H. pylori Virulence Factors: Genetic Polymorphism and Disease

6

Young Sun Kim

Abstract

Helicobacter pylori (*H. pylori*) virulence factors are important for clarifying the role of *H. pylori* in the regional differences in the gastric cancer distribution and the pathogenesis of clinically significant diseases such as gastric cancer or peptic ulcer. Genetic polymorphism of *H. pylori* virulence factors differs by geographic region, in which East-Asian-type *cagA* is known to be more virulent than Western type. As there are more repetitions of Glu-Pro-Ile-Tyr-Ala (EPIYA)-C segment in *cagA*, *H. pylori* becomes more virulent, which is associated with gastric cancers in the West. Between genotype m1 and m2 of *vacA* middle region, m1 is more virulent, which is thought to be the cause of the increased prevalence rate of gastric cancers in many East-Asian regions. If all the studies to date are put together, *cagA*, *vacA*, and *oipA* are the factors associated with gastric cancer and *dupA* can be considered to be an important virulent factor for duodenal ulcer, but because different studies have showed different results and particularly results were different by geographic region, more research is needed.

Keywords

Helicobacter pylori • Virulence factor • Genetic polymorphism

6.1 Introduction

Helicobacter pylori (*H. pylori*) has a genome of 1,600 genes, which are well conserved, but its variants are very diverse and complex in terms of combination and structure of the genes by region depending on the physiological and ecological changes in the strains and hosts, which makes an interesting subject as a model for adaptation and evolution of microorganisms [1, 2].

Y.S. Kim, MD, PhD
Division of Gastroenterology, Healthcare System Gangnam Center Seoul National University Hospital, 152 Teheran-ro, Gangnam-gu, Seoul 06236, South Korea
e-mail: yspanda@gmail.com

N. Kim (ed.), *Helicobacter pylori*, DOI 10.1007/978-981-287-706-2_6

H. pylori strains have evolved continuously within the human body when mankind left Africa and moved to America and Oceania approximately 58,000 years ago, and it is predicted that the virulence of *H. pylori* has been changed along [1, 3] (Fig. 6.1). For example, a genetic analysis of a strain isolated from Peruvians in South America demonstrated that while *cag* pathogenicity island (PAI) gene of Peruvian natives did not contain cytotoxin-associated gene A (*cagA*) gene, *cagA* gene of Europeans was newly inserted after the arrival of Europeans. And by a comparison of *cagA* DNA sequences, it was also confirmed that genes of Indo-Aryans and Neolithic man in the Crescent region coexist in the Indian strain. These genetic diversities of virulence factors including *cagA* and vacuolating cytotoxin (*vacA*) depending on the region suggest a possibility that pathogenicity of *H. pylori* is an acquired characteristic for survival and adaptation through the process of evolution and transfer [3–5].

H. pylori live in the human stomach for a long time, but most of those infected do not have symptoms and only some of those infected are taken with diseases, which are not necessarily same diseases. Thus, the reason why those infected show different results is because bacterial factors,

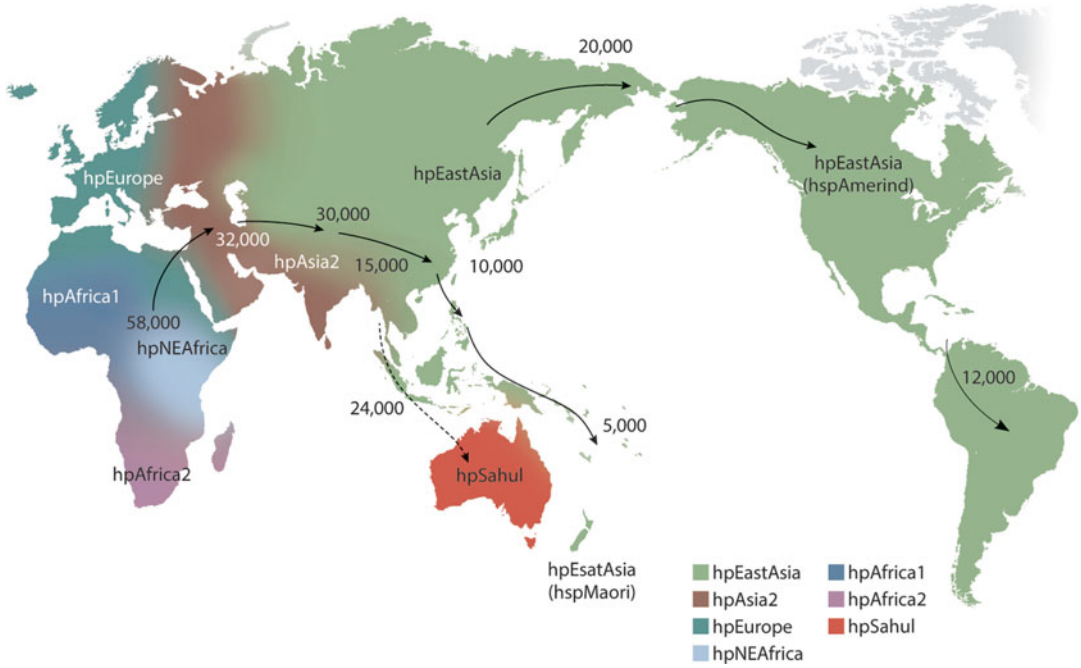

Fig. 6.1 Distribution of *H. pylori* genotypes before Columbus found the New World and human migration to America and Oceania began. There are seven modern *H. pylori* population types: hpEurope, hpEastAsia, hpAfrica1, hpAfrica2, hpAsia2, hpNEAfrica, and hpSahul. hpEurope includes almost all *H. pylori* strains isolated from ethnic Europeans, including people from countries colonized by Europeans. Most *H. pylori* isolates from East Asia belong to hpEastAsia, which includes hspMaori (Polynesians, Melanesians, and native Taiwanese), hspAmerind (American Indians), and hspEAsia (East Asia) subpopulations. hpAsia2 strains are isolated in South, Southeast, and Central Asia; hpAfrica1 in West Africa, South Africa, and African Americans. hpNEAfrica is predominantly made up of isolates from Northeast Africa. hpAfrica2 is very distinct from any other type and has currently only been isolated in South Africa. hpSahul is a novel group specific to *H. pylori* strains isolated from Australian Aborigines and Highlanders of New Guinea. *H. pylori* is predicted to have spread from East Africa over the same time period as anatomically modern humans (~58,000 years ago) and has remained intimately associated with their human hosts ever since. Estimated global patterns of *H. pylori* migration are indicated by *arrows*, and the *numbers* show the estimated time since they migrated (years ago). The *broken arrow* indicates an unconfirmed migration pattern (Adapted from Yamaoka et al. [9])

in particular virulence factors, as well as host and environmental factors play an important role. Although genetic studies of virulence factors have been actively conducted based on the already known DNA sequence of *H. pylori*, crucial virulence factor genes responsible for inducing diseases have not been fully established yet, which can be explained by high degree of variations in the DNA sequences of each *H. pylori* (genetic polymorphism). Such diverse variations are caused by point mutation, substitution, insertion, or deletion, and occasionally several strains of *H. pylori* with different genetic backgrounds are observed in a single individual. Through these observations, it is thought that not only endogenous mutations but also chromosomal rearrangements or recombination occur between each strain [1]. So far, there have been many studies to examine the relationship between diseases and known virulence factors, such as CagA, VacA, induced by contact with epithelium (IceA), outer inflammatory protein (OipA), duodenal ulcer promoting gene (*dupA*) and blood group A antigen-binding adhesion (BabA) [6–14] (Table 6.1). In this chapter, we will discuss the relationship of the genetic polymorphism of these virulence factors and gastroduodenal diseases with geographic differences.

6.2 Cytotoxin-Associated Gene A (*cagA*)

CagA is the most studied virulence factor of *H. pylori*, which is located at one end of the *cag* PAI. The *cag* PAI encodes a type IV secretion system, responsible for the injection of the CagA protein into the host cells [15–17].

CagA-producing strains are reported to be associated with severe clinical outcomes, especially in Western countries. Approximately 60–70 % of isolated *H. pylori* strains from Western countries are known to be positive for *cagA*, which cause more severe inflammatory reactions with increased interleukin (IL)-8 production. It has been reported that individuals infected with *cagA*-positive strains of *H. pylori* are at a higher risk of peptic ulcer or gastric cancer than those infected with *cagA*-negative strains [6, 18]. The studies conducted in Western

Table 6.1 Association of *Helicobacter pylori* virulence factors with host responses and disease outcome

Virulence factor	Colonization/cell damage	Inflammation/immunity	Disease outcome
cag PAI	Increased bacterial density	Increased production of cytokines and antimicrobials Infiltration of immune cells	Gastritis Peptic ulcer Gastric cancer
CagA	Disruption of epithelial junctions Epithelial motility and scattering	Cytokine production Up regulation of oncogenic proteins	Atrophic gastritis Gastric cancer
VacA	Colonization Epithelial permeability Epithelial erosion and necrosis Vacuolation Apoptosis	Disruption of antigen processing Inhibition of T-cell proliferation	Ulceration
OipA	Adherence to epithelial cells Colonization	Cytokine production	Dysplasia Gastric cancer Duodenal ulcer
DupA		Cytokine production	Duodenal ulcer Decreased gastric cancer

Modified from Allison and Ferrero [14]
cag PAI cag pathogenicity island, *CagA* cytotoxin-associated gene A, *VacA* vacuolating cytotoxin, *OipA* outer inflammatory protein, *DupA* duodenal ulcer promoting gene

countries showed that the prevalence of CagA antibodies was significantly higher in peptic ulcer patients or duodenal ulcer patients compared to control, that is, 100 % for peptic ulcer disease, 85–100 % for duodenal ulcer, and 30–60 % for control [7, 8].

A study using Western blotting method for serological detection of antibodies against *cagA* reported that serum antibodies to *cagA* were detected more frequently in gastric carcinoma patients (91 %) than control (72 %) [19].

A cohort study (mean follow-up period of 11.5 years) for 58 subjects infected with *H. pylori* showed that infection with *cagA*-positive *H. pylori* strains is associated with an increased risk for the eventual development of atrophic gastritis and intestinal metaplasia [20]. However, it is difficult to determine the importance of *cagA* in clinical outcomes in East-Asian countries including Korea because nearly all *H. pylori* strains possess *cagA* [8, 10, 12, 13].

6.2.1 *cagA* Type: Western Versus East Asian

There has been an increasing tendency in the last decade to explain the higher incidence of gastric cancer in East Asia using the concept of East-Asian-type *cagA* and Western-type *cagA* [9]. There are different numbers of repeat sequences located in the 3′ region of the *cagA* gene of different *H. pylori* strains. Each repeat region of the *cagA* protein contains Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs, including a tyrosine phosphorylation site. It has now become more common to name the first repeat region as EPIYA-A and EPIYA-B segments and to name the second repeat region as EPIYA-C or EPIYA-D segments [21]. Western-type *cagA* contain EPIYA-A, EPIYA-B, and EPIYA-C segments. By contrast, East-Asian-type *cagA* contain the EPIYA-A, EPIYA-B, and EPIYA-D segments, but not the EPIYA-C segment (Fig. 6.2). Individuals infected with East-Asian-type *cagA* strains were reported to have an increased risk of peptic ulcer or gastric cancer compared with those with Western-type *cagA* strains [9, 11].

However, there are limitations to explain the higher incidence of gastric cancer in East Asia using the concept of East-Asian-type *cagA* and Western-type *cagA* because the incidence of gastric cancer is also high in some regions where Western-type *cagA* strains are reported to account for the majority of *H. pylori* strains (e.g., in Peru and Columbia (age standardized rate [ASR] per 100,000 population 21.2 and 17.4, respectively)) [22]. In addition, in Africa the rate of *H. pylori* infection is high (e.g., 70–97 % of patients with dyspepsia are infected with *H. pylori*, as are 80 % of asymptomatic volunteers), but gastric cancer is generally uncommon; this seemingly contradictory situation is known as the "African enigma" [23]. The incidence of gastric cancer is extremely high in Mali, West Africa (ASR per 100,000 population 20.3), and the frequency of gastric cancer among women in this country is higher than it is among women in Japan (ASR per 100,000 population 19.3 vs. 18.2) [9]. Accordingly, these facts cannot be explained by the presence of East-Asian-type *cagA* versus Western-type *cagA* alone. In a study comparing the *cagA* gene repeat sequences found in Columbia (ASR per 100,000 population 17.4) with those found in the USA (ASR per 100,000 population 4.1) to explain the geographic difference in the incidence of gastric cancer, 100 *H. pylori* isolates from patients with simple gastritis (30 from Columbia and 70 from the USA) were analyzed; 57 % of the isolates from Columbia had two EPIYA-C segments, whereas only 4 % of the isolates from the USA had two EPIYA-C segments (Y. Yamaoka, unpublished data) [9, 11]. Overall, the number of EPIYA-C segments may explain, to some extent, the geographic difference in the incidence of gastric cancer in Western countries [9]. Further research is required to determine whether these subtypes are involved in the pathogenesis of gastric cancer.

In the meta-analysis of case control studies with age- and sex-matched controls, which provided raw data in East-Asian countries including Japan, Korea, and China, in eight studies, the pooled prevalence of CagA seropositivity was 71.6 % (1,019 out of 1,423) in cases and 62.7 % (1,595 out of 2,542) in controls. The estimated

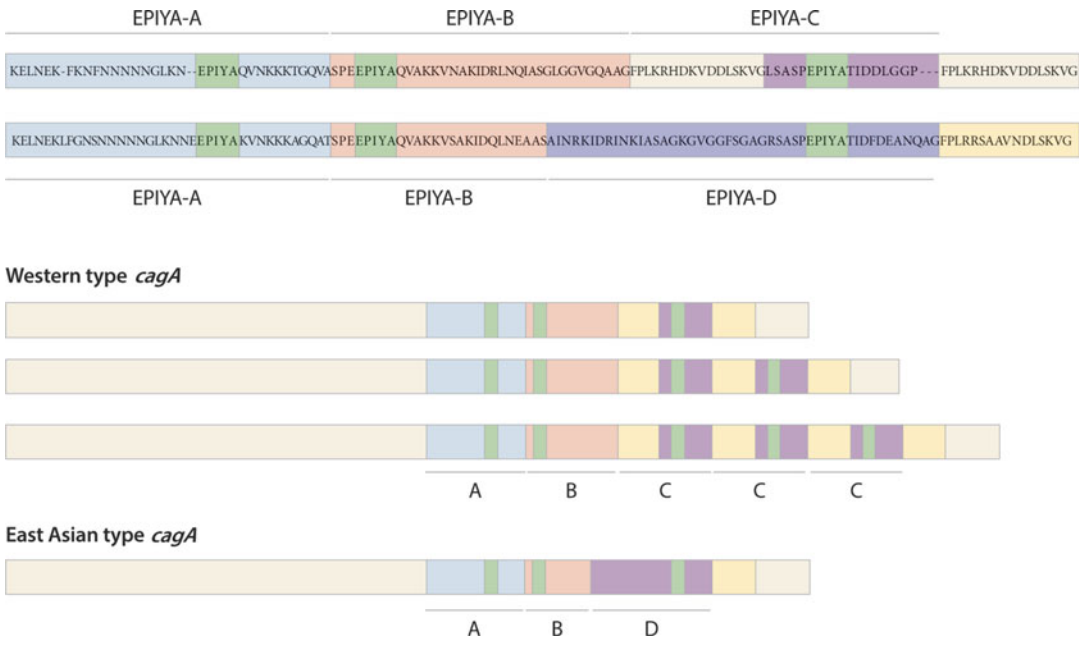

Fig. 6.2 Structural polymorphism in *cagA*. Western-type *cagA* contain EPIYA-A, EPIYA-B, and EPIYA-C segments. By contrast, East-Asian-type *cagA* contain the EPIYA-A, EPIYA-B, and EPIYA-D segments, but not the EPIYA-C segment. The EPIYA motif in each segment (shown in *green*) represents the tyrosine phosphorylation sites of *cagA*. The sequence flanking the tyrosine phosphorylation site of the EPIYA-D segment (EPIYATIDF), but not the EPIYA-C segment (EPIYATIDD), matches perfectly the consensus high-affinity binding sequence for the SH2 domains of SHP2. In Western countries, the incidence of gastric cancer is significantly higher in patients infected with strains containing multiple EPIYA-C segments than in patients infected with strains containing a single EPIYA-C segment (i.e., ABCCC vs. ABC). By contrast, almost all East-Asian strains contain a single EPIYA-D segment. *cagA* forms dimers in cells in a phosphorylation-independent manner, and the CagA multimerization (CM) sequence (also named the conserved repeat responsible for phosphorylation-independent activity [CRPIA] or MARK2/PAR1b kinase inhibitor [MKI]) in *yellow* was identified as the site responsible for dimerization, for inhibition of MARK2/PAR1b kinase, and for the interaction of *cagA* with activated c-Met (Adapted from Yamaoka [9])

overall OR was 1.50 (95 % confidence interval [CI], 1.30–1.72). In meta-analysis in a random effect model, overall OR was 1.81 (95 % CI, 1.30–2.11). This shows that the gastric cancer risk for *cagA*-positive cases was higher overall than in *H. pylori*-infected subjects; however, the OR in East-Asian countries was smaller than the result of the meta-analysis that included Western countries (1.81 vs. 2.64). In addition, the presence of anti-CagA antibodies increases the risk of gastric cancer in the *H. pylori*-negative population. The prevalence of anti-CagA antibodies ranged from 18.2 % to 81.8 % in gastric cancer patients and 9.8–60.2 % in controls [24]. The lower frequency of higher titer IgG antibody in advanced cancer may be due to the increasing extent of intestinal metaplasia associated with transition from the intestinal type of early gastric cancer to advanced cancer, such that the local environment is no longer ideal for the growth of *H. pylori* [9, 25]. CagA antibodies may be positive in patients who have a negative *H. pylori* serologic test since CagA antibodies can potentially remain positive for a longer period of time than the anti-*H. pylori* antibody [9, 24]. This evidence confirms that CagA antibodies can potentially remain positive for a longer period of time than the anti-*H. pylori* antibody [9]. Accordingly, anti-CagA antibody was related to gastric cancer in both *H. pylori*-positive and *H. pylori*-negative populations in East-Asian population [9]. However, it is necessary to evaluate the availability of anti-*H. pylori* antibody plus anti-CagA antibody for screening for risk of gastric cancer.

6.3 Vacuolating Cytotoxin (*vacA*)

All the *H. pylori* strains have a functional *vacA*, which encodes a vacuolating cytotoxin. However, there is significant sequence diversity in *vacA* genes across the many *H. pylori* isolate strains [26–30] (Fig. 6.3). There is variation in the vacuolating activity of different *H. pylori* strains, primarily due to differences in the *vacA* gene structure at the signal (s)-region (s1 and s2) and the middle (m)-region (m1 and m2) [9, 26]. In vitro experiments demonstrated that s1/m1 strains are the most cytotoxic, followed by s1/m2 strains, whereas s2/m2 strains have no cytotoxic activity and s2/m1 strains are rare [26]. *vacA* s1/m1 is the most common strain in East Asia including Korea [27].

6.3.1 Geographic Differences in *vacA* Genotypes

It has been known that there are geographic differences in the distribution of both the *vacA* s- and m-region subtypes [26] (Fig. 6.4). Subtype s1a is predominant in Northern Europe and Australia, whereas subtype s1b is prevalent in South America. Subtype s1c is the major subtype in East Asia, but is extremely rare in Western Europe. The most common *vacA* genotypes in Korea are s1c for the s-region and m1 for the m-region [26–28].

There have been many reports that individuals infected with s1 or m1 *H. pylori* strains have an increased risk of peptic ulcer or gastric cancer compared with individuals infected with s2 or m2 strains [26–28]. In East Asia including Korea, however, most *H. pylori* strains have an s1-type s-region; therefore, the pathogenic difference cannot be explained by the type of s region present [9].

The clear geographic differences in the distribution of both the *vacA* s- and m-region subtypes strongly suggest a geographic structure of *H. pylori* populations throughout the world [26]. The distribution of both s- and m-region allelic types in Central and South America is similar to that in the Iberian Peninsula. This distribution of *H. pylori vacA* genotypes may reflect the extensive cultural and socioeconomic relationships between these parts of the world during past centuries. Particular *H. pylori* strains may have been spread through Central and South America by colonization of these areas by Spanish and Portuguese descendants. Similarly, subtype s1a is more common in the northern European countries and its predominance in Commonwealth countries (Canada and Australia) again may reflect historic relationships. It is unknown whether the evolution of both subtypes occurred in Europe. Within Europe, the distribution gradient of subtypes s1a (northern and eastern Europe) and s1b (Iberian Peninsula) was highly consistent among all tested strains from 12 different countries. If subtypes s1a and s1b already existed for a long time in Europe and were freely movable, a more homogeneous distribution would have been expected. On the other hand, if transmission

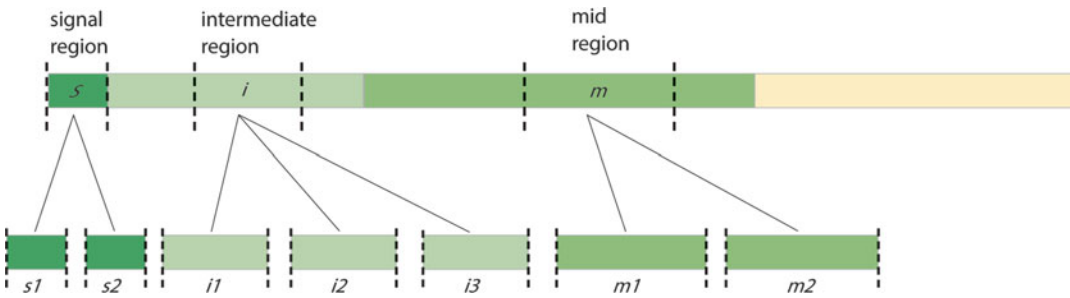

Fig. 6.3 *vacA* allelic diversity and structure. Significant allelic diversity exists in three regions of the *vacA* gene: the signal region (*s1* and *s2*), the intermediate region (*i1*, *i2*, and *i3*), and the mid-region (*m1* and *m2*) (Adapted from Palframan et al. [30])

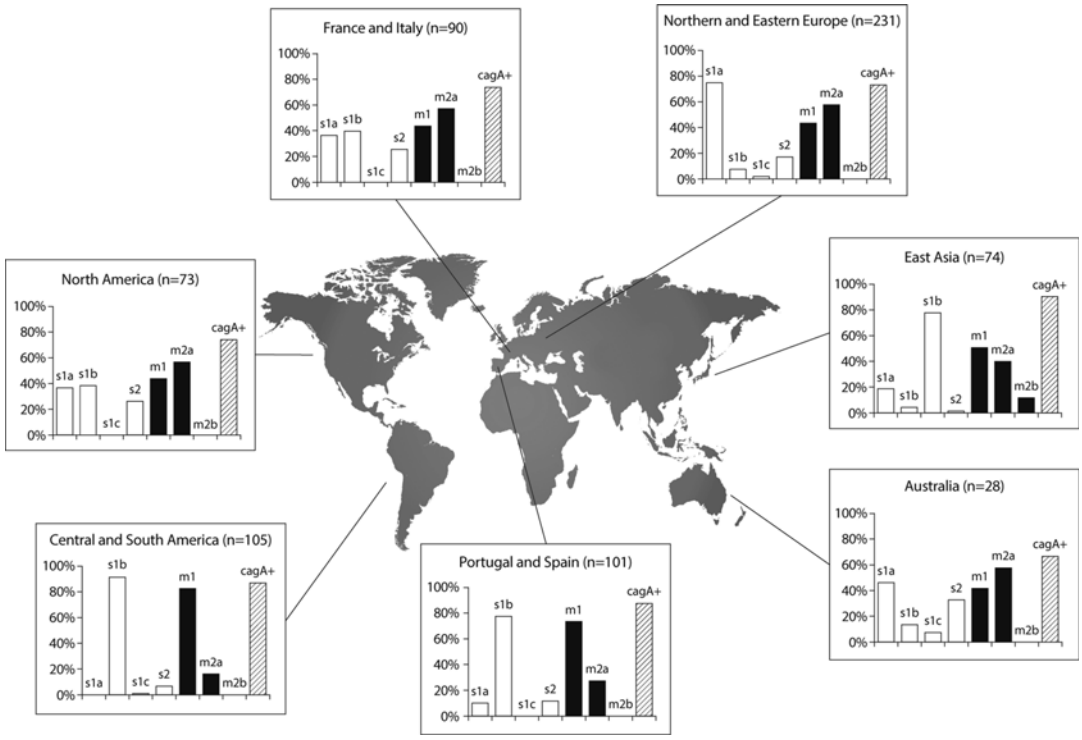

Fig. 6.4 Distribution of *vacA* s- and m-region and *cagA* genotypes of *H. pylori* strains from different parts of the world. For each region, the prevalence of each type (s1a, s1b, s1c, s2, m1, m2a, and m2b; *cagA* positive) is given as a percentage of the total number of strains (shown in *parentheses*). Only strains containing a single *vacA* genotype are represented (Adapted from Van Doorn et al. [26])

of *H. pylori* is highly local, only occurring over very short distances during childhood, this may have prevented a broader geographic distribution of each subtype. An alternative hypothesis would be that the distribution of different subtypes reflects particular adaptation of *H. pylori* to specific host populations [26–28, 31, 32].

In Korean *H. pylori* strain, a wide diversity has been observed in *vacA* s1-region. In the study performed by Kim et al. [12], *vacA* s1a–s1c was determined as the most common subtype in South Korea and considering the positivity of genotypes, *vacA* s1c and s1a were identified as the major genotypes. s1a–s1c was significantly frequent in benign gastric ulcer (73.2 %, $p=0.023$), gastric cancer (73.6 %, $p=0.018$), and dysplasia (71.9 %, $p=0.048$) than control (58. 7 %) (Tables 6.2 and 6.3).

On the other hand, a study performed in Korea for peptic ulcer and gastritis patients by Park et al. [31] showed that s1c, s1a, and s1b were found in 66.1 %, 35.6 %, and 0 % of *H. pylori* isolates, respectively. Another study [8] analyzed *H. pylori* isolates from Seoul, Korea; s1c and s1a were found in 90 % and 7 %, respectively. In both studies, s1 subtypes were not related to clinical outcome. This explanation may support the reason for the possible infection of multiple strains in Korea and other countries. However, further studies will be required to clarify these diversities.

Regarding the combination of the *vacA* s and m genotypes, Japanese strain from Okinawa showed that the *vacA* s1/m1 genotype was significantly higher in strains from gastric ulcer (79.2 %) and gastric cancer (87.5 %) than those from gastritis (59.2 %) ($p=0.002$ and 0.006, respectively). The prevalence of the *vacA* s1/m2 genotype tended to be higher in strains from patients with duodenal ulcer than those from

Table 6.2 Positivity of *vacA*, *cagA*, *iceA*, *oipA*, and *dupA* of *H. pylori* in 401 colonies from Korea

	Control ($n=75$[a])	BGU ($n=71$[a])	DU ($n=102$[a])	Stomach cancer ($n=121$[a])	Dysplasia ($n=32$[a])	Total ($n=401$[a])	p value
vacA s1 (%)	75/75 (100.0)	71/71 (100.0)	102/102 (100.0)	121/121 (100.0)	32/32 (100.0)	401/401 (100.0)	0.999
s1a	61/75 (81.3)	67/71 (94.4)*	84/102 (82.4)	108/121 (89.3)	31/34 (96.9)*	351/401 (87.5)	0.026
s1b	9/75 (12.0)	6/71 (8.5)	15/102 (14.7)	17/121 (14.0)	7/32 (21.9)	54/401 (13.5)	0.436
s1c	67/75 (89.3)	58/71 (81.7)	83/102 (81.4)	113/121 (93.4)	28/32 (87.5)	349/401 (87.0)	0.052
vacA m1	70/75 (93.3)	69/71 (97.2)	89/102 (87.3)	116/121 (95.9)	32/32 (100.0)	376/401 (92.9)	0.018
vacA m2	5/75 (6.7)	2/71 (2.8)	13/102 (12.7)	5/121 (4.1)	0/32 (0.0)	25/401 (6.2)	0.018
vacA i1	75/75 (100.0)	71/71 (100.0)	102/102 (100.0)	121/121 (100.0)	32/32 (100.0)	401/401 (100.0)	0.999
vacA i2	10/75 (13.3)	14/71 (19.7)	10/102 (9.8)	15/121 (12.4)	1/32 (3.1)	50/401 (12.5)	0.153
cagA	59/66 (89.4)	53/64 (82.8)	82/96 (85.4)	96/106 (90.6)	23/27 (85.2)	313/359 (87.2)	0.586
EPIYA-C	1/46 (2.2)	1/39 (2.6)	3/52 (5.8)	2/78 (2.6)	0/19 (0.0)	7/234 (3.0)	0.709
EPIYA-D	45/46 (97.8)	38/39 (97.4)	49/52 (94.2)	76/78 (97.4)	19/19 (100.0)	227/234 (97.0)	0.709
*iceA*1	74/75 (98.7)	68/71 (95.8)	96/102 (94.1)	114/121 (94.2)	32/32 (100.0)	384/401 (95.8)	0.359
*iceA*2	27/75 (36.0)	27/71 (38.0)	45/102 (44.1)	67/119 (56.3)*	16/32 (50.0)	182/399 (45.6)	0.036
oipA	63/75 (84.0)	63/71 (88.7)	96/100 (96.0)*	114/120 (95.0)*	27/32 (84.4)	363/398 (91.2)	0.015
dupA	12/72 (16.7)	52/64 (81.3)*	74/99 (74.7)*	22/95 (23.2)	10/24 (41.7)*	170/354 (48.0)	<0.001

Adapted from Kim et al. [12]
Missing values are not included. Each number behind the dash is the total number of colonies which were analyzed
BGU benign gastric ulcer, *DU* duodenal ulcer, *H. pylori Helicobacter pylori*, *EPIYA-C* Western-type *cagA*, *EPIYA-D* East-Asian-type *cagA*
*$p<0.05$, comparing with control group
[a]Total number of each group

patients with gastritis (27.2% vs. 17.3%), although the difference did not reach statistical significance ($p=0.08$). The prevalence of the *vacA* s2/m2 genotype was significantly higher in strains from gastritis patients than in those from gastric ulcer, duodenal ulcer, and gastric cancer patients (22.4% vs. 11.9%, 10.5%, and 4.2%, $p=0.04$, 0.01, and 0.04, respectively) [33–35]. These results suggested that diverse *vacA* genotypes contribute to the clinical outcomes in Okinawa and low incidence of gastric cancer in Okinawa [9, 11].

With respect to the m-region, there is variation within East Asia. Although m1 strains are common in parts of north East Asia, such as Japan and South Korea, m2 strains are predominant in parts of south East Asia, such as Taiwan and Vietnam [35–37]. As the incidence of gastric cancer is higher in the north than in the south of East Asia, the m-region may play a role in the regional difference in disease pattern [9]. Even within Vietnam, the incidence of gastric cancer is approximately 1.5 times higher in Hanoi in the north than in Ho Chi Minh in the south of the

Table 6.3 *vacA* and *iceA* subtypes of *H. pylori* in 401 colonies from Korea

	Control ($n=75^a$)	BGU ($n=71^a$)	DU ($n=102^a$)	Stomach cancer ($n=121^a$)	Dysplasia ($n=32^a$)	Total ($n=401^a$)
vacA s1 (%)						
a	8/75 (10.7)	9/71 (12.7)	12/102 (11.8)	4/121 (3.3)	1/32 (3.1)	34/401 (8.5)
b	–	–	–	–	–	–
c	14/75 (18.7)	4/71 (5.6)*	18/102 (17.6)	11/121 (9.1)	1/32 (3.1)*	48/401 (12.0)
a–b	–	4/71 (5.6)	7/102 (6.9)	2/121 (1.7)	3/32 (9.4)	16/401 (4.0)
a–c	44/75 (58.7)	52/71 (73.2)*	57/102 (55.9)	89/121 (73.6)*	23/32 (71.9)*	265/401 (66.1)
others	9/75 (12.0)	2/71 (2.8)	8/102 (7.8)	15/121 (12.4)	4/32 (12.5)	38/401 (9.5)
vacA m						
m1	70/75 (93.3)	69/71 (97.2)	89/102 (87.3)	116/121 (95.9)	32/32 (100.0)	376/401 (92.9)
m2	5/75 (6.7)	2/71 (2.8)	13/102 (12.7)	5/121 (4.1)	0/32 (0.0)	25/401 (6.2)
vacA i						
i1	65/75 (86.7)	57/71 (80.3)	92/102 (90.2)	106/121 (87.6)	31/32 (96.9)	351/401 (87.5)
i2	–	–	–	–	–	–
i1 and i2	10/75 (13.3)	14/71 (19.7)	10/102 (9.8)	15/121 (12.4)	1/32 (3.1)	50/401 (12.5)
vacA						
s1i1m1	60/75 (80.0)	56/71 (78.9)	83/102 (81.4)	103/121 (85.1)*	31/32 (96.9)*	333/401 (83.0)
s1i1m2	5/75 (6.7)	1/71 (1.4)	9/102 (8.8)	3/121 (2.5)	–	18/401 (4.5)
s1i1 and i2m1	10/75 (13.3)	13/71 (18.3)	6/102 (5.9)	13/121 (10.7)	1/32 (3.1)	43/401 (10.7)
s1i1 and i2m2	–	1/71 (1.4)	4/102 (3.9)	2/121 (1.7)	–	7/401 (1.7)
iceA						
1	48/75 (64.0)	44/71 (62.0)	57/102 (55.9)	51/119 (42.9)*	16/32 (50.0)	216/399 (54.1)
2		3/71 (4.2)	6/102 (5.9)	7/119 (5.9)	–	16/399 (4.0)
1 and 2	27/75 (36.0)	24/71 (33.8)	39/102 (38.2)	61/119 (51.3)*	16/30 (50.0)	167/399 (41.9)

Adapted from Kim et al. [12]
Missing values are not included. Each number behind the dash is the total number of colonies which were analyzed
BGU benign gastric ulcer, *DU* duodenal ulcer, *H. pylori Helicobacter pylori*
*$p<0.05$, comparing with control group
[a]Total number of each group

country. Comparison of two geographically distant cities in Vietnam, Hanoi and Ho Chi Minh, showed that the *vacA* m1 genotype, thought to be more toxic than the *vacA* m2 type, is more prevalent in Hanoi, where the incidence of gastric cancer is higher than in Ho Chi Minh [36, 37]. These data support the hypothesis that the *vacA* m1 type is closely associated with gastric carcinogenesis [9].

In 2007, a third disease-related region of *vacA* was identified between the s-region and the m-region; it was named the intermediate (i)-region [38]. Yamaoka et al. [9] reported that all s1/m1 strains were classified as type i1, and all s2/m2 strains were classified as type i2, but s1/m2 strains were classified as either type i1 or i2, and i1 strains were shown to be more pathogenic. Typing of the i-region was also reported to be more effective for determining the risk of gastric cancer in Iranian patients than typing of the s-region or m-region [39]. However, in a study of patients from East and Southeast Asia, there was no association between the i-region and disease [11, 40].

More recently, a fourth disease-related region – the deletion (d) region – was identified between the i-region and the m- region [9]. The d-region is divided into d1 (no deletion) and d2 (a 69–81 bp deletion). The study of Western strains demonstrated that

d1 was a risk factor for gastric mucosal atrophy; however, almost all East-Asian strains are classified as s1/i1/d1. Therefore, further researches are needed to clarify association between d- or i-region and clinical outcome [11, 39, 40].

6.4 Induced by Contact with Epithelium (*iceA*)

An initial series of studies showed that *iceA* has two main allelic variants, *iceA*1 and *iceA*2. The expression of *iceA*1 was upregulated on contact between *H. pylori* and human epithelial cells, and the *iceA*1 genotype was linked with enhanced mucosal IL-8 expression and acute antral inflammation [9, 11, 41]. The *iceA* type 1 allele is reported to be predominant in Japan and Korea, and the *iceA* type 2 allele in the United States and Colombia [8, 9, 35, 41].

In a meta-analysis [42] including 50 studies with a total of 5,357 patients to confirm the relationship between the *iceA* allelic type and clinical outcomes, the overall prevalence of *iceA*1 was significantly higher by 64.6 % (1,791/2,771) in Asian countries than in Western countries (64.6 % vs. 42.1 %), whereas the prevalence of *iceA2* was more prevalent in Western countries than in Asian countries (45.1 % vs. 25.8 %). Sensitivity analysis showed that the presence of *iceA1* was significantly associated with peptic ulcer (OR 1.25; 95 % CI, 1.08–1.44); however, the presence of *iceA2* was inversely associated with peptic ulcer (OR 0.76; 95 % CI, 0.65–0.89). These findings were significant in Western countries. And the presence of *iceA* was not associated with gastric cancer. Most studies examined the *cagA* status; however, only 15 studies examined the correlation and only 2 showed a positive correlation between the presence of *cagA* and *iceA1*. It is possible that *iceA* is a discriminating factor for peptic ulcer which is independent of *cagA* [9, 11]. However, it is a result that has not been confirmed in other countries, such as Japan and Korea [8, 11, 41]. Kim et al. [12] reported that the positivity of *iceA*1 in Korean *H. pylori* isolates was more than 95 %, and *iceA*2 was variable from 35 % to 55 % among clinical disease. In addition, strains expressed with *iceA*2 alone were only about 5 %, and most strains were detected with *iceA*1 along with *iceA*2. This result could support a wide diversity of *H. pylori* infection in South Korea.

In summary, despite numerous attempts to relate *vacA* genotypes to outcome or disease pathogenesis, no consistent associations or demonstrable biologic basis for the putative associations has appeared. Further studies are warranted.

6.5 Outer Membrane Protein

Outer membrane protein has been shown to act as an adhesion that facilitates bacterial attachment to the host epithelium. Approximately 4 % of the *H. pylori* genome is predicted to encode outer membrane proteins. There have been many studies that investigate the expression status of outer membrane protein such as OipA, BabA, or BabB in different clinical outcomes [9, 43–45].

6.5.1 Outer Inflammation Protein (*oipA*)

oipA was initially identified as a proinflammatory response-inducing protein based, in part, on the fact that *oipA* isogenic mutants reduced the production of IL-8 from gastric epithelial cell lines [9, 43–45]. Transcription of *IL-8* genes in both *oipA* and *cag* PAI dependent through interactions with different binding sites are involved, such as transcription factors within the IL-8 promoter for nuclear factor-κB (NF-κB), activator protein 1 (AP-1), and interferon-stimulated responsive element (ISRE)-like element [9]. *oipA* functional status was related to clinical presentation, *H. pylori* density, and gastric inflammation. *cag* PAI, *babA2*, or *vacA* status appears important only as surrogate markers for a functional *oipA* gene. It is also important to reconfirm that the presence of *cagA*, *vacA*, and *oipA* are linked such that typically *H. pylori* either produce all of these proteins or none of them and that clinical outcomes, such as peptic ulcer and

gastric cancer, are associated with strains with and without these virulence factors. However, strains with recognized virulence factors tend to produce more severe inflammation and are associated with higher risk of these important clinical outcomes [9, 11].

In a study [45] analyzed *H. pylori* isolates from the United States and Colombia, an independent univariate analysis, showed that the *oipA* "on," *cag* PAI-positive, *vacA* s1 genotype, and the *babA*-positive type were all related to a risk of duodenal ulcer. Importantly, a multiple logistic regression analysis showed that only the *oipA* "on" status was an independent determinant predictor of duodenal ulcer from gastritis. This finding was confirmed in another study [46] based on a non-overlapping cohort of 200 patients who were examined for four outer membrane proteins, OipA, BabA, BabB, and sialic acid-binding adhesion (SabA), by immunoblot, in which multiple logistic regression analysis showed that only *oipA*-positive status was an independent determinant predictor of gastric cancer vs. gastritis and duodenal ulcer vs. gastritis.

However, strains in Asia appeared to be different from those in Western countries in the aspect of outer membrane proteins and their actions [9, 11]. Kim et al. [12] reported that *oipA* was more frequently detected in duodenal ulcer and gastric cancer, but significant effect on gastroduodenal diseases was not found in Korean *H. pylori* isolates. These results could also be an evidence of the different distribution of virulence factors according to geographic differences.

The *H. pylori oipA* has been demonstrated to be a potential antigen for a vaccine. Recently, *oipA* have been tested in mice and vector-based approaches and/or multicomponent vaccines have been investigated [46]. The study showed that *H. pylori oipA* encoding construct is capable of inducing humoral and cellular responses in immunized mice. The antibody response profiles elicited by the DNA vaccine alone administered intradermally (the gene gun method) showed that it produced a Th2 immune response, while co-delivery of *IL-2* and *LTB* gene encoding constructs promoted a Th1-biased immune response. Further studies warranted for developing vaccination for *H. pylori*.

6.5.2 Duodenal Ulcer Promoting Gene A (*dupA*)

In 2005, the first disease-specific *H. pylori* virulence factor that induced duodenal ulcer and had a suppressive action on gastric cancer was identified and was named duodenal ulcer promoting gene A (*dupA*) [47, 48]. The presence of *dupA* was associated with elevated IL-8 production in the antrum (i.e., antrum-predominant gastritis – a feature of duodenal ulcer disease) and has been reported to induce IL-12 production from monocytes [49].

In an initial study of a total of 500 *H. pylori* isolates, including 160 from Japan, 175 from South Korea, and 165 from Colombia, the positive rate for the *dupA* gene was high in patients with duodenal ulcer and low in patients with gastric cancer, regardless of a patients' nationality (42% vs. 9% on average) [47]. In the study analyzed 401 Korean *H. pylori* isolates by Kim et al. [12], the prevalence of *dupA* was 48.0%. Infection by *dupA*-positive *H. pylori* showed an increased risk of gastric ulcer (OR 33.06; 95% CI, 11.91–91.79) and duodenal ulcer (OR 15.60; 95% CI, 6.49–37.49). More than 75% of colonies with gastric ulcer and duodenal ulcer expressed *dupA*, which suggests that *dupA* may be a fundamental factor for the development of peptic ulcer diseases in South Korea.

However, Brazil, Singapore, Malaysia, and Japan failed to demonstrate a correlation between the presence of the *dupA* gene and disease [9]. An academic report on Brazilian strains by Queiroz et al. [50] showed that a *dupA* gene mutation (deletion or insertion) was found in 50% of patients with gastric cancer, whereas it was found in only approximately 20% of patients with duodenal ulcer. As a result, the positive rate for the functional *dupA* gene was considerably higher in patients with duodenal ulcer than in patients with gastric cancer. Further investigation might clarify the effect of functional *dupA* on various gastroduodenal diseases.

6.5.3 Blood Group A Antigen-Binding Adhesion (*babA*)

babA-mediated adherence of *H. pylori* to the gastric epithelium plays a critical role in the efficient

delivery of bacterial virulence factors that damage host tissue [51, 52]. Interactions between BabA and Lewis b (Leb)-related antigens are the best characterized adhesion receptor interactions in *H. pylori*. The *babA* gene was initially cloned from strain CCUG17875, which contains a silent *babA*1 gene and an expressed *babA*2 gene [9, 51]. A number of studies have suggested a relation between *babA2*-positive *H. pylori* and increased cellular mucosal inflammations and an increased risk of developing clinical outcomes [9].

Gerhard et al. [51] reported that the presence of *babA2* could be regarded as a good indicator of the ability of strains to express the Lewis b antigen-binding adhesion and that *babA2* is significantly associated with duodenal ulcer in *H. pylori* isolated from a German population. The incidence of the *babA2* genotype was about 72 % in their study (duodenal ulcer 100 %, gastric cancer 77.8 %, and gastritis 51.4 %).

However, Kim et al. [53] reported that the incidence of *babA* was low and was not related to peptic ulcer disease in Korea. The presence of the *babA* genes in *H. pylori* isolates from peptic ulcer and gastritis patients was 27.3 % and 26.3 %, respectively ($p=0.578$). In addition, the four pathogenicity-related genes, *cagA*, *vacA* s1c/m1, *iceA1*, and *babA*, did not correlate with other genes. In a Japanese study by Fujimoto et al. [54], all strains from East Asia expressed BabA protein, and 24 (9.8 %) of the Western strains were *babA* negative. For these strains, the *babA*-negative status was correlated inversely with *cagA* or *vacA* s status (i.e., only 1 [4.2 %] and none [0 %] of these *babA*-negative strains were *cagA*- or *vacA* s1-positive, respectively). Most (91 %) Western strains were classified as either *cagA*-positive/*vacA* s1-positive/BabA-H (triple positive, 76 %), *cagA*-positive/*vacA* s1-positive/BabA-L (6.1 %), or *cagA*-negative/*vacA* s2-positive/*babA*-negative strains (9.4 %). However, there was no relationship between the triple-positive strains and clinical outcome. *babA*-negative status is associated with mild gastric injury and lower *H. pylori* density. *babA*-negative strains also are associated infrequently with duodenal ulcer or gastric cancer.

However, because *babA*-negative status is linked closely to *cagA*-negative/*vacA* s2 status, potential interactions between these different putative virulence factors cannot be ruled out.

In summary, it remains unclear how BabA expression is regulated or if expressing low levels of BabA has a direct role in the pathogenesis of duodenal ulcer or gastric cancer [11]. Further studies are warranted.

Conclusions

The *H. pylori* virulence factors are important for clarifying the role of *H. pylori* in the regional differences in the gastric cancer distribution and the pathogenesis of clinically significant diseases such as gastric cancer or peptic ulcer. If all the studies to data are put together, *cagA*, *vacA*, and *oipA* are the factors associated with gastric cancer, and *dupA* can be considered to be an important virulent factor for duodenal ulcer, but because different studies have showed different results and particularly results were different by geographic region, more research is needed.

The biggest reasons why there is a limit in clarifying the relationship between *H. pylori* and diseases only by the virulence factors are as follows: *H. pylori* is composed of nearly 1,600 genes; thus, it is possible that a pathogenic gene which has not yet been identified plays a critical role. Moreover, pathogenesis of gastroduodenal diseases including gastric cancer involves several factors such as diet, environmental changes caused by human movement, host factors, or duration of *H. pylori* infection, which should be taken into account along with the virulent factors in the pathogenesis of diseases. In addition, it is needed to better understand and interpret the research methods and terminology when the study results associated with *H. pylori* virulence factors are comprehended and analyzed. Tests for virulence factors are to be made through the use of the strains isolated from the host in vitro or an animal model; however, the actual results caused by these virulence factors in the human body can be different from the results obtained in the laboratory.

Therefore, it should be kept in mind that actual working mechanisms of the *H. pylori* virulence factors in the host can be much more complicated and divers than we imagine.

References

1. Moodley Y, Linz B, Bond RP, Nieuwoudt M, Soodyall H, Schlebusch CM, et al. Age of the association between *Helicobacter pylori* and man. PLoS Pathog. 2012;8:e1002693.
2. Peek Jr RM, Blaser MJ. *Helicobacter pylori* and gastrointestinal tract adenocarcinomas. Nat Rev Cancer. 2002;2:28–37.
3. Linz B, Balloux F, Moodley Y, Manica A, Liu H, Roumagnac P, et al. An African origin for the intimate association between humans and *Helicobacter pylori*. Nature. 2007;445:915–8.
4. Devi SM, Ahmed I, Khan AA, Rahman SA, Alvi A, Sechi LA, et al. Genomes of *Helicobacter pylori* from native Peruvians suggest admixture of ancestral and modern lineages and reveal a western type cag-pathogenicity island. BMC Genomics. 2006;7:191.
5. Yamaoka Y. *Helicobacter pylori* typing as a tool for tracking human migration. Clin Microbiol Infect. 2009;15:829–34.
6. Ching CK, Wong BC, Kwok E, Ong L, Covacci A, Lam SK. Prevalence of *CagA*-bearing *Helicobacter pylori* strains detected by the anti-*CagA* assay in patients with peptic ulcer disease and in controls. Am J Gastroenterol. 1996;91:946–53.
7. Weel JF, van der Hulst RW, Gerrits Y, Roorda P, Feller M, Dankert J, et al. The interrelationship between cytotoxin-associated gene A, vacuolating cytotoxin, and *Helicobacter pylori*-related diseases. J Infect Dis. 1996;173:1171–5.
8. Yamaoka Y, Kodama T, Gutierrez O, Kim JG, Kashima K, Graham DY. Relationship between *Helicobacter pylori iceA*, *cagA*, *and vacA* status and clinical outcome: studies in four different countries. J Clin Microbiol. 1999;37:2274–9.
9. Yamaoka Y. Mechanisms of disease: *Helicobacter pylori* virulence factors. Nat Rev Gastroenterol Hepatol. 2010;7:629–41.
10. Crabtree JE, Taylor JD, Wyatt JI, Heatley RV, Shallcross TM, Tompkins DS, et al. Mucosal IgA recognition of *Helicobacter pylori* 120 kDa protein, peptic ulceration and gastric pathology. Lancet. 1991;338:332–5.
11. Shiota S, Suzuki R, Yamaoka Y. The significance of virulence factors in *Helicobacter pylori*. J Dig Dis. 2013;14:341–9.
12. Kim JY, Kim N, Nam RH, Suh JH, Chang H, Lee JW, et al. Association of polymorphisms in virulence factor of *Helicobacter pylori* and gastroduodenal diseases in South Korea. J Gastroenterol Hepatol. 2014;29:984–91.
13. Kim YS, Kim N, Kim JM, Kim MS, Park JH, Lee MK, et al. *Helicobacter pylori* genotyping findings from multiple cultured isolates and mucosal biopsy specimens: strain diversities of *Helicobacter pylori* isolates in individual hosts. Eur J Gastroenterol Hepatol. 2009;21:522–8.
14. Allison CC, Ferrero RL. Role of virulence factors and host cell signaling in the recognition of *Helicobacter pylori* and the generation of immune responses. Future Microbiol. 2010;5:1233–55.
15. Censini S, Lange C, Xiang Z, Crabtree JE, Ghiara P, Borodovsky M, et al. *Cag*, a pathogenicity island of *Helicobacter pylori*, encodes type 1-specific an disease-associated virulence factors. Proc Natl Acad Sci U S A. 1996;93:14648–53.
16. Crabtree JE, Covacci A, Farmery SM, Xiang Z, Tompkins DS, Perry S, et al. *Helicobacter pylori* induced interleukin-8 expression in gastric epithelial-cells is associated with *CagA* positive phenotype. J Clin Pathol. 1995;48:41–5.
17. Sharma SA, Tummuru MKR, Miller GG, Blaser MJ. Interleukin-8 response of gastric epithelial cell lines to *Helicobacter pylori* stimulation in vitro. Infect Immun. 1995;63:1681–7.
18. Cover TL, Glupczynski Y, Lage AP, Burette A, Tummuru MK, Perez-Perez GI, et al. Serologic detection of infection with *CagA*$^+$ *Helicobacter pylori* strains. J Clin Microbiol. 1995;33:1496–500.
19. Crabtree JE, Wyatt JI, Sobala GM, Miller G, Tompkins DS, Primrose JN, et al. Systemic and mucosal response to *Helicobacter pylori* in gastric mucosa. Gut. 1993;34:1339–43.
20. Kuipers EJ, Pérez-Pérez GI, Meuwissen SG, Blaser MJ. *Helicobacter pylori* and atrophic gastritis: importance of the *CagA* status. J Natl Cancer Ins. 1995;87:1777–80.
21. Hatakeyama M. Oncogenic mechanisms of the *Helicobacter pylori CagA* protein. Nat Rev Cancer. 2004;4:688–94.
22. Kersulyte D, Mukhopadhyay AK, Velapatiño B, Su W, Pan Z, Garcia C, et al. Differences in genotypes of *Helicobacter pylori* from different human populations. J Bacteriol. 2000;182:3210–8.
23. Holcombe C. *Helicobacter pylori*: the African enigma. Gut. 1992;33:429–31.
24. Shiota S, Matsunari O, Watada M, Yamaoka Y. Serum *Helicobacter pylori CagA* antibody as a biomarker for gastric cancer in east-Asian countries. Future Microbiol. 2010;5:1885–93.
25. Asaka M, Kimura T, Kato M, Kudo M, Miki K, Ogoshi K, et al. Possible role of *Helicobacter pylori* infection in early gastric cancer development. Cancer. 1994;73:2691–4.
26. Van Doorn LJ, Figueiredo C, Mégraud F, Pena S, Midolo P, Queiroz DM, et al. Geographic distribution of *vacA* allelic types of *Helicobacter pylori*. Gastroenterology. 1999;116:823–30.
27. Atherton JC, Peek Jr RM, Tham KT, Cover TL, Blaser MJ. Clinical and pathological importance of heterogeneity in *vacA*, the vacuolating cytotoxin gene of

Helicobacter pylori. Gastroenterology. 1997;112: 92–9.
28. van Doorn LJ, Figueriedo C, Carneiro F, Sanna R, Pena S, Midolo P, et al. Worldwide heterogeneity of the *Helicobacter pylori vacA* gene. Gut. 1997;41 suppl 1:A34.
29. Atherton JC. The clinical relevance of strain types of *Helicobacter pylori*. Gut. 1997;40:701–3.
30. Palframan SL, Kwok T, Gabriel K. Vacuolating cytotoxin A (*VacA*), a key toxin for *Helicobacter pylori* pathogenesis. Front Cell Infect Microbiol. 2012;12:92.
31. Park SK, Kim GH, Jeong EJ, Bae YM, Heo J, Chu HJ, et al. Clinical relevance between the *cagA*, *vacA*, and *iceA* status of *Helicobacter pylori* and Benign gastroduodenal diseases. Korean J Gastroenterol. 2002;40: 23–31.
32. Cover TL, Blaser MJ. Purification and characterization of the vacuolating toxin from *Helicobacter pylori*. J Biol Chem. 1992;267:10570–5.
33. Nogueira C, Figueiredo C, Carneiro F, Gomes AT, Barreira R, Figueira P, et al. *Helicobacter pylori* genotypes may determine gastric histopathology. Am J Pathol. 2001;158:647–54.
34. Matsunari O, Shiota S, Suzuki R, Watada M, Kinjo N, Murakami K, et al. Association between *Helicobacter pylori* virulence factors and gastroduodenal diseases in Okinawa. Japan J Clin Microbiol. 2012;50: 876–83.
35. Nguyen TL, Uchida T, Tsukamoto Y, Trinh DT, Ta L, Mai BH, et al. *Helicobacter pylori* infection and gastroduodenal diseases in Vietnam: a cross-sectional, hospital-based study. BMC Gastroenterol. 2010; 10:114.
36. Sahara S, Sugimoto M, Vilaichone RK, Mahachai V, Miyajima H, Furuta T, et al. Role of *Helicobacter pylori cagA* EPIYA motif and *vacA* genotypes for the development of gastrointestinal diseases in Southeast Asian countries: a meta-analysis. BMC Infect Dis. 2012;12:223.
37. Uchida T, Nguyen LT, Takayama A, Okimoto T, Kodama M, Murakami K, et al. Analysis of virulence factors of *Helicobacter pylori* isolated from a Vietnamese population. BMC Microbiol. 2009;9:175.
38. Ogiwara H, Sugimoto M, Ohno T, Vilaichone RK, Mahachai V, Graham DY, et al. Role of deletion located between the intermediate and middle regions of the *Helicobacter pylori vacA* gene in cases of gastroduodenal diseases. J Clin Microbiol. 2009;47: 3493–500.
39. Rhead JL, Letley DP, Mohammadi M, Hussein N, Mohagheghi MA, Eshagh Hosseini M, et al. A new *Helicobacter pylori* vacuolating cytotoxin determinant, the intermediate region, is associated with gastric cancer. Gastroenterology. 2007;133:926–36.
40. Ogiwara H, Graham DY, Yamaoka Y. *VacA* i-region subtyping. Gastroenterology. 2008;134:1267. author reply 1268.
41. van Doorn LJ, Figueiredo C, Sanna R, Plaisier A, Schneeberger P, de Boer W, et al. Clinical relevance of the *cagA*, *vacA*, *and iceA* status of *Helicobacter pylori*. Gastroenterology. 1998;115:58–66.
42. Shiota S, Watada M, Matsunari O, Iwatani S, Suzuki R, Yamaoka Y. *Helicobacter pylori iceA*, clinical outcomes, and correlation with *cagA*: a meta-analysis. PLoS ONE. 2012;7, e30354.
43. Yamaoka Y, Ojo O, Fujimoto S, Odenbreit S, Haas R, Gutierrez O, et al. *Helicobacter pylori* outer membrane proteins and gastroduodenal disease. Gut. 2006;55:775–81.
44. Yamaoka Y, Kwon DH, Graham DY. A M(r) 34,000 proinflammatory outer membrane protein (*oipA*) of *Helicobacter pylori*. Proc Natl Acad Sci U S A. 2000;97:7533–8.
45. Yamaoka Y, Kikuchi S, el-Zimaity HM, Gutierrez O, Osato MS, Graham DY. Importance of *Helicobacter pylori* oipA in clinical presentation, gastric inflammation, and mucosal interleukin 8 production. Gastroenterology. 2002;123:414–24.
46. Chen J, Lin L, Li N, She F. Enhancement of *Helicobacter pylori* outer inflammatory protein DNA vaccine efficacy by co-delivery of interleukin-2 and B subunit heat-labile toxin gene encoded plasmids. Microbiol Immunol. 2012;56:85–92.
47. Lu H, Hsu PI, Graham DY, Yamaoka Y. Duodenal ulcer promoting gene of *Helicobacter pylori*. Gastroenterology. 2005;128:833–48.
48. Schmidt HM, Andres S, Kaakoush NO, Engstrand L, Eriksson L, Goh KL, et al. The prevalence of the duodenal ulcer promoting gene (*dupA*) in *Helicobacter pylori* isolates varies by ethnic group and is not universally associated with disease development: a case-control study. Gut Pathog. 2009;1:5.
49. Hussein NR, Argent RH, Marx CK, Patel SR, Robinson K, Atherton JC. *Helicobacter pylori dupA* is polymorphic, and its active form induces proinflammatory cytokine secretion by mononuclear cells. J Infect Dis. 2010;202:261–9.
50. Queiroz DM, Rocha GA, Rocha AM, Moura SB, Saraiva IE, Gomes LI, et al. *DupA* polymorphisms and risk of *Helicobacter pylori*-associated diseases. Int J Med Microbiol. 2011;301:225–8.
51. Gerhard M, Lehn N, Neumayer N, Borén T, Rad R, Schepp W, et al. Clinical relevance of the *Helicobacter pylori* gene for blood-group antigen-binding adhesin. Proc Natl Acad Sci U S A. 1999;96:12778–83.
52. Ilver D, Arnqvist A, Ogren J, Frick IM, Kersulyte D, Incecik ET, et al. *Helicobacter pylori* adhesin binding fucosylated histo-blood group antigens revealed by retagging. Science. 1998;279:373–7.
53. Kim YS, Woo CW, Lee YM, Son BR, Kim JW, Chae HB, et al. Genotyping *CagA*, *VacA* Subtype, *IceA*1, and *BabA* of *Helicobacter pylori* isolates from Korean patients, and their association with gastroduodenal diseases. J Korean Med Sci. 2001;16:579–84.
54. Fujimoto S, Ojo OO, Arnqvist A, Wu JY, Odenbreit S, Haas R, et al. *Helicobacter pylori babA* expression, gastric mucosal injury, and clinical outcome. Clin Gastroenterol Hepatol. 2007;51:49–58.