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Review

Helicobacter pylori CagA protein variation associated with gastric cancer in Asia

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Recent molecular analysis has provided the pathological actions of CagA on gastric epithelial cells. CagA is injected into epithelial cells via the type IV secretion system and undergoes tyrosine phosphorylation in the cells. In addition, translocated CagA forms a physical complex with SHP-2. There are two major CagA subtypes; the East Asian and the Western type. The East Asian CagA protein possesses stronger SHP-2 binding activity than the Western CagA. The grades of inflammation, activity of gastritis, and atrophy are significantly higher in gastritis patients infected with the East Asian CagA-positive strain than in gastritis patients infected with the *cagA*-negative or Western CagA-positive strains. The prevalence of the East Asian CagA-positive strain is associated with the mortality rate of gastric cancer in Asia. Endemic circulation of *H. pylori* populations carrying biologically more active CagA proteins in East Asian countries, where the mortality rate of gastric cancer is among the highest in the world, may be involved in increasing the risk of gastric cancer in these populations.

Key words: *Helicobacter pylori*, CagA, gastric cancer, pathogenicity island, type IV secretion system

Introduction

Helicobacter pylori is a gram-negative microaerophilic bacterium that chronically colonizes the gastric epithelium of more than half of all people worldwide. It is a human pathogen responsible for chronic active gastritis, and infection with this organism is an important risk factor for peptic ulcer, gastric cancer, and gastric mucosa-associated lymphoid tissue lymphoma.[1–4] *H. pylori* has been implicated in gastric carcinogenesis on the basis of various epidemiological studies.[3,5,6] A Working Group of the World Health Organization International Agency for Research on Cancer concluded that *H. pylori* is a group I carcinogen in humans.[7] CagA protein, encoded by the *cagA* gene, is one of the most studied virulence factors of *H. pylori* and is a highly immunogenic protein. The *cagA* gene is one of the genes in a pathogenicity island (PAI) known as the *cag* PAI. The presence of *cagA* is considered a marker for the presence of *cag* PAI.[8] The *cag* PAI is a 40-kb locus in the chromosomal glutamate racemase gene. Its G + C content (35%) differs from the G + C content of the rest of the genome (39%), suggesting that it was acquired from another organism by horizontal transfer.[9–11] At some point during evolution, IS605, a mobile sequence encoding two transposases, entered the *H. pylori* genome and in some strains interrupted, multilated, or deleted parts of the PAI.[10] The severity of *H. pylori*-related disease is correlated with the presence of *cag* PAI. Infection with *cag* PAI-positive *H. pylori* is statistically associated with duodenal ulceration, gastric mucosal atrophy, and gastric cancer.[8,10,12] Recent studies have provided a molecular basis for the pathological actions of CagA on gastric epithelial cells. In this review, our recent molecular analysis of *cagA* and *cag* PAI and the relationship between the variations of these genes and clinical outcome in Asia are summarized.

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Type IV secretion system of *H. pylori*

H. pylori attaches specifically and tightly to gastric epithelial cells. The adherence of *H. pylori* to the gastric epithelial cells is an important determinant of pathogenesis. Bacterial attachment causes microvilli effacement, actin rearrangement, pedestal formation, and induction of IL-8 release. Segal et al. first reported that attachment of *H. pylori* to cultured gastric epithelial

cells such as AGS cells can induce tyrosine phosphorylation of a 145-kDa host protein and accumulation of F-actin beneath the bacterium, and also the subsequent evoked activation of nuclear factor (NF)-κB and release of IL-8.[13] The ability of bacteria to induce protein tyrosine phosphorylation, IL-8 production, and the rearrangement of actin cytoskeletons is closely correlated with the presence of the *cag* PAI. The *cag* PAI contains 31 genes, 6 of which are thought to encode a putative type IV secretion system, which specializes in the transfer of a variety of multimolecular complexes across the bacterial membrane to the extracellular space or into other cells.[11] For example, the *cag* homologs of VirB4 (CagE), VirB7 (CagT), VirB9 (*cag*ORF528), VirB10 (*cag*ORF527), VirB11 (*cag*ORF525), and VirD4 (*cag*ORF524) of *Argobacterium tumefaciens* have been shown to be assembled as a complex and form the type IV transport machinery. The potential of *H. pylori* to deliver bacterial effector molecules through the putative type IV secretion system into the attached host cells has been suggested, thus enabling the bacteria to alter host cell signaling such as that required for protein tyrosine phosphorylation, stimulation of IL-8 release, and induction of actin dynamics.[11]

H. pylori CagA is translocated from the bacteria to gastric epithelial cells and receives tyrosine phosphorylation

Adherence of *H. pylori* to gastric epithelial cells can induce host cellular responses, including the reorganization of actin cytoskeletons, the tyrosine phosphorylation of a 145-kDa protein, and release of IL-8. The 145-kDa phosphorylated protein induced in gastric epithelial cells infected with *H. pylori* strain 87A300 was originally reported by Segal and colleagues, who proposed that the 145-kDa protein was a host cellular component.[13] We investigated the origin of the 145-kDa protein induced in gastric epithelial cells infected with *H. pylori* and identified the phosphorylated protein as CagA of *H. pylori*. Epithelial cells infected with various *H. pylori* clinical isolates resulted in generation of tyrosine-phosphorylated proteins ranging from 130 to 145 kDa in size that were also induced in vitro by mixing host cell lysate with bacterial lysate. When epithelial cells were infected with [^{35}S]methionine-labeled *H. pylori*, a radioactive 145-kDa protein was detected in the immunoprecipitates with an antiphosphotyrosine antibody or an anti-CagA antibody. The 145-kDa protein recognized by the anti-CagA and antiphosphotyrosine antibodies was induced in epithelial cells after infection of wild-type *H. pylori*, but not the *cagA* knockout mutant. Furthermore, the amino acid sequence of the phosphorylated 145-kDa protein induced by *H. pylori* infection was identified in the CagA sequence. These data revealed that tyrosine-phosphorylated 145-kDa protein is *H. pylori* CagA protein. After attachment of *cagA*-positive *H. pylori* to gastric epithelial cells, CagA is directly injected from the bacteria into the cells via the bacterial type IV secretion system and undergoes tyrosine phosphorylation in the host cells.[14] Several other investigators also discovered these important findings regarding CagA.[15–17]

SHP-2 tyrosine phosphatase is an intracellular target of *H. pylori* CagA protein

After attachment of *cagA*-positive *H. pylori* to gastric epithelial cells, CagA is directly injected from the bacteria into the cells via the bacterial type IV secretion system and undergoes tyrosine phosphorylation in the host cells. The *cagA*-positive *H. pylori*–host cell interaction also triggers morphological changes (hummingbird phenotype) similar to those induced by hepatocyte growth factor (HGF).[16] It has been reported that activation of SHP-2, a cytoplasmic tyrosine phosphatase, plays a major role in HGF-induced cellular morphological changes.[18] SHP-2 positively regulates signal transduction events from a variety of activated receptor tyrosine kinases.[19–21] Because SH2 domains are phosphotyrosine-binding modules, we investigated the capacity of CagA to bind SHP-2. In lysates from AGS cells transfected with the CagA expression vector, CagA coimmunoprecipitated endogenous SHP-2 and vice versa. In contrast, the phosphorylation-resistant CagA, which was mutated in the phosphorylation sites, and SHP-2 did not coimmunoprecipitate each other. Thus, CagA binds SHP-2 in gastric epithelial cells in a tyrosine phosphorylation-dependent manner.[22]

H. pylori virulence factor CagA, which is translocated from bacteria into gastric epithelial cells, can perturb mammalian signal transduction machineries and modify cellular functions by physically interacting with a host cell protein, SHP-2. SHP-2, similarly to its *Drosophila* homolog Corkscrew, is known to play an important positive role in the mitogenic signal transduction that connects receptor tyrosine kinases and *ras*. Also, SHP-2 is actively involved in the regulation of spreading, migration, and adhesion of cells.[21,23] Deregulation of SHP-2 by CagA may induce abnormal proliferation and movement of gastric epithelial cells (Fig. 1).

CagA is translocated into epithelial cells and binds to SHP-2 in human gastric mucosa

Recent molecular analysis has indicated that CagA is injected into epithelial cells via the type IV secretion

system and undergoes tyrosine phosphorylation in cells and that translocated CagA binds SHP-2. We also demonstrated these phenomena in human gastric mucosa in vivo. The tyrosine phosphorylated CagA and CagA coimmunoprecipitated SHP-2 were detected in the gastric mucosa from *H. pylori*-positive gastritis patients.[24] Therefore, CagA is translocated into the gastric epithelial cells, receives tyrosine phosphorylation, and indeed binds SHP-2 in human gastric mucosa in vivo. Interestingly, CagA protein, tyrosine phosphorylated CagA, and CagA coimmunoprecipitated endogenous SHP-2 were not detected in the gastric mucosa with intestinal metaplasia or cancer, although they were detected in the noncancer mucosal tissue from *H. pylori*-positive early gastric cancer patients.[24] Correa's model of gastric carcinogenesis suggests that the process may be a continuous progression whereby lesions of increasing severity develop over two or three decades: acute gastritis progresses to chronic gastritis, then to chronic atrophic gastritis with the development of intestinal metaplasia, and finally to frank gastric carcinoma.[25] It has been estimated that 10% of patients with chronic atrophic gastritis develop gastric cancer over a 15 year period. Intestinal metaplasia with goblet cells has been associated with gastric cancer in up to 90% of cases and is held to be a good histological marker of premalignancy. Chronic gastritis induced by *H. pylori* infection usually progresses to atrophic gastritis. The risk for gastric cancer increases with the degree and extent of atrophic gastritis. Severe glandular atrophy develops, and intestinal metaplasia occurs, accompanied by the disappearance of *H. pylori* colonization. It is therefore suspected that *H. pylori* infection plays a causative role at a relatively early phase in gastric carcinogenesis. Deregulation of SHP-2 by CagA may play a role in the acquisition of a cellular transformed phenotype at a relatively early stage of multistep gastric carcinogenesis.

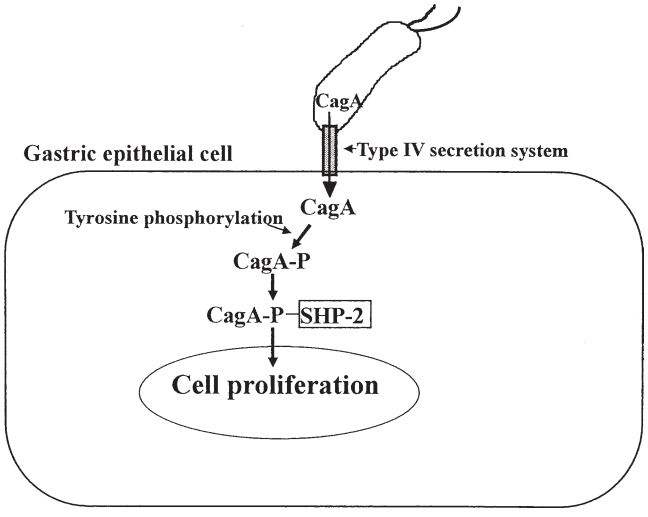

Fig. 1. Scheme of *Helicobacter pylori*–cell interaction. CagA is injected into the gastric epithelial cells via the type IV secretion system, receives tyrosine phosphorylation, and binds SHP-2 in the gastric epithelial cell

Diversity of CagA

H. pylori is genetically more diverse than most bacterial species. Strain-specific genetic diversity has been proposed to be involved in the organism's ability to cause different diseases. There are also indications of significant geographic differences among strains. Only one-half to two-thirds of Western isolates carry *cagA*. In contrast, nearly all East Asian strains carry *cagA*.[26,27] The CagA protein is a highly immunogenic protein with a molecular weight of 128 to 140 kDa. Variation in the size of the protein has been correlated with the presence of a variable number of repeat sequences located in the 3′ region of the gene.[28,29] The phosphorylation sites are located in the repeat region of CagA.[30] The sequence variation raises an intriguing possibility that the biological activity of CagA can vary from one strain to the next, which may influence the pathogenicities of different *cagA*-positive *H. pylori* strains. The alignment of the deduced amino acid sequence in the 3′ region of the *cagA* gene among strains is shown in Fig. 2.

We previously reported that NCTC11637 possesses five Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs that are po-

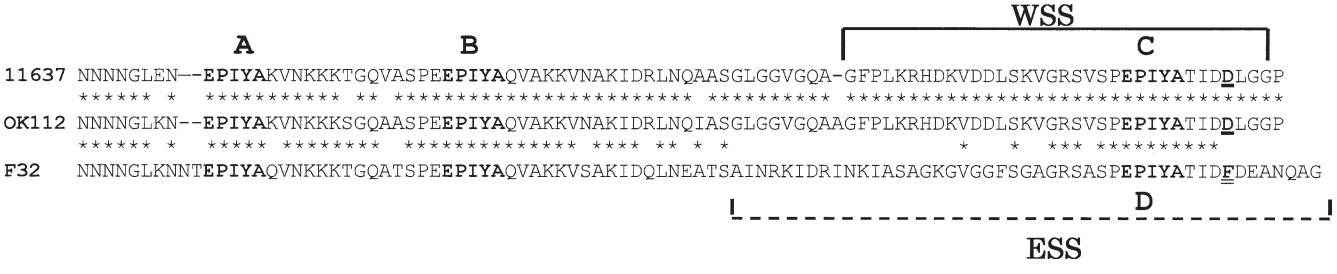

Fig. 2. Alignment of the deduced amino acid sequence in the 3′ region of the *cagA* gene among strains (NCTC11637, OK112, and F32). The pattern of diversity of the CagA phosphorylation site was "A-B-C-C-C" in NCTC11637, "A-B-C" in OK112, and "A-B-D" in F32

tential targets of tyrosine phosphorylation by Src family kinases.[31] These EPIYA motifs are involved in the interaction of CagA with SHP-2. The first and the second EPIYA motifs (which we designated EPIYA-A and EPIYA-B, respectively) are present in almost all CagA proteins, whereas the remaining three EPIYA motifs (which we designated EPIYA-C) were made by duplication of an EPIYA-containing 34-amino-acid sequence. Because the sequence exists in various numbers ranging from 1 to 3 in most CagA proteins from *H. pylori* isolated in Western countries, we designated it the *W*estern CagA-specific, *S*HP-2 binding *S*equence (WSS).[31] The WSS contains D1, D2, and D3 motifs as defined by Covacci et al.[28] or R1 and WSR regions as defined by Yamaoka et al.[32] OK112 had a single WSS and was thus classified as the "A-B-C" type, whereas 11637-CagA was classified as the "A-B-C-C-C" type because it had three WSS repeats.

The amino acid sequence of CagA from *H. pylori* isolated in East Asian countries is quite different from that of Western CagA. Predominant East Asia CagA proteins do not have the WSS, but instead possess a distinct sequence that we designated *E*ast Asian the CagA-specific, *S*HP-2-binding *S*equence (ESS) in the corresponding region.[31] ESS contains a JSR sequence previously defined by Yamaoka et al.[32] and also possesses an EPIYA motif, denoted EPIYA-D. F32 had a single ESS and was thus classfied as the "A-B-D" type. Upon tyrosine phosphorylation, this East Asian-specific sequence confers stronger SHP-2 binding and transforming activities to Western CagA (Fig. 3).[31] The CagA-SHP-2 interaction requires the SH2 domains of SHP-2. De Souza et al. reported that the two SH2 domains from SHP-2 bind to highly related sequences, and the consensus ligand-binding motif for the N- and C-SH2 domains of SHP-2 is pY-(S/T/A/V/I)-X-(V/I/L)-X-(W/F).[33] Intriguingly, the consensus motif perfectly matches the SHP-2-binding site of East Asian CagA, pY-A-T-I-D-F. Furthermore, replacement of the pY + 5 position from W/F with any other amino acids, such as aspartic acid in the case of WSS in Western CagA, reduces the binding affinity to SHP-2. Hence, differential SHP-2-binding activities observed between WSS and ESS of CagA proteins are caused by the difference in a single amino acid at the pY + 5 position. The potential of CagA to disturb host cell functions as a virulence factor could be determined by the degree of SHP-2-binding activity. The diversity of the CagA phosphorylation site, which collectively determines binding affinity of CagA to SHP-2, may be an important variable in determining the clinical outcome of infection with different *H. pylori* strains.

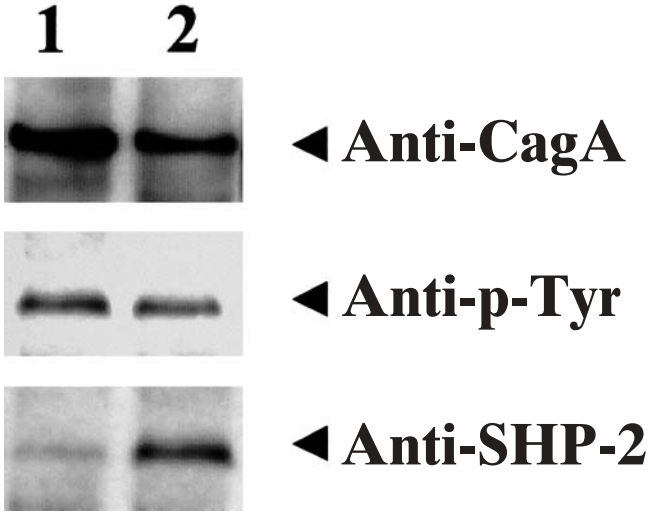

Fig. 3. Immunoblot analysis of AGS cells infected with OK112 (*lane 1*) or F32 (*lane 2*) by anti-CagA, antiphosphotyrosine, and anti-SHP-2 antibodies. East Asian type CagA protein of strain F32 conferred stronger SHP-2 binding to the Western type CagA protein of OK112

Distribution of CagA protein diversity and association between the CagA protein diversity and clinical outcome

The incidence and mortality rate due to gastric cancer in Japan is high compared with that in other developed countries. However, large intracountry differences in the mortality rates of gastric cancer have been reported.[34] Fukui is a typical rural prefecture located on the central Japanese mainland (Honshu), while Okinawa consists of islands in the southwestern part of Japan and has a history and food culture different from those of other parts of Japan. The two areas are separated by more than 1300 km. The prevalence of atrophic gastritis, a precursor lesion of gastric cancer, is more frequent in Fukui, and the mortality rate from gastric cancer is more than 2.4 times higher in Fukui (43.7/100 000 in 1999) than in Okinawa (18.2/100 000 in 1999).

We previously investigated the diversity of CagA phosphorylation sites in isolates from two different areas in Japan (Fukui and Okinawa) where the gastric cancer risk is different to examine the association between diversity and gastric cancer. We demonstrated that the prevalence of *cagA*-positive *H. pylori* was significantly different between Fukui and Okinawa. All isolates examined from Fukui (64 strains) were *cagA*-positive strains. In contrast, 12.0% (6/50) isolates from Okinawa were *cagA*-negative strains. All *cagA*-negative strains were isolated from patients with chronic gastritis. In addition, the distribution of CagA protein diversity was different in Fukui and Okinawa. Almost all strains isolated from Fukui were East Asian CagA-positive strains containing the ESS sequence. Predomi-

Table 1. Distribution of the diversity of the CagA protein

	Fukui			Okinawa		
	cagA (−)	East Asian	Western	*cagA* (−)	East Asian	Western
Chronic gastritis	0	35	0	6	28	8
Gastric cancer	0	29	0	0	8	0
Total	0	64	0	6	36	8*

*The prevalence of Western CagA-positive strain was significantly higher in Okinawa than in Fukui ($P = 0.001$)
Source: Modified from Ref. 35

Table 2. Distribution of CagA protein diversity in the world: analysis using data in GenBank

	CagA type		Mortality rate of gastric cancer
Country	East Asian	Western	(/100000 males in 2000)
The West			
Ireland	0	3	13.07
Austria	0	1	14.12
Italy	0	1	27.84
England	0	1	17.66
USA	0	6	6.15
Australia	0	3	8.64
East Asia			
Japan	52	0	58.39
Korea	5	0	36.71
China	8	0	24.56
Asia			
Vietnam	4	0	12.83
Thailand	3	2	3.31
India	0	3	3.83

nant Fukui strains had a single ESS region and were classified "A-B-D." In contrast, 16.0% (8/50) of Okinawa strains were Western CagA-positive strains containing a WSS sequence. The prevalence of the Western CagA-positive strain was significantly higher in Okinawa than in Fukui. All gastric cancer strains (29 Fukui and 8 Okinawa strains) were East Asian CagA-positive strains in both Fukui and Okinawa (Table 1).[35]

Distribution of CagA protein diversity in the world

We investigated the distribution of CagA protein diversity in the world using the data deposited in the GenBank. All Western strains (3 Irish, 1 Austrian, 1 Italian, 1 English, 6 American, and 3 Australian stains) had Western CagA. In contrast, all East Asian strains (52 Japanese, 5 Korean, and 8 Chinese strains) had East Asian CagA. In Vietnam, all 4 strains had East Asian CagA. In Thailand, 3 strains had East Asian and 2 strains had Western CagA. In India, all 3 strains had Western CagA. Although the clinical characteristics of most strains were not available in the GenBank, the prevalence of the East Asian CagA-positive strain appears to be associated with the mortality rate of gastric cancer in Asia (Table 2).[35]

Relationship between histological features and diversity of CagA

The diversity of CagA may influence the pathogenicities of different *cagA*-positive *H. pylori* strains. The grade of inflammation and activity of gastritis was significantly higher in chronic gastritis patients with the East Asian CagA-positive strain than in chronic gastritis patients infected with the *cagA*-negative strain or Western CagA-positive strain in both gastric antral and fundic mucosa. The grade of gastric mucosal atrophy was also significantly higher in chronic gastritis patients infected with the East Asian CagA-positive strain than in chronic gastritis patients infected with the *cagA*-negative strain or Western CagA-positive strain in both gastric antral and fundic mucosa (Table 3).[35]

Table 3. Relationship between histological features and diversity of CagA in chronic gastritis patients

		Inflammation		Activity		Atrophy	
Okinawa	*n*	Antrum	Body	Antrum	Body	Antrum	Body
cagA (−)	6	1 (1.0 ± 0.6)	1 (0.8 ± 0.4)	1 (0.8 ± 0.4)	1 (0.7 ± 0.5)	1 (1.2 ± 0.4)	1 (0.7 ± 0.5)
Western	8	1 (1.0 ± 0.8)	1 (1.0 ± 0.5)	1 (1.0 ± 0.5)	1 (0.9 ± 0.6)	1 (1.5 ± 0.5)	1 (1.1 ± 0.4)
East Asian	28	2 (2.2 ± 0.5)[a]	2 (1.9 ± 0.5)[b]	2 (2.0 ± 0.6)[c]	2 (1.7 ± 0.5)[d]	2 (2.3 ± 0.5)[e]	2 (2.1 ± 0.5)[f]

Median score (mean ± SD)
[a] Significantly higher than *cagA* (−) ($P = 0.002$) or Western group ($P = 0.0012$)
[b] Significantly higher than *cagA* (−) ($P = 0.0025$) or Western group ($P = 0.008$)
[c] Significantly higher than *cagA* (−) ($P = 0.002$) or Western group ($P = 0.0025$)
[d] Significantly higher than *cagA* (−) ($P = 0.003$) or Western group ($P = 0.007$)
[e] Significantly higher than *cagA* (−) ($P = 0.001$) or Western group ($P = 0.01$)
[f] Significantly higher than *cagA* (−) ($P = 0.0003$) or Western group ($P = 0.0004$)
Source: Modified from Ref. 35

Conclusions

Strain-specific diversity has been proposed to be involved in the ability of *H. pylori* to cause different diseases. The *cagA* gene product CagA was demonstrated to be injected into the host cytoplasm through a type IV secretion system and phosphorylated by the host cellular kinases.[11,14–17] In addition, CagA forms a physical complex with SHP-2, which is known to play an important positive role in mitogenic signal transduction, and stimulates phosphatase activity.[22] In Japan, nearly 100% of the strains possess functional *cag* PAI,[27,36] and the incidence of atrophic gastritis and gastric cancer is quite high compared with Western countries.[37] There are two major CagA subtypes, the East Asian and the Western type. We recently discovered that East Asian CagA has a distinct sequence at the SHP-2-binding site, and that the East Asian-specific sequence confers stronger SHP-2 binding and transforming activities than Western CagA.[31]

East Asian CagA-positive *H. pylori* is associated with atrophic gastritis and gastric cancer, and persistent active inflammation induced by the East Asian CagA-positive strain may play a role in its pathogenesis.[35] Endemic circulation of *H. pylori* populations carrying biologically more active CagA proteins in East Asian countries, where the mortality rate of gastric cancer is among the highest in the world, may be involved in increasing the risk of gastric cancer in these populations.

References

1. Waren JR, Marshall BJ. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet 1983;1:1273–5.
2. Marshall BJ, Goodwin CS, Warren JR, Murray R, Blincow ED, Blackbourn SJ, et al. Prospective double blind trial of duodenal ulcer relapse after eradication of *Campylobacter pylori*. Lancet 1988;2:1437–42.
3. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al. *Helicobacter pylori* infection and the risk of gastric carcinoma. N Engl J Med 1991;325:11127–31.
4. Wotherspoon AC, Ortiz-Hidalgo C, Falzon MR, Isaacson PG. *Helicobacter pylori*-associated gastritis and primary B-cell gastric lymphoma. Lancet 1991;338:1175–6.
5. The Eurogast Study Group. An association between *Helicobacter pylori* infection and gastric cancer. Lancet 1993;341:1359–62.
6. Kikuchi S, Wada O, Nakajima T, Nishi T, Kobayashi O, Konishi T, et al. Serum anti-*Helicobacter pylori* antibody and gastric carcinoma among young adults. Research group on prevention of gastric carcinoma among young adults. Cancer (Phila) 1995;75:2789–93.
7. IARC. Schistosomes, liver flukes and *Helicobacter pylori*. Monographs on the evaluation of carcinogenic risks to humans. IARC Sci Publ 1994;61:1–241.
8. Covacci A, Censini S, Bugnoli M, Petracca R, Burroni D, Macchia G, et al. Molecular characterization of the 128-kDa immunodominant antigen of *Helicobacter pylori* associated with cytotoxicity and duodenal ulcer. Proc Natl Acad Sci U S A 1993;90:5791–5.
9. Tomb JF, White O, Kerlavage AR, Clayton RA, Sutton GG, Fleischmann RD, et al. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature (Lond) 1997;388:539–47.
10. Censini S, Lange C, Xiang Z, Crabtree JE, Ghiara P, Borodovsky M, et al. *Cag*, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. Proc Natl Acad Sci U S A 1996;93:14648–53.
11. Covacci A, Telford J L, Del Giudice G, Parsonnet J, Rappuoli R. *Helicobacter pylori* virulence and genetic geography. Science 1999;284:1328–33.
12. Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, et al. Infection with *Helicobcter pylori* strains possessing *cagA* is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer Res 1995;55:2111–5.
13. Segal ED, Falkow S, Tompkins LS. *Helicobacter pylori* attachment to gastric cells induces cytoskeletal rearrangements and tyrosine phosphorylation of host cell protein. Proc Natl Acad Sci U S A 1996;93:1259–64.
14. Asahi M, Azuma T, Ito S, Suto H, Nagai Y, Tsubokawa M, et al. *Helicobacter pylori* CagA protein can be tyrosine phosphorylated in gastric epithelial cells. J Exp Med 2000;191:593–602.
15. Stein M, Rappuoli R, Covacci A. Tyrosine phosphorylation of the *Helicobacter pylori* CagA antigen after *cag*-driven host cell translocation. Proc Natl Acad Sci U S A 2000;97:1263–8.
16. Segal ED, Cha J, Lo J, Falkow S, Tompkins LS. Altered states: involvement of phosphorylated CagA in the induction of host

cellular growth changes by *Helicobacter pylori*. Proc Natl Acad Sci U S A 1999;96:14559–64.
17. Odenbreit S, Puls J, Sedlmaier B, Gerland E, Fischer W, Haas R. Translocation of *Helicobacter pylori* CagA into gastric epithelial cells by type IV secretion. Science 2000;287:1497–500.
18. Kodama A, Matozaki T, Fukuhara A, Kikyo M, Ichihashi M, Takai Y. Involvement of an SHP-2-Rho small G protein pathway in hepatocyte growth factor/scatter factor-induced cell scattering. Mol Biol Cell 2000;11:2565–75.
19. Feng GS, Hui CC, Pawson T. SH2-containing phosphotyrosine phosphatase as a target of protein-tyrosine kinases. Science 1993;259:1607–11.
20. Freeman RM Jr, Plutzky J, Neel BG. Identification of a human src homology 2-containing protein-tyrosine-phosphatase: a putative homolog of *Drosophila* corkscrew. Proc Natl Acad Sci U S A 1992;89:11239–43.
21. Ahmad S, Banville D, Zhao Z, Fischer EH, Shen SH. A widely expressed human protein-tyrosine phosphatase containing src homology 2 domains. Proc Natl Acad Sci U S A 1993;90:2197–201.
22. Higashi H, Tsutsumi R, Muto S, Sugiyama T, Azuma T, Asaka M, et al. SHP-2 tyrosine phosphatase as an intracellular target of *Helicobacter pylori* CagA protein. Science 2002;295:683–6.
23. Yu D, Qu C, Henegariu O, Lu X, Feng G. Protein-tyrosine phosphatase Shp-2 regulates cell spreading, migration, and focal adhesion. J Biol Chem 1998;273:21125–31.
24. Yamazaki S, Yamakawa A, Ito Y, Ohtani M, Higashi H, Hatakeyama M, et al. The CagA protein of *Helicobacter pylori* is translocated into epithelial cells and binds to SHP-2 in human gastric mucosa. J Infect Dis 2003;187:334–7.
25. Correa P. A human model of gastric carcinogenesis. Cancer Res 1988;48:3554–60.
26. van Doorn LJ, Figueiredo C, Megraud F, Pena S, Midolo P, Queiroz DM, et al. Geographic distribution of *vacA* allelic types of *Helicobacter pylori*. Gastroenterology 1999;116:823–30.
27. Ito Y, Azuma T, Ito S, Miyaji H, Hirai M, Yamazaki Y, et al. Analysis and typing of the *vacA* gene from *cagA*-positive strains of *Helicobacter pylori* isolated in Japan. J Clin Microbiol 1997;35:1710–4.
28. Covacci A, Censini S, Bugnoli M, Petracca R, Burroni D, Macchia G, et al. Molecular characterization of the 128-kDa immunodominant antigen of *Helicobacter pylori* associated with cytotoxicity and duodenal ulcer. Proc Natl Acad Sci U S A 1993;90:5791–5.
29. 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;36:2258–63.
30. Backert S, Moese S, Selbach M, Brinkmann V, Meyer TF. Phosphorylation of tyrosine 972 of the *Helicobacter pylori* CagA protein is essential for induction of a scattering phenotype in gastric epithelial cells. Mol Microbiol 2001;42:631–44.
31. Higashi H, Tsutsumi R, Fujita A, Yamazaki S, Asaka M, Azuma T, et al. 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 2002;99:14428–33.
32. Yamaoka Y, El-Zimaity HM, Gutierrez O, Figura N, Kim JG, Kodama T, et al. Relationship between the *cagA* 3′ repeat region of *Helicobacter pylori*, gastric histology, and susceptibility to low pH. Gastroenterology 1999;117:342–9.
33. De Souza D, Fabri LJ, Nash A, Hilton DJ, Nicola NA, Baca M. SH2 domains from suppressor of cytokine signaling-3 and protein tyrosine phosphatase SHP-2 have similar binding specificities. Biochemistry 2002;41:9229–36.
34. Ito S, Azuma T, Murakita H, Hirai M, Miyaji H, Ito Y, et al. Profile of *Helicobacter pylori* cytotoxin derived from two areas of Japan with different prevalence of atrophic gastritis. Gut 1996;39:800–6.
35. Azuma T, Yamazaki S, Yamakawa A, Ohtani M, Muramatsu A, Suto H, et al. Variation in the SHP-2 binding site of *Helicobacter pylori* CagA protein is associated with gastric atrophy and cancer. J Infect Dis 2004; in press.
36. Azuma T, Yamakawa A, Yamazaki S, Fukuta K, Ohtani M, Ito Y, 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;186:1621–30.
37. Correa P. A human model of gastric carcinogenesis. Cancer Res 1988;48:3554–60.