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Clinical Relevance of the *babA2* Genotype of *Helicobacter pylori* in Japanese Clinical Isolates

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Genotypic variation of *Helicobacter pylori* is speculated to associate with different clinical outcomes. In Western countries, the gene encoding blood group antigen-binding adhesin (BabA), *babA2*, is of high clinical relevance and is a useful marker to identify patients who are at higher risk for peptic ulceration and gastric adenocarcinoma, as are *vacA* and *cagA*. We investigated the presence of *babA2* and *cagA* in 179 Japanese clinical isolates by PCR and Southern blot analysis and looked for correlations with various clinical outcomes (nonulcer dyspepsia, duodenal ulcers, gastric ulcers gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma). The prevalence of the *babA2* genotype was 84.9% and that of the *cagA* genotype was 96.1%. There was no correlation between the *babA2* and *cagA* genotypes, and there was no association between the *babA2* or *cagA* status and clinical outcome. These results indicate that *babA2* status is not of high clinical relevance in Japan and that Japanese strains are different from those infecting Western populations.

Helicobacter pylori infection in the stomach activates a mucosal inflammatory response and leads to diverse clinical outcomes in humans (3). Most infected persons are asymptomatic with histologic gastritis (22), or they may develop a gastric or duodenal ulcer (1), adenocarcinoma (13), or mucosa-associated lymphoid tissue (MALT) lymphoma (27, 32) of the stomach.

Numerous studies indicate that both bacterial and host factors may be important in leading to particular clinical and pathological sequelae of the infection. Among the bacterial factors, the role of the ability to adhere to epithelial cells is crucial in the initiation of a gastric inflammatory response (17, 24, 28). The blood group antigen-binding adhesin BabA has been shown to mediate adherence of *H. pylori* to human Lewis b (α-1,3/4-difucosylated) blood group antigens on gastric epithelial cells (5). In vitro adherence assays revealed that *H. pylori* bound in a lineage-specific manner to gastric surface mucous cells mediated by fucosylated blood group antigens (11). Furthermore, a study using transgenic mice expressing the human Lewis b epitope in gastric epithelial cells indicated that Lewis b antigens function as receptors for an *H. pylori* adhesin and mediate its attachment to gastric pit and surface mucous cells (12). The attachment of *H. pylori* to gastric epithelial cells in such transgenic mice resulted in the development of chronic gastritis and gastric atrophy (15). In a recent study, the gene encoding BabA was cloned (and named *babA2*), thus allowing the identification of *H. pylori* strains harboring the *babA2* genotype by PCR (18).

The *cag* pathogenicity island is one of the major virulence factors of *H. pylori*, and there is a high frequency of the presence of the *cagA* gene in the *cag* pathogenicity island in patients with duodenal ulcers (16), atrophic gastritis (21, 22), gastric carcinomas (4), and MALT lymphomas (9) in Western countries. However, in Japan *H. pylori* strains harboring the *cagA* gene have not been related to clinical outcomes because of the very high prevalence of strains harboring the *cagA* gene in Japanese clinical isolates (25, 29). A recent study has shown that the presence of the *babA2* gene was significantly associated with duodenal ulcers and gastric carcinomas and with the presence of the *cagA* genotype in a Western population (14).

The clinical relevance of the *H. pylori babA2* genotype has not yet been determined in a large series of clinical isolates in Japan. Therefore, we investigated the presence of *babA2* and *cagA* in Japanese clinical *H. pylori* isolates and their correlation with clinical outcomes (nonulcer dyspepsia, duodenal ulcer, gastric ulcer, gastric adenocarcinoma, and MALT lymphoma).

MATERIALS AND METHODS

Subjects. One hundred seventy-nine *H. pylori* strains were obtained from antral and corpus biopsies from Japanese patients between 1997 and 1999 at Hokkaido University Hospital, Sapporo, Japan. None of the patients had received nonsteroidal antiinflammatory drugs or antibiotics within the previous 3 months. The patient population consisted of 179 patients (117 men and 62 women) with a mean age of 48.6 years (range, 21 to 74 years). The patients were classified at the time of endoscopy into those having gastric ulcers ($n = 45$), duodenal ulcers ($n = 41$), gastric adenocarcinomas ($n = 40$), MALT lymphomas ($n = 11$), or no evidence of mucosal ulceration but with chronic gastritis (nonulcer dyspepsias) ($n = 42$). The classification of patients was based on the results of endoscopic and histological examinations.

Bacterial strains. Biopsy specimens were cultured on *H. pylori*-selective agar plates (Eiken Chemical Co., Ltd., Tokyo, Japan) under microaerophilic conditions (5% O_2, 10% CO_2, 85% N_2 at 37°C; AaeroPack Systems, Mitsubishi Gas Chemical, Osaka, Japan) for up to 5 days. The organisms were identified as *H. pylori* by Gram staining, colony morphology, and positive oxidase, catalase, and urease reactions. A single colony on the agar was collected and cultured again under the same microaerophilic conditions in brain heart infusion broth (Nissui, Osaka, Japan) containing 5% (vol/vol) horse serum for up to 3 days. Aliquots were stored at −80°C in 10% phosphate-buffered saline containing 20% (vol/vol) glycerol. After thawing of the aliquots of the frozen culture, bacterial suspensions were cultured at 37°C in brain heart infusion broth containing 5% fetal calf serum (GIBCO BRL, Rockville, Md.) under microaerobic conditions as de-

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TABLE 1. Correlation between the *babA2* and *cagA* genotypes and disease prevalence

Clinical outcome	No. (%) of positive strains	
	babA2	*cagA*
Gastric ulcer (n = 45)	38 (84.4)	43 (95.6)
Duodenal ulcer (n = 41)	35 (85.4)	39 (95.1)
Nonulcer dyspepsia (n = 42)	34 (81.0)	41 (97.6)
Gastric adenocarcinoma (n = 40)	36 (90.0)	40 (100)
MALT lymphoma (n = 11)	9 (81.8)	9 (81.8)
Total (n = 179)	152 (84.9)	172 (96.1)

scribed above on a gyratory shaker at 160 rpm for 24 to 36 h to the plateau phase. The bacterial suspensions were centrifuged at 2,000 × *g* for 5 min, and the bacterial pellets were used for genomic DNA extraction. Genomic DNA was extracted by using a SepaGene kit (Nippon Gene, Toyama, Japan) according to the manufacturer's instructions.

PCR. PCR was performed according to a previously reported method (2). An aliquot (0.5 μl) of *Taq* DNA polymerase and deoxynucleoside triphosphates (Takara Shuzou Co., Ltd., Shiga, Japan) was mixed with 1 μl of a genomic DNA sample of each strain and primer. The *babA2* primers were designed on the basis of the recently published signal sequence of the *babA2* gene (14). The primers used were *babA2*F (5′-AATCCAAAAAGGAGAAAAAGTATGAAA-3′) and *babA2*R (5′-TGTTAGTGATTTCGGTGTAGGACA-3′) for *babA2* amplification, *ureA*F (5′-GCCAATGGTAAATTAGTT-3′) and *ureA*R (5′-CTCCTTAA TTGTTTTTAC-3′) for *ureA* amplification, and *cagA*F (5′-GGGGATCCATGA CTAACGAAACC-3′) and *cagA*R (5′-GGCTTAAGTGATGGGACACCCAA-3′) for *cagA* amplification. These base sequences corresponded to the nucleotide sequences of strain NTCC 11638 or strain J99 (31). PCR was performed using a thermal cycler (Takara Shuzou) under the following conditions: an initial denaturation for 5 min at 92°C; 35 cycles of 1 min at 92°C, 1 min at 52 to 58°C, and 1 min at 72°C; and a final extension at 72°C for 10 min. PCR amplification of the *H. pylori ureA* gene was performed as a positive control.

Southern blot analysis. Ten micrograms of genomic DNA of *H. pylori* was digested by the restriction enzyme *Mbo*II (New England BioLabs, Beverly, Mass.), electrophoresed on a 1% agarose gel, and then transferred onto a nylon membrane. The *cagA* probe was made from the same primers as those described above and labeled with digoxigenin (DIG) using a PCR DIG probe synthesis kit (Boehringer Mannheim GmbH, Mannheim, Germany). The *cagA* probe was located in the upstream region of the total *cagA* gene (381 of 4,043 bp). These base sequences corresponded to the nucleotide sequences of the *cagA* gene of strain NTCC 11638. The membrane was hybridized with the labeled probe for 20 h at 42°C in DIG Easy Hyb (Boehringer Mannheim GmbH). After being washed sequentially in 2× SSC–0.1% sodium dodecyl sulfate (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and 0.2× SSC–0.1% sodium dodecyl sulfate, the detection of the membrane was done by using a DIG nucleic acid detection kit (Boehringer Mannheim GmbH) according to the manufacturer's instructions.

Statistical analysis. Fisher's exact test was used for the analysis of categorical data (Table 1). Analyses were done using Stat View Software, version 4.5 (SAS Institute Inc., Cary, N.C.). A P value of <0.05 was accepted as statistically significant.

RESULTS

PCR and Southern blot analysis. The *babA2* genotype was detected in 152 of the 179 isolates (84.9%) by PCR. The *cagA* genotype was detected in 172 of the 179 isolates (96.1%) by PCR and Southern blot analysis. The *babA2*-positive strains showed the presence of *cagA* (146 of 152 isolates [96.7%]). Twenty-six of the 27 *babA2*-negative strains showed the presence of *cagA* (96.3%). Only one of the 27 *babA2*-negative strains (3.7%) did not show the presence of *cagA*. There was no correlation between the *babA2* genotype and the *cagA* genotype.

Relationship between prevalence of *cagA* and *babA2* genotypes and clinical outcome. Table 1 shows the relationship between the prevalence of the *cagA* and *babA2* genotypes and clinical outcome. The overall prevalence of the *babA2* genotype was 84.9% (152 of 179 isolates). No significant correlation was obtained between the *babA2* genotype and clinical outcome.

The overall prevalence of the *cagA* genotype was 96.1% (172 of 179 isolates). The presence of the *cagA* genotype was not correlated with any clinical outcomes (Table 1).

DISCUSSION

The adherence of *H. pylori* to epithelial cells is a crucial factor in the specific tropism and pathogenicity of the organism in the stomach (4, 16, 17, 24, 28). Biochemical studies have identified a protein from *H. pylori*, an adhesin named BabA, that allows binding to the blood group antigen Lewis b present on the surface of gastric epithelial cells (6). Two corresponding genes encoding BabA have been cloned and named *babA1* and *babA2* (18). Only the *babA2* gene is functionally active (18). These two genes have almost complete sequence homology, with the exception of a translational initiation codon in the signal peptide sequence found only in *babA2* (18). A recent study has shown that a mismatch PCR method that amplifies the signal peptide sequence is an efficient method for selective identification of the presence of the *babA2* gene (14). The distribution of the presence of the *babA2* genotype in Western countries has been shown to be about 66 to 72% in recent studies (14, 18). One of these studies has suggested that the presence of *babA2, vacAs1*, and *cagA* ("triple-positive" strains) showed a highly significant correlation to the prevalence of duodenal ulcers and gastric adenocarcinomas (14). Although a previous study in Japan showed that almost all Japanese strains (97.7%) harbored the *vacAs1* genotype (19), we tested for the presence of the *vacAs1* genotype by PCR (19). In our study, it was confirmed that 172 of 179 strains (96.1%) showed the *vacAs1* genotype. In addition, there was no correlation between the *babA2* and *vacAs1* genotypes (data not shown).

The present study suggested that the prevalence of the *babA2* genotype is higher in Japan than in Western countries and that there is not a significant correlation between the *babA2* genotype and clinical outcome in Japan. These results are not in accordance with those of a recent study in a Western population (14). In Japan, only a few patients infected with *babA2*-, *cagA*-, or *vacAs1*-positive strains will suffer from peptic ulcers or gastric adenocarcinomas. It is therefore difficult to explain the different clinical outcomes from virulence factors only, such as *babA2, cagA*, and *vacAs1* of *H. pylori*.

We tested the adherence abilities of *babA2*-negative strains compared with those of *babA2*-positive strains in an in vitro study in which a flow-cytometric assay was performed by using Lewis b-positive gastric epithelial cells (KATOIII cells). Our findings showed that the *babA2*-positive strains adhered more strongly than did the *babA2*-negative strains but that the *babA2*-negative strains adhered weakly to the cells (data not shown). It is still a fact that both the *babA2*-positive strains and the *babA2*-negative strains colonize in the stomach and that there are adherence factors other than the *babA2* gene. We therefore investigated the presence of the *hpaA* gene, one of the bacterial adhesins (8, 10, 17, 23, 26), in *babA2*-negative strains by PCR. The *hpaA* gene was found to be present in all

babA2-negative strains (data not shown). We speculate that the *babA2*-negative strains can colonize in the human stomach because of the presence of other bacterial adhesins.

An explanation of the different clinical outcomes from the host factors, including histopathology of Lewis b expression in gastric tissue, may be possible, but a previous study in Japan showed that the majority of gastric biopsies from patients (95% of cases of normal foveolar epithelium, 75% of intestinal metaplasias, and 75% of intestinal types of gastric cancer) expressed Lewis b (20), and other previous studies in Western countries showed that the expression of the Lewis b antigen on primary gastric cells was about 95% (7, 30). One of those studies demonstrated that adherence of *H. pylori* to gastric epithelial cells was not dependent on the expression of either Lewis a or Lewis b on primary cells isolated from the biopsy and that incubation of primary gastric cells with monoclonal antibodies to either Lewis a or Lewis b had no effect on *H. pylori* binding (7). Thus, it seems to be difficult to explain the different clinical outcomes in Japan in terms of host factors such as the expression of Lewis b antigen on gastric epithelial cells and the presence of BabA.

In conclusion, our current data do not support the hypothesis that the virulence factors of *H. pylori*, BabA and CagA, are strongly associated with peptic ulcer disease and gastric adenocarcinoma in Western countries. We speculate that the prevalences of *babA2* and *cagA* genotypes in Japan are much higher than those in Western countries.

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REFERENCES

1. **Anonymous.** 1994. NIH consensus conference. *Helicobacter pylori* in peptic ulcer disease. NIH consensus development panel on *Helicobacter pylori* in peptic ulcer disease. JAMA **272:**65–69.
2. **Awakawa, T., T. Sugiyama, K. Hisano, M. Karita, and A. Yachi.** 1995. Detection and identification of *cagA* of *Helicobacter pylori* by polymerase chain reaction. Eur. J. Gastroenterol. Hepatol. **7:**S75–S78.
3. **Blaser, M. J.** 1992. Hypothesis on the pathogenesis and natural history of *Helicobacter pylori*-induced inflammation. Gastroenterology **102:**720–727.
4. **Blaser, M. J., G. I. Perez-Perez, H. Kleanthous, T. L. Cover, R. M. Peek, P. H. Chyou, G. N. Stemmermann, and A. Nomura.** 1995. Infection with *Helicobacter pylori* strains possessing *cagA* is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer Res. **55:**2111–2115.
5. **Boren, T., P. Falk, K. A. Roth, G. Larson, and S. Normark.** 1993. Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens. Science **262:**1892–1895.
6. **Boren, T., S. Normark, and P. Falk.** 1994. *Helicobacter pylori*: molecular basis for host recognition and bacterial adherence. Trends Microbiol. **2:**221–228.
7. **Clyne, M., and B. Drumm.** 1997. Absence of effect of Lewis A and Lewis B expression on adherence of *Helicobacter pylori* to human gastric cells. Gastroenterology **113:**72–80.
8. **Doig, P., J. W. Austin, M. Kostrzynska, and T. J. Trust.** 1992. Production of a conserved adhesin by the human gastroduodenal pathogen *Helicobacter pylori*. J. Bacteriol. **174:**2539–2547.
9. **Eck, M., B. Schmausser, R. Haas, A. Greiner, S. Czub, and H. K. Muller-Hermelink.** 1997. MALT-type lymphoma of the stomach is associated with *Helicobacter pylori* strains expressing the CagA protein. Gastroenterology **112:**1482–1486.
10. **Evans, D. G., D. J. Evans, Jr., J. J. Moulds, and D. Y. Graham.** 1988. *N*-acetylneuraminyllactose-binding fibrillar hemagglutinin of *Campylobacter pylori*: a putative colonization factor antigen. Infect. Immun. **56:**2896–2906.
11. **Falk, P. G., K. A. Roth, T. Boren, T. U. Westblom, J. I. Gordon, and S. Normark.** 1993. An in vitro adherence assay reveals that *Helicobacter pylori* exhibits cell lineage-specific tropism in the human gastric epithelium. Proc. Natl. Acad. Sci. USA **90:**2035–2039.
12. **Falk, P. G., L. Bry, J. Holgersson, and J. I. Gordon.** 1995. Expression of a human alpha-1, 3/4-fucosyltransferase in the pit cell lineage of FVB/N mouse stomach results in production of Leb-containing glycoconjugates: a potential transgenic mouse model for studying *Helicobacter pylori* infection. Proc. Natl. Acad. Sci. USA **92:**1515–1519.
13. **Forman, D., D. G. Newell, F. Fullerton, J. W. Yarnell, A. R. Stacey, N. Wald, and F. Sitas.** 1991. Association between infection with *Helicobacter pylori* and risk of gastric cancer: evidence from a prospective investigation. BMJ **302:**1302–1305.
14. **Gerhard, M., N. Lehn, N. Neumayer, T. Boren, R. Rad, W. Schepp, S. Miehlke, M. Classen, and C. Prinz.** 1999. Clinical relevance of the *Helicobacter pylori* gene for blood-group antigen-binding adhesin. Proc. Natl. Acad. Sci. USA **96:**12778–12783.
15. **Guruge, J. L., P. G. Falk, R. G. Lorenz, M. Dans, H. P. Wirth, M. J. Blaser, D. E. Berg, and J. I. Gordon.** 1998. Epithelial attachment alters the outcome of *Helicobacter pylori* infection. Proc. Natl. Acad. Sci. USA **95:**3925–3930.
16. **Hamlet, A., A. C. Thoreson, O. Nilsson, A. M. Svennerholm, and L. Olbe.** 1999. Duodenal *Helicobacter pylori* infection differs in *cagA* genotype between asymptomatic subjects and patients with duodenal ulcers. Gastroenterology **116:**259–268.
17. **Hessey, S. J., J. Spencer, J. I. Wyatt, G. Sobala, B. J. Rathbone, A. T. Axon, and M. F. Dixon.** 1990. Bacterial adhesion and disease activity in *Helicobacter*-associated chronic gastritis. Gut **31:**134–138.
18. **Ilver, D., A. Arnqvist, J. Ogren, I. M. Frick, D. Kersulyte, E. T. Incecik, D. E. Berg, A. Covacci, L. Engstrand, and T. Boren.** 1998. *Helicobacter pylori* adhesin binding fucosylated histo-blood group antigens revealed by retagging. Science **279:**373–377.
19. **Ito, Y., T. Azuma, S. Ito, H. Miyaji, M. Hirai, Y. Yamazaki, F. Sato, T. Kato, Y. Kohli, and M. Kuriyama.** 1997. Analysis and typing of the *vacA* gene from *cagA*-positive strains of *Helicobacter pylori* isolated in Japan. J. Clin. Microbiol. **35:**1710–1714.
20. **Kobayashi, K., J. Sakamoto, T. Kito, Y. Yamamura, T. Koshikawa, M. Fujita, T. Watanabe, and H. Nakazato.** 1993. Lewis blood group-related antigen expression in normal gastric epithelium, intestinal metaplasia, gastric adenoma, and gastric carcinoma. Am. J. Gastroenterol. **88:**919–924.
21. **Kuipers, E. J., G. I. Perez-Perez, S. G. Meuwissen, and M. J. Blaser.** 1995. *Helicobacter pylori* and atrophic gastritis: importance of the *cagA* status. J. Natl. Cancer Inst. **87:**1777–1780.
22. **Kuipers, E. J., A. M. Uyterlinde, A. S. Pena, R. Roosendaal, G. Pals, G. F. Nelis, H. P. Festen, and S. G. Meuwissen.** 1995. Long-term sequelae of *Helicobacter pylori* gastritis. Lancet **345:**1525–1528.
23. **Lingwood, C. A., G. Wasfy, H. Han, and M. Huesca.** 1993. Receptor affinity purification of a lipid-binding adhesin from *Helicobacter pylori*. Infect. Immun. **61:**2474–2478.
24. **Logan, R. P.** 1996. Adherence of *Helicobacter pylori*. Aliment. Pharmacol. Ther. **10:**S3–S15.
25. **Maeda, S., K. Ogura, H. Yoshida, F. Kanai, T. Ikenoue, N. Kato, Y. Shiratori, and M. Omata.** 1998. Major virulence factors, VacA and CagA, are commonly positive in Helicobacter pylori isolates in Japan. Gut **42:**338–343.
26. **Odenbreit, S., M. Till, D. Hofreuter, G. Faller, and R. Haas.** 1999. Genetic and functional characterization of the *alpAB* gene locus essential for the adhesion of *Helicobacter pylori* to human gastric tissue. Mol. Microbiol. **31:**1537–1548.
27. **Parsonnet, J., S. Hansen, L. Rodriguez, A. B. Gelb, R. A. Warnke, E. Jellum, N. Orentreich, J. H. Vogelman, and G. D. Friedman.** 1994. *Helicobacter pylori* infection and gastric lymphoma. N. Engl. J. Med. **330:**1267–1271.
28. **Segal, E. D., S. Falkow, and L. S. Tompkins.** 1996. *Helicobacter pylori* attachment to gastric cells induces cytoskeletal rearrangements and tyrosine phosphorylation of host cell proteins. Proc. Natl. Acad. Sci. USA **93:**1259–1264.
29. **Shimoyama, T., S. Fukuda, M. Tanaka, T. Mikami, Y. Saito, and A. Munakata.** 1997. High prevalence of the CagA-positive *Helicobacter pylori* strains in Japanese asymptomatic patients and gastric cancer patients. Scand. J. Gastroenterol. **32:**465–468.
30. **Taylor, D. E., D. A. Rasko, R. Sherburne, C. Ho, and L. D. Jewell.** 1998. Lack of correlation between Lewis antigen expression by *Helicobacter pylori* and gastric epithelial cells in infected patients. Gastroenterology **115:**1113–1122.
31. **Tomb, J. F., O. White, A. R. Kerlavage, R. A. Clayton, G. G. Sutton, R. D. Fleischmann, K. A. Ketchum, H. P. Klenk, S. Gill, B. A. Dougherty, K. Nelson, J. Quackenbush, L. Zhou, E. F. Kirkness, S. Peterson, B. Loftus, D. Richardson, R. Dodson, H. G. Khalak, A. Glodek, K. McKenney, L. M. Fitzegerald, N. Lee, M. D. Adams, J. D. Gocayne, T. R. Utterback, J. D. Peterson, J. M. Kelley, M. D. Cotton, J. M. Weidman, C. Fujii, C. Bowman, L. Watthey, E. Wallin, W. S. Hayes, M. Borodovsky, P. D. Karp, H. O. Smith, C. M. Fraser, K. Hickey, D. E. Berg, and J. C. Venter.** 1997. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature **388:**539–547.
32. **Wotherspoon, A. C., C. Ortiz-Hidalgo, M. R. Falzon, and P. G. Isaacson.** 1991. *Helicobacter pylori*-associated gastritis and primary B-cell gastric lymphoma. Lancet **338:**1175–1176.