Evaluation of the Anti-East Asian CagA-Specific Antibody for CagA Phenotyping

Lam Tung Nguyen,1,2† Tomohisa Uchida,1,3† Akiko Kuroda,1,2† Yoshiyuki Tsukamoto,1 Tuan Dung Trinh,4 Long Ta,3 Hong Bang Mai,4 Dang Quy Dung Ho,5 Hoa Hai Hoang,5 Rathai-Korn Vilaiichone,5 Varocha Mahachai,7 Takeshi Matsuhashi,8 Yoko Kudo,2 Tadayoshi Okimoto,2 Masaaki Kodama,2 Kazunori Murakami,2 Toshio Fujioka,2 Yoshio Yamaoka,9 and Masatsugu Moriyama1*

Department of Molecular Pathology,1 Department of Gastroenterology,2 Department of Human Environmental and Social Medicine,3 and Department of Environmental and Preventive Medicine,9 Faculty of Medicine, Oita University, Oita, Japan; 108 Hospital, Hanoi, Vietnam4; Cho Ray Hospital, Ho Chi Minh, Vietnam5; Gastroenterology Unit, Department of Medicine, Thammasat University Hospital, Pathumthani, Thailand6; Gastroenterology Unit, Department of Medicine, Chulalongkorn University Hospital, Bangkok, Thailand7; and Department of Gastrointestinal Endoscopy, Tama-Nagayama Hospital, Nippon Medical School, Tokyo, Japan8

Received 12 May 2009/Returned for modification 13 August 2009/Accepted 15 September 2009

The determination of the cagA genotype is generally based on sequencing the variable 3’ region of the cagA gene. In a previous study, we successfully generated an anti-East Asian CagA-specific antibody (anti-EAS Ab) immunoreactive only with the East Asian CagA and not with the Western CagA. In a small number of Japanese patients, anti-EAS Ab appeared to be a useful tool for phenotyping CagA immunohistochemically. The present study was conducted to validate the anti-EAS Ab immunohistochemistry method in a larger number of patients from Vietnam and Thailand. A total of 385 Vietnamese and Thais were recruited. Helicobacter pylori status was determined by a combination of three methods, including culture, histology, and immunohistochemistry with anti-H. pylori antibody. The sensitivity, specificity, and accuracy of the anti-EAS Ab immunohistochemistry method for the diagnosis of CagA phenotype were calculated based on the results of the cagA sequencing as the gold standard. The sensitivity, specificity, and accuracy of our immunohistochemistry method were 96.7%, 97.9%, and 97.1%, respectively. Moreover, anti-EAS Ab was not cross-reactive with noninfected gastric mucosa. In conclusion, immunohistochemistry with anti-EAS Ab appears to be a good method for determination of CagA phenotype.

Helicobacter pylori is a spiral, gram-negative bacterium that chronically infects the human stomach and plays a causative role in the pathogenesis of gastritis, gastroduodenal ulcer, gastric cancer, and mucosa-associated lymphoid tissue lymphoma (19, 22). It is well recognized that H. pylori strains possessing an approximately 40-kb cluster of genes named the cag pathogenicity island (cag PAI) are more virulent and more strongly associated with severe clinical outcomes, such as peptic ulcer and gastric cancer (3, 5). The cag PAI consists of approximately 30 genes, several of which encode component proteins of the type IV secretion system and are essential for the induction of proinflammatory cytokines, such as interleukin-8, from gastric epithelial cells (3, 5). Moreover, the cag PAI contains cagA, the gene encoding the CagA protein, which is currently believed to have oncogenic potential (8, 9).

CagA has several repeated 5-amino-acid sequences (glutamic acid-proline-isoleucine-tyrosine-alanine), named EPIYA motifs, located at the C terminus of the protein. The EPIYA motif is divided into EPIYA-A, EPIYA-B, EPIYA-C, and EPIYA-D stretches, based on the amino acid sequences flanking each of them. According to the combinations of these EPIYA motifs, two major types of CagA protein have been observed. The Western CagA has EPIYA-A and EPIYA-B followed by one to five repeats of the EPIYA-C sequence. The East Asian CagA also has EPIYA-A and EPIYA-B, but the third motif is EPIYA-D (8, 9).

After H. pylori adheres to the gastric epithelium, CagA is translocated via the type IV secretion system into the host cell cytoplasm, where it undergoes phosphorylation by several Src family kinases at the tyrosine residues of all EPIYA motifs (10, 11). Phosphorylated CagA is then able to interact with and dysregulate several cellular transduction signal pathways. Notably, phosphorylated CagA binds to the Src homology 2 (SHP-2) domain containing tyrosine phosphatase via the EPIYA-C or EPIYA-D motif (10, 11, 14). Compared to the Western CagA, the East Asian type exhibits stronger binding affinity for SHP-2, due to a specific sequence overlying EPIYA-D (Y-A-T-I-D-F) that perfectly matches the consensus ligand binding motif for SHP-2 (Y-[V/I/A/S]-X-[L/I/V]-X-[F/W]) (10, 14). As a result, the East Asian CagA is considered to be more toxic than its Western homologue and more strongly associated with severe clinical outcomes, including gastric cancer (2, 8, 9). Therefore, the accurate diagnosis of the CagA phenotype would be useful, especially for molecular epidemiologic surveys.

† L.T.N., T.U., and A.K. contributed equally to this work.
* Corresponding author. Mailing address: Department of Molecular Pathology, Faculty of Medicine, Oita University, Yufu-city, Oita 879-5593, Japan. Phone: 81-97-586-5690. Fax: 81-97-586-5699. E-mail: mmoriyam@med.oita-u.ac.jp.
* Corresponding author. Mailing address: Department of Molecular Pathology, Faculty of Medicine, Oita University, Yufu-city, Oita 879-5593, Japan. Phone: 81-97-586-5690. Fax: 81-97-586-5699. E-mail: mmoriyam@med.oita-u.ac.jp.
In a previous study, we successfully generated an anti-East Asian CagA-specific antibody (anti-EAS Ab) which was immunoreactive only with the East Asian CagA and not with the Western CagA (23). We have also shown that anti-EAS Ab might be a useful tool for genotyping CagA immunohistochemically (23). However, in the previous study, with only Japanese patients, most strains analyzed possessed East Asian CagA; therefore, the accuracy of the test when using anti-EAS Ab was not confirmed. Due to the geographical genomic diversity of H. pylori, the value of our method in other populations needs to be validated. Therefore, the present study was carried out using a large number of patients from countries other than Japan, including Thailand, where the presence of Western CagA would be expected.

### Materials and Methods

**Patients.** We recruited a total of 385 patients (225 female, 160 male; mean age of 45.6 ± 13.4 years [mean ± standard deviation]) undergoing upper endoscopy at six hospitals in Thailand (Thammasat University Hospital, Chulalongkorn University Hospital, Rajavithi Hospital, and Bangkok Hospital) and Vietnam (Choray Hospital and 108 Hospital) (Table 1; Fig. 1). All participants provided written informed consent, and the study was approved by the local ethics committees. During each endoscopy session, five gastric biopsy specimens were obtained (two from the antrum, two from the corpus, and one from the upper part of the lesser curvature). Two specimens (one from the antrum and one from the corpus) were used for H. pylori culture, and the remaining three were used for histological examination. Clinical diagnosis was determined by endoscopic observation. Details of the disease distribution are shown in Table 1. Peptic ulcer was significantly more common in Vietnamese patients than in Thai patients \((P < 0.05)\).

**H. pylori isolation, subculture, and extraction of genomic DNA.** For H. pylori isolation, biopsy specimens were homogenized in sterile 0.9% saline solution and passed onto Mueller-Hinton II agar medium (Becton Dickinson, NJ) supplemented with 7% horse blood without antibiotics. The culture plates were incubated for up to 10 days at 37°C under microaerophilic conditions (10% O\(_2\), 5% CO\(_2\), and 85% N\(_2\)). H. pylori was identified based on colony morphology, Gram staining, and positive reactions for oxidase, catalase, and urease. Isolated strains were stored at −80°C in brucella broth (Difco, NJ) containing 10% dimethyl sulfoxide and 10% horse serum.

For DNA extraction, H. pylori was subcultured from the stock and multiple colonies from agar plates were collected together, followed by extraction of genomic DNA as described previously (23).

**Histology and immunohistochemistry.** Hematoxylin and eosin (HE) and Giemsa staining were performed according to the standard protocol. Immunohistochemistry was performed as described previously using polyclonal anti-H. pylori antibody (Dako, Glostrup, Denmark), polyclonal anti-CagA antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), and home-made anti-EAS Ab (23). The results were judged by an experienced pathologist (T.U.), who was blinded to all the patients’ information.

**H. pylori infection status.** To determine H. pylori status, we used a combination of three methods, culture, histology (HE and Giemsa staining), and immunohistochemistry with anti-H. pylori antibody. Patients were designated as H. pylori negative only if all the results were negative, whereas H. pylori-positive status required at least one positive result. However, due to the necessity of purified DNA for sequencing, only patients with successful isolation of H. pylori were chosen from the infected group.

**Sequencing of the \(3’\) end of the cagA gene.** The 3’ end of the cagA gene containing EPIYA motifs was amplified by PCR using several sets of primers (listed in Table 2). PCR products were purified with an Illustra GFX PCR DNA and gel band purification kit (Amersham Biosciences, UK), and the amplified fragments were sequenced with a Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) using an ABI Prism 310 genetic analyzer (Applied Biosystems) in accordance with the manufacturer’s instructions.

**Investigation of the cagA gene promoter region.** The promoter region of the cagA gene was detected by PCR using two sets of primers designed by Ikemou et al. (12) (Table 2). To confirm the result of PCR, dot blot analysis was performed as described previously (16). Briefly, 200 ng of each sample DNA was mixed with denaturing buffer and spotted onto a Hybond N\(^+\) membrane (Amersham Biosciences) via a 96-well Bio-Dot apparatus (Bio-Rad, Ivry-sur-Seine, France). DNA of the reference strain ATCC 43504 and human DNA were also transferred to the membrane as positive and negative controls, respectively. The cagA promoter region of the ATCC 43504 strain was amplified by PCR using the above-mentioned primer sets. The amplified fragments were purified with Illustra GFX PCR DNA and a gel band purification kit and used as probes. The probes were labeled with horseradish peroxidase, hybridized overnight at 42°C to the membranes, and finally exposed to Hyperfilm ECL using enhanced chemiluminescence (ECL) direct nucleic acid labeling and detection systems (Amersham Biosciences).

**Western blot assay.** H. pylori total protein was extracted by sonication, separated electrophoretically on a 10% sodium dodecyl sulfate-polyacrylamide gel, and transferred to an Amersham Hybond cellulose membrane (Amersham Biosciences). The membrane was incubated with polyclonal anti-CagA antibody as the first antibody and then with horseradish peroxidase-conjugated secondary antibody (Dako, Glostrup, Denmark). The membrane was incubated with horseradish peroxidase-conjugated secondary antibody (Dako, Glostrup, Denmark). The membrane was incubated with horseradish peroxidase-conjugated secondary antibody (Dako, Glostrup, Denmark).
antibody (Dako). Finally, the membrane was exposed to Hyperfilm ECL using an ECL Western blotting detection kit (Amersham Biosciences) in accordance with the manufacturer’s instructions.

**RNA extraction and reverse transcriptase PCR (RT-PCR).** H. pylori total RNA was isolated with ISOGEN (Nippongene, Toyama, Japan) and treated with DNase I (Takara Biotechnology, Shiga, Japan) to remove DNA contamination. The absence of DNA contamination was verified by PCR with a 16S rRNA primer set (6) (Table 2) using RNA solution as the template.

For RT-PCR, 500 ng of total RNA was reverse transcribed into cDNA with random primers using a Transcriptor first-strand cDNA synthesis kit (Roche, Mannheim, Germany) in accordance with the manufacturer’s instructions. The synthesized cDNA was then subjected to PCR amplification with the CagA-Set1 and CagA-Set2 for cagA and 16S rRNA as the positive control (Table 2).

**Nucleotide sequence accession numbers.** Representative sequences were deposited into the DNA Data Bank of Japan under the accession numbers AB469561 to AB469640.

### RESULTS

**Determination of cagA genotype by sequencing.** To determine the cagA genotypes, the DNA fragment covering the whole EPIYA motif region of the cagA gene was amplified and then the PCR products were purified and sequenced. The amplification was successful in the majority of cases with CagA-Set1 primers that had worked well with the Japanese samples (unpublished data). However, in about 20% of cases, several “trial and error” attempts with other primer sets were made before the DNA fragment of interest was finally amplified, reflecting the geographic variations in the H. pylori cagA gene.

As a result, 44 of 53 (83%) samples from Thailand had the Western-type cagA gene containing the three EPIYA motifs A, B, and C, and the remaining 9 (17%) had the East Asian type. In contrast, 88 (95.7%) of the H. pylori strains from Vietnam possessed the East Asian CagA with the three EPIYA motifs A, B, and D, and only 4 (4.3%) had the Western CagA with the A, B, and C motifs. These data indicate that the distributions of the cagA genotypes differ significantly between Vietnam and Thailand (Table 1).

**Lack of cagA expression due to deletion of its promoter region.** All H. pylori strains isolated from the 145 infected patients in this study possessed the cagA gene as revealed by PCR. However, immunohistochemistry with anti-CagA antibody gave negative results in seven patients, even though the presence of H. pylori in their gastric biopsy specimens was confirmed by both HE/Giemsa staining and immunohistochemistry with anti-H. pylori antibody.

To validate the negative results with immunohistochemistry, the protein of H. pylori strains isolated from these seven patients was used for the Western blotting with anti-CagA antibody. The experiment was repeated several times, but CagA protein was not detected on the blotting membranes. Further-

### TABLE 2. Primers used in this study

| Gene and DNA region | Primer | Primer sequence (5‘–3‘) | PCR product size (bp) |
|--------------------|--------|-------------------------|----------------------|
| cagA 3’ region     | CagA-Set1 | GAATTGTCTGATATAACTTTGAAA | 1,125                |
|                    | CagA-F1  | GCGTATGTTGGGCTTACGTTAGGC |                     |
|                    | CagA-R1  | CTAAAAAGACCTGGAAAGAAG   | 870                  |
|                    | CagA-F2  | GTTGCCAAAATGCCCTGTT     |                     |
|                    | CagA-R2  | AACCCGAGATGGATTTCAAAAA  | 870                  |
|                    | CagA-F3  | ATCTTTGAGTTGCTATCTCATCT |                     |
|                    | CagA-R3  | GAATTGTCTGATAACTTTGAAA  | 990                  |
|                    | CagA-F3  | GTTGCCAAAATGCCCTGTT     |                     |
|                    | CagA-R3  | AACCCGAGATGGATTTCAAAAA  |                     |
| cagA promoter regiona | CagAP-Set1 | GTTGGTAAAATGTTGAAACGC  | 730                  |
|                    | CagAP-F1 | CTCACTTGTTCCAACCCATTTC |                     |
|                    | CagAP-R1 | CTGCAAAGATGGTTGGCAGA    |                     |
| 16S rRNAb          | 16S RNA-Set  | TGGGACATCCGCAGGTAAATG  | 520                  |
|                    | 16S RNA-F  | GCTAAGAGATCAGGCTATG    |                     |
|                    | 16S RNA-R  | GCTAAGAGATCAGGCTATG    |                     |

* From Ikenoue et al. (12).

### TABLE 3. Diagnostic accuracy of immunohistochemistry with anti-EAS Ab

| Immunohistochemistry result with anti-EAS Ab | No. of specimens with cagA genotype | East Asian | Western |
|---------------------------------------------|-------------------------------------|------------|---------|
| Positive                                    | 87                                  | 1          |         |
| Negative                                    | 3                                   | 47         |         |
| Total                                       | 90                                  | 48         |         |

* Sensitivity, 96.7% (87/90); specificity, 97.9% (47/48); accuracy, 97.1% [(87 + 47)/(90 + 48)].
more, the absence of cagA mRNA in the total RNA solution extracted from these seven strains was also confirmed by RT-PCR. These results prompted us to suspect that a change in the promoter of the cagA gene might be responsible for the abolition of its transcription. Therefore, we amplified the cagA promoter region by PCR with two primer sets, CagAP-Set1 and CagAP-Set2. All of the above-mentioned seven strains gave a negative PCR result which was subsequently confirmed by dot blot analysis. In contrast, for those H. pylori strains capable of producing CagA protein, the promoter region was clearly detected by both PCR and dot blotting (Fig. 2).

Taken together, it can be concluded that the lack of CagA protein in the seven strains was entirely due to the deletion of the cagA promoter region. These strains were excluded from further analysis.

Accuracy of anti-EAS immunohistochemistry for diagnosis of CagA phenotypes. To determine the accuracy of our immunohistochemistry test, first we performed immunohistochemistry with the anti-EAS Ab on gastric biopsy specimens from 240 patients whose H. pylori infection status was confirmed to be negative. Positive staining was not observed in any of the specimens, confirming that the anti-EAS Ab was not cross-reactive with noninfected gastric mucosa.

Next, for H. pylori-infected patients, we excluded seven cases in which cagA failed to be expressed due to deletion of its promoter region, as described above. We compared the results of immunohistochemistry using anti-EAS Ab with that of the cagA sequencing, which was regarded as the gold standard in this study. Of 90 cases of infection with H. pylori strains possessing East Asian CagA as determined by cagA sequencing, 87 were immunoreactive with anti-EAS Ab. Among 48 cases of infection with Western CagA-producing strains, only 1 was positively stained, while the remaining 47 were not. For all cases, the presence of H. pylori in gastric biopsy specimens was confirmed by immunohistochemistry with anti-H. pylori antibody (Fig. 3). These results indicated that the sensitivity, specificity, and accuracy of our immunohistochemistry method were 96.7%, 97.9%, and 97.1%, respectively (Table 3).

DISCUSSION

To determine the cagA genotype, it is usually necessary to sequence its 3’ region containing the EPIYA motifs. Several authors have described simple and accurate PCR methods for characterizing the number and the type of EPIYA motif, whereby cagA genotyping can be greatly facilitated (1, 17, 26). However, the interstrain variations in the primer annealing site might be problematic for DNA-based methods. We also had difficulty with PCR for several H. pylori strains, which makes the diagnostic process very time-consuming and frustrating. Immunohistochemistry with anti-EAS Ab provides more options for the diagnosis of CagA phenotype and has a number of
potential merits. First, it is very accurate for both Western and East Asian CagA, showing no cross-reactivity with noninfected gastric mucosa. Second, the immunohistochemistry method does not require genomic DNA, and therefore it is particularly useful in cases where isolation of *H. pylori* is unsuccessful. Third, our method may allow considerable saving of diagnosis-related costs and time, since it can be performed rapidly without any requirement for *H. pylori* isolation, subculture, and DNA preparation or expensive equipment for sequencing. Finally, immunohistochemistry with anti-EAS Ab can be widely applied in many hospitals because it is technically simple. Because of these merits, our method may be useful in countries or regions where both phenotypes are present. Such regions are found mostly in East Asia, including Okinawa, Thailand, Malaysia, and Singapore. (13, 20, 21, 24). It has been reported that in multiethnic societies, the distribution of *cagA* genotypes is not similar among different ethnic groups. For example, in Thailand, Malaysia, and Singapore, Eastern CagA is predominant among isolates from Chinese groups while Western CagA is predominant among isolates from Thais, Indians, and Malays (13, 20, 21, 24). However, our method also has some disadvantages. First, it is not feasible if the biopsy specimen does not contain *H. pylori*. Additionally, our method is not able to diagnose the number and type of EPIYA motifs. Finally, if the result is negative, our method cannot tell whether the patient is infected with Western CagA-producing *H. pylori* or a CagA-negative strain. In such cases, it is necessary to perform immunohistochemistry with anti-CagA antibody for differentiation.

In our study, among the 48 subjects infected with Western CagA-producing *H. pylori*, one was immunohistochemically positive based on anti-EAS Ab. The reason for the false positivity in this case is difficult to clarify but may have resulted from mixed infection. It is likely that the biopsy specimens used for culture were colonized by the Western CagA-producing strain, while those used for immunohistochemistry were infected with the Eastern CagA-producing one or both, leading to inconsistency between sequencing and immunohistochemistry. Otherwise, the false positivity may have originated from the cross-reactivity of anti-EAS Ab with Western CagA or technical errors.

We accidentally found a number of *H. pylori* strains in which the promoter region of the *cagA* gene was deleted and thus could not produce CagA protein. These strains might be functionally regarded as *cagA* negative even though they possess the *cagA* gene. Therefore, we suggest that the promoter region of *cagA* may serve as a better marker for *cagA*-positive strains. This finding also indicates that genotyping of the *cagA* gene by

FIG. 3. Diagnosis of *cagA* genotype by immunohistochemistry. Sections from gastric biopsy specimens VA49 (A and B) and VB84 (C and D) were subjected to immunohistochemistry with anti-*H. pylori* antibody (α-Hp Ab) (A and C) and anti-East Asian-specific antibody (α-EAS Ab) (B and D). Sample VA49, which was infected by East-Asian CagA-producing *H. pylori*, was stained with anti-EAS Ab (B), whereas VB84, infected with Western CagA-producing *H. pylori*, was not (D). The presence of *H. pylori* in both specimens was confirmed by positive immunoreactivity with anti-*H. pylori* Ab (A and C). Original magnification, ×100.
than in Thailand, despite the fact that the age-standardized incidence rate among the general population is similar in the two countries (7, 18). It is currently believed that East Asian CagA is more toxic and more strongly associated with gastric cancer than Western CagA (8, 9). Molecular epidemiology also showed that in the geographic areas where the incidence of gastric cancer is high, such as Japan, South Korea, and China, most of the strains have East Asian cagA while Western cagA is very rare (4, 15, 23, 25, 27, 28). From these facts, it is tempting to speculate that the distribution of the cagA genotype may partly contribute to the difference in the gastric cancer incidence rates between Vietnam and Thailand.

In conclusion, our results indicate that immunohistochemistry using the anti-EAS Ab is a useful tool for the diagnosis of CagA phenotype in terms of its high accuracy, rapidity, and ease of performance.

ACKNOWLEDGMENTS

This work was supported in part by Grants-in-Aid from the Japan Society for the Promotion of Science.

We thank Tsyusyo Iwao for his technical assistance.

REFERENCES

1. Argent, R. H., Y. Zhang, and J. C. Atherton. 2005. Simple method for determination of the number of Helicobacter pylori CagA variable-region EPIYA tyrosine phosphorylation motifs by PCR. J. Clin. Microbiol. 43:791–795.
2. Azuma, T., S. Yamazaki, A. Yamakawa, M. Ohtani, A. Muramatsu, H. Suto, Y. Ito, M. Dojo, Y. Yamazaki, K. Kurimoto, Y. Keida, H. Higashi, and M. Hatakeyama. 2004. Association between diversity in the Src homology 2 domain-containing tyrosine phosphatase binding site of Helicobacter pylori CagA protein and gastric atrophy and cancer. J. Infect. Dis. 189:820–827.
3. Censini, S., C. Lange, Z. Xiang, J. E. Crabtree, P. Ghiara, M. Borodovsky, R. Rappuoli, and A. Covacci. 1996. cag, a pathogenicity island of Helicobacter pylori, encodes type I-specific and disease-associated virulence factors. Proc. Natl. Acad. Sci. USA 93:14648–14653.
4. Choi, K. D., N. Kim, D. H. Lee, J. M. Kim, J. S. Kim, H. C. Jung, and I. S. Song. 2007. Analysis of the 3′ variable region of the cagA gene of Helicobacter pylori isolated in Koreans. Dig. Sci. 52:960–966.
5. Covacci, A., J. L. Telford, G. Del Giudice, J. Parsonnet, and R. Rappuoli. 1999. Helicobacter pylori virulence and genetic geography. Science 284:1328–1333.
6. Engstrand, L., A. M. Nguyen, D. Y. Graham, and F. A. el-Zantari. 1992. Reverse transcription and polymerase chain reaction amplification of rRNA for detection of Helicobacter species. J. Clin. Microbiol. 30:2295–2301.
7. Ferlay, J., F. Bray, P. Pisani, and D. M. Parkin. 2004. GLOBOCAN 2002: cancer incidence, mortality and prevalence worldwide, version 2.0. IARC CancerBase no 5. IARC Press, Lyon, France.
8. Hatakeyama, M. 2006. Helicobacter pylori CagA: a bacterial intruder conspiring gastric carcinogenesis. Int. J. Cancer 119:1217–1223.
9. Hatakeyama, M. 2004. Oncogenic mechanisms of the Helicobacter pylori CagA protein. Nat. Rev. Cancer 4:688–694.
10. Higashi, H., R. Tsutsui, A. Fujita, S. Yamazaki, M. Asaka, T. Azuma, and M. Hatakeyama. 2002. Biological activity of the Helicobacter pylori virulence factor CagA is determined by variation in the tyrosine phosphorylation sites. Proc. Natl. Acad. Sci. USA 99:14428–14433.
11. Higashi, H., R. Tsutsui, S. Muto, T. Sugiyama, T. Azuma, M. Asaka, and M. Hatakeyama. 2002. SHP-2 tyrosine phosphatase as an intracellular target of Helicobacter pylori CagA protein. Science 295:683–686.
12. Ikenoue, T., S. Maeda, K. Ogura, M. Akanuma, Y. Mitsuno, I. Imai, H. Yoshida, Y. Shiratori, and M. Omata. 2001. Determination of Helicobacter pylori virulence by single gene analysis of the cag pathogenicity island. Clin. Diagn. Lab. Immunol. 8:181–186.
13. Mohamed, R., A. Hanafiah, I. M. Rose, M. R. Manaf, S. A. Abdullah, I. Sagap, A. Van Belkum, and J. A. Yaacob. 2009. Helicobacter pylori cagA gene variants in Malaysians of different ethnicity. Eur. J. Clin. Microbiol. Infect. Dis. 28:865–869.
14. Naito, M., T. Yamazaki, R. Tsutsui, H. Higashi, K. Oono, S. Yamazaki, T. Azuma, and M. Hatakeyama. 2006. Influence of EPIYA-repeat polymorphism on the phosphorylation-dependent biological activity of Helicobacter pylori CagA. Gastroenterology 130:1181–1190.
15. Nguyen, L. T., T. Uchida, K. Murakami, T. Fujioka, and M. Moriyama. 2008. Helicobacter pylori virulence and the diversity of gastric cancer in Asia. J. Med. Microbiol. 57:1445–1453.
16. Occhialini, A., A. Marais, R. Alm, F. Garcia, R. Sierra, and F. Megraud. 2000. Distribution of open reading frames of plasticity region of strain J99 in Helicobacter pylori strains isolated from gastric carcinoma and gastritis patients in Costa Rica. Infect. Immun. 68:6240–6249.
17. Panayotopoulou, E. G., D. N. Ngoukas, K. Kalliaropoulou, G. Papatheodoris, A. F. Mentis, and A. J. Archimandritis. 2007. Strategy to characterize the number and type of repeating EPIYA phosphorylation motifs in the carboxyl terminus of CagA protein in Helicobacter pylori clinical isolates. J. Clin. Microbiol. 45:488–495.
18. Parkin, D. M. 2006. The global health burden of infection-associated cancers in the year 2002. Int. J. Cancer 118:3030–3044.
19. Peek, R. M., Jr., and M. J. Blaser. 2002. Helicobacter pylori and gastrointestinal tract adenocarcinomas. Nat. Rev. Cancer 2:28–37.
20. Ramel, M., A. Aminuddin, H. Altzh, M. R. Isa, A. Y. Jami, H. J. Tan, A. J. Rahman, A. M. Rizal, and M. Z. Mazlum. 2005. cagA gene variants in Malaysian Helicobacter pylori strains isolated from patients of different ethnic groups. FEMS Immunol. Med. Microbiol. 44:239–242.
21. Schmidt, H. K., B. L. Goh, K. M. Fock, I. Hilmii, S. Damodaran, D. Forman, and H. Mitchell. 2009. Distinct cagA EPIYA motifs are associated with ethnic diversity in Malaysia and Singapore. Helicobacter 14:256–263.
22. Suerbaum, S., and P. Michetti. 2002. Helicobacter pylori infection. N. Engl. J. Med. 347:1175–1186.
23. Uchida, T., R. Kanada, Y. Tsukamoto, N. Hijiya, K. Matsuura, Y. Yamaoka. 2004. Association between diversity in the Src homology 2 EPIYA tyrosine phosphorylation motifs by PCR. J. Clin. Microbiol. 40:197–204.
24. Vilaichone, R. K., V. Mahachai, S. Tumwasorn, J. Y. Wu, D. Y. Graham, and F. Megraud. 2000. Distribution of open reading frames of plasticity region of strain J99 in Helicobacter pylori strains isolated from gastric carcinoma and gastritis patients in Costa Rica. Infect. Immun. 68:6240–6249.
25. Whitehead, J., J. Zhang, C. Xu, and L. He. 2004. GLOBOCAN 2002: cancer incidence, mortality and prevalence worldwide, version 2.0. IARC CancerBase no 5. IARC Press, Lyon, France.
26. Zhou, W., S. Yamazaki, A. Yamakawa, M. Ohtani, Y. Ito, Y. Keida, H. Higashi, M. Hatakeyama, J. Si, and T. Azuma. 2004. Oncogenic mechanisms of the Helicobacter pylori CagA protein. Nat. Rev. Cancer 4:688–694.
27. Zhou, W., S. Yamazaki, A. Yamakawa, M. Ohtani, Y. Ito, Y. Keida, H. Higashi, M. Hatakeyama, J. Si, and T. Azuma. 2004. The diversity of cagA and cagA genes of Helicobacter pylori in East Asia. FEMS Immunol. Med. Microbiol. 40:81–87.