol.* 2010; 17:130–132. [PubMed: 19966800]
46. Suzuki M, et al. *Helicobacter pylori* CagA phosphorylation-independent function in epithelial proliferation and inflammation. *Cell Host Microbe.* 2009; 5:23–34. [PubMed: 19154985]
47. Yamaoka Y, Kita M, Kodama T, Sawai N, Imanishi J. *Helicobacter pylori* *cagA* gene and expression of cytokine messenger RNA in gastric mucosa. *Gastroenterology.* 1996; 110:1744–1752. [PubMed: 8964399]
48. Yamaoka Y, et al. Chemokines in the gastric mucosa in *Helicobacter pylori* infection. *Gut.* 1998; 42:609–617. [PubMed: 9659152]
49. Yamaoka Y, et al. Relation between clinical presentation, *Helicobacter pylori* density, interleukin 1 β and 8 production, and *cagA* status. *Gut.* 1999; 45:804–811. [PubMed: 10562576]
50. Yamaoka Y, et al. Relation between cytokines and *Helicobacter pylori* in gastric cancer. *Helicobacter.* 2001; 6:116–124. [PubMed: 11422466]
51. Censini S, et al. *cag*, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. *Proc Natl Acad Sci USA.* 1996; 93:14648–14653. [PubMed: 8962108]
52. Fischer W, et al. Systematic mutagenesis of the *Helicobacter pylori* *cag* pathogenicity island: essential genes for CagA translocation in host cells and induction of interleukin-8. *Mol Microbiol.* 2001; 42:1337–1348. [PubMed: 11886563]
53. Al-Ghoul L, et al. Analysis of the type IV secretion system-dependent cell motility of *Helicobacter pylori*-infected epithelial cells. *Biochem Biophys Res Commun.* 2004; 322:860–866. [PubMed: 15336542]

54. Brandt S, Kwok T, Hartig R, Konig W, Backert S. NF-kappaB activation and potentiation of proinflammatory responses by the *Helicobacter pylori* CagA protein. *Proc Natl Acad Sci USA*. 2005; 102:9300–9305. [PubMed: 15972330]
55. Kim SY, Lee YC, Kim HK, Blaser MJ. *Helicobacter pylori* CagA transfection of gastric epithelial cells induces interleukin-8. *Cell Microbiol*. 2006; 8:97–106. [PubMed: 16367869]
56. Kersulyte D, et al. Differences in genotypes of *Helicobacter pylori* from different human populations. *J Bacteriol*. 2000; 182:3210–3218. [PubMed: 10809702]
57. Holcombe C. *Helicobacter pylori*: the African enigma. *Gut*. 1992; 33:429–431. [PubMed: 1582581]
58. Atherton JC. The pathogenesis of *Helicobacter pylori*-induced gastro-duodenal diseases. *Annu Rev Pathol Mech Dis*. 2006; 1:63–96.
59. Cover TL, Blanke SR. *Helicobacter pylori* VacA, a paradigm for toxin multifunctionality. *Nat Rev Microbiol*. 2005; 3:320–332. [PubMed: 15759043]
60. Kusters JG, van Vliet AH, Kuipers EJ. Pathogenesis of *Helicobacter pylori* infection. *Clin Microbiol Rev*. 2006; 19:449–490. [PubMed: 16847081]
61. Boncristiano M, et al. The *Helicobacter pylori* vacuolating toxin inhibits T cell activation by two independent mechanisms. *J Exp Med*. 2003; 198:1887–1897. [PubMed: 14676300]
62. Gebert B, Fischer W, Weiss E, Hoffmann R, Haas R. *Helicobacter pylori* vacuolating cytotoxin inhibits T lymphocyte activation. *Science*. 2003; 301:1099–1102. [PubMed: 12934009]
63. Sundrud MS, Torres VJ, Unutmaz D, Cover TL. Inhibition of primary human T cell proliferation by *Helicobacter pylori* vacuolating toxin (VacA) is independent of VacA effects on IL-2 secretion. *Proc Natl Acad Sci USA*. 2004; 101:7727–7732. [PubMed: 15128946]
64. Mimuro H, et al. *Helicobacter pylori* dampens gut epithelial self-renewal by inhibiting apoptosis, a bacterial strategy to enhance colonization of the stomach. *Cell Host Microbe*. 2007; 2:250–263. [PubMed: 18005743]
65. Oldani A, et al. *Helicobacter pylori* counteracts the apoptotic action of its VacA toxin by injecting the CagA protein into gastric epithelial cells. *PLoS Pathog*. 2009; 5:e1000603. [PubMed: 19798427]
66. Tegtmeyer N, et al. Importance of EGF receptor, HER2/Neu and Erk1/2 kinase signaling for host cell elongation and scattering induced by the *Helicobacter pylori* CagA protein: antagonistic effects of the vacuolating cytotoxin VacA. *Cell Microbiol*. 2009; 11:488–505. [PubMed: 19046339]
67. Yokoyama K, et al. Functional antagonism between *Helicobacter pylori* CagA and vacuolating toxin VacA in control of the NFAT signaling pathway in gastric epithelial cells. *Proc Natl Acad Sci USA*. 2005; 102:9661–9666. [PubMed: 15980153]
68. Cover TL, Blaser MJ. Purification and characterization of the vacuolating toxin from *Helicobacter pylori*. *J Biol Chem*. 1992; 267:10570–10575. [PubMed: 1587837]
69. Leunk RD. Production of a cytotoxin by *Helicobacter pylori*. *Rev Infect Dis*. 1991; 13 (Suppl 8):S686–S689. [PubMed: 1925309]
70. Atherton JC, et al. Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. Association of specific vacA types with cytotoxin production and peptic ulceration. *J Biol Chem*. 1995; 270:17771–17777. [PubMed: 7629077]
71. Letley DP, Lastovica A, Louw JA, Hawkey CJ, Atherton JC. Allelic diversity of the *Helicobacter pylori* vacuolating cytotoxin gene in South Africa: rarity of the vacA s1a genotype and natural occurrence of an s2/m1 allele. *J Clin Microbiol*. 1999; 37:1203–1205. [PubMed: 10074554]
72. Sugimoto M, Zali MR, Yamaoka Y. The association of vacA genotypes and *Helicobacter pylori*-related gastroduodenal diseases in the Middle East. *Eur J Clin Microbiol Infect Dis*. 2009; 28:1227–1236. [PubMed: 19551413]
73. Sugimoto M, Yamaoka Y. The association of vacA genotype and *Helicobacter pylori*-related disease in Latin American and African populations. *Clin Microbiol Infect*. 2009; 15:835–842. [PubMed: 19392900]
74. Rhead JL, et al. A new *Helicobacter pylori* vacuolating cytotoxin determinant, the intermediate region, is associated with gastric cancer. *Gastroenterology*. 2007; 133:926–936. [PubMed: 17854597]

75. Hussein NR, et al. Differences in virulence markers between *Helicobacter pylori* strains from Iraq and those from Iran: potential importance of regional differences in *H. pylori*-associated disease. *J Clin Microbiol.* 2008; 46:1774–1779. [PubMed: 18353934]
76. Basso D, et al. Clinical relevance of *Helicobacter pylori* *cagA* and *vacA* gene polymorphisms. *Gastroenterology.* 2008; 135:91–99. [PubMed: 18474244]
77. Ogiwara H, Graham DY, Yamaoka Y. *vacA* i-region subtyping. *Gastroenterology.* 2008; 134:1267. [PubMed: 18395110]
78. Ogiwara H, et al. Role of deletion located between the intermediate and middle regions of the *Helicobacter pylori* *vacA* gene in cases of gastroduodenal diseases. *J Clin Microbiol.* 2009; 47:3493–3500. [PubMed: 19726606]
79. Figueiredo C, Machado JC, Yamaoka Y. Pathogenesis of *Helicobacter pylori* Infection. *Helicobacter.* 2005; 10 (Suppl 1):14–20. [PubMed: 16178966]
80. Fujimoto S, et al. *Helicobacter pylori* BabA expression, gastric mucosal injury, and clinical outcome. *Clin Gastroenterol Hepatol.* 2007; 5:49–58. [PubMed: 17157077]
81. Lu H, Yamaoka Y, Graham DY. *Helicobacter pylori* virulence factors: facts and fantasies. *Curr Opin Gastroenterol.* 2005; 21:653–659. [PubMed: 16220040]
82. Yamaoka Y, et al. *Helicobacter pylori* outer membrane proteins and gastroduodenal disease. *Gut.* 2006; 55:775–781. [PubMed: 16322107]
83. Yamaoka, Y.; Alm, R. *Helicobacter pylori*: Molecular Genetics and Cellular Biology. Yamaoka, Y., editor. Caister Academic Press; 2008. p. 37–60.
84. Yamaoka Y. Increasing evidence of the roles of *Helicobacter pylori* SabA in the pathogenesis of gastroduodenal disease. *J Infect Dev Ctries.* 2008; 2:174–181. [PubMed: 19738347]
85. Yamaoka Y. Roles of *H. pylori* BabA in gastroduodenal pathogenesis. *World J Gastroenterol.* 2008; 14:4265–4272. [PubMed: 18666312]
86. Yamaoka Y, Kwon DH, Graham DY. A M(r) 34,000 proinflammatory outer membrane protein (*oipA*) of *Helicobacter pylori*. *Proc Natl Acad Sci USA.* 2000; 97:7533–7538. [PubMed: 10852959]
87. Odenbreit S, Kavermann H, Puls J, Haas R. CagA tyrosine phosphorylation and interleukin-8 induction by *Helicobacter pylori* are independent from alpAB, HopZ and bab group outer membrane proteins. *Int J Med Microbiol.* 2002; 292:257–266. [PubMed: 12398216]
88. Ando T, et al. Polymorphisms of *Helicobacter pylori* HP0638 reflect geographic origin and correlate with *cagA* status. *J Clin Microbiol.* 2002; 40:239–246. [PubMed: 11773122]
89. Dossumbekova A, et al. *Helicobacter pylori* HopH (OipA) and bacterial pathogenicity: genetic and functional genomic analysis of *hopH* gene polymorphisms. *J Infect Dis.* 2006; 194:1346–1355. [PubMed: 17054063]
90. Akanuma M, et al. The evaluation of putative virulence factors of *Helicobacter pylori* for gastroduodenal disease by use of a short-term Mongolian gerbil infection model. *J Infect Dis.* 2002; 185:341–347. [PubMed: 11807716]
91. Yamaoka Y. *Helicobacter pylori* outer membrane proteins and gastric inflammation: author's reply. *Gut.* 2006; 55:1361. [PubMed: 16905703]
92. Yamaoka Y, et al. Role of interferon-stimulated responsive element-like element in interleukin-8 promoter in *Helicobacter pylori* infection. *Gastroenterology.* 2004; 126:1030–1043. [PubMed: 15057743]
93. Choi IJ, Fujimoto S, Yamauchi K, Graham DY, Yamaoka Y. *Helicobacter pylori* environmental interactions: effect of acidic conditions on *H pylori*-induced gastric mucosal interleukin-8 production. *Cell Microbiol.* 2007; 9:2457–2469. [PubMed: 17517062]
94. Lu H, et al. Functional and intracellular signaling differences associated with the *Helicobacter pylori* AlpAB adhesin from Western and East Asian strains. *J Biol Chem.* 2007; 282:6242–6254. [PubMed: 17202133]
95. Kudo T, et al. Pattern of transcription factor activation in *Helicobacter pylori*-infected Mongolian gerbils. *Gastroenterology.* 2007; 132:1024–1038. [PubMed: 17383425]
96. Wu JY, et al. Balance between polyoma enhancing activator 3 and activator protein 1 regulates *Helicobacter pylori*-stimulated matrix metalloproteinase 1 expression. *Cancer Res.* 2006; 66:5111–5120. [PubMed: 16707434]

97. Lu H, et al. Regulation of interleukin-6 promoter activation in gastric epithelial cells infected with *Helicobacter pylori*. *Mol Biol Cell*. 2005; 16:4954–4966. [PubMed: 16030249]
98. Tabassam FH, Graham DY, Yamaoka Y. OipA plays a role in *Helicobacter pylori*-induced focal adhesion kinase activation and cytoskeletal re-organization. *Cell Microbiol*. 2008; 10:1008–1020. [PubMed: 18067607]
99. Tabassam FH, Graham DY, Yamaoka Y. *Helicobacter pylori* activate epidermal growth factor receptor- and phosphatidylinositol 3-OH kinase-dependent Akt and glycogen synthase kinase 3 β phosphorylation. *Cell Microbiol*. 2009; 11:70–82. [PubMed: 18782353]
100. Yamaoka Y, et al. *Helicobacter pylori* infection in mice: Role of outer membrane proteins in colonization and inflammation. *Gastroenterology*. 2002; 123:1992–2004. [PubMed: 12454856]
101. Lu H, Hsu PI, Graham DY, Yamaoka Y. Duodenal ulcer promoting gene of *Helicobacter pylori*. *Gastroenterology*. 2005; 128:833–848. [PubMed: 15825067]
102. Hussein NR, et al. *Helicobacter pylori dupA* is polymorphic, and its active form induces proinflammatory cytokine secretion by mononuclear cells. *J Infect Dis*. 2010; 202:261–269. [PubMed: 20533870]
103. Schmidt HM, et al. The prevalence of the duodenal ulcer promoting gene (*dupA*) in *Helicobacter pylori* isolates varies by ethnic group and is not universally associated with disease development: a case-control study. *Gut Pathog*. 2009; 1:5. [PubMed: 19338650]
104. Yamaoka Y. Roles of the plasticity regions of *Helicobacter pylori* in gastroduodenal pathogenesis. *J Med Microbiol*. 2008; 57:545–553. [PubMed: 18436586]
105. Arachchi HSJ, et al. Prevalence of duodenal ulcer-promoting gene (*dupA*) of *Helicobacter pylori* in patients with duodenal ulcer in North Indian population. *Helicobacter*. 2007; 12:591–597. [PubMed: 18001398]
106. Zhang Z, et al. The *Helicobacter pylori* duodenal ulcer promoting gene, *dupA* in China. *BMC Gastroenterol*. 2008; 8:49. [PubMed: 18950522]
107. Douraghi M, et al. *dupA* as a risk determinant in *Helicobacter pylori* infection. *J Med Microbiol*. 2008; 57:554–562. [PubMed: 18436587]
108. Gomes LI, et al. Lack of association between *Helicobacter pylori* infection with *dupA*-positive strains and gastroduodenal diseases in Brazilian patients. *Int J Med Microbiol*. 2007; 298:223–230. [PubMed: 17897881]
109. Pacheco AR, et al. Involvement of the *Helicobacter pylori* plasticity region and *cag* pathogenicity island genes in the development of gastroduodenal diseases. *Eur J Clin Microbiol Infect Dis*. 2008; 27:1053–1059. [PubMed: 18560912]
110. Nguyen LT, et al. *Helicobacter pylori dupA* gene is not associated with clinical outcomes in the Japanese population. *Clin Microbiol Infect*. 2010; 16:1264–1269. [PubMed: 19832706]
111. Argent RH, Burette A, Miendje Deyi VY, Atherton JC. The presence of *dupA* in *Helicobacter pylori* is not significantly associated with duodenal ulceration in Belgium, South Africa, China, or North America. *Clin Infect Dis*. 2007; 45:1204–1206. [PubMed: 17918084]
112. Hussein NR. The association of *dupA* and *Helicobacter pylori*-related gastroduodenal diseases. *Eur J Clin Microbiol Infect Dis*. 2010; 29:817–821. [PubMed: 20419465]
113. Queiroz DM, et al. *dupA* polymorphisms and risk of distal gastric cancer. *Gastroenterology*. 2008; 134:A-85.
114. Kersulyte D, et al. Cluster of type IV secretion genes in *Helicobacter pylori*'s plasticity zone. *J Bacteriol*. 2003; 185:3764–3772. [PubMed: 12813069]
115. Kersulyte D, et al. *Helicobacter pylori*'s plasticity zones are novel transposable elements. *PLoS ONE*. 2009; 4:e6859. [PubMed: 19727398]
116. Fischer W, et al. Strain-specific genes of *Helicobacter pylori*: genome evolution driven by a novel type IV secretion system and genomic island transfer. *Nucleic Acids Res*. 10.1093/nar/gkq378
117. Couturier MR, Tasca E, Montecucco C, Stein M. Interaction with CagF is required for translocation of CagA into the host via the *Helicobacter pylori* type IV secretion system. *Infect Immun*. 2006; 74:273–281. [PubMed: 16368981]
118. Pattis I, Weiss E, Laugks R, Haas R, Fischer W. The *Helicobacter pylori* CagF protein is a type IV secretion chaperone-like molecule that binds close to the C-terminal secretion signal of the CagA effector protein. *Microbiology*. 2007; 153:2896–2909. [PubMed: 17768234]

119. Jimenez-Soto LF, et al. *Helicobacter pylori* type IV secretion apparatus exploits beta1 integrin in a novel RGD-independent manner. *PLoS Pathog.* 2009; 5:e1000684. [PubMed: 19997503]

Key points

- Experimental evidence suggests that East-Asian-type CagA is more virulent than Western-type CagA, but the concept that this explains geographic differences in the incidence of gastric cancer is still controversial
- The number of CagA Glu-Pro-Ile-Tyr-Ala (EPIYA)-C segments (with multiple repeats increasing the virulence) may explain, to some extent, geographic differences in the incidence of gastric cancer in Western countries
- The genotype of the *vacA* middle region (m1 versus m2; m1 being more virulent) may, in part, explain geographic differences in the incidence of gastric cancer in East Asian countries
- CagA, OipA and VacA are thought to interact synergistically with each other to induce serious disease
- The presence of functional DupA with an intact, full size *dupA* cluster (*tfs3a*) is associated with duodenal ulcers
- Bacterial factors, environmental factors and host factors are thought to form complex interactions with each other in the actual development of disease

Box 1*Helicobacter pylori* plasticity zones

- There are several regions of the *H. pylori* genome in which the G+C content is lower than in the rest of the genome (33–34% vs 39%)
- One region of such low G+C content is the *cag* PAI—the others have been named collectively as plasticity zones on the basis of the variability in gene content among different *H. pylori* isolates
- Nearly half of the strain-specific genes of *H. pylori* are located in plasticity zones

Review criteria

The references cited in this review were identified by searching the MEDLINE database. No date restrictions were applied to the search, which was limited to articles published in the English language. The search terms used were “*Helicobacter pylori*”, “CagA”, “cag pathogenicity island”, “VacA”, “OipA”, “DupA”, and “plasticity”. The reference lists of the articles identified were also searched to identify additional relevant references.

Figure 1.

Distribution of *Helicobacter pylori* genotypes before Columbus found the New World and human migration to America and Oceania began. There are seven modern *H. pylori* population types—hpEurope, hpEastAsia, hpAfrica1, hpAfrica2, hpAsia2, hpNEAfrica and hpSahul.^{7–9} hpEurope includes almost all *H. pylori* strains isolated from ethnic Europeans, including people from countries colonized by Europeans. Most *H. pylori* isolates from East Asia belong to hpEastAsia, which includes hspMaori (Polynesians, Melanesians, and native Taiwanese), hspAmerind (American Indians) and hspEAsia (East Asia) subpopulations. hpAsia2 strains are isolated in South, Southeast and Central Asia; hpAfrica1 in West Africa, South Africa and African Americans. hpNEAfrica is predominantly made up of isolates from Northeast Africa. hpAfrica2 is very distinct from any other type and has currently only been isolated in South Africa. hpSahul is a novel group specific to *H. pylori* strains isolated from Australian Aborigines and Highlanders of New Guinea. *H. pylori* is predicted to have spread from East Africa over the same time period as anatomically modern humans (~58,000 years ago), and has remained intimately associated with their human hosts ever since. Estimated global patterns of *H. pylori* migration are indicated by arrows and the numbers show the estimated time since they migrated (years ago). The broken arrow indicates an unconfirmed migration pattern. Detailed analyses of migration patterns have been explained previously.^{8,9}

Figure 2.

Structural polymorphism in CagA. Western-type CagA contain EPIYA-A, EPIYA-B, and EPIYA-C segments. By contrast, East-Asian-type CagA contain the EPIYA-A, EPIYA-B and EPIYA-D segments, but not the EPIYA-C segment. The EPIYA motif in each segment (shown in green) represents the tyrosine phosphorylation sites of CagA. The sequence flanking the tyrosine phosphorylation site of the EPIYA-D segment (EPIYATIDF), but not the EPIYA-C segment (EPIYATIDD), matches perfectly the consensus high-affinity binding sequence for the SH2 domains of SHP2. In Western countries, the incidence of gastric cancer is significantly higher in patients infected with strains containing multiple EPIYA-C segments than in patients infected with strains containing a single EPIYA-C segment (that is, ABCCC versus ABC). By contrast, almost all East Asian strains contain a single EPIYA-D segment. CagA forms dimers in cells in a phosphorylation-independent manner, and the CagA multimerization (CM) sequence (also named the conserved repeat responsible for phosphorylation-independent activity [CRPIA] or MARK2/PAR1b kinase inhibitor [MKI]) in yellow was identified as the site responsible for dimerization, for inhibition of MARK2/PAR1b kinase and for the interaction of CagA with activated c-Met.

Figure 3.

The pathogenesis of CagA-related signaling. In the early steps of CagA recognition, CagF binds CagA.^{117,118} CagL, CagY and probably CagI utilize host integrin $\beta 1$ as a cell-surface receptor, which triggers delivery of CagA into target cells.^{41,119} Injected CagA can then undergo tyrosine phosphorylation by Src and Abl family kinases at the EPIYA motifs located near the C-terminal end (in EPIYA-A [A], EPIYA-B [B], EPIYA-C [C] or EPIYA-D [D] segments). CagA binds to and activates or inactivates multiple signaling proteins in both a phosphorylation-dependent and phosphorylation-independent manner. There are currently 20 known cellular binding partners of CagA, 10 of which form phosphorylation-dependent interactions with CagA.³⁵ CagA itself forms dimers in a phosphorylation-independent manner, via the CagA multimerization (CM) sequence⁴² (also known as MKI or CRPIA). CM sequence is also essential for formation of the CagA-Par1⁴² and CagA-c-Met complexes.⁴⁶ The proposed functions of complexes formed by CagA with Grb7, SHP1, Ras-GAP, PI3K, α -Pix and integrin $\beta 1$ are unknown. Experimental data obtained via natural infection versus transfection of CagA are conflicting—PI3K, Grb2 and Crk did not interact with CagA in transfection experiments.³⁴

Figure 4.

The pathogenesis of OipA-related signaling. Phosphorylation by OipA reportedly regulates various epithelial cell signaling pathways, most of which are also important for *cag* PAI-related signaling. p38/STAT1 pathways are regulated independently of the *cag* PAI. OipA and the *cag* PAI are both necessary for full activation of the IL-8 promoter, but act via different pathways that diverge upstream of IRF1: only OipA is involved in the STAT1/IRF1/ISRE pathway.⁹² OipA is believed to function as an adhesin; but its target receptors have not been identified. OipA phosphorylation sites are shown for all molecules. EGFR contains more than 10 phosphorylation sites but only the two investigated as part of OipA-related signaling pathways^{98,99} are shown. OipA is reportedly involved in phosphorylation of FAK at tyrosine (Y) 397, Y576, Y577, Y861 and Y925, but not Y407; whereas the *cag* PAI is involved in phosphorylation of FAK at Y407 only.⁹⁸ However, another study reported that FAK phosphorylation at Y397 was *cag* PAI dependent.⁴¹ Broken arrows indicate unconfirmed interactions. Abbreviations: S, serine; T, threonine.

Figure 5.

Type IV secretion system in the *Helicobacter pylori* plasticity zone. Gene arrangement in the plasticity region of *H. pylori* strain Shi470, which has a complete *dupA* cluster (named *tfs3a* [type IV secretion 3a]) and functional *dupA* gene sequence, in comparison with corresponding regions of genome sequences of other *H. pylori* strains (G27, J99, P12 and 26695). Genes encoding type IV secretion system (T4SS) components are represented by arrows, with frameshift mutations indicated by asterisks. Genes encoding proteins that have 90–95% sequence similarity to the Shi470 proteins are shown in green and genes encoding proteins that have 50–75% sequence similarity are shown in pink. It is possible that only those *H. pylori* strains that have both a functional *dupA* gene sequence and a complete *tfs3a* are pathogenic and have the same action as the *cag* PAI and ComB T4SS. The numbers shown represent the gene number of each strain deposited in GenBank (GenBank accession numbers for Shi470, G27, J99, P12 and 26695 are CP001072, CP001173, AE001439, CP001217 and AE000511, respectively).

Table 1

Frequency of CagA phenotype and incidence of gastric cancer in 2008

Geographic region/country	Number of strains studied	Number of sequences by CagA type			Age-standardized incidence rates per 100,000 population for gastric cancer		
		Western	East Asian	Overall incidence	Male	Female	
World total	NA	NA	NA	14.1	19.8*	9.1	
Asia	NA	NA	NA	18.6*	25.9*	11.7	
East Asia	NA	NA	NA	30.0*	42.4*	18.3*	
South Korea	175	1	174	41.4*	62.2*	24.6*	
Japan	419	21	398	31.1*	46.8*	18.2*	
China	65	4	61	29.9*	41.3*	18.5*	
Taiwan	34	0	34	10.4	13.5	7.6	
West Asia	NA	NA	NA	9.4	12.6	6.7	
Southeast Asia	NA	NA	NA	8.6	10.9	6.7	
Vietnam	122	4	118	18.9*	24.4*	14.6	
Malaysia	3	2	1	8.4	10.7	6.4	
Thailand	106	54	52	3.5	4.2	3.0	
South-Central Asia	NA	NA	NA	5.3	6.7	3.9	
Kazakhstan	4	4	0	20.6*	31.7*	13.7	
Iran	91	91	0	15.6*	21.9*	9.0	
Turkey	51	51	0	13.5	18.9*	8.8	
Pakistan	26	26	0	6.3	8.0	4.5	

Geographic region/country	Number of strains studied	Number of sequences by CagA type			Age-standardized incidence rates per 100,000 population for gastric cancer		
		Western	East Asian		Overall incidence	Male	Female
India	4	4	0		3.8	4.7	2.9
<i>Latin America and Caribbean</i>	NA	NA	NA		11.7	15.7*	8.4
South America	NA	NA	NA		12.4	17.3*	8.4
Colombia	147	147	0		17.4*	23.4*	12.5
Chile	1	1	0		17.9*	27.3*	10.3
Brazil	10	10	0		10.9	16.2*	6.6
Central America	NA	NA	NA		10.9	12.7	9.3
Costa Rica	33	33	0		21.8*	28.5*	15.6*
Caribbean	NA	NA	NA		8.5	11.2	6.1
<i>Europe</i>	NA	NA	NA		10.3	14.7	7.0
Central-East Europe	NA	NA	NA		14.7	22.2*	9.7
West Europe	NA	NA	NA		6.5	9.0	4.4
Italy	57	57	0		10.9	14.8	7.7
Germany	1	1	0		7.7	10.3	5.5
Ireland	3	3	0		7.3	10.1	4.8
Greece	100	100	0		5.7	7.9	3.9
France	63	63	0		4.9	7.5	2.8
Sweden	5	5	0		4.3	5.7	3.1
North Europe	NA	NA	NA		6.2	8.6	4.2
<i>Oceania</i>	NA	NA	NA		5.5	7.5	3.7

Geographic region/country	Number of strains studied	Number of sequences by CagA type			Age-standardized incidence rates per 100,000 population for gastric cancer		
		Western	East Asian	Overall incidence	Male	Female	
Australia	1	1	0	5.2	7.3	3.2	
<i>North America</i>	NA	NA	NA	4.2	5.8	2.8	
Canada	20	20	0	4.8	6.7	3.1	
USA	152	152	0	4.1	5.7	2.8	
<i>Africa</i>	NA	NA	NA	4.0	4.7	3.3	
South Africa	12	12	0	3.2	4.5	2.3	

CagA sequencing data were initially obtained from the NCBI (April 16, 2007).²⁶ Data from previous studies on genotyping of the *cagA* repeat region were also included for South Korea, Japan, China, Taiwan, Kazakhstan, Pakistan, Italy, France, South Africa, Canada, USA, Colombia and Brazil,²⁰ in addition to Vietnam,^{20,24} Thailand,^{20,25} Iran²³ and Turkey.²² Data on age-standardized incidence rates (ASRs) per 100,000 population obtained from the GLOBOCAN database, which provides access to the most recent (2008) estimates of the incidence of, and mortality from, 27 major cancers worldwide; organized by the International Agency for Research on Cancer (IARC).¹

* Regions and countries with an ASR ≥ 15.0 . Abbreviation: NA, not available.

Table 2

*vacA*m region genotype and incidence of gastric cancer in 2008

Country/city	Number of strains studied	<i>vacA</i> genotypes (%)		Age-standardized incidence rates per 100,000 population for gastric cancer	
		m1	m2	Overall incidence	Male
South Korea	87	83 (95.4)	4 (4.6)	41.4	62.2
Japan	83	79 (95.2)	4 (4.8)	31.1	46.8
China	27	18 (66.7)	9 (33.3)	29.9	41.3
Vietnam*	78	29 (37.1)	45 (57.9)	18.9	24.4
Hanoi*†	38	19 (50.0)	17 (44.7)	NA	27
Ho Chi Minh*†	40	10 (25.0)	28 (70.0)	NA	18.7
Taiwan	28	9 (32.1)	19 (67.9)	10.4	13.5
					7.6

Data for *vacA* genotypes were obtained from previous studies by Yamaoka *et al.* (for South Korea, Japan, China and Taiwan)²⁰ and by Uchida *et al.* (for Vietnam).²⁴ To exclude the possible bias among countries, only simple gastritis cases were extracted from the published data. Data for gastric cancer incidence by country were obtained from the GLOBOCAN database, which provides access to the most recent estimates (for 2008) of the incidence of, and mortality from, 27 major cancers worldwide; organized by the International Agency for Research on Cancer (IARC).¹

* For four strains from Vietnam (two from Hanoi and two from Ho Chi Minh) neither m1 nor m2 could be detected; therefore, the sum of m1 and m2 does not match the number of strains studied.

† Data for gastric cancer incidence in two cities in Vietnam were obtained from the CI5 (Cancer Incidence in Five Continents) database, which provides access to detailed information on the incidence of cancer recorded by cancer registries (regional or national) worldwide; organized by the IARC (<http://ci5.iarc.fr/>). Abbreviations: m, middle; NA, not analyzed.