

# *Helicobacter pylori* Diversity and Gastric Cancer Risk

 Timothy L. Cover<sup>a,b,c</sup>

Department of Medicine<sup>a</sup> and Department of Pathology, Microbiology and Immunology,<sup>b</sup> Vanderbilt University School of Medicine, Nashville, Tennessee, USA; Veterans Affairs Tennessee Valley Healthcare System, Nashville, Tennessee, USA<sup>c</sup>

**ABSTRACT** Gastric cancer is a leading cause of cancer-related death worldwide. *Helicobacter pylori* infection is the strongest known risk factor for this malignancy. An important goal is to identify *H. pylori*-infected persons at high risk for gastric cancer, so that these individuals can be targeted for therapeutic intervention. *H. pylori* exhibits a high level of intraspecies genetic diversity, and over the past two decades, many studies have endeavored to identify strain-specific features of *H. pylori* that are linked to development of gastric cancer. One of the most prominent differences among *H. pylori* strains is the presence or absence of a 40-kb chromosomal region known as the *cag* pathogenicity island (PAI). Current evidence suggests that the risk of gastric cancer is very low among persons harboring *H. pylori* strains that lack the *cag* PAI. Among persons harboring strains that contain the *cag* PAI, the risk of gastric cancer is shaped by a complex interplay among multiple strain-specific bacterial factors as well as host factors. This review discusses the strain-specific properties of *H. pylori* that correlate with increased gastric cancer risk, focusing in particular on secreted proteins and surface-exposed proteins, and describes evidence from cell culture and animal models linking these factors to gastric cancer pathogenesis. Strain-specific features of *H. pylori* that may account for geographic variation in gastric cancer incidence are also discussed.

About 2 million new cancer cases each year worldwide are attributable to infections (1). Hepatitis viruses, papillomavirus, and *Helicobacter pylori* are responsible for most of these malignancies (arising in the liver, cervix, and stomach, respectively). Since *H. pylori* is the only bacterium known to be a common cause of cancer in humans, the relationship between *H. pylori* and gastric cancer is of particular interest.

A large body of evidence links *H. pylori* to two types of stomach cancer—gastric adenocarcinoma and gastric lymphoma. This review focuses on gastric adenocarcinoma, the most common type of stomach cancer. Epidemiological studies have shown that the risk of gastric cancer is higher in *H. pylori*-infected persons than in *H. pylori*-negative persons and that *H. pylori* infection precedes the development of gastric cancer (2–4). *H. pylori* is associated with adenocarcinoma of the distal (noncardia) stomach but not cancer of the proximal stomach. Experimental orogastric infection of Mongolian gerbils with *H. pylori* can result in the development of gastric cancer (5), which provides further evidence of a causative role. Consequently, the International Agency for Research on Cancer (World Health Organization) classifies *H. pylori* as a group I carcinogen (4), a category that includes well-known carcinogens such as tobacco smoke and asbestos.

*H. pylori* colonizes the stomach and elicits a gastric mucosal inflammatory response termed “gastritis” in both humans and experimentally infected animals. Once established in the human stomach, *H. pylori* and gastric inflammation can persist for many decades in the absence of antimicrobial treatment. Longitudinal studies indicate that gastritis is one of the first detectable changes in a stepwise pathway of histologic abnormalities that can ultimately culminate in gastric cancer: inflammation, gastric atrophy (loss of specialized cell types such as parietal cells and chief cells), intestinal metaplasia (presence of intestinal-type epithelium in the stomach), and dysplasia (6, 7). The development of gastric cancer in the setting of *H. pylori* infection is thought to be a long-term consequence of many alterations, including chronic inflammation (which contributes to the pathogenesis of many types of malignancy) (8), DNA damage, activation of gastric stem cells,

changes in cell proliferation and apoptosis, changes in epithelial differentiation and polarity, degradation of tumor suppressors, and impaired gastric acidification, leading to bacterial overgrowth with species not found in the normal acidic stomach (6, 7).

## EPIDEMIOLOGY OF GASTRIC CANCER

The incidence of gastric cancer varies markedly throughout the world, and it occurs about twice as commonly in males than females (3). The highest incidence rates are currently observed in East Asia (about 60 cases per 100,000 males in Japan and Korea) (3). In all parts of the world, *H. pylori* is the strongest known risk factor for gastric cancer (3, 4). Regions of the world with a low prevalence of *H. pylori* infection tend to have a relatively low incidence of gastric cancer, but geographic variation in gastric cancer rates cannot be explained entirely by variations in *H. pylori* prevalence. For example, populations in many parts of Africa and India have a high prevalence of *H. pylori* infection but a relatively low incidence of gastric cancer (3).

Although *H. pylori* is the strongest known risk factor for gastric cancer, most *H. pylori*-infected persons tolerate the presence of this organism over an entire lifetime without any adverse effects, and some persons may even derive health benefits from *H. pylori* (9). An important goal is to define the factors that determine whether gastric cancer will develop, so that the subset of *H. pylori*-infected persons with the highest risk of gastric cancer can be identified and targeted for therapeutic interventions.

Published 26 January 2016

**Citation** Cover TL. 2016. *Helicobacter pylori* diversity and gastric cancer risk. mBio 7(1): e01869-15. doi:10.1128/mBio.01869-15.

**Copyright** © 2016 Cover. This is an open-access article distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported license](https://creativecommons.org/licenses/by-nc-sa/4.0/), which permits unrestricted noncommercial use, distribution, and reproduction in any medium, provided the original author and source are credited.

Address correspondence to timothy.l.cover@vanderbilt.edu.

TABLE 1 Strain-specific *H. pylori* features that correlate with gastric cancer risk

Gene or region	Encoded protein(s)	Feature of gene in <i>H. pylori</i> strains <sup>a</sup>	
		Higher gastric cancer risk	Lower gastric cancer risk
<i>cag</i> PAI	CagA and T4SS	Present	Absent
<i>cagA</i>	Effector protein	More EPIYA motifs*	Fewer EPIYA motifs
		EPIYA-D motif*	Lack of EPIYA-D motif
		EPIYA-B motif*	EPIYT-B motif
		High levels of CagA production*	Lower levels of CagA production
<i>vacA</i>	Secreted toxin	s1, i1, m1 forms	s2, i2, m2 forms
<i>babA</i>	OMP	Present	Absent
<i>sabA</i>	OMP	In frame	Out of frame
<i>homB</i>	OMP	Present	Absent
<i>oipA</i> ( <i>hopH</i> )	OMP	In frame	Out of frame
<i>hopQ</i>	OMP	Type I form	Absence of type I form
<i>dupA</i>	VirB4 homolog	Absent	Present

<sup>a</sup> *cagA*-positive strains are associated with a higher risk of gastric cancer than are *cagA*-negative strains. Among *cagA*-positive strains, those producing CagA with the features indicated with an asterisk are associated with a higher risk of gastric cancer.

### H. PYLORI STRAIN-SPECIFIC PROPERTIES ASSOCIATED WITH GASTRIC CANCER

*H. pylori* is characterized by a high level of intraspecies genetic diversity (10, 11). Diversity in the nucleotide sequences of individual genes is attributable to a high mutation rate, as well as a high rate of intraspecies recombination (12, 13). Strains from unrelated persons not only differ in the sequences of individual genes but also exhibit differences in gene content and chromosomal organization. The core genome of *H. pylori* consists of about 1,100 genes (present in all *H. pylori* strains), and each strain typically contains several hundred additional genes that are not universally present.

Over the past two decades, numerous studies have analyzed *H. pylori* isolates from persons with different disease states in an effort to identify strain-specific features that correlate with the presence of gastric cancer or premalignant histologic lesions. This review focuses on strain-specific variations in secreted proteins or surface-exposed proteins that correlate with increased gastric cancer risk (Table 1) and describes the actions of these factors in cell culture and animal models that link these proteins to gastric cancer pathogenesis.

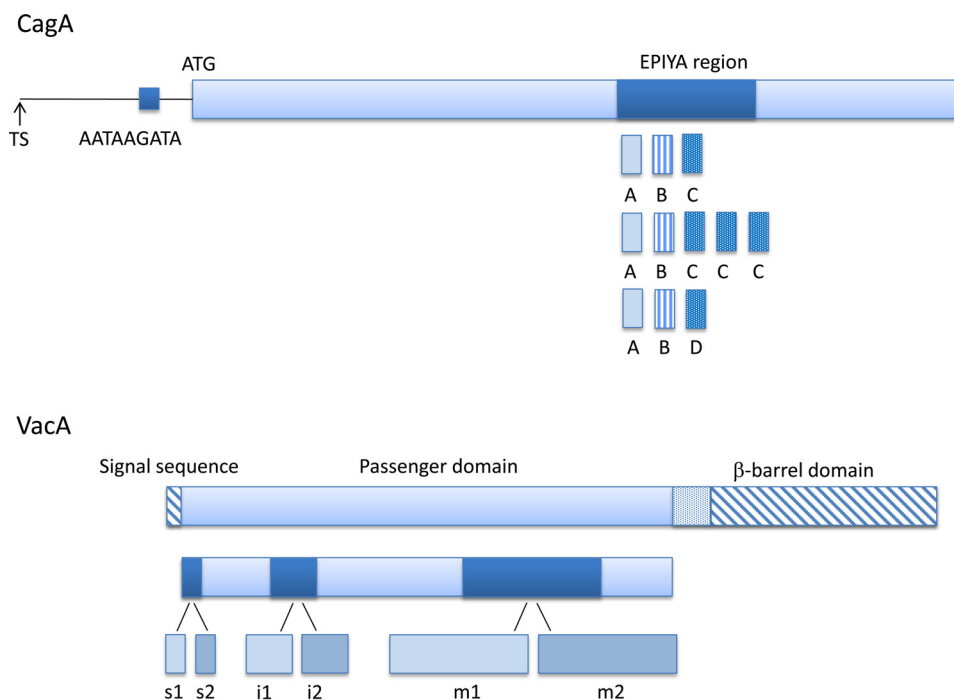
**The *cag* pathogenicity island.** One of the most striking variations among *H. pylori* strains from unrelated persons is the presence or absence of a chromosomal region known as the *cag* pathogenicity island (PAI). Individual strains may contain an intact *cag* PAI (about 40 kb), a *cag* PAI that has undergone chromosomal rearrangements, or an incomplete *cag* PAI that lacks one or more genes (14). The *cag* PAI encodes an antigenic effector protein (CagA) and contains about 18 genes required for the entry of CagA into host cells through a type IV secretion system (T4SS)-mediated process (15–17). In addition to its role in translocating CagA into host cells, the type IV secretion system is required for *H. pylori*-induced upregulation of proinflammatory cytokine secretion by gastric epithelial cells (18).

Upon entry into host cells, CagA undergoes tyrosine phosphorylation by Src and Abl family kinases at sites known as EPIYA motifs (16, 17, 19). CagA in either its phosphorylated form or its nonphosphorylated form can interact with at least 10 host cell components, resulting in a complex assortment of cellular alterations (16, 17). These include changes in epithelial cell shape and polarity (20, 21), disruption of apical epithelial junctional com-

plexes (22), changes in epithelial differentiation (resulting in an invasive phenotype, resembling an epithelial to mesenchymal transition) (23), enhanced proliferation and inhibited apoptosis (24, 25), activation of gastric stem cells (26), and degradation of the p53 tumor suppressor (27). When experimentally introduced into the stomachs of Mongolian gerbils, *H. pylori* strains containing *cagA* and an intact *cag* PAI promote the development of premalignant changes and gastric cancer, whereas *cagA* mutant strains or mutant strains with a defective *cag* T4SS fail to cause gastric cancer (28–31). Similarly, strains containing *cagA* and an intact *cag* PAI cause more severe gastric inflammation in gerbils than do *cagA* mutant strains or mutant strains with a defective *cag* T4SS (28–31). Transgenic mice expressing CagA spontaneously develop gastric epithelial hyperplasia, adenocarcinoma of the stomach and small intestine, and hematologic malignancies (32). Therefore, CagA has been designated a bacterial oncoprotein (17).

A large body of evidence indicates that the risk of gastric cancer or premalignant lesions is higher in persons infected with *cagA*-positive *H. pylori* strains than in persons infected with *cagA*-negative strains (33–35). The increased risk of gastric cancer observed with *cagA*-positive strains (which often contain the entire *cag* PAI) is attributed to the cellular effects of CagA (described above), combined with an enhanced gastric mucosal inflammatory response (36).

Most studies linking CagA-producing strains to increased gastric risk have analyzed *H. pylori* isolates to determine whether the *cagA* gene is present or absent or have assessed the presence of anti-CagA serum antibody responses as an indication of infection with CagA-producing strains. The risk of gastric cancer among persons infected with CagA-producing strains can be further stratified by analyzing CagA amino acid sequence variations that influence the protein's activity. For example, the level of CagA tyrosine phosphorylation in host cells is influenced by the number of EPIYA motifs within the protein that can undergo phosphorylation (Fig. 1). CagA proteins containing higher numbers of EPIYA motifs exhibit enhanced binding to intracellular targets and enhanced activity within host cells (37–39), and strains harboring higher numbers of EPIYA motifs (Fig. 1) are associated with greater gastric cancer risk (40, 41). The intracellular activity of CagA is also influenced by amino acid sequence variations within the tyrosine phosphorylation motifs. For example, strains



**FIG 1** Diversity in amino acid sequences of CagA and VacA proteins. Dark blue coloration indicates regions of diversity that influence gastric cancer risk. (A) CagA is a translocated into host cells through a type IV secretion system-dependent process. Within host cells, CagA undergoes tyrosine phosphorylation at sites known as EPIYA motifs. Most CagA proteins contain multiple EPIYA motifs, which are designated EPIYA-A, EPIYA-B, EPIYA-C, or EPIYA-D based on the flanking sequences. Among CagA proteins produced by different *H. pylori* strains, there is variation in the number and type of EPIYA motifs. EPIYA-D motifs are found almost exclusively in CagA proteins produced by *H. pylori* strains from East Asia. Nucleotide sequence variation in an untranslated region upstream of the ATG start codon in the vicinity of an AATAAGATA motif influences levels of CagA production. TS, transcription start site. (B) VacA is secreted by a type V (autotransporter) secretion system. A 140-kDa VacA precursor protein undergoes cleavage of an amino-terminal signal sequence and C-terminal proteolytic processing, resulting in an 88-kDa secreted passenger domain, a small secreted peptide, and a  $\beta$ -barrel domain localized to the outer membrane. There are three main regions of diversity within the 88-kDa passenger domain, designated the s, i, and m regions; within each region, sequences can be classified into one of two main types (s1 or s2, i1 or i2, and m1 or m2). Multiple possible combinations (s1/i1/i1, s2/i2/i2, s1/i2/m2, etc.) can be present in different *H. pylori* strains as a result of recombination.

containing EPIYT-B motifs are less frequently associated with gastric cancer than are strains containing EPIYA-B motifs (42).

Among *H. pylori* strains that produce CagA, there is variation in the levels of CagA production. This variation is attributable to nucleotide sequence variation downstream of the *cagA* transcriptional start site, within the 5' untranslated region of the *cagA* transcript (Fig. 1) (43, 44). Two recent studies showed that strains producing high levels of CagA are linked to an increased risk of premalignant lesions compared to strains producing lower levels of CagA (43, 44).

**Vacuolating toxin.** *H. pylori* secretes a protein known as VacA through an autotransporter or type V secretion pathway (45–47). VacA was originally identified based on its capacity to cause vacuolation of epithelial cells (48) but is now known to have a much broader range of activities (45–47). The amino acid sequence, structure, and cellular effects of VacA are unrelated to those of any other known bacterial toxins (49). Most VacA-induced cellular alterations are attributable to its capacity for pore formation in cell membranes (45–47).

All *H. pylori* strains contain a *vacA* gene, and nearly all secrete a VacA protein, but there is considerable variation among strains in VacA-mediated effects on host cells. This is due to differences among strains in the levels of VacA produced or secreted (50), as well as amino acid sequence variation among VacA proteins. Several families of *vacA* alleles have been described based on se-

quences differences in regions of *vacA* designated the s, i, and m regions (Fig. 1) (51–53). Type s1/i1/m1 forms of VacA are more active in assays of cell-vacuolating activity than are forms of VacA designated s2, i2, or m2, and type s2 forms of VacA lack detectable activity in this assay (51, 52, 54, 55). The differences in activity of type s1 and s2 forms of VacA are attributable to impaired channel-forming properties of the type s2 protein (54).

Thus far, most studies of VacA cellular effects have been conducted with type s1/i1/m1 forms of VacA. Studies with this form of the protein indicate that it enters host cells through a clathrin-independent process and can ultimately localize in endosomes as well as mitochondria (56–58). Consequences of VacA intoxication in epithelial cells include altered endosomal function (59), changes in mitochondrial membrane permeability (56), stimulation of autophagy (60), reactive oxygen species accumulation (61), and sometimes cell death (45–47). VacA inhibits the acid-secretory capacity of parietal cells (62) and causes functional alterations in a variety of immune cells. Inhibitory effects of VacA on T cells have been studied in the most detail (63, 64), but B cells, eosinophils, mast cells, and dendritic cells are also targeted (45–47).

Strains containing *vacA* alleles classified as s1, i1, or m1 (encoding the more active forms of VacA) are associated with a higher risk of gastric cancer or premalignant lesions (such as intestinal metaplasia) than strains classified as s2, i2, or m2 (34, 41, 52, 65). In a mouse model, *H. pylori* strains producing s1/i1 forms of VacA

induced more severe and extensive intestinal metaplasia and inflammation in the stomach than strains producing s2/i2 toxins (65). The increased risk of gastric cancer associated with strains producing more active forms of VacA may be a consequence of several actions, including the capacity of VacA to stimulate gastric epithelial cell injury, alter parietal cell function and gastric acidification, and interfere with immune cell function.

**Outer membrane proteins and DupA.** The *H. pylori* genome contains about 60 genes that are predicted to encode outer membrane proteins (OMPs). While many OMP-encoding genes are conserved among all *H. pylori* strains, others may be present or absent in individual strains, and production of OMPs can be regulated by slipped-strand mispairing within polynucleotide repeat regions. Two of the most extensively studied OMPs, BabA and SabA, function as adhesins that mediate *H. pylori* binding to gastric epithelial cells. BabA binds to the fucosylated Lewis b histo-blood group antigen on host cells, and SabA binds to the sialyl dimeric Lewis x glycosphingolipid (66, 67). In addition to its role in mediating *H. pylori* adherence to epithelial cells, SabA can function as a sialic acid-dependent hemagglutinin and has a role in nonopsonic activation of neutrophils (68).

Several studies have reported that infection with *H. pylori* strains containing in-frame *babA* or *sabA* genes is associated with an increased risk of gastric cancer, premalignant changes, or enhanced gastric inflammation, compared to infection with strains that lack these genes or that harbor out-of-frame genes (69–72). When challenged with a BabA-producing *H. pylori* strain, transgenic mice expressing the BabA receptor developed more severe gastritis, atrophy, and anti-parietal cell antibodies than infected wild-type animals (73). These results indicate that BabA-mediated adherence modulates the outcome of infection.

Three other OMPs (HomB, HopQ and HopH [OipA]) have been linked to gastric cancer. HomA and HomB are two closely related *H. pylori* OMPs; individual strains may contain one or both of the corresponding genes (74). Similarly, two forms of HopQ (designated type I and type II) have been described; individual strains may contain one or both of the corresponding genes (75). Strains containing *homB* or type I *hopQ* or in-frame *hopH* (*oipA*) alleles have been associated with an increased risk of gastric cancer, compared to strains lacking these features (72, 76–78). The presence of an in-frame *hopH* (*oipA*) allele contributes to development of gastric cancer in Mongolian gerbils and has been linked to increased gastric inflammation in mice (29, 79). In comparison to a wild-type strain, a *homB* mutant strain exhibited diminished adherence to gastric epithelial cells and reduced capacity to stimulate production of the proinflammatory cytokine interleukin 8 (IL-8) (74). HopQ is required for maximal activity of the *cag* T4SS (80). Cell culture experiments suggest that HopH (OipA) can contribute to *H. pylori* adherence (81) and also may have a role in regulating expression of proinflammatory cytokines and other processes in gastric epithelial cells (82). HopH (OipA) does not contribute to stimulation of cytokine expression unless the *cag* PAI is also present (81, 83).

Finally, a gene designated *dupA* (for “duodenal-ulcer-promoting gene”), located within a nonconserved region of the *H. pylori* chromosome known as the plasticity region, is reported to be a marker of gastric cancer risk. Strains containing this gene have been associated with a reduced risk of gastric atrophy and gastric cancer compared to strains lacking this gene (84, 85). There is variation among strains in the length of the *dupA* ORF, and this

may also influence the risk of gastric cancer (86, 87). The *dupA* gene exhibits weak sequence similarity to *virB4* components of type IV secretion systems, but at present it is not known whether *dupA* has a similar activity in *H. pylori*. DupA is reported to have a role in stimulating IL-8 production in gastric epithelial cells and promoting *H. pylori* survival at low pH (84), and strains containing active forms of the *dupA* gene induce proinflammatory cytokine production in mononuclear cells (86).

Associations of specific OMPs or *dupA* with gastric cancer have been detected less consistently than associations between the *cag* PAI or *vacA* and gastric cancer. Nevertheless, a substantial body of experimental evidence indicates that several OMPs and *dupA* influence *H. pylori*-host interactions, and as discussed below, there is an association between the presence of several of these genes and the presence of the *cag* PAI and type s1 *vacA*. These OMPs and DupA may contribute to gastric cancer pathogenesis by augmenting *H. pylori* adherence, enhancing the activity of the *cag* T4SS, influencing *H. pylori*-induced signaling in host cells, or stimulating proinflammatory immune responses.

#### RELATIONSHIPS AMONG STRAIN-SPECIFIC DETERMINANTS OF GASTRIC CANCER RISK

Intraspecies genetic recombination occurs commonly in *H. pylori*, and the species is considered to have a recombinational population structure (12, 13). Therefore, most polymorphisms are distributed randomly among individual strains (12). Interestingly, the strain-specific features associated with gastric cancer (Table 1) tend to be distributed nonrandomly, even though these genes are localized at unlinked sites in the *H. pylori* chromosome. For example, the majority of *cag* PAI-positive strains contain type s1 *vacA*, *babA*, *homB*, type I *hopQ*, and in-frame *oipA* alleles, and the majority of *cag* PAI-negative strains contain type s2 *vacA*, type II *hopQ*, and out-of-frame *oipA* alleles and lack *babA* and *homB* (51, 69, 74, 75, 81, 83, 88, 89).

The nonrandom distribution of strain-specific features associated with gastric cancer (Table 1) is presumably attributable to selective forces that favor certain combinations. Recent studies in cell culture models have revealed functional interactions among these proteins that may account for the observed associations. For example, both CagA and VacA are used by *H. pylori* to acquire nutrients such as iron from host cells (90), and this process may require a balanced activity of the two proteins. In addition, the effects of CagA on gastric epithelial cells are attenuated by the presence of VacA, and the actions of VacA are attenuated by the presence of CagA (91–94). VacA stimulates degradation of CagA through autophagy (61), which may account for some of the observed antagonism. Functional interactions between OMPs and the T4SS also have been reported (80, 95). For example, BabA and HopQ can potentiate *cag* T4SS-dependent phenotypes (80, 95). In addition, *cag*-positive strains stimulate expression of a host cell gene required for synthesis of sialyl Lewis x (the receptor for the SabA adhesin) (96). By inducing higher levels of gastric inflammation, *cag*-positive strains can indirectly stimulate increased SabA-mediated attachment of the bacteria to epithelial cells.

Several studies have analyzed the correlation between multiple strain-specific features and the development of gastric cancer. These studies indicate that the risk of gastric cancer is highest in persons infected with strains harboring multiple constituents listed in Table 1 (e.g., *cagA*, type s1 *vacA* and *babA*) (34, 69). Strains harboring few or none of these features are less frequently



associated with gastric cancer. Collectively, these studies suggest that there is a spectrum of strains, ranging from those associated with a very high incidence of gastric cancer to those associated with a very low risk of gastric cancer.

### GEOGRAPHIC VARIATION IN GASTRIC CANCER INCIDENCE

The incidence of gastric cancer varies markedly throughout the world, for reasons that are not well understood. Since the incidence of gastric cancer is particularly high in East Asia (3), efforts have been directed toward comparing *H. pylori* isolates from East Asia with isolates from other parts of the world. One of the most striking observations is that several of the strain-specific features linked to high gastric cancer risk (including the *cagA* PAI, type s1 forms of *vacA* and *babA*) are present in nearly all East Asian *H. pylori* isolates (88, 97, 98). Conversely, *cagA*-negative strains containing type s2 *vacA* alleles and lacking *babA* are commonly found in the United States and Western Europe but are rarely isolated in East Asia (51, 88, 89). The predominance of strains harboring *cagA*, type s1 *vacA*, and other strain-specific markers linked to gastric cancer (Table 1) may be one of the factors contributing to a high rate of gastric cancer in East Asia.

Most *H. pylori* isolates from East Asia constitute a distinct group based on multilocus sequence typing of housekeeping genes (99), and genes under positive selection (including *cagA* and *vacA*) are highly divergent in East Asian strains compared to strains isolated elsewhere in the world (100). The distinctive properties of CagA in East Asian strains have been studied in the most detail. Specifically, a CagA tyrosine phosphorylation motif (EPIYA-D) found exclusively in East Asian strains is associated with a higher level of CagA tyrosine phosphorylation within cells and greater cellular effects than are seen with non-Asian forms of CagA (37, 101, 102). Correspondingly, strains producing CagA proteins with EPIYA-D motifs have been associated with a higher risk of gastric cancer than strains producing other forms of CagA (103). There has been relatively little effort to analyze the functions of other proteins besides CagA in East Asian strains compared to non-Asian strains, but East Asian forms of AlpA and AlpB outer membrane proteins are reported to have different effects on signaling in gastric epithelial cells than forms of AlpA/AlpB found elsewhere in the world (104). Thus, specialized properties of CagA and other constituents in East Asian *H. pylori* strains may contribute to the high incidence of gastric cancer in that part of the world. Variations in host genetics and environmental factors are also likely to contribute to geographic differences in gastric cancer incidence.

### RELATIONSHIP BETWEEN BACTERIAL AND HOST RISK FACTORS FOR GASTRIC CANCER

Multiple host-related factors are known to be determinants of gastric cancer risk (105). In the context of *H. pylori* infection, polymorphisms in genes involved in cytokine production have been studied in the most detail (6, 105, 106). An association between gastric cancer risk and polymorphisms linked to IL-1 $\beta$  production is relevant because this cytokine not only contributes to gastric inflammation but also regulates gastric acid secretion. Several studies have shown that the contribution of specific bacterial factors to gastric cancer risk is augmented in persons who have specific genetic risk factors. For example, the risk of gastric cancer is particularly high in persons harboring certain polymorphisms in genes encoding cytokines (IL-1 $\beta$ , tumor necrosis factor [TNF], and IL-10) who are infected with *H. pylori* strains containing type

s1 *vacA* and *cagA* (34, 106, 107). These studies indicate that the risk of gastric cancer is determined by both bacterial and host factors.

The composition of the human diet is another factor that influences gastric cancer risk. For example, a high-salt diet and a diet low in fruits and vegetables have been associated with increased gastric cancer risk (3, 108). A relationship between composition of the diet and strain-specific *H. pylori* risk factors for gastric cancer also has been observed in experiments with animal models of gastric cancer. Specifically, Mongolian gerbils infected with a *cag* PAI-positive strain and fed high-salt or low-iron diets had an increased incidence of gastric cancer compared to infected animals fed a regular diet, whereas the high-salt and low-iron diets did not confer an increased risk of gastric cancer in animals infected with a *cagA* mutant strain or uninfected animals (30, 31). The mechanisms by which dietary composition influences gastric cancer risk are not yet well understood. Changes in *H. pylori* gene transcription in response to the composition of the diet may be one mechanism. For example, *H. pylori* produces increased levels of CagA when exposed to high-salt conditions (109), and low-iron conditions stimulate enhanced activity of the *cag* T4SS (30). These results in animal models suggest that certain dietary risk factors for gastric cancer are relevant mainly in persons who are infected with *cag* PAI-positive strains.

Multilocus sequence typing of housekeeping genes in *H. pylori* isolates from human populations throughout the world has allowed the identification of groups of strains with distinct geographic distributions (99). The observed patterns of geographic diversity suggest that *H. pylori* has been present in humans for at least 100,000 years (110), that *H. pylori* accompanied humans out of Africa in multiple waves of migration beginning about 60,000 years ago (111), and that *H. pylori* strains subsequently diversified in relative isolation in various parts of the world (111). Evolutionary theory posits that prolonged association of pathogenic organisms with hosts should lead to a progressive loss of virulence. Since *H. pylori* has been associated with humans for at least 100,000 years, one might anticipate a gradual reduction in the capacity of these bacteria to cause disease.

The development of a commensal or symbiotic relationship between *H. pylori* and humans is presumably dependent on coevolution of the bacteria and human hosts over a prolonged time period. In many parts of Africa, a high proportion of humans carry *H. pylori*, but the rate of gastric cancer is relatively low (a phenomenon known as the African enigma) (3). Similarly, a recent study in the country of Colombia revealed that humans of African origin, when infected with *H. pylori* strains of African origin, had relatively benign gastric pathology with little evidence of progression to gastric cancer (112). The low incidence of disease in these populations illustrates a relationship between *H. pylori* and humans reflecting coevolution of both species over a prolonged period of time. Introduction of *H. pylori* into a noncoevolved human population may result in a less favorable outcome. In support of this hypothesis, mismatch between the geographic origin of *H. pylori* strains and the geographic ancestry of human hosts has been associated with more severe gastric pathology and development of premalignant gastric lesions (112). These findings bolster the view that gastric cancer risk is influenced by both bacterial and host factors and suggest that disruptions in coevolved bacterial-human relationships may contribute to elevated gastric cancer risk.

## FUTURE PROSPECTS

Comparison of *H. pylori* strains isolated from patients with gastric cancer to isolates from patients with non-malignant gastric histology has led to the identification of multiple strain-specific constituents that contribute to gastric cancer pathogenesis (Table 1). Most previous studies have analyzed *H. pylori* strains isolated from single gastric biopsy specimens obtained at the time when gastric cancer was diagnosed. In future studies, more robust sampling approaches will allow analysis of *H. pylori* strains at earlier time points (prior to the development of gastric cancer) and will allow the detection of multiple strains of *H. pylori* within individual stomachs. Inhibition of T4SS-mediated phenomena has been observed *in vitro* when cell lines are coinfecting with two different *H. pylori* strains (113), and similarly, diminished severity of disease has been observed in human subjects coinfecting with *cagA*-positive and *cagA*-negative strains (114). Therefore, further studies of the functional consequences of coinfection with multiple strains of *H. pylori* are warranted.

Thus far, only a few candidate strain-specific *H. pylori* genes have been evaluated to detect possible links to gastric cancer, and often the analysis has been limited to a determination of whether a gene is present or absent. In future studies, it will be important to use a more comprehensive approach for analyzing *H. pylori* genetic variation to permit analysis of a larger number of strain-specific bacterial factors. In addition to determining whether genes are present or absent, important insights will be gained by investigating whether the encoded proteins are produced, analyzing levels of gene transcription or protein production, and analyzing variations in gene sequences that are linked to variations in protein function. It will also be important to investigate further the functions of the strain-specific *H. pylori* features listed in Table 1 as well as newly identified strain-specific constituents in cell culture and animal models and thereby elucidate the mechanisms by which these factors contribute to gastric cancer pathogenesis.

Recent studies have revealed functional interactions among several bacterial factors linked to gastric cancer pathogenesis (91–94). Developing an improved understanding of these functional interactions and the effects of such interactions on gastric cancer pathogenesis is also an important goal. For example, since VacA can inhibit the actions of CagA in cell culture models, it will be important to determine whether imbalances in VacA and CagA production can influence gastric cancer risk.

Further investigation of the geographic variation in gastric cancer incidence is also warranted. In particular, there has been relatively little effort thus far to evaluate whether the variations in amino acid sequences of *H. pylori* proteins in strains from different geographic regions are accompanied by alterations in protein function and whether this sequence variation influences the development of gastric cancer. It will also be important to investigate further the coevolved relationships between *H. pylori* and humans that minimize the risk of gastric cancer, as well as the molecular basis by which disruption in these relationships leads to increased cancer risk.

In summary, a large body of evidence indicates that there is a correlation between the risk of gastric cancer and strain-specific features of *H. pylori* strains, and studies in cell culture systems and animal models provide mechanistic support for the observed correlations. In future studies, it should be possible to define more clearly the role of strain-specific *H. pylori* constituents in gastric carcinogenesis. A better understanding of this topic may lead to

the development of improved methods to identify *H. pylori*-infected persons at high risk for development of gastric cancer, so they can be targeted for therapeutic interventions.

## ACKNOWLEDGMENTS

This work was supported by NIH AI039657, CA116087, and the Department of Veterans Affairs (2I01BX000627).

## FUNDING INFORMATION

HHS | National Institutes of Health (NIH) provided funding to Timothy L Cover under grant numbers AI039657 and CA116087. U.S. Department of Veterans Affairs (VA) provided funding to Timothy L Cover under grant number 2I01BX000627.

## REFERENCES

- de Martel C, Ferlay J, Franceschi S, Vignat J, Bray F, Forman D, Plummer M. 2012. Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. *Lancet Oncol* 13:607–615. [http://dx.doi.org/10.1016/S1470-2045\(12\)70137-7](http://dx.doi.org/10.1016/S1470-2045(12)70137-7).
- Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. 2001. *Helicobacter pylori* infection and the development of gastric cancer. *N Engl J Med* 345:784–789. <http://dx.doi.org/10.1056/NEJMoa001999>.
- de Martel C, Forman D, Plummer M. 2013. Gastric cancer: epidemiology and risk factors. *Gastroenterol Clin North Am* 42:219–240. <http://dx.doi.org/10.1016/j.gtc.2013.01.003>.
- IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 2012. Biological agents. Volume 100 B. A review of human carcinogens. IARC Monogr Eval Carcinog Risks Hum 100:1–441.
- Watanabe T, Tada M, Nagai H, Sasaki S, Nakao M. 1998. *Helicobacter pylori* infection induces gastric cancer in Mongolian gerbils. *Gastroenterology* 115:642–648. [http://dx.doi.org/10.1016/S0016-5085\(98\)70143-X](http://dx.doi.org/10.1016/S0016-5085(98)70143-X).
- Peek RM, Jr., Blaser MJ. 2002. *Helicobacter pylori* and gastrointestinal tract adenocarcinomas. *Nat Rev Cancer* 2:28–37. <http://dx.doi.org/10.1038/nrc703>.
- Fox JG, Wang TC. 2007. Inflammation, atrophy, and gastric cancer. *J Clin Invest* 117:60–69. <http://dx.doi.org/10.1172/JCI30111>.
- Elinav E, Nowarski R, Thaiss CA, Hu B, Jin C, Flavell RA. 2013. Inflammation-induced cancer: crosstalk between tumours, immune cells and microorganisms. *Nat Rev Cancer* 13:759–771. <http://dx.doi.org/10.1038/nrc3611>.
- Cover TL, Blaser MJ. 2009. *Helicobacter pylori* in health and disease. *Gastroenterology* 136:1863–1873. <http://dx.doi.org/10.1053/j.gastro.2009.01.073>.
- Blaser MJ, Berg DE. 2001. *Helicobacter pylori* genetic diversity and risk of human disease. *J Clin Invest* 107:767–773. <http://dx.doi.org/10.1172/JCI12672>.
- Suerbaum S, Josenhans C. 2007. *Helicobacter pylori* evolution and phenotypic diversification in a changing host. *Nat Rev Microbiol* 5:441–452. <http://dx.doi.org/10.1038/nrmicro1658>.
- Suerbaum S, Smith JM, Bapumia K, Morelli G, Smith NH, Kunstmann E, Dyrek I, Achtman M. 1998. Free recombination within *Helicobacter pylori*. *Proc Natl Acad Sci U S A* 95:12619–12624. <http://dx.doi.org/10.1073/pnas.95.21.12619>.
- Morelli G, Didelot X, Kusecek B, Schwarz S, Bahlawane C, Falush D, Suerbaum S, Achtman M. 2010. Microevolution of *Helicobacter pylori* during prolonged infection of single hosts and within families. *PLoS Genet* 6:e1001036. <http://dx.doi.org/10.1371/journal.pgen.1001036>.
- Olbermann P, Josenhans C, Moodley Y, Uhr M, Stamer C, Vauterin M, Suerbaum S, Achtman M, Linz B. 2010. A global overview of the genetic and functional diversity in the *Helicobacter pylori* cag pathogenicity island. *PLoS Genet* 6:e1001069. <http://dx.doi.org/10.1371/journal.pgen.1001069>.
- Fischer W. 2011. Assembly and molecular mode of action of the *Helicobacter pylori* Cag type IV secretion apparatus. *FEBS J* 278:1203–1212. <http://dx.doi.org/10.1111/j.1742-4658.2011.08036.x>.
- Tegtmeyer N, Wessler S, Backert S. 2011. Role of the cag-pathogenicity island encoded type IV secretion system in *Helicobacter pylori* pathogenesis. *FEBS J* 278:1190–1202. <http://dx.doi.org/10.1111/j.1742-4658.2011.08035.x>.

17. Hatakeyama M. 2014. *Helicobacter pylori* CagA and gastric cancer: a paradigm for hit-and-run carcinogenesis. *Cell Host Microbe* 15: 306–316. <http://dx.doi.org/10.1016/j.chom.2014.02.008>.
18. Viala J, Chaput C, Boneca IG, Cardona A, Girardin SE, Moran AP, Athman R, Mémet S, Huerre MR, Coyle AJ, DiStefano PS, Sansonetti PJ, Labigne A, Bertin J, Philpott DJ, Ferrero RL. 2004. Nod1 responds to peptidoglycan delivered by the *Helicobacter pylori* cag pathogenicity island. *Nat Immunol* 5:1166–1174. <http://dx.doi.org/10.1038/nri1131>.
19. Odenbreit S, Püls J, Sedlmaier B, Gerland E, Fischer W, Haas R. 2000. Translocation of *Helicobacter pylori* CagA into gastric epithelial cells by type IV secretion. *Science* 287:1497–1500. <http://dx.doi.org/10.1126/science.287.5457.1497>.
20. Segal ED, Cha J, Lo J, Falkow S, Tompkins LS. 1999. Altered states: involvement of phosphorylated CagA in the induction of host cellular growth changes by *Helicobacter pylori*. *Proc Natl Acad Sci U S A* 96: 14559–14564. <http://dx.doi.org/10.1073/pnas.96.25.14559>.
21. Saadat I, Higashi H, Obuse C, Umeda M, Murata-Kamiya N, Saito Y, Lu H, Ohnishi N, Azuma T, Suzuki K, Ohno S, Hatakeyama M. 2007. *Helicobacter pylori* CagA targets PARI/MARK kinase to disrupt epithelial cell polarity. *Nature* 447:330–333. <http://dx.doi.org/10.1038/nature05765>.
22. Amieva MR, Vogelmann R, Covacci A, Tompkins LS, Nelson WJ, Falkow S. 2003. Disruption of the epithelial apical-junctional complex by *Helicobacter pylori* CagA. *Science* 300:1430–1434. <http://dx.doi.org/10.1126/science.1081919>.
23. Bagnoli F, Buti L, Tompkins L, Covacci A, Amieva MR. 2005. *Helicobacter pylori* CagA induces a transition from polarized to invasive phenotypes in MDCK cells. *Proc Natl Acad Sci U S A* 102:16339–16344. <http://dx.doi.org/10.1073/pnas.0502598102>.
24. Suzuki M, Mimuro H, Kiga K, Fukumatsu M, Ishijima N, Morikawa H, Nagai S, Koyasu S, Gilman RH, Kersulyte D, Berg DE, Sasakawa C. 2009. *Helicobacter pylori* CagA phosphorylation-independent function in epithelial proliferation and inflammation. *Cell Host Microbe* 5:23–34. <http://dx.doi.org/10.1016/j.chom.2008.11.010>.
25. Neal JT, Peterson TS, Kent ML, Guillemin K. 2013. *H. pylori* virulence factor CagA increases intestinal cell proliferation by Wnt pathway activation in a transgenic zebrafish model. *Dis Models Mech* 6:802–810. <http://dx.doi.org/10.1242/dmm.011163>.
26. Sigal M, Rothenberg ME, Logan CY, Lee JY, Honaker RW, Cooper RL, Passarelli B, Camorlinga M, Bouley DM, Alvarez G, Nusse R, Torres J, Amieva MR. 2015. *Helicobacter pylori* activates and expands Lgr5(+) stem cells through direct colonization of the gastric glands. *Gastroenterology* 148: 1392–1404.e21. <http://dx.doi.org/10.1053/j.gastro.2015.02.049>.
27. Buti L, Spooner E, Van der Veen AG, Rappuoli R, Covacci A, Ploegh HL. 2011. *Helicobacter pylori* cytotoxin-associated gene A (CagA) subverts the apoptosis-stimulating protein of p53 (ASPP2) tumor suppressor pathway of the host. *Proc Natl Acad Sci U S A* 108:9238–9243. <http://dx.doi.org/10.1073/pnas.1106200108>.
28. Rieder G, Merchant JL, Haas R. 2005. *Helicobacter pylori* cag-type IV secretion system facilitates corpus colonization to induce precancerous conditions in Mongolian gerbils. *Gastroenterology* 128:1229–1242. <http://dx.doi.org/10.1053/j.gastro.2005.02.064>.
29. Franco AT, Johnston E, Krishna U, Yamaoka Y, Israel DA, Nagy TA, Wroblewski LE, Piazzuelo MB, Correa P, Peek RM, Jr. 2008. Regulation of gastric carcinogenesis by *Helicobacter pylori* virulence factors. *Cancer Res* 68:379–387. <http://dx.doi.org/10.1158/0008-5472.CAN-07-0824>.
30. Noto JM, Gaddy JA, Lee JY, Piazzuelo MB, Friedman DB, Colvin DC, Romero-Gallo J, Suarez G, Loh J, Slaughter JC, Tan S, Morgan DR, Wilson KT, Bravo LE, Correa P, Cover TL, Amieva MR, Peek RM, Jr. 2013. Iron deficiency accelerates *Helicobacter pylori*-induced carcinogenesis in rodents and humans. *J Clin Invest* 123:479–492. <http://dx.doi.org/10.1172/JCI64373>.
31. Gaddy JA, Radin JN, Loh JT, Zhang F, Washington MK, Peek RM, Jr., Algood HM, Cover TL. 2013. High dietary salt intake exacerbates *Helicobacter pylori*-induced gastric carcinogenesis. *Infect Immun* 81: 2258–2267. <http://dx.doi.org/10.1128/IAI.01271-12>.
32. Ohnishi N, Yuasa H, Tanaka S, Sawa H, Miura M, Matsui A, Higashi H, Musashi M, Iwabuchi K, Suzuki M, Yamada G, Azuma T, Hatakeyama M. 2008. Transgenic expression of *Helicobacter pylori* CagA induces gastrointestinal and hematopoietic neoplasms in mouse. *Proc Natl Acad Sci U S A* 105:1003–1008. <http://dx.doi.org/10.1073/pnas.0711183105>.
33. Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, Stemmermann GN, Nomura A. 1995. Infection with *Helicobacter pylori* strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. *Cancer Res* 55:2111–2115.
34. Figueiredo C, Machado JC, Pharoah P, Seruca R, Sousa S, Carvalho R, Capelinda AF, Quint W, Caldas C, van Doorn LJ, Carneiro F, Sobrinho-Simões M. 2002. *Helicobacter pylori* and interleukin 1 genotyping: an opportunity to identify high-risk individuals for gastric carcinoma. *J Natl Cancer Inst* 94:1680–1687. <http://dx.doi.org/10.1093/jnci/94.22.1680>.
35. Plummer M, van Doorn LJ, Franceschi S, Kleter B, Canzian F, Vivas J, Lopez G, Colin D, Muñoz N, Kato I. 2007. *Helicobacter pylori* cytotoxin-associated genotype and gastric precancerous lesions. *J Natl Cancer Inst* 99:1328–1334. <http://dx.doi.org/10.1093/jnci/djm120>.
36. Suzuki N, Murata-Kamiya N, Yanagiya K, Suda W, Hattori M, Kanda H, Bingo A, Fujii Y, Maeda S, Koike K, Hatakeyama M. 2015. Mutual reinforcement of inflammation and carcinogenesis by the *Helicobacter pylori* CagA oncoprotein. *Sci Rep* 5:10024. <http://dx.doi.org/10.1038/srep10024>.
37. Higashi H, Tsutsumi R, Fujita A, Yamazaki S, Asaka M, Azuma T, Hatakeyama M. 2002. Biological activity of the *Helicobacter pylori* virulence factor CagA is determined by variation in the tyrosine phosphorylation sites. *Proc Natl Acad Sci U S A* 99:14428–14433. <http://dx.doi.org/10.1073/pnas.222375399>.
38. Argent RH, Kidd M, Owen RJ, Thomas RJ, Limb MC, Atherton JC. 2004. Determinants and consequences of different levels of CagA phosphorylation for clinical isolates of *Helicobacter pylori*. *Gastroenterology* 127:514–523. <http://dx.doi.org/10.1053/j.gastro.2004.06.006>.
39. Nagase L, Hayashi T, Senda T, Hatakeyama M. 2015. Dramatic increase in SHP2 binding activity of *Helicobacter pylori* western CagA by EPIYA-C duplication: its implications in gastric carcinogenesis. *Sci Rep* 5:15749. <http://dx.doi.org/10.1038/srep15749>.
40. Azuma T, Yamakawa A, Yamazaki S, Fukuta K, Ohtani M, Ito Y, Dojo M, Yamazaki Y, Kuriyama M. 2002. Correlation between variation of the 3' region of the cagA gene in *Helicobacter pylori* and disease outcome in Japan. *J Infect Dis* 186:1621–1630. <http://dx.doi.org/10.1086/345374>.
41. Basso D, Zambon CF, Letley DP, Stranges A, Marchet A, Rhead JL, Schiavon S, Guariso G, Ceroti M, Nitti D, Rugge M, Plebani M, Atherton JC. 2008. Clinical relevance of *Helicobacter pylori* cagA and vacA gene polymorphisms. *Gastroenterology* 135:91–99. <http://dx.doi.org/10.1053/j.gastro.2008.03.041>.
42. Zhang XS, Tegtmeyer N, Traube L, Jindal S, Perez-Perez G, Sticht H, Backert S, Blaser MJ. 2015. A specific A/T polymorphism in western tyrosine phosphorylation B-motifs regulates *Helicobacter pylori* CagA epithelial cell interactions. *PLoS Pathog* 11:e1004621. <http://dx.doi.org/10.1371/journal.ppat.1004621>.
43. Loh JT, Shaffer CL, Piazzuelo MB, Bravo LE, McClain MS, Correa P, Cover TL. 2011. Analysis of cagA in *Helicobacter pylori* strains from Colombian populations with contrasting gastric cancer risk reveals a biomarker for disease severity. *Cancer Epidemiol Biomarkers Prev* 20: 2237–2249. <http://dx.doi.org/10.1158/1055-9965.EPI-11-0548>.
44. Ferreira RM, Pinto-Ribeiro I, Wen X, Marcos-Pinto R, Dinis-Ribeiro M, Carneiro F, Figueiredo C. 23 September 2015. *Helicobacter pylori* cagA promoter region sequences influence CagA expression and interleukin 8 secretion. *J Infect Dis*. <http://dx.doi.org/10.1093/infdis/jiv467>.
45. Cover TL, Blanke SR. 2005. *Helicobacter pylori* VacA, a paradigm for toxin multifunctionality. *Nat Rev Microbiol* 3:320–332. <http://dx.doi.org/10.1038/nrmicro1095>.
46. Boquet P, Ricci V. 2012. Intoxication strategy of *Helicobacter pylori* VacA toxin. *Trends Microbiol* 20:165–174. <http://dx.doi.org/10.1016/j.tim.2012.01.008>.
47. Kim IJ, Blanke SR. 2012. Remodeling the host environment: modulation of the gastric epithelium by the *Helicobacter pylori* vacuolating toxin (VacA). *Front Cell Infect Microbiol* 2:37. <http://dx.doi.org/10.3389/fcimb.2012.00037>.
48. Cover TL, Blaser MJ. 1992. Purification and characterization of the vacuolating toxin from *Helicobacter pylori*. *J Biol Chem* 267: 10570–10575.
49. Gangwer KA, Mushrush DJ, Stauff DL, Spiller B, McClain MS, Cover TL, Lacy DB. 2007. Crystal structure of the *Helicobacter pylori* vacuolating toxin p55 domain. *Proc Natl Acad Sci U S A* 104:16293–16298. <http://dx.doi.org/10.1073/pnas.0707447104>.
50. Forsyth MH, Atherton JC, Blaser MJ, Cover TL. 1998. Heterogeneity in levels of vacuolating cytotoxin gene (vacA) transcription among *Helicobacter pylori* strains. *Infect Immun* 66:3088–3094.



51. Atherton JC, Cao P, Peek RM, Jr., Tummuru MK, Blaser MJ, Cover TL. 1995. Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. Association of specific vacA types with cytotoxin production and peptic ulceration. *J Biol Chem* 270:17771–17777.
52. Rhead JL, Letley DP, Mohammadi M, Hussein N, Mohagheghi MA, Eshagh Hosseini M, Atherton JC. 2007. A new *Helicobacter pylori* vacuolating cytotoxin determinant, the intermediate region, is associated with gastric cancer. *Gastroenterology* 133:926–936. <http://dx.doi.org/10.1053/j.gastro.2007.06.056>.
53. Gangwer KA, Shaffer CL, Suerbaum S, Lacy DB, Cover TL, Bordenstein SR. 2010. Molecular evolution of the *Helicobacter pylori* vacuolating toxin gene vacA. *J Bacteriol* 192:6126–6135. <http://dx.doi.org/10.1128/JB.01081-10>.
54. McClain MS, Cao P, Iwamoto H, Vinion-Dubiel AD, Szabo G, Shao Z, Cover TL. 2001. A 12-amino-acid segment, present in type s2 but not type s1 *Helicobacter pylori* VacA proteins, abolishes cytotoxin activity and alters membrane channel formation. *J Bacteriol* 183:6499–6508. <http://dx.doi.org/10.1128/JB.183.22.6499-6508.2001>.
55. Letley DP, Rhead JL, Twells RJ, Dove B, Atherton JC. 2003. Determinants of non-toxicity in the gastric pathogen *Helicobacter pylori*. *J Biol Chem* 278:26734–26741. <http://dx.doi.org/10.1074/jbc.M304071200>.
56. Willhite DC, Blanke SR. 2004. *Helicobacter pylori* vacuolating cytotoxin enters cells, localizes to the mitochondria, and induces mitochondrial membrane permeability changes correlated to toxin channel activity. *Cell Microbiol* 6:143–154. <http://dx.doi.org/10.1046/j.1462-5822.2003.00347.x>.
57. Gauthier NC, Monzo P, Gonzalez T, Doye A, Oldani A, Gounon P, Ricci V, Cormont M, Boquet P. 2007. Early endosomes associated with dynamic F-actin structures are required for late trafficking of *H. pylori* VacA toxin. *J Cell Biol* 177:343–354. <http://dx.doi.org/10.1083/jcb.200609061>.
58. Calore F, Genisset C, Casellato A, Rossato M, Codolo G, Esposti MD, Scorrano L, de Bernard M. 2010. Endosome-mitochondria juxtaposition during apoptosis induced by *H. pylori* VacA. *Cell Death Differ* 17:1707–1716. <http://dx.doi.org/10.1038/cdd.2010.42>.
59. Satin B, Norais N, Telford J, Rappuoli R, Murgia M, Montecucco C, Papini E. 1997. Effect of *Helicobacter pylori* vacuolating toxin on maturation and extracellular release of procathepsin D and on epidermal growth factor degradation. *J Biol Chem* 272:25022–25028. <http://dx.doi.org/10.1074/jbc.272.40.25022>.
60. Terebiznik MR, Raju D, Vázquez CL, Torbrück K, Kulkarni R, Blanke SR, Yoshimori T, Colombo MI, Jones NL. 2009. Effect of *Helicobacter pylori*'s vacuolating cytotoxin on the autophagy pathway in gastric epithelial cells. *Autophagy* 5:370–379. <http://dx.doi.org/10.4161/auto.5.3.7663>.
61. Tsugawa H, Suzuki H, Saya H, Hatakeyama M, Hirayama T, Hirata K, Nagano O, Matsuzaki J, Hibi T. 2012. Reactive oxygen species-induced autophagic degradation of *Helicobacter pylori* CagA is specifically suppressed in cancer stem-like cells. *Cell Host Microbe* 12:764–777. <http://dx.doi.org/10.1016/j.chom.2012.10.014>.
62. Wang F, Xia P, Wu F, Wang D, Wang W, Ward T, Liu Y, Aikhionbare F, Guo Z, Powell M, Liu B, Bi F, Shaw A, Zhu Z, Elmoselhi A, Fan D, Cover TL, Ding X, Yao X. 2008. *Helicobacter pylori* VacA disrupts apical membrane-cytoskeletal interactions in gastric parietal cells. *J Biol Chem* 283:26714–26725. <http://dx.doi.org/10.1074/jbc.M800527200>.
63. Gebert B, Fischer W, Weiss E, Hoffmann R, Haas R. 2003. *Helicobacter pylori* vacuolating cytotoxin inhibits T lymphocyte activation. *Science* 301:1099–1102. <http://dx.doi.org/10.1126/science.1086871>.
64. Sundrud MS, Torres VJ, Unutmaz D, Cover TL. 2004. 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 U S A* 101:7727–7732. <http://dx.doi.org/10.1073/pnas.0401528101>.
65. Winter JA, Letley DP, Cook KW, Rhead JL, Zaitoun AA, Ingram RJ, Amilon KR, Croxall NJ, Kaye PV, Robinson K, Atherton JC. 2014. A role for the vacuolating cytotoxin, VacA, in colonization and *Helicobacter pylori*-induced metaplasia in the stomach. *J Infect Dis* 210:954–963. <http://dx.doi.org/10.1093/infdis/jiu154>.
66. I liver D, Arnqvist A, Ogren J, Frick IM, Kersulyte D, Incecik ET, Berg DE, Covacci A, Engstrand L, Borén T. 1998. *Helicobacter pylori* adhesin binding fucosylated histo-blood group antigens revealed by retagging. *Science* 279:373–377. <http://dx.doi.org/10.1126/science.279.5349.373>.
67. Mahdavi J, Sonden B, Hurtig M, Olfat FO, Forsberg L, Roche N, Angstrom J, Larsson T, Teneberg S, Karlsson KA, Altraja S, Wadstrom T, Kersulyte D, Berg DE, Dubois A, Petersson C, Magnusson KE, Norberg T, Lindh F, Lundskog BB, Arnqvist A, Hammarstrom L, Boren T. 2002. *Helicobacter pylori* SabA adhesin in persistent infection and chronic inflammation. *Science* 297:573–578. <http://dx.doi.org/10.1126/science.1069076>.
68. Aspholm M, Olfat FO, Nordén J, Sondén B, Lundberg C, Sjöström R, Altraja S, Odenbreit S, Haas R, Wadström T, Engstrand L, Semino-Mora C, Liu H, Dubois A, Teneberg S, Arnqvist A, Borén T. 2006. SabA is the *H. pylori* hemagglutinin and is polymorphic in binding to sialylated glycans. *PLoS Pathog* 2:e110. <http://dx.doi.org/10.1371/journal.ppat.0020110>.
69. Gerhard M, Lehn N, Neumayer N, Borén T, Rad R, Schepp W, Miehle S, Classen M, Prinz C. 1999. Clinical relevance of the *Helicobacter pylori* gene for blood-group antigen-binding adhesin. *Proc Natl Acad Sci U S A* 96:12778–12783. <http://dx.doi.org/10.1073/pnas.96.22.12778>.
70. Prinz C, Schöniger M, Rad R, Becker I, Keiditsch E, Wagenpfeil S, Classen M, Rösch T, Schepp W, Gerhard M. 2001. Key importance of the *Helicobacter pylori* adherence factor blood group antigen binding adhesin during chronic gastric inflammation. *Cancer Res* 61:1903–1909.
71. Yu J, Leung WK, Go MY, Chan MC, To KF, Ng EK, Chan FK, Ling TK, Chung SC, Sung JJ. 2002. Relationship between *Helicobacter pylori* babA2 status with gastric epithelial cell turnover and premalignant gastric lesions. *Gut* 51:480–484. <http://dx.doi.org/10.1136/gut.51.4.480>.
72. Yamaoka Y, Ojo O, Fujimoto S, Odenbreit S, Haas R, Gutierrez O, El-Zimaity HM, Reddy R, Arnqvist A, Graham DY. 2006. *Helicobacter pylori* outer membrane proteins and gastroduodenal disease. *Gut* 55:775–781. <http://dx.doi.org/10.1136/gut.2005.083014>.
73. Guruge JL, Falk PG, Lorenz RG, Dans M, Wirth HP, Blaser MJ, Berg DE, Gordon JI. 1998. Epithelial attachment alters the outcome of *Helicobacter pylori* infection. *Proc Natl Acad Sci U S A* 95:3925–3930. <http://dx.doi.org/10.1073/pnas.95.7.3925>.
74. Oleastro M, Cordeiro R, Ferrand J, Nunes B, Lehours P, Carvalho-Oliveira I, Mendes AI, Penque D, Monteiro L, Mégraud F, Ménard A. 2008. Evaluation of the clinical significance of homB, a novel candidate marker of *Helicobacter pylori* strains associated with peptic ulcer disease. *J Infect Dis* 198:1379–1387. <http://dx.doi.org/10.1086/592166>.
75. Cao P, Cover TL. 2002. Two different families of hopQ alleles in *Helicobacter pylori*. *J Clin Microbiol* 40:4504–4511. <http://dx.doi.org/10.1128/JCM.40.12.4504-4511.2002>.
76. Jung SW, Sugimoto M, Graham DY, Yamaoka Y. 2009. homB status of *Helicobacter pylori* as a novel marker to distinguish gastric cancer from duodenal ulcer. *J Clin Microbiol* 47:3241–3245. <http://dx.doi.org/10.1128/JCM.00293-09>.
77. Talebi Bezin Abadi A, Rafiei A, Ajami A, Hosseini V, Taghvaei T, Jones KR, Merrell DS. 2011. *Helicobacter pylori* homB, but not cagA, is associated with gastric cancer in Iran. *J Clin Microbiol* 49:3191–3197. <http://dx.doi.org/10.1128/JCM.00947-11>.
78. Yakoob J, Abbas Z, Khan R, Salim SA, Awan S, Abrar A, Jafri W. 2016. *Helicobacter pylori* outer membrane protein Q allele distribution is associated with distinct pathologies in Pakistan. *Infect Genet Evol* 37:57–62. <http://dx.doi.org/10.1016/j.meegid.2015.10.027>.
79. Yamaoka Y, Kita M, Kodama T, Imamura S, Ohno T, Sawai N, Ishimaru A, Imanishi J, Graham DY. 2002. *Helicobacter pylori* infection in mice: role of outer membrane proteins in colonization and inflammation. *Gastroenterology* 123:1992–2004. <http://dx.doi.org/10.1053/gast.2002.37074>.
80. Belogolova E, Bauer B, Pombaiah M, Asakura H, Brinkman V, Ertl C, Bartfeld S, Nechitaylo TY, Haas R, Machuy N, Salama N, Churin Y, Meyer TF. 2013. *Helicobacter pylori* outer membrane protein HopQ identified as a novel T4SS-associated virulence factor. *Cell Microbiol* 15:1896–1912. <http://dx.doi.org/10.1111/cmi.12158>.
81. Dossumbekova A, Prinz C, Mages J, Lang R, Kusters JG, van Vliet AH, Reindl W, Backert S, Saur D, Schmid RM, Rad R. 2006. *Helicobacter pylori* HopH (OipA) and bacterial pathogenicity: genetic and functional genomic analysis of hopH gene polymorphisms. *J Infect Dis* 194:1346–1355. <http://dx.doi.org/10.1086/508426>.
82. Yamaoka Y. 2010. Mechanisms of disease: *Helicobacter pylori* virulence factors. *Nat Rev Gastroenterol Hepatol* 7:629–641. <http://dx.doi.org/10.1038/nrgastro.2010.154>.
83. Odenbreit S, Swoboda K, Barwig I, Ruhl S, Borén T, Koletzko S, Haas R. 2009. Outer membrane protein expression profile in *Helicobacter py-*



- lori clinical isolates. *Infect Immun* 77:3782–3790. <http://dx.doi.org/10.1128/IAI.00364-09>.
84. Lu H, Hsu PI, Graham DY, Yamaoka Y. 2005. Duodenal ulcer promoting gene of *Helicobacter pylori*. *Gastroenterology* 128:833–848. <http://dx.doi.org/10.1053/j.gastro.2005.01.009>.
  85. Abadi AT, Taghvaei T, Wolfram L, Kusters JG. 2012. Infection with *Helicobacter pylori* strains lacking dupA is associated with an increased risk of gastric ulcer and gastric cancer development. *J Med Microbiol* 61:23–30. <http://dx.doi.org/10.1099/jmm.0.027052-0>.
  86. Hussein NR, Argent RH, Marx CK, Patel SR, Robinson K, Atherton JC. 2010. *Helicobacter pylori* dupA is polymorphic, and its active form induces proinflammatory cytokine secretion by mononuclear cells. *J Infect Dis* 202:261–269. <http://dx.doi.org/10.1086/653587>.
  87. Queiroz DM, Rocha GA, Rocha AM, Moura SB, Saraiva IE, Gomes LI, Soares TF, Melo FF, Cabral MM, Oliveira CA. 2011. dupA polymorphisms and risk of *Helicobacter pylori*-associated diseases. *Int J Med Microbiol* 301:225–228. <http://dx.doi.org/10.1016/j.ijmm.2010.08.019>.
  88. Van Doorn LJ, Figueiredo C, Mégraud F, Pena S, Midolo P, Queiroz DM, Carneiro F, Vanderborght B, Pegado MD, Sanna R, De Boer W, Schneeberger PM, Correa P, Ng EK, Atherton J, Blaser MJ, Quint WG. 1999. Geographic distribution of vacA allelic types of *Helicobacter pylori*. *Gastroenterology* 116:823–830. [http://dx.doi.org/10.1016/S0016-5085\(99\)70065-X](http://dx.doi.org/10.1016/S0016-5085(99)70065-X).
  89. Hennig EE, Allen JM, Cover TL. 2006. Multiple chromosomal loci for the babA gene in *Helicobacter pylori*. *Infect Immun* 74:3046–3051. <http://dx.doi.org/10.1128/IAI.74.5.3046-3051.2006>.
  90. Tan S, Noto JM, Romero-Gallo J, Peek RM, Jr., Amieva MR. 2011. *Helicobacter pylori* perturbs iron trafficking in the epithelium to grow on the cell surface. *PLoS Pathog* 7:e1002050. <http://dx.doi.org/10.1371/journal.ppat.1002050>.
  91. Argent RH, Thomas RJ, Letley DP, Rittig MG, Hardie KR, Atherton JC. 2008. Functional association between the *Helicobacter pylori* virulence factors VacA and CagA. *J Med Microbiol* 57:145–150. <http://dx.doi.org/10.1099/jmm.0.47465-0>.
  92. Yokoyama K, Higashi H, Ishikawa S, Fujii Y, Kondo S, Kato H, Azuma T, Wada A, Hirayama T, Aburatani H, Hatakeyama M. 2005. 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 U S A* 102:9661–9666. <http://dx.doi.org/10.1073/pnas.0502529102>.
  93. Oldani A, Cormont M, Hofman V, Chiozzi V, Oregioni O, Canonici A, Sciuolo A, Sommi P, Fabbri A, Ricci V, Boquet P. 2009. *Helicobacter pylori* counteracts the apoptotic action of its VacA toxin by injecting the CagA protein into gastric epithelial cells. *PLoS Pathog* 5:e1000603. <http://dx.doi.org/10.1371/journal.ppat.1000603>.
  94. Tegtmeyer N, Zabler D, Schmidt D, Hartig R, Brandt S, Backert S. 2009. 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* 11:488–505. <http://dx.doi.org/10.1111/j.1462-5822.2008.01269.x>.
  95. Ishijima N, Suzuki M, Ashida H, Ichikawa Y, Kanegae Y, Saito I, Borén T, Haas R, Sasakawa C, Mimuro H. 2011. BabA-mediated adherence is a potentiator of the *Helicobacter pylori* type IV secretion system activity. *J Biol Chem* 286:25256–25264. <http://dx.doi.org/10.1074/jbc.M111.233601>.
  96. Marcos NT, Magalhães A, Ferreira B, Oliveira MJ, Carvalho AS, Mendes N, Gilmartin T, Head SR, Figueiredo C, David L, Santos-Silva F, Reis CA. 2008. *Helicobacter pylori* induces beta3GnT5 in human gastric cell lines, modulating expression of the SabA ligand sialyl-Lewis x. *J Clin Invest* 118:2325–2336. <http://dx.doi.org/10.1172/JCI34324>.
  97. Ito Y, Azuma T, Ito S, Miyaji H, Hirai M, Yamazaki Y, Sato F, Kato T, Kohli Y, Kuriyama M. 1997. Analysis and typing of the vacA gene from cagA-positive strains of *Helicobacter pylori* isolated in Japan. *J Clin Microbiol* 35:1710–1714.
  98. Lai CH, Kuo CH, Chen YC, Chao FY, Poon SK, Chang CS, Wang WC. 2002. High prevalence of cagA- and babA2-positive *Helicobacter pylori* clinical isolates in Taiwan. *J Clin Microbiol* 40:3860–3862. <http://dx.doi.org/10.1128/JCM.40.10.3860-3862.2002>.
  99. Falush D, Wirth T, Linz B, Pritchard JK, Stephens M, Kidd M, Blaser MJ, Graham DY, Vacher S, Perez-Perez GI, Yamaoka Y, Mégraud F, Otto K, Reichard U, Katzowitzsch E, Wang X, Achtman M, Suerbaum S. 2003. Traces of human migrations in *Helicobacter pylori* populations. *Science* 299:1582–1585. <http://dx.doi.org/10.1126/science.1080857>.
  100. Duncan SS, Valk PL, McClain MS, Shaffer CL, Metcalf JA, Bordenstein SR, Cover TL. 2013. Comparative genomic analysis of East Asian and non-Asian *Helicobacter pylori* strains identifies rapidly evolving genes. *PLoS One* 8:e55120. <http://dx.doi.org/10.1371/journal.pone.0055120>.
  101. Azuma T, Yamazaki S, Yamakawa A, Ohtani M, Muramatsu A, Suto H, Ito Y, Dojo M, Yamazaki Y, Kuriyama M, Keida Y, Higashi H, Hatakeyama M. 2004. Association between diversity in the Src homology 2 domain—containing tyrosine phosphatase binding site of *Helicobacter pylori* CagA protein and gastric atrophy and cancer. *J Infect Dis* 189:820–827. <http://dx.doi.org/10.1086/381782>.
  102. Suzuki M, Kiga K, Kersulyte D, Cok J, Hooper CC, Mimuro H, Sanada T, Suzuki S, Oyama M, Kozuka-Hata H, Kamiya S, Zou QM, Gilman RH, Berg DE, Sasakawa C. 2011. Attenuated CagA oncoprotein in *Helicobacter pylori* from Amerindians in Peruvian Amazon. *J Biol Chem* 286:29964–29972. <http://dx.doi.org/10.1074/jbc.M111.263715>.
  103. Jones KR, Joo YM, Jang S, Yoo YJ, Lee HS, Chung IS, Olsen CH, Whitmire JM, Merrell DS, Cha JH. 2009. Polymorphism in the CagA EPIYA motif impacts development of gastric cancer. *J Clin Microbiol* 47:959–968. <http://dx.doi.org/10.1128/JCM.02330-08>.
  104. Lu H, Wu JY, Beswick EJ, Ohno T, Odenbreit S, Haas R, Reyes VE, Kita M, Graham DY, Yamaoka Y. 2007. Functional and intracellular signaling differences associated with the *Helicobacter pylori* AlpAB adhesin from Western and East Asian strains. *J Biol Chem* 282:6242–6254. <http://dx.doi.org/10.1074/jbc.M611178200>.
  105. McLean MH, El-Omar EM. 2014. Genetics of gastric cancer. *Nat Rev Gastroenterol Hepatol* 11:664–674. <http://dx.doi.org/10.1038/nrgastro.2014.143>.
  106. El-Omar EM, Rabkin CS, Gammon MD, Vaughan TL, Risch HA, Schoenberg JB, Stanford JL, Mayne ST, Goedert J, Blot WJ, Fraumeni JF, Jr., Chow WH. 2003. Increased risk of noncardia gastric cancer associated with proinflammatory cytokine gene polymorphisms. *Gastroenterology* 124:1193–1201. [http://dx.doi.org/10.1016/S0016-5085\(03\)00157-4](http://dx.doi.org/10.1016/S0016-5085(03)00157-4).
  107. Kato I, van Doorn LJ, Canzian F, Plummer M, Franceschi S, Vivas J, Lopez G, Lu Y, Gioia-Patricola L, Severson RK, Schwartz AG, Muñoz N. 2006. Host-bacterial interaction in the development of gastric precancerous lesions in a high risk population for gastric cancer in Venezuela. *Int J Cancer* 119:1666–1671. <http://dx.doi.org/10.1002/ijc.21979>.
  108. Cover TL, Peek RM, Jr. 2013. Diet, microbial virulence, and *Helicobacter pylori*-induced gastric cancer. *Gut Microbes* 4:482–493. <http://dx.doi.org/10.4161/gmic.26262>.
  109. Loh JT, Torres VJ, Cover TL. 2007. Regulation of *Helicobacter pylori* cagA expression in response to salt. *Cancer Res* 67:4709–4715. <http://dx.doi.org/10.1158/0008-5472.CAN-06-4746>.
  110. Moodley Y, Linz B, Bond RP, Nieuwoudt M, Soodyall H, Schlebusch CM, Bernhöft S, Hale J, Suerbaum S, Mugisha L, van der Merwe SW, Achtman M. 2012. Age of the association between *Helicobacter pylori* and man. *PLoS Pathog* 8:e1002693. <http://dx.doi.org/10.1371/journal.ppat.1002693>.
  111. Linz B, Balloux F, Moodley Y, Manica A, Liu H, Roumagnac P, Falush D, Stamer C, Prugnolle F, van der Merwe SW, Yamaoka Y, Graham DY, Perez-Trallero E, Wadstrom T, Suerbaum S, Achtman M. 2007. An African origin for the intimate association between humans and *Helicobacter pylori*. *Nature* 445:915–918. <http://dx.doi.org/10.1038/nature05562>.
  112. Kodaman N, Pazos A, Schneider BG, Piazuolo MB, Mera R, Sobota RS, Scincinchi LA, Shaffer CL, Romero-Gallo J, de Sablet T, Harder RH, Bravo LE, Peek RM, Wilson KT, Cover TL, Williams SM, Correa P. 2014. Human and *Helicobacter pylori* coevolution shapes the risk of gastric disease. *Proc Natl Acad Sci U S A* 111:1455–1460. <http://dx.doi.org/10.1073/pnas.1318093111>.
  113. Jiménez-Soto LF, Clausen S, Sprenger A, Ertl C, Haas R. 2013. Dynamics of the Cag-type IV secretion system of *Helicobacter pylori* as studied by bacterial co-infections. *Cell Microbiol* 15:1924–1937. <http://dx.doi.org/10.1111/cmi.12166>.
  114. Secka O, Antonio M, Berg DE, Tapgun M, Bottomley C, Thomas V, Walton R, Corrah T, Thomas JE, Adegbola RA. 2011. Mixed infection with cagA positive and cagA negative strains of *Helicobacter pylori* lowers disease burden in The Gambia. *PLoS One* 6:e27954. <http://dx.doi.org/10.1371/journal.pone.0027954>.