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The significance of virulence factors in *Helicobacter pylori*

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Abstract

Helicobacter pylori (*H. pylori*) infection is linked to various gastroduodenal diseases; however, only a small fraction of these patients develop associated diseases. Despite the high prevalence of *H. pylori* infection in Africa and South Asia, the incidence of gastric cancer in these areas is much lower than those in other countries. The incidence of gastric cancer tends to decrease from north to south in East Asia. Such geographic differences in the pathology can be explained, at least in part, by the presence of different types of *H. pylori* virulence factors in addition to the host and environmental factors. Virulence factors of *H. pylori*, such as *cagA*, *vacA*, *dupA*, *iceA*, *oipA* and *babA*, have been demonstrated to be predictors of severe clinical outcomes. Interestingly, meta-analysis showed that CagA seropositivity was associated with gastric cancer compared with gastritis even in East Asian countries where almost of the strains possessing *cagA*. Meta-analysis also confirmed the significance of *vacA*, *dupA* and *iceA*. However, there remains the possibility that additional important pathogenic genes can be existed because *H. pylori* consists of approximately 1 600 genes. Despite advances in our understanding of the development of *H. pylori*-related diseases, further work is required to clarify the roles of *H. pylori* virulence factors.

Keywords

Helicobacter pylori; virulence factors; stomach neoplasms

Introduction

Helicobacter pylori (*H. pylori*) infection is now regarded as a high risk factor for severe gastritis-associated diseases, including peptic ulcer (PU) and gastric cancer (GC).¹ The infection persists for a long period of time in most infected patients, but only a small proportion of these patients develop associated diseases.² The transmission mechanism of *H. pylori* has not been fully clarified, but human-to-human spread via the oral-oral or fecal-oral routes is thought to be the most plausible.³ Studies have shown the presence of *H. pylori* in drinking water as well as the influence of poor living conditions and sanitation on *H. pylori* infection, which support the oral-fecal spread route.

In addition to the host factor and diet, virulence factors of *H. pylori* such as CagA, VacA, DupA, IceA, OipA, and BabA have been revealed to be the predictors of gastric atrophy, intestinal metaplasia and severe clinical outcomes.⁴ Although gastric cancer and duodenal

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ulcer are at the opposite ends of the disease spectrum, *H. pylori* infection promotes the development of both diseases, and the most putative *H. pylori* virulence factors have been found to be associated with an increased risk of these diseases. However, these virulence factors cannot be used as disease-specific markers in East Asian countries such as Japan because the majority of *H. pylori* strains possess these virulence factors in these regions.⁴ This paper describes the current knowledge about the pathogenesis of *H. pylori*-related diseases from the aspect of *H. pylori* virulence factors.

Cytotoxin-associated gene A (CagA)

CagA is the most studied virulence factor of *H. pylori*, which is located at one end of the *cag* pathogenicity island (PAI), an approximately 40-kb-region which is thought to have been incorporated into the *H. pylori* genome by horizontal transfer from an unknown source.⁵ The *cag* PAI encodes a type IV secretion system, through which CagA is delivered into host cells.^{6,7} Although *cagA*-positive strains are reported to be associated with severe clinical outcomes, particularly in Western countries, it is difficult to determine the importance of *cagA* in clinical outcomes in East Asian countries because nearly all *H. pylori* strains possess *cagA*.⁴ However, those infected with *H. pylori* containing *cagA* do not always induce serum CagA antibody. Although most Japanese *H. pylori* strains possess *cagA*, CagA seropositivity in individuals with normal gastric appearances and/or gastritis ranges from 53.7% to 81.1% in Japan.^{8,9} The association between serum anti-CagA antibody and gastric cancer in East Asian countries (Japan, Korea and China) was clarified in our meta-analysis of case-control studies with age- and gender-matched controls,¹⁰ in which 10 studies with a total of 4 325 patients were identified. CagA seropositivity was associated with gastric cancer compared with gastritis even in East Asian countries, although the odds ratio (OR) in East Asian countries was smaller than that of the meta-analysis included Western countries. Furthermore, even in the *H. pylori*-negative population, the presence of serum CagA antibody increases the risk of gastric cancer. This evidence confirms that CagA antibody can potentially remain positive for a longer period of time than the anti-*H. pylori* antibody. Accordingly, serum CagA antibody was related to gastric cancer in both *H. pylori*-positive and -negative populations in East Asian countries.

CagA can be divided to two types, East-Asian-type and Western-type, according to the repeat sequences of the 3' region of *cagA*.⁴ The repeat regions contain the Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs. Sequences have been annotated according to segments (20–50 amino acids) flanking the EPIYA motifs (*i.e.*, segments EPIYA-A, -B, -C or -D).⁴ East-Asian-type CagA, containing EPIYA-D segments, exhibits a stronger binding affinity for Src homology 2 domain of Src homology 2-containing protein-tyrosine phosphatase (SHP-2) and a greater ability to induce morphological changes in epithelial cells than Western-type CagA, which contains EPIYA-C segments.¹¹ 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.¹² Nearly all the *H. pylori* strains in Japan possess East-Asian-*cagA*;^{13,14} therefore, it is difficult to explain the outcomes based on the *cagA* genotype in Japan. However, interestingly, we found a relatively low rate (70.3%) of East-Asian-type *cagA* strains in Okinawa compared with other parts of Japan.¹⁵ Okinawa consists of small islands (2 276 km²) in Southwestern Japan, with the lowest incidence of gastric cancer in Japan. In Okinawa, Western-type *cagA* and *cagA*-negative strains were found to be 16.0% and 13.6%, respectively. Furthermore, we found that East-Asian-type *cagA* was significantly associated with severe gastroduodenal diseases. Okinawa remained under the rule of the United States after the World War II (WWII) until 1972. Thus, Western-type *cagA* and *cagA*-negative strains may have spread horizontally from the United States populations living in Okinawa. However, Western-type *cagA* sequence in Okinawa was different from that of the typical Western-type *cagA* present in Western countries,^{15,16}

which has been named as J-Western-type *cagA*. Therefore, it is probable that these strains were not derived from the United States populations after WWII. We also found specific EPIYA-like motifs (ESIYA and ESIYT) in the C-terminal region of CagA in strains isolated in Okinawa.^{15,17} Interestingly, ESIYT sequences were also found in Amerindian strains.¹⁸ Additionally, the CagA multimerization (CM) sequence, which has been reported to promote proliferation and inflammation,⁴ was also similar to that in the strains of Amerindian and Okinawa^{15,18}. These findings suggest that these strains share the same ancestry.

Amerindian CagA was reported in populations of the Peruvian Amazon.¹⁹ Amerindian CagA can be divided into 2 types, *i.e.*, AM-I and AM-II.¹⁸ Amerindian CagAs have altered or degenerate “EPIYA-B” motifs: ESIYT and GSIYD in AM-I and AM-II CagAs, respectively. The N-terminus of AM-II CagA lacks two large internal segments containing 180 amino acids. Interestingly, AM-II CagA has attenuated abilities to stimulate gastric epithelial proliferation and inflammation during infection compared with those of the Western-type CagA or East-Asian-type CagA. The Amerindian CagA CM segment plays an important role in those findings.¹⁸

We also examined the *cagA* sequence data deposited in the GenBank and found that J-Western-type *cagA* possessed a 12-bp insertion located in the 5' region of *cagA* sequence compared to the original Western-type *cagA* strains.²⁰ We found that 12-bp insertion was more prevalent in strains with *vacA* m2 genotypes. Duncan *et al.*²¹ stated that J-Western-type *cagA* strains were found not only in Okinawa but also in other parts of the world. Therefore, we examined the prevalence of 12-bp insertion in the strains from Okinawa and the United States,²² and found that even in cases of Western-type *cagA/vacA* s1m2 strains, the prevalence of 12-bp insertion was significantly higher in strains from Okinawa than that in strains from the United States (83.9% vs 37.5%). Intriguingly, the 12-bp insertion was found in 50.0% Western-type *cagA/vacA* s1m1 strains in Okinawa, which was higher than that of Western-type-*cagA/vacA* s1m2 strains in the United States (37.5%). These findings suggest that 12-bp insertion was more specific in strains in Okinawa irrespective of the status of *vacA*, although it was more common in strains with *vacA* m2 genotype. Phylogenetic tree showed that all strains with 12-bp insertion were in the J-Western-type *cagA* cluster, which was distinct from that of East-Asian-type *cagA* and typical Western-type *cagA* in Western countries. Interestingly, strains from Okinawa with 12-bp insertion formed one cluster different from the United States strains with 12-bp insertion. We then designed the primer pair which can detect the 12-bp insertion only by polymerase chain reaction (PCR). The prevalence of strains with 12-bp insertion was examined in 336 strains isolated from Okinawa by PCR, and the 12-bp insertion was found in 23.7%, 14.3%, 4.2% and 4.0% of strains with duodenal ulcer, gastritis, gastric cancer and gastric ulcer, respectively. Low biological activities of *cagA* including 12-bp insertion may be inversely associated with gastric ulcer. Further *in vitro* studies are needed to clarify the role of the J-Western-type *cagA* sequences or 12-bp insertion.

Vacuolating cytotoxin (VacA)

VacA is the second most extensively studied *H. pylori* virulence factor. Unlike *cagA*, virtually all the *H. pylori* strains have a functional *vacA*, which encodes a vacuolating cytotoxin. VacA can induce vacuolation and multiple cellular activities, including membrane-channel formation, cytochrome *c* release from mitochondria which leads to apoptosis, and binding to cell membrane receptors followed by the initiation of a proinflammatory response.^{2,23,24} The differences in *vacA* structure at the s region (s1 and s2) and the m region (m1 and m2) led to variations in the vacuolating activity of different *H. pylori* strains.^{25–27} *In vitro* experiment showed that s1m1 strains are the most cytotoxic, followed by s1m2 strains, whereas s2m2 strains have no cytotoxic activity; s2m1 strains are

rare.²⁵ In agreement with *in vitro* data, many studies in Western countries^{25,26,28,29} showed that individuals infected with *vacA* s1 or m1 *H. pylori* strains have an increased risk of peptic ulcer or gastric cancer compared with those with s2 or m2 strains. The m1 strains are common in areas of Northeast Asia, *e.g.*, Japan and South Korea, whereas m2 strains are predominant in Southeast Asian regions such as Taiwan (China) and Vietnam.^{14,30} As the incidence of gastric cancer is higher in Northeast Asia than in Southeast Asia, the m region may play a role in the regional difference in the disease pattern. In Vietnam, the *vacA* m2 strain is more prevalent in Ho Chi Minh City (south of Vietnam) than in Hanoi (north of Vietnam), and the incidence of peptic ulcer is lower in Ho Chi Minh City than in Hanoi.³⁰ A recent meta-analysis³¹ showed that *vacA* m1 was significantly associated with peptic ulcer in Southeast Asian countries including Vietnam, Thailand, Malaysia and Singapore.

Although most of the *H. pylori* strains possess the *vacA* s1m1 genotype in the mainland of Japan,³² the high prevalence of other *vacA* genotype was also found in Okinawa compared with other part of Japan¹⁵. Furthermore, the *vacA* s1m1 genotype was significantly prevalent in strains from gastric ulcer (79.2%) and gastric cancer (87.5%) than those from gastritis (59.2%); whereas the *vacA* s1m2 genotype was more common in strains from gastritis than those from gastric ulcer (17.3% *vs.* 7.9%, *P* = 0.04). The prevalence of the *vacA* s2m2 genotype was significantly higher in strains from gastritis than those from gastric ulcer, duodenal ulcer and gastric cancer (22.4% *vs.* 11.9%, 10.5% and 4.2%, *P* = 0.04, 0.01 and 0.04, respectively). Therefore, even in areas of East Asia where there are many cases with non-s1/m1 strains, both s and m genotypes of *vacA* can be used as markers for *H. pylori*-related diseases.

A third disease-related region of *vacA* named the intermediate (i) region was identified between the s region and the m region.³³ Although s1m1 and s2m2 strains were exclusively classified as i1 and i2, respectively, s1m2 strains were classified as either type i1 or i2, and i1 strains were shown to be more pathogenic. A recent study³⁴ showed that the amino acid at position 196 of the i region was significantly associated with severe outcomes. In addition, the authors also found that the amino acid at position 231 of the i region was linked to severe disease pathologies only within strains that carry a non-EPIYA-ABD CagA allele. The fourth and possibly disease-related region is the deletion (d) region located between the i region and the m region.³² The d region can also be subdivided into d1 (no deletion) and d2 (a 69-to 81-bp deletion). The role of the status of the i region or d region is still controversial.^{32,33,35} At present, therefore, the type of the m region may be the best overall marker for gastric cancer, especially in East Asia.

Duodenal ulcer promoting gene (*dupA*)

dupA is the first genetic factor of *H. pylori* detected to be associated with a differential susceptibility to duodenal ulcer and gastric cancer, and thus, it can be considered as a disease-specific virulence marker even in East Asian countries such as Japan and South Korea.³⁶ The prevalence of *dupA* was significantly higher in strains from patients with duodenal ulcer but lower in those with gastric cancer, regardless of the nationality (Japan, Korea and Colombia) (42% *vs.* 9% on average).³⁶ In a review, the prevalence of *dupA* in patients with gastritis worldwide was stated as 44.88% and differed significantly among nationality and ethnicity.³⁷ An association between *dupA* status and disease development can be observed in mostly Asian countries. A meta-analysis³⁸ including 17 studies with a total of 2 466 patients was performed to confirm the relationship between the *dupA* and clinical outcomes, and we revealed that the overall prevalence of the *dupA* gene was 31.0% (496/1 600) in Asian countries and 64.1% (526/820) in Western countries. The infection with *dupA*-positive *H. pylori* increased the risk for duodenal ulcer (OR 1.41, 95% confidence interval [CI] 1.12–1.76), particularly in Asian countries (OR 1.57, 95% CI 1.19–

2.06) but not in Western countries (OR 1.09, 95% CI 0.73–1.62). There was no association between the presence of *dupA* and gastric cancer and gastric ulcer. Additionally, we also reported that the presence of *dupA* was significantly associated with eradication failure.³⁹

Recent full-sequenced data of *H. pylori* revealed that the length of the *dupA* open reading frame (ORF) depends on the strains, as that of strains Shi470 and G27 have approximately 600 bp longer ORF (approximately 2 500 bp) than that of strain J99 due to the additional 5' region of *dupA*.^{19,40} This suggests that *dupA* has two genotypes according to the sequence of the putative 5' region (presence; long-type and absence; short-type); however, most previous studies did not take the additional 5' region into account. In addition, several studies indicated that some strains have a presence of mutations in *dupA*, which created a premature stop codon.^{41–43} Gomes *et al.*⁴¹ reported that there was a frameshift mutation in 16.28% (14/86) of *dupA* which created a premature stop codon which might have considerable effects on protein expression or function. It is clear that strains with these mutated sequences would not produce intact DupA protein. Intriguingly, the presence of *dupA* without stop codon was more frequently found in strains from patients with duodenal ulcer than in strain from those with gastritis and gastric cancer in their following study.⁴³ Hussein *et al.*⁴² classified a *dupA* allele with 1 884 bp (short-type) as *dupA1* and a truncated version by the mutations as *dupA2*. We found that the prevalence of intact long-type *dupA* was 26.3% (84/319) in the strains isolated from Okinawa;⁴⁴ using PCR-based sequencing, point mutation leading to stop codon was only found in 6 out of 84 (7.1%) long-type *dupA* strains. Interestingly, intact long-type *dupA* without frameshift mutation, but not short-type *dupA*, was significantly related with gastric ulcer and gastric cancer than gastritis. Even after adjusted by age, gender and *cagA*, the presence of intact long-type *dupA* was significantly associated with gastric ulcer and gastric cancer compared with gastritis (OR 3.35, 95% CI 1.55–7.24 and OR 4.14, 95% CI 1.23–13.94, respectively), suggesting that intact long-type *dupA* might produce a functional DupA protein and intact long-type *dupA* can be a real virulence marker for severe outcomes. In contrast, non-intact long-type *dupA* may not produce functional DupA protein. This might be the reason why the importance of *dupA* on clinical outcomes was conflicting in different studies. The previous information gained from PCR-based methods without taking long-type *dupA* into account must be interpreted with caution. It will be better to detect intact *dupA* by measuring intact DupA protein using immunoblotting techniques, which has not been reported previously.

In addition, the *vir* genes exist before and after the region of the *dupA* locus.⁴⁵ In strain Shi470, for example, *virB2*, *virB3*, *virB4* (*dupA*), *virB8*, *virB9*, *virB10*, *virB11*, *virD4* and *virD2* were detected.⁴⁰ These are structurally similar to the T4SS called *cag* PAI and ComB, and are thought to be the third T4SS. These observations suggest that only strains that are intact *dupA*-positive and form a novel T4SS might be involved in gastroduodenal diseases. We recently reported that complete *dupA* cluster (possessing *dupA* and all adjacent *vir* genes) was significantly associated with duodenal ulcer in the United States.⁴⁶ Complete *dupA* cluster with intact long-type *dupA* might be true virulence factors. Further studies from other geographic area will help to elucidate of the importance of intact long-type *dupA* and complete *dupA* cluster.

Induced by contact with epithelium (*IceA*)

An initial series of studies showed that *iceA* has 2 main allelic variants, *iceA1* and *iceA2*.^{47,48} *iceA1* demonstrated sequence homology with a gene from *Neisseria lactamica*, *nlaIIIR*, which encodes a CTAG-specific restriction endonuclease.⁴⁸ On the other hand, *iceA2* has no homology to known genes and the function of the *iceA2* product remains unclear. van Doorn *et al.*⁴⁷ reported that the *iceA* allelic type was independent of the *cagA* and *vacA* status, and there was a significant association between the presence of the *iceA1*

allele and peptic ulcer. The expression of *iceA1* was upregulated on contact between *H. pylori* and human epithelial cells, and the *iceA1* genotype was linked with enhanced mucosal interleukin (IL)-8 expression and acute antral inflammation.^{48,49} However, the role of *iceA* was subsequently controversial since several studies were unable to reproduce the observation in other populations including Japanese.^{50–54} We performed a meta-analysis including 50 studies with a total of 5 357 patients to confirm the relationship between the *iceA* allelic type and clinical outcomes,⁵⁵ and revealed that the overall prevalence of *iceA1* was significant higher 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*.

Outer inflammatory protein (OipA)

OipA is one of the outer membrane proteins, functions in adhesion.⁵⁶ Its functional status is regulated by slipped-strand mispairing that is based on the number of CT dinucleotide repeats in the 5' region of the genes (switch “on” = functional and switch “off” = nonfunctional).⁵⁶ OipA was initially identified as a proinflammatory response-inducing protein based on the fact that *oipA* isogenic mutants reduced the induction of IL-8 from gastric epithelial cell lines.⁵⁹ Several studies revealed that OipA can induce inflammation and actin dynamics through the phosphorylation of multiple signaling pathways that usually interact with *cag* PAI (CagA)-related pathways.^{57–62} *H. pylori*-related inflammatory signaling related to gastric carcinogenesis is regulated by the activation of the phosphoinositide-3 kinase (PI3K)/Akt signaling pathway.⁶² OipA-regulated IL-8 release through PI3K/Akt is recently reported to be dependent on forkhead transcription factors of class O (FoxO) 1/3a inactivation.⁶³

We previously examined the expression status or presence of multiple virulence factors (*cag* PAI, *vacA*, *iceA*, *oipA*, and *babA*) in different clinical outcomes,⁶⁴ in which *H. pylori* isolates were obtained from 247 patients in 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 study⁶⁵ based on a non-overlapping cohort of 200 patients who were examined for 4 outer-membrane proteins, OipA, BabA, BabB and 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. The importance of *oipA* was also confirmed by infection model. A challenge of human volunteers with *oipA* “on”/whole *cag* PAI-negative clinical isolate (Baylor challenge strain 100; ATCC BAA-945) caused severe inflammation.⁶⁶ In addition, the *oipA* mutants did not induce gastric mucosal inflammation in mice infected for 12 weeks, whereas *cagE* mutants did induce mucosal inflammation, although the levels were lower than those in the parental strains (*cagE* is an important component of *cag* PAI).⁶⁷ Moreover, *oipA* mutant induced a milder inflammation compared with wild type in Mongolian gerbil model.^{68,69} IL-1 β , IL-17, IL-18, and TNF- α mRNA level was significantly lower in gerbils infected with *oipA* mutant strains.⁶⁸

Abovementioned findings suggest that the presence of OipA is a better marker of severe clinical outcomes than *cag* PAI. However, it is important to note that clinical isolates containing *cag* PAI typically have an *oipA* “on” status^{64,65} despite the *oipA* gene being physically located approximately 100 kb from the *cag* PAI on the *H. pylori* chromosome. *oipA* status is also linked to the *vacA* s region type, and it is further linked closely to the presence of *babA* gene.⁷⁰ These linkages of the virulence factors may have a certain biological significance, and they may somehow interact with each other; therefore, it might be better to hypothesize that these factors interact synergistically with each other and induce serious diseases, rather than to discuss which of these factors is the most virulent.⁴ It is interesting to note that most East Asian strains are classified as *oipA* status “on”, and the CT-repeat sequences in the signal region of *oipA* were half-collapsed (e.g., CTGCCTTTCT repeat sequence), suggesting that this may result from an intentional change in the status in the course of evolution of the bacteria in order to prevent the switch from being turned “off” easily.⁵⁶ Interestingly, a recent study⁷¹ using whole genome sequences revealed that two *oipA* genes were found in the different loci in the strains mainly from East Asian countries but not Western countries. This increase of the secondary locus can be explained by a novel DNA duplication mechanism associated with inversion.⁷²

Blood group antigen binding adhesin (BabA)

Interactions between BabA and Lewis b (Le^b)-related antigens are the best characterized adhesin-receptor interactions in *H. pylori*.⁷³ The *babA* gene was initially cloned from strain CCUG17875, which contains a silent *babA1* gene and an expressed *babA2* gene. The sequence of these two genes differs only by the presence of a 10 bp deletion in the signal peptide sequence of *babA1* which eliminates its translational initiation codon. However, we found that all 80 strains from a panel of Western and East Asian isolates containing an intact ATG start codon in the *babA* gene,⁷⁰ and another groups also reported the absence of the *babA1* type deletion⁷⁴. Overall, the absence of a translational initiation codon, as described for *babA1* from CCUG17875 appears to be very rare. Therefore, it seems to be better to discuss the presence of *babA* but not *babA2*. Importantly, the BabA protein levels do not always match the presence of the *babA* gene.^{70,75} Several mechanisms for the regulation of BabA expression are predicted, including at both transcriptional and translational levels.⁷³ Altogether, the information gained from currently used PCR-based methods must be interpreted with caution. In addition, we propose that researchers should not use current PCR-based methods in future studies. Interestingly, the genomic locations of *babA* and *babB* genes are completely different among strains (Fig.1). For simplicity, the chromosomal locations downstream of *hypD* and *s18* will be referred to as locus A and locus B, respectively.⁷⁴ *H. pylori* 26695 contains *babC* gene whose sequence is closely related to *babA* and *babB*.⁷⁴ The chromosomal locations of *babC* in strain 26695 will be referred to as locus C. The *babC* gene does not exist in strain J99. Strain HPAG-1 have *babC* gene at locus B and *babB* gene at locus C. The locus C was mainly empty in the strains isolated from East Asian countries as well as from Western African countries.⁷¹ Strain B8 and SouthAfrica7 have *babA* gene at both locus A and locus B. For binding with Le^b, at least one copy of *babA* is necessary irrespective of Locus.⁷⁴ However, expression of a BabA protein and the Lewis ^b-binding phenotype were not dependent on the chromosomal locus of *babA*.⁷⁵

A recent research⁷⁶ reported that *H. pylori* infection introduced DNA double-strand breaks (DSBs) in primary and transformed murine and human epithelial and mesenchymal cells. The *babA* mutant was clearly less capable of inducing DSBs, suggesting that bacterial adhesion via *babA* is required to induce DSBs. On the other hand, *cagA* and *vacA* were dispensable for DSB induction. It is possible that DSB induction contributes to the genetic instability and frequent chromosomal aberrations that are the hallmarks of gastric cancer.

In conclusion, although we focused on six *H. pylori* virulence factors in this review, there remains the possibility that additional important pathogenic genes can be existed because *H. pylori* consists of approximately 1 600 genes. A recent study⁷⁷ showed that groupings by multi-locus sequence typing (MLST) using seven housekeeping genes were also associated with the prevalence of gastric cancer, although we reported a problem of this interpretation⁷⁸. Whole genome analyses are useful for the investigation of genetic factors that are related to differences in the virulence among strains. We believe that larger amounts of data will become available at lower costs in the near future, and other important novel virulence factors might be discovered.

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Figure 1.
bab gene pattern in the full-sequenced strains.