JOURNAL OF CLINICAL MICROBIOLOGY, May 2003, p. 1942–1945 Vol. 41, No. 5
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.5.1942–1945.2003

Regional Variation among *vacA* Alleles of *Helicobacter pylori* in China

Jingtong Wang,[1,2] Leen-Jan van Doorn,[3] Philip A. Robinson,[1] Xuhuai Ji,[4,5] Dong Wang,[1,5] Yu Wang,[6] Lianying Ge,[7] John L. Telford,[8] and Jean E. Crabtree[1]*

Molecular Medicine Unit, St. James's University Hospital, Leeds, LS9 7TF, United Kingdom[1]; Gastroenterology Department[2] and Hepatology Department, People's Hospital, Beijing University,[6] Beijing, Department of Gastroenterology, Changhai Hospital, Second Military Medical University, Shanghai,[5] and The First Hospital of Guangxi Medical University, Guangxi,[7] People's Republic of China; Delft Diagnostic Laboratory, Delft, The Netherlands[3]; Department of Medicine, Stanford Medical School, California[4]; and Chiron Vaccines, Siena, Italy[8]

Received 4 December 2002/Returned for modification 31 January 2003/Accepted 20 February 2003

Allelic variation among *Helicobacter pylori vacA* occurs in the signal and middle region of the gene. The aim of the study was to investigate alleleic variation of *vacA* among *H. pylori* strains from three regional areas of China and the relationship between *vacA* alleles and PUD. DNA extracted from 88 clinical isolates of *H. pylori* was analyzed by type-specific PCR and reverse hybridization line probe assay (LiPA) to determine the genotype of *vacA* and presence of *cagA*. In 87 isolates, all of the *vacA* alleles could be classified as either type s1c or type s1a, and all could be classified as m1, m2a, or m2b. One strain could not be typed. In all, 41% of patients were infected with multiple *vacA* genotypes, with the highest level being observed in Shanghai (63%). In strains from Beijing, s1a was dominant; by contrast, s1c was dominant in Guangxi and Shanghai. The prevalence of m2b strains in Shanghai (63%) was significantly higher than that in Beijing (83%) or Guangxi (0%). Thirty of the 87 patients had peptic ulcers. However, there was no association between *vacA* genotype and PUD. This study demonstrates that there is significant geographic diversity of genotype of *vacA* within China. The absence of *vacA* s2 genotypes precluded analysis of an association of *vacA* s genotypes and clinical disease.

Helicobacter pylori is an important human pathogen which chronically infects the human gastric mucosa (21). The clinical outcome of long-term infection is variable and is considered to relate to both bacterial virulence factors (1, 11, 26, 27) and host genotype (9). The vacuolating cytotoxin VacA (3, 6, 7, 23) and the *cag* pathogenicity island (2, 5) are two identified virulence factors that are considered to have an important role in the pathogenesis of *H. pylori* infection.

VacA, which in vitro induces vacuolation in epithelial cells (6, 7), is encoded by *vacA*, which has distinct allelic types (3, 6, 15, 25, 27). Genomic differences in *vacA* are located in both the signal sequences (s region) and the midregion (m region) of the gene. To date, four families of *vacA* alleles can be differentiated based on analysis of the s region (s1a, s1b, s1c, and s2), and three families can be differentiated based on the m region (m1, m2a, and m2b). The different combination of s and m regions determines the production of cytotoxic activity. Strains with the genotype s1 m1 produce high levels of vacuolating cytotoxin in vitro (3, 4, 6). Strains with the genotype s2 produce an inactive toxin (18), whereas strains with the genotype m2 produce toxic activity with a different target cell specificity from those of m1 genotype (16, 19). The m2 genotype has been classified into two separate phylogenetically distinct subtypes: m2a and m2b (25). There is global variation in the distribution of *vacA* alleles in different ethnic populations (27). The prevalence of s1c and s1a is high in strains from Asia; however, s1b is frequent in Southern Europe, South America, South Africa, and the United States (14, 22, 27, 29).

* Corresponding author. Mailing address: Level 7 Clinical Sciences Building, St. James's University Hospital, Leeds LS9 7TF, United Kingdom. Phone: 44-113-2065267. Fax: 44-113-2429722. E mail: msjjc@stjames.leeds.ac.uk.

In North American and Western Europe, infection with *H. pylori* strains containing the s1 *vacA* allele is associated with peptic ulcer disease (PUD), and there is a significant association between the presence of *vacA* s1 and *cagA* (4, 24, 26, 27). However in Japan, South Korea, and China, where s1 alleles predominate, *vacA* genotypes have not been associated with more severe clinical outcome (20, 22, 29). Global variation in the distribution of *vacA* alleles may explain diverse reports linking *vacA* genotype to clinical disease from different geographic areas.

China is a country with wide area, large population, and high rate of *H. pylori* infection. In addition, the incidence rate of gastric cancer is high in certain areas. VacA is an important immunogenic antigen candidate for *H. pylori* vaccines, and the efficacy of prophylactic immunization with VacA against *H. pylori* infection has been demonstrated in the murine model (12). Genomic variation among *vacA* is relevant to the optimization of vaccine preparations for different populations. The purposes of this study was to investigate *vacA* diversity in different areas of China and to determine whether the *vacA* genotypes are associated with clinical outcome in *H. pylori* infection.

METHODS AND MATERIALS

***H. pylori* culture.** *H. pylori* isolates were obtained from patients with PUD or nonulcer dyspepsia (NUD) undergoing routine upper gastrointestinal endoscopy at the People's Hospital of Beijing University (Beijing, China), the First Hospital of Guangxi University (Nanning, Guangxi Province, China), and the Changhai Hospital (Shanghai, China). The patients included in this study were all long-term residents of the local regions. The study was ethically approved by the People's Hos-

pital of Beijing University, the First Hospital of Guangxi University and the Changhai Hospital. *H. pylori* were grown on 5% (vol/vol) horse blood agar plates at 37°C for 3 to 5 days in a microaerophillic atmosphere using CampyPaks (Oxoid, Basingstoke, United Kingdom). The first passage of cultured isolates was frozen at −70°C and not subcultured to single colonies. Bacteria were identified as *H. pylori* based on their urease, oxidase, and catalase activities.

DNA isolation from bacteria. For DNA extraction 1.0 μl of bacterial suspension was incubated in 200 μl of 10 mM Tris buffer, pH 8.0 containing 1 mM EDTA, 100 mM NaCl and 100 μg/ml lysozyme at 4°C for 5 min and 37°C for 10 min. Sodium dodecyl sulfate was added to a final concentration of 1% (wt/vol). After incubation at 65°C for 10 min, samples were incubated at 50°C for 2 h with proteinase K at a concentration of 25 μg/ml. An equal volume of phenol-chloroform (1:1) was added and the mixture vortexed. After centrifuging at 13,600 × *g* for 4 min, DNA was precipitated from the aqueous phase with 0.1 volume 3 M sodium acetate solution, pH 5.2, and 2.5 volumes of 100% ethanol. After sedimentation of the DNA (13,600 × *g* for 10 min), the supernatant was discarded and DNA pellets were dissolved in 100 μl of sterile deionized water.

Multiplex PCR and LiPA analysis for *vacA* genotypes and presence of *cagA*. The genotyping of *vacA* s and m regions and *cagA* was undertaken as previously described (25, 27, 28). A multiplex PCR using type-specific primers for *vacA* s1a, s1b, s1c, s2, *vacA* m1, m2a, m2b, and *cagA* (25, 28) was undertaken on DNA from each culture. PCR products were denatured and reverse hybridized onto a line probe assay (LiPA) containing oligonucleotide probes for type-specific detection of amplified *vacA* and detection of *cagA* immobilized on nitrocellulose strips. Isolates were classified as multiple infections if the PCR-LiPA hybridization was to multiple probes of the *vacA* s region or m region or both.

Statistical analysis. The chi-square test was used to assess the relation between *H. pylori vacA* genotype and geographic origin and endoscopic diagnosis. Data were analyzed by SPSS for Windows, version 10.0.

RESULTS

Patients and endoscopic diagnosis. A total of 88 clinical *H. pylori* isolates were obtained, of which 24 isolates were from Guangxi, 40 isolates were from Beijing, and 24 isolates were from Shanghai. The age of patients (mean ± standard error of the mean) from whom the *H. pylori* isolates were obtained was 46.6 ± 2.45 years (16 males and 8 females) in Guangxi, 46.0 ± 2.13 years (20 males and 20 females) in Beijing, and 47.9 ± 3.2 years (16 males and 8 females) in Shanghai. The 88 isolates were typed for *vacA* alleles and *cagA* by multiplex PCR and LiPA. One isolate from Beijing, which showed no result for *vacA* s region but was positive for *vacA* m2a and *cagA*, was excluded from further analysis. At endoscopy 30 patients presented with peptic ulceration. The remaining 57 patients were diagnosed as having NUD. No patients with gastric cancer were included in the study. There was no significant difference between the mean age of *H. pylori* infected patients with PUD (46.5 ± 13.1 years) or NUD (47.5 ± 13.9 years).

TABLE 1. *H. pylori* genotypes as determined by PCR-LiPA hybridization for 87 clinical isolates from China[a]

Genotype(s)	No. of isolates[b]			
	GX (24 isolates)	BJ (40 isolates)	SH (24 isolates)	Total
s1a	1	9	0	10
s1b	0	0	0	0
s1c	19	15	16	50
s1a + s1c	4	15	8	27
m1	13	10	3	26
m2a	10	26	4	40
m2b	0	1	4	5
m1 + m2a	1	2	2	5
m2a + m2b	0	0	2	2
m1 + m2a + m2b	0	0	8	8
m2b + m1	0	0	1	1
cagA	22 (92)	39 (100)	24 (100)	85 (98)
Multiple *vacA*[c]	5 (21)	16 (40)	15 (63)	36 (41)

[a] Isolates were from three regional areas of China: Guangxi (GX), Beijing (BJ), and Shanghai (SH). The analysis of *vacA* allelic types included both multiple- and single-strain infections.
[b] Values in parentheses are percentages.
[c] Rates of multiple-genotype infection are equivalent to those shown for infection with multiple *vacA* genotypes.

Molecular analysis of *H. pylori* strains. The frequency of *vacA* s and m-alleles and *cagA* in the three regional areas of China is shown in Table 1. The dominant *vacA* genotype in the eighty seven isolates was s1c (88%) and m2a (65%). Neither s1b nor s2 alleles were detected. Thirty-six isolates (41%) had more than one type of s or m *vacA* allele, indicating multiple infection. The prevalence of multiple *vacA* alleles in the cultures varied from 21 to 63% between the three regions. The patients from Shanghai had the highest rate of infection with multiple *vacA* genotypes. In Beijing, 14 of the 16 multiple *vacA* genotypes were in the s region, 1 was in the m region, and 1 was in both. In contrast, in Shanghai only 2 of the 15 isolates with multiple *vacA* genotypes were in the s region, 6 were in the m region, and 7 were in both (Yates corrected χ^2, $P < 0.005$ for comparison of s region versus m region and s and m region). There was no significant age difference between patients with multiple-strain infection (48.8 ± 14.0 years) or single-strain infection (45.8 ± 12.9 years).

The prevalence of *cagA* among the eighty seven clinical isolates was 98%. The prevalence rates in Beijing, Shanghai and Guangxi were 100, 100, and 92%, respectively and there was no significant difference in prevalence of *cagA*.

Geographic distribution *vacA* s and m alleles in three areas in China. The geographic distribution of the subtypes of *vacA* s1 and m (including both single-strain isolates and multistrain isolates) was different in the three areas of China (Table 2). The prevalence of s1a subtype in the isolates from Beijing was 62%, significantly higher ($P < 0.01$) than that in Shanghai (33%) and Guangxi (21%). Conversely, the s1c subtype was significantly ($P < 0.05$) more frequent in isolates from Guangxi (96%) and Shanghai (100%) than Beijing (77%). The prevalence of m1 type in Guangxi (58%) was significantly higher ($P < 0.05$) than in Beijing (31%). The frequency of the m2a subtype in Beijing (72%) was significantly higher ($P < 0.05$) than in Guangxi (46%). No m2b subtypes were found in the isolates from Guangxi, and only one m2b type was observed in

TABLE 2. Prevalence of *vacA* subtypes of s- and m-region determined by PCR-LiPA[a]

vacA genotype	% Prevalence of (no. with) subtype[b]			
	GX (24 isolates)	BJ (40 isolates)	SH (24 isolates)	Total
s1a	21 (5)	**62 (24)**	33 (8)	43 (37)
s1b	0	0	0	0
s1c	96 (23)	**77 (30)**	100 (24)	89 (77)
m1	58 (14)	**31 (12)**	58 (14)	46 (40)
m2	**46 (11)**	74 (29)	88 (21)	70 (61)
m2a	**46 (11)**	72 (28)	67 (16)	63 (55)
m2b	0 (0)	3 (1)	**63 (15)**	18 (16)

[a] A total of 87 *H. pylori* isolates from three regions of China were analyzed (regions: Guangxi [GX], Beijing [BJ], and Shanghai [SH]). The analysis included both multiple- and single-strain infections.
[b] Numbers in boldface type indicate a significant difference compared with other regional areas.

Beijing. In contrast, the frequency of m2b subtype in Shanghai (63%) was significantly higher ($P < 0.01$).

The relationship between *vacA* alleles and PUD. Nine (30%) of the thirty patients with PUD and 27 (47%) of the fifty seven patients with NUD had multiple *vacA* genotypes. The frequency of infection with strains with multiple *vacA* genotype in patients with or without PUD was not significantly different ($P = 0.12$). The relationship between *H. pylori vacA* genotype and PUD was assessed in the fifty one patients whose *H. pylori* isolates contained a single genotype *vacA* for s and m regions. Fifty of these 51 isolates were *cagA* positive. The prevalence rates of s1a and s1c were 20 and 80%, respectively. The prevalence rates of m1, m2a and m2b were 47, 47, and 6%, respectively. The frequencies of different *vacA* alleles among patients with PUD and NUD are shown in Table 3. No relationship was found between infection with strains with differing *vacA* alleles and PUD.

DISCUSSION

This PCR-LiPA typing study of *H. pylori* isolates demonstrates that *vacA* subtypes s1c, m2a and m1 are dominant in China, similar to other East Asian countries (27). However, there is marked geographic diversity of *vacA* genotypes within China, emphasizing that even within one Asian country genotypic diversity exists. The *H. pylori* isolates were cultured from three populations selected from three distinct geographic regions of China, Guangxi in the south, Beijing in the north, and Shanghai in the east. All subjects included were long-term residents of the local regions. In addition, the frequency of infection with multiple *vacA* genotypes (41%) in China was considerably higher than that observed in northern Europe (11%) using similar PCR-LiPA typing (27). Higher infection levels with multiple *vacA* genotypes have been observed in Portugal and South America (8, 10, 13); however, the multiple infection rate of 63% in isolates from Shanghai is higher than that previously observed in other countries.

Genotypic analysis on cultured isolates may result in underestimation of multiple infections due to possible selective growth of strains. Additionally, the PCR-LiPA does not discriminate between different strains with the same *vacA* genotype, thereby potentially further underestimating multiple infections. The high levels of multiple infection in developing countries such as China may reflect the high incidence of *H. pylori* within such populations. In China the use of chopsticks may also play an important role in bacterial transmission (17). The reasons for the variation in frequency of multiple *vacA* types in the three geographic regions in China is unclear. There were no significant differences in the age of patients with multiple and single *vacA* genotypes. Microbial culture from the gastric biopsies from the three regions in China was under identical conditions in one United Kingdom laboratory, arguing against differences in selection of *H. pylori* isolates in vitro.

No association was observed in this study between *vacA* genotype and the presence of PUD, concurring with an earlier study in China (20). These findings are discrepant with earlier studies in North American, Dutch and German populations (4, 24, 26), but consistent with reports from Japan (29). The reason for the conflicting results is likely to relate to geographic diversity in the distribution of *vacA* genotypes. The high frequency of *vacA* s1 and *cagA* genotypes in Asian strains precludes identification of an association with clinical disease. The frequency of *vacA* m1 strains was significantly higher in Guangxi, an area with a high incidence of gastric cancer, than Beijing. Infection with *vacA* m1 genotypes is associated with increased risk of gastric ulcer and gastric carcinoma in the Portuguese population (10). Further analysis of *H. pylori vacA* genotypes in larger cohorts of patients from Guangxi, and examination of the association between *vacA* genotypes and gastric carcinoma, would be of interest.

Earlier studies in Mexican and Portuguese populations have identified that patients with PUD are more frequently infected with multiple *vacA* genotypes (10, 13). In the present study, no association between multiple infection and peptic ulcers was observed in the Chinese patients. The reason of these differences is unclear. The clinical importance of multiple strain infections and the dynamics of colonization with multiple strains requires further investigation.

The protective effect of therapeutic vaccination with VacA has been demonstrated in the murine *H. pylori* model (12). The immunogenicity of VacA makes it an important antigen in *H. pylori* subunit vaccines. Whether vaccines based on m1-type VacA will protect against m2-type *H. pylori* strains is not clear. Given the high frequency of *vacA* m2 alleles in the Chinese population, this is a question which should be urgently addressed.

TABLE 3. Frequency of *H. pylori vacA* alleles in patients with PUD and NUD[a]

Allele	No. of isolates							
	BJ ($n = 23$)		GX ($n = 19$)		SH ($n = 9$)		Total ($n = 51$)	
	PUD (6)	NUD (17)	PUD (11)	NUD (8)	PUD (4)	NUD (5)	PUD (21)	NUD (30)
s1a	3	6	1	0	0	0	4	6
s1c	3	11	10	8	4	5	17	24
m1	3	6	7	5	0	3	10	14
m2a	3	11	4	3	3	1	10	14
m2b	0	1	0	0	1	1	1	2

[a] Isolates were from three regional areas of China: Guangxi (GX), Beijing (BJ), and Shanghai (SH). Only isolates with single *vacA* s and m regions are included.

ACKNOWLEDGMENTS

This study was supported by and the European Commission (contract ICA4-CT-1999-10010) and Yorkshire Cancer Research.

REFERENCES

1. **Achtman, M., T. Azuma, D. E. Berg, Y. Ito, G. Morelli, Z. J. Pan, S. Suerbaum, S. A. Thompson, A. van der Elde, and L. J. van Doorn.** 1999. Recombination and clonal groupings within *Helicobacter pylori* from different geographical regions. Mol. Microbiol. **32:**459–470.
2. **Akopypants, N. S., S. W. Clifton, D. Kersulyte, J. E. Crabtree, B. E. Youree, C. A. Reece, N. O. Bukanov, S. E. Drazek, B. A. Roe, and D. E. Berg.** 1998. Analyses of the *cag* pathogenicity island of *Helicobacter pylori*. Mol. Microbiol. **28:**37–54.
3. **Atherton, J. C., P. Cao, R. M. J. Peek, M. K. Tummuru, M. J. Blaser, and T. L. Cover.** 1995. Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. Association of specific *vacA* types with cytotoxin production and peptic ulceration. J. Biol. Chem. **270:**17771–17777.
4. **Atherton, J. C., R. M. J. Peek, K. T. Tham, T. L. Cover, and M. J. Blaser.** 1997. Clinical and pathological importance of heterogeneity in *vacA,* the vacuolating cytotoxin gene of *Helicobacter pylori.* Gastroenetrology **112:**92–99.
5. **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 1-specific and disease-associated virulence factors. Proc. Natl. Acad. Sci. USA **93:**14648–14653.
6. **Cover, T. L., M. K Tummuru, P. Cao, S. A. Thompson, and M. J. Blaser.** 1994. Divergence of genetic sequences for the vacuolating cytotoxin among *Helicobacter pylori* strains. J. Biol. Chem. **269:**10566–10573.
7. **Cover, T. L.** 1996. The vacuolating toxin of *Helicobacter pylori*. Mol. Microbiol. **20:**241–246.
8. **de Gusmao, V. R., E. N. Mendes, D. M. Queiroz, G. A. Rocha, A. M. C. Rocha, A. A. R. Ashour, and A. S. T. Carvalho.** 2000. *vacA* genotypes in *Helicobacter pylori* strains isolated from children with and without duodenal ulcer in Brazil. J. Clin. Microbiol. **38:**2853–2857.
9. **El-Omar, E. M., M. Carrington, W. H. Cho, K. E. McColl, J. H. Bream, H. A. Young, J. Herrera, J. Lissowska, C. C. Yuan, N. Rothman, G. Lanyon, M. Martin, J. F. Fraumeni, and C. S. Rabkin.** 2000. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature **404:**398–402.
10. **Figueiredo, C., L. J. van Doorn, C. Nogueira, J. M. Soares, C. Pinho, P. Figueira, W. G. V. Quint, and F. Carneiro.** 2001. *Helicobacter pylori* genotypes are associated with clinical outcome in Portuguese patients and show a high prevalence of infections with multiple strains. Scand. J. Gastroenterol. **36:**128–135.
11. **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.
12. **Ghiara, P., M. Rossi, M. Marchetti, A. di Tommaso, C. Vindigni, F. Ciampolini, A. Covvaci, J. L. Telford, M. T. de Magistris, M. Pizza, R. Rappuoli, and G. del Giudice.** 1997. Therapeutic intragastric vaccination against *Helicobacter pylori* in mice eradictaes an otherwise chronic infection and confers protection against reinfection. Infect. Immun. **65:**4996–5002.
13. **Gonzalez-Valencia, G., J. C. Atherton, O. Munoz, M. Dehesa, A. Madrazo-de la Garza, and J. Torres.** 2000. *Helicobacter pylori vacA* and *cagA* genotypes in Mexican adults and children. J. Infect. Dis. **182:**1450–1454.
14. **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 *vacA* gene from *cagA* positive strains of *Helicobacter pylori* isolated in Japan. J. Clin. Microbiol. **35:**1710–1714.
15. **Ji, X., F. Frati, S. Barone, C. Pagliaccia, D. Burroni, G. Xu, R. Rappuoli, J. M. Reyrat, and J. L. Telford.** 2002. Evolution of functional polymorphism in the gene coding for the *Helicobacter pylori* cytotoxin. FEMS Microbiol. Lett. **206:**253–258.
16. **Ji, X., T. Fernandez, D. Burroni, J. C. Atherton, J. M. Reyrat, R. Rappuoli, and J. L. Telford.** 2000. Cell specificity of the *Helicobacter pylori* cytotoxin is determined by a short region in the polymorphic mid-region. Infect. Immun. **68:**3754–3757.
17. **Leung, W. K., J. Y. Sung, T. K. W. Ling, K. L. K. Siu, and A. F. B. Cheng.** 1999. Use of chopsticks for eating and *Helicobacter pylori* infection. Dig. Dis. Sci. **44:**1173–1176.
18. **McClain, M. S., P. Cao, H. Iwamoto, A. D. Vinion-Dubiel, G. Szabo, and T. L. Cover.** 2001. A 12-amino-acid segment, present in type s2 but not type s1 *Helicobacter pylori* VacA proteins, abolishes cytotoxin activity and alters membrane channel formation. J. Bacteriol. **183:**6499–6508.
19. **Pagliaccia, C., M. De Bernard, P. Lupetti, X. Ji, D. Burroni, T. L. Cover, E. Papini, R. Rappuoli, J. L. Telford, and J. M. Reyrat.** 1998. The m2 form of *Helicobacter pylori* cytotoxin has cell-type specific vacuolating activity. Proc. Natl. Acad. Sci. USA **95:**10212–10217.
20. **Pan, Z. J., D. E. Berg, R. W. van de Hulst, W. W. Su, A. Raudonikiene, S. D. Xiao, J. Dankert, G. N. J. Tytgat, and A. van der Ende.** 1998. Prevalence of vacuolating cytotoxin production and distribution of distinct *vacA* alleles in *Helicobacter pylori* from China. J. Infect. Dis. **178:**220–226.
21. **Peek, R. M., and M. J. Blaser.** 2002. *Helicobacter pylori* and gastrointestinal tract adenocarcinomas. Nat. Rev. Cancer **2:**28–37.
22. **Shimoyama, T., T. Yoshimura, T. Mikami, S. Fukada, J. E. Crabtree, and A. Munakata.** 1998. Evaluation of *Helicobacter pylori vacA* genotype in Japanese patients with gastric cancer. J. Clin. Pathol. **51:**299–301.
23. **Telford, J. L., P. Ghiara, M. Dell'Orco, M. Comanducci, D. Burroni, M. Bugnoli, M. F. Tecce, S. Censini, A. Covacci, Z. Xiang, E. Papini, C. Montecucco, L. Parrente, and R. Rappuoli.** 1994. Gene structure of *Helicobacter pylori* cytotoxin and evidence of its key role in gastric disease. J. Exp. Med. **179:**1653–1658.
24. **Strobel, S., S. Bereswill, P. Balig, P. Allgaier, H. G. Sonntag, and M. Kist.** 1998. Identification and analysis of a new *vacA* genotype variant of *Helicobacter pylori* in different patients groups in Germany. J. Clin. Microbiol. **36:**1285–1289.
25. **van Doorn, L. J., C. Figueiredo, R. Sanna, S. Pena, P. Midolo, E. K. Ng, J. C. Atherton, M. J. Blaser, and W. Quint.** 1998. Expanding allelic diversity of *Helicobacter pylori vacA*. J. Clin. Microbiol. **36:**2597–2603.
26. **van Doorn, L. J., C. Figueiredo, R. Sanna, A. Plaisier, P. Schineeberger W. der Bore, and W. Quint.** 1998. Clinical relevance of the *cagA, vacA* and *iceA* status of *Helicobacter pylori*. Gastroenterology **115:**58–66.
27. **van Doorn, L. J., C. Figueiredo, F. Megraud, A. S. Pena, P. Midolo, D. M. Queiroz, F. Carneiro, B. Vanderborght, M. G. F. Pegado, R. Sanna, W. de Boer, P. M. Schneeberger, P. Correa, E. K. W. Ng, J. Atherton, M. J. Blaser, and W. G. V. Quint.** 1999. Geographic distribution of *vacA* allelic types of *Helicobacter pylori*. Gastroenterology **116:**823–830.
28. **van Doorn, L. J., C. Figueiredo, R. Rossau, G. Jannes, M. van Asboeck, J. C. Sousa, F. Carneiro, and W. G. V. Quint.** 1998. Typing of *Helicobacter pylori vacA* gene and detection of *cagA* gene by PCR and reverse hybridization. J. Clin. Microbiol. **36:**1271–1276.
29. **Yamaoka, Y., T. Kodama, O. Gutierrez, J. G. Kim, K. Kashima, and D. Y. Graham.** 1999. Relationship between *Helicobacter pylori iceA*, *cagA* and *vacA* status and clinical outcomes: Studies in four different countries. J. Clin. Microbiol. **37:**2274–2279.