JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2002, p. 239–246 Vol. 40, No. 1
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.1.239–246.2002

Polymorphisms of *Helicobacter pylori* HP0638 Reflect Geographic Origin and Correlate with *cagA* Status

T. Ando,[1,2,3]* R. M. Peek,[2] D. Pride,[1,4] S. M. Levine,[1] T. Takata,[1] Y.-C. Lee,[1] K. Kusugami,[3] A. van der Ende,[5] E. J. Kuipers,[6] J. G. Kusters,[6] and M. J. Blaser[1,2,7]

Department of Medicine, New York University School of Medicine,[1] and Department of Veterans Affairs Medical Center,[7] New York, New York; Department of Medicine[2] and Department of Microbiology and Immunology,[4] Vanderbilt University School of Medicine, Nashville, Tennessee; First Department of Internal Medicine, Nagoya University School of Medicine, Nagoya, Japan[3]; and Department of Medical Microbiology, Academic Medical Center, University of Amsterdam, Amsterdam,[5] and Department of Gastroenterology and Hepatology, University Hospital Dijkzigt, Rotterdam,[6] The Netherlands

Received 12 June 2001/Returned for modification 27 August 2001/Accepted 15 October 2001

Since the associations between *Helicobacter pylori* genotype and disease differ in Asia and the West, we investigated the correlation between HP0638, encoding an outer membrane protein, and potential markers of virulence (*cagA*, *vacA*, and *iceA*). For 109 strains from nine countries, the status of *cagA*, *vacA*, and *iceA* was determined by PCR and/or a line probe assay. We also studied 18 strains from 8 patients (parents and 6 daughters) from a Dutch family and paired strains collected on average 8 years apart from 11 patients. When the HP0638 signal sequences were amplified by PCR and DNA sequence determinations were performed, 89 (96%) of 93 *cagA*-positive strains had HP0638 in frame, versus none (0%) of 16 *cagA*-negative strains ($P < 0.001$). Among strains in which HP0638 was in frame, a six-CT dinucleotide repeat pattern was dominant in Western countries (23 of 33 strains [70%]), while a pattern of three CT repeats with another CT after four T's (3 + 1-CT-repeat pattern) was dominant in East Asia (31 of 46 strains [67%]); however, specific CT repeat patterns did not correlate with clinical outcome. HP0638 phylogenetic trees also showed geographic characters. The HP0638 frame status and CT dinucleotide repeat patterns were identical for 9 of 11 pairs of strains obtained on average 8 years apart from individuals and the 15 strains obtained from the mother and all six daughters. Thus, HP0638 frame status and *cagA* status are strongly correlated. The CT dinucleotide repeat pattern in the putative HP0638 signal sequence has geographic characters and appears stable in particular patients and families over a period of years. Analysis of HP0638 CT polymorphisms may serve as a new typing system to discriminate *H. pylori* isolates for epidemiological purposes.

Helicobacter pylori, a microaerophilic, gram-negative bacterium that colonizes the human stomach, is involved in the pathogenesis of peptic ulcer disease and gastric cancer (GCA) (8, 9). *H. pylori* is highly genetically diverse, with isolates being easily distinguishable by various fingerprinting techniques (2, 3, 32) or by sequencing of representative gene segments (16). Extensive interstrain gene transfer and recombination are important causes of such diversity (1, 18, 31).

Recently, several *H. pylori* genes related to the risk of disease were identified (22). The cytotoxin-associated gene (*cagA*) is a marker for the *cag* island, whose presence is associated with a more severe clinical outcome (9, 12, 13, 15, 20, 36). The *cag* island genes encode proteins that enhance the virulence of the strain, for example, by increasing host cell cytokine production (5, 12, 35) and by altering protein tyrosine phosphorylation (24). A protein that induces vacuoles in epithelial cells is encoded by *vacA* (14, 21, 29, 33). *vacA* is present in all *H. pylori* strains and contains at least two variable regions (6). The s region (encoding the signal peptide) exists as s1 (including s1a, s1b, and s1c) or s2 allelic types (39). The m region (middle) occurs as m1 or m2 allelic types. Most *vacA* s1 strains are *cagA* positive, and most *vacA* s2 strains are *cagA* negative (6), even though these two genetic elements do not have any physical linkage on the *H. pylori* chromosome (10). The *vacA* and *cagA* DNA sequences in strains from the United States and Europe differ from those of strains in China and Japan (7, 17, 26, 38, 39, 41, 46). For example, in Western countries, most s1 strains are s1a or s1b, while about 80% of the s1 strains from East Asia are s1c (39). The allelic groupings of *babA* and *babB*, other outer membrane protein genes, are independent of one another, and each allelic group has geographic variations (28). *cagA*-positive strains frequently are *vacA* s1, *vacA* m1, and *iceA1*. The *iceA1* genotype also is associated with *vacA* s1 and *vacA* m1 (42).

Several previous studies addressed whether *H. pylori* clusters geographically by examining sequences of housekeeping genes (1), *ureAB* (11), and *cagA* (23, 41, 45); these reports indicated that *H. pylori* sequences have geographic characters. However, Asian and Western strains remain difficult to distinguish, and further genetic markers are needed to reliably differentiate strains from different regions of the world.

Gram-negative bacterial outer membranes mediate the interaction with the surrounding environment. For *H. pylori* to survive and persist in the gastric milieu, specific adaptations involving its outer membrane proteins would be expected. The expression of HP0638, encoding the OipA outer membrane protein, has been reported to correlate with interleukin 8

* Corresponding author. Mailing address: First Department of Internal Medicine, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, Aichi 466-8550, Japan. Phone: 81-52-744-2144. Fax: 81-52-744-2157. E-mail: andot01@mcmbox.med.nyu.edu.

TABLE 1. Characteristics of the 109 *H. pylori* strains studied

Origin	Clinical diagnosis	No. of strains
Asian countries (n = 54)		
Japan	DU	9
	GU	16
	GCA	6
	NUD	7
China	DU	4
	GCA	4
India	DU	1
	NUD	7
Western countries (n = 55)		
United States		
Caucasian	DU	10
	GU	1
	NUD	17
African-Americans	DU	3
	GU	2
	NUD	5
Europe	DU	4
	GU	1
	NUD	8
Colombia	DU	1
	GU	3

(IL-8) induction (44). Variations in dinucleotide repeats located in the region encoding the signal sequence determine whether or not the complete open reading frame (ORF) is in frame. Since the associations between *H. pylori* genotype and disease differ in Asia and the West, we hypothesized that the expression of HP0638 might correlate with that of other genes associated with inflammation, such as *cagA*, and that geographically based alleles might exist. We also sought to determine whether HP0638 frame status is stable in isolates from individuals over time and among members of a family.

MATERIALS AND METHODS

Bacterial strains. We studied *H. pylori* isolates from 109 patients undergoing upper gastrointestinal tract endoscopy in six different areas (38 in the United States, 13 in Europe, 4 in Colombia, 38 in Japan, 8 in China, and 8 in India) (Table 1). For each patient, clinical status had been determined as described previously (5). From the 109 patients, biopsy specimens were homogenized with a glass rod and incubated on brucella agar plates with 10% newborn calf serum (BS agar; Intergen) for 5 to 7 days at 37°C in a 5% CO_2 atmosphere. A single colony was picked and streaked for isolation on a BS agar plate for 3 days, and *H. pylori* strains were identified by Gram staining and catalase, oxidase, and urease assays. We also examined a group of 18 strains previously collected from 8 patients (parents and 6 adult daughters) from one extended Dutch family (37) and paired strains collected on average 8 years apart from 11 patients as described previously (19).

Assessment of *H. pylori cagA*, *vacA*, and *iceA1* status. A chloroform-phenol extraction method was used to obtain DNA from the *H. pylori* isolates as described previously (43). Analyses for the presence of *cagA*, *vacA* s- and m-region allelic types, and *iceA* subtypes were done by PCR with established primers (6, 36, 40). PCRs were performed by standard methods with a reaction volume of 50 μl containing 0.5 U of *Taq* (Qiagen), 1.5 mM $MgCl_2$, and 200 ng of each primer. The PCR protocol (30 cycles) included a denaturing step at 94°C for 1 min, annealing at 5°C below the predicted melting temperature of the primers for 1 min, and extension at 72°C for 1 min/kb of amplification product. *cagA* and *vacA* status also had been confirmed by reverse hybridization with a line probe assay (40).

Assessment of HP0638 status. According to the complete genome sequence of *H. pylori* strains 26695 and J99 (4, 34), CT dinucleotide repeats are located in the region encoding the signal sequence of HP0638. The region of the signal sequence of HP0638 including the repeats was amplified by PCR with primers AN9260 (5′-CAAGCGCTTAACAGATAGGC-3′) and AN9261 (5′AAGGCGT TTTCTGCTGAAGC-3′) (44). PCR products were purified by using a QiaQuick PCR purification kit and a QiaQuick gel extraction kit (both kits from Qiagen); directly sequenced on both strands by using an automated Applied Biosystems, Inc., sequencer at the New York University Medical Center DNA Sequencing Core Facility; and analyzed by using Sequencer 3.1.1 (Gene Code Corp., Inc., Ann Arbor, Mich.).

Phylogenetic analysis. Alignments of HP0638 nucleotides 1 to 150 derived from the PCR with primer pair AN9260-AN9261 were created by using GCG Pileup (Wisconsin Package version 9.1), and phylograms were constructed.

RESULTS

***cagA*, *vacA*, and HP0638 status of *H. pylori* strains from different geographic areas.** The 109 strains studied could be divided into dichotomous groups by *cagA* status (positive, 93; negative, 16), *vacA* s allelic type status (s1, 95; s2, 14), *vacA* m allelic type status (m1, 80; m2, 29), and *iceA* status (*iceA1*, 61; *iceA2*, 48). HP0638 in-frame status was more correlated with *cagA*-positive (100%; $P < 0.001$), *vacA* s1 (100%; $P < 0.001$), *vacA* m1 (83%; $P < 0.001$), and *iceA1* (62%; $P < 0.01$) than HP0638 out-of-frame status (*cagA* positive, 20%; *vacA* s1, 20%; *vacA* m1, 30%; and *iceA1*, 30%). HP0638 was in frame in all 54 strains from East Asia and India and in 35 (64%) of the 55 strains from western countries, essentially mirroring *cagA* status (Table 2). Of the 109 patients studied, those carrying strains in which HP0638 was in frame had the more severe diseases (duodenal ulcer [DU], 30%; gastric ulcer [GU], 25%; nonulcer dyspepsia [NUD], 34%; and GCA, 11%) than did those with strains in which HP0638 was out of frame (DU, 25%; GU, 5%; and NUD, 70%). Since all 54 strains from Asian patients both were *cagA* positive and had HP0638 in frame, no analysis of independent relationships with clinical findings was possible. For the 55 patients from western countries, the effects of *cagA* positivity, HP0638 frame status, and clinical outcome also could not be dissociated (Table 2). All 16 *cagA*-negative strains had *vacA* s2 alleles, and all 93 *cagA*-positive strains had s1 alleles. Among the 93 *cagA*-positive strains, 54 Asian strains had the s1c (43) or s1a (11) allele, whereas 39 western strains had the s1a (14) or s1b (25) allele.

Analysis of HP0638 variants and *vacA* s-region allelic types. The sequence heterogeneity of nucleotides 1 to 150 of the HP0638 PCR products was examined. From each sample, the PCR amplicon was of the expected size. Phylogenetic analysis of these sequences revealed the existence of three major groupings based on geographic origin (Fig. 1). Strains from East Asia appeared exclusively in group A, six of the eight Indian strains constituted group B, and Western strains comprised group C. Group C was further subdivided into subgroups C1 and C2. C1 consisted of strains from Caucasian individuals only, whereas strains from African-Americans appeared only in subgroup C2. Phylogenetic analysis of the HP0638 fragment retained geographic characters even when the CT repeat region was removed (data not shown).

CT dinucleotide repeats in the signal peptide coding region of HP0638. CT repeat patterns differed between the groups of strains based on their geographic origin (Tables 2 and 3).

TABLE 2. Variations in HP0638 CT repeat region sequences in 109 *H. pylori* strains from six geographic regions

Origin	*cagA* genotype[a]	No. of CT repeats	HP0638 CT repeat region sequence	Frame status	Total	No. of isolates by diagnosis			
						DU	GU	NUD	GCA
Asian countries									
Japan (n = 38)	+	1 + 1 + 1	CTTT**CT**GT**CT**TT**CT**CGTT	In	3	2	1	0	0
		1 + 1 + 2	CTAA**CT**TT**CT**TT(**CT**)$_2$CGTT	In	1	0	1	0	0
		1 + 3	CTTT**CT**GT(**CT**)$_3$CGTT	In	2	0	2	0	0
		2 + 1	CTTT(**CT**)$_2$TTTT**CT**CGTT	In	1	0	0	1	0
		3 + 1	CTAA(**CT**)$_3$TTTT**CT**CGTT	In	26	6	10	6	4
		3 + 2	CTAA(**CT**)$_3$TT(**CT**)$_2$CGTT	In	1	0	1	0	0
		5	CTTTTACTAA(**CT**)$_5$TTCGTT[b]	In	4	1	1	0	2
China (n = 8)	+	2 + 1	CTTTTA(**CT**)$_2$TT**CT**GTCT	In	1	1	0	0	0
		3	CTAA(**CT**)$_3$TTTTATCGTT	In	1	1	0	0	0
		3 + 1	CTAA(**CT**)$_3$TTTT**CT**CGTT	In	5	2	0	0	3
		3 + 2	CTAA(**CT**)$_3$TT(**CT**)$_2$CGTT	In	1	0	0	0	1
India (n = 8)	+	1 + 1 + 1	CTTT**CT**GT**CT**TT**CT**CGTT	In	1	0	0	1	0
		2 + 3	CTAA(**CT**)$_2$TT(**CT**)$_3$CGTT	In	1	0	0	1	0
		3 + 1	CTAA(**CT**)$_3$TTTT**CT**CGTT	In	2	0	0	2	0
		6	CTTACTAA(**CT**)$_6$CGTT	In	4	1	0	3	0
Total	+			In	54	14	16	14	10
Western countries									
Europe (n = 13)	+	6	CTTACTAA(**CT**)$_6$CGTT	In	5	2	1	2	0
		8	CTTA(**CT**)$_8$CGTT[c]	In	1	0	0	1	0
		9	CTTACTAA(**CT**)$_9$CGTT[d]	In	2	1	0	1	0
		7	CTTA(**CT**)$_7$CGTT	Out	1	0	0	1	0
		8	CTTACTAA(**CT**)$_8$CGTT[c]	Out	1	0	0	1	0
	−	9	CTTA(**CT**)$_9$CGTT[d]	Out	1	0	0	1	0
		10	CTTTTA(**CT**)$_{10}$CGTT	Out	2	1	0	1	0
United States									
Caucasians (n = 28)	+	6	CTTACTAA(**CT**)$_6$CGTT	In	13	7	0	6	0
		9	CTTACTAA(**CT**)$_9$CGTT[d]	In	3	0	1	2	0
		4	ATAA(**CT**)$_4$CGTT	Out	1	0	0	1	0
		12	CTTA(**CT**)$_{12}$CGTT[e]	Out	1	1	0	0	0
	−	5	CTTACTAA(**CT**)$_5$CGTT[b]	Out	3	0	0	3	0
		7	CTTACTAA(**CT**)$_7$CGTT	Out	5	1	0	4	0
		8	CTTACTAA(**CT**)$_8$CGTT[c]	Out	1	0	0	1	0
		9	CTTA(**CT**)$_9$CGTT[d]	Out	1	1	0	0	0
African-Americans (n = 10)	+	5	CTTACTAACC(**CT**)$_5$CGTT[b]	In	2	1	0	1	0
		6	CTTACTAA(**CT**)$_6$CGTT	In	5	2	1	2	0
		9	CTTACTAA(**CT**)$_9$CGTT[d]	In	1	0	1	0	0
		12	CTTACTAA(**CT**)$_{12}$CGTT[e]	In	1	0	0	1	0
	−	7	CTTACTAA(**CT**)$_7$CGTT	Out	1	0	0	1	0
Colombia (n = 4)	+	6	CTTACTAA(**CT**)$_6$CGTT	In	1	0	1	0	0
		9	CTTACTAA(**CT**)$_9$CGTT[d]	In	1	0	1	0	0
	−	8	CTTACTAA(**CT**)$_8$CGTT[c]	Out	2	1	1	0	0
Total	+			In	35	13	6	16	0
				Out	4	1	0	3	0
	−			Out	16	4	1	11	0

[a] +, positive; −, negative.
[b] ORF with five CT repeats is in frame due to insertion of TT sequence 6 bp upstream of CT repeats or insertion of CC sequence immediately upstream of CT repeats.
[c] ORF with eight CT repeats is in frame due to deletion of CTAA sequence immediately upstream of CT repeats.
[d] ORF with nine CT repeats is out of frame due to deletion of CTAA sequence immediately upstream of CT repeats.
[e] ORF with 12 CT repeats is out of frame due to deletion of CTAA sequence immediately upstream of CT repeats.

Among the 46 strains isolated from East Asia, all had ≤5 CT repeats (Table 3, values in boldface), and the pattern of three CT repeats with another after four T's (3 + 1-CT-repeat pattern) was the most common (67%). Conversely, among the 35 strains in which HP0638 was in frame and which were isolated from western countries, all but 2 had ≥6 CT repeats (Table 3, values in boldface), and the six-CT-repeat pattern was the most common (24 of 35 strains [69%]). The strains from India had mixed CT repeat patterns (Tables 2 and 3). Twenty strains in which HP0638 was out of frame showed variable CT repeat

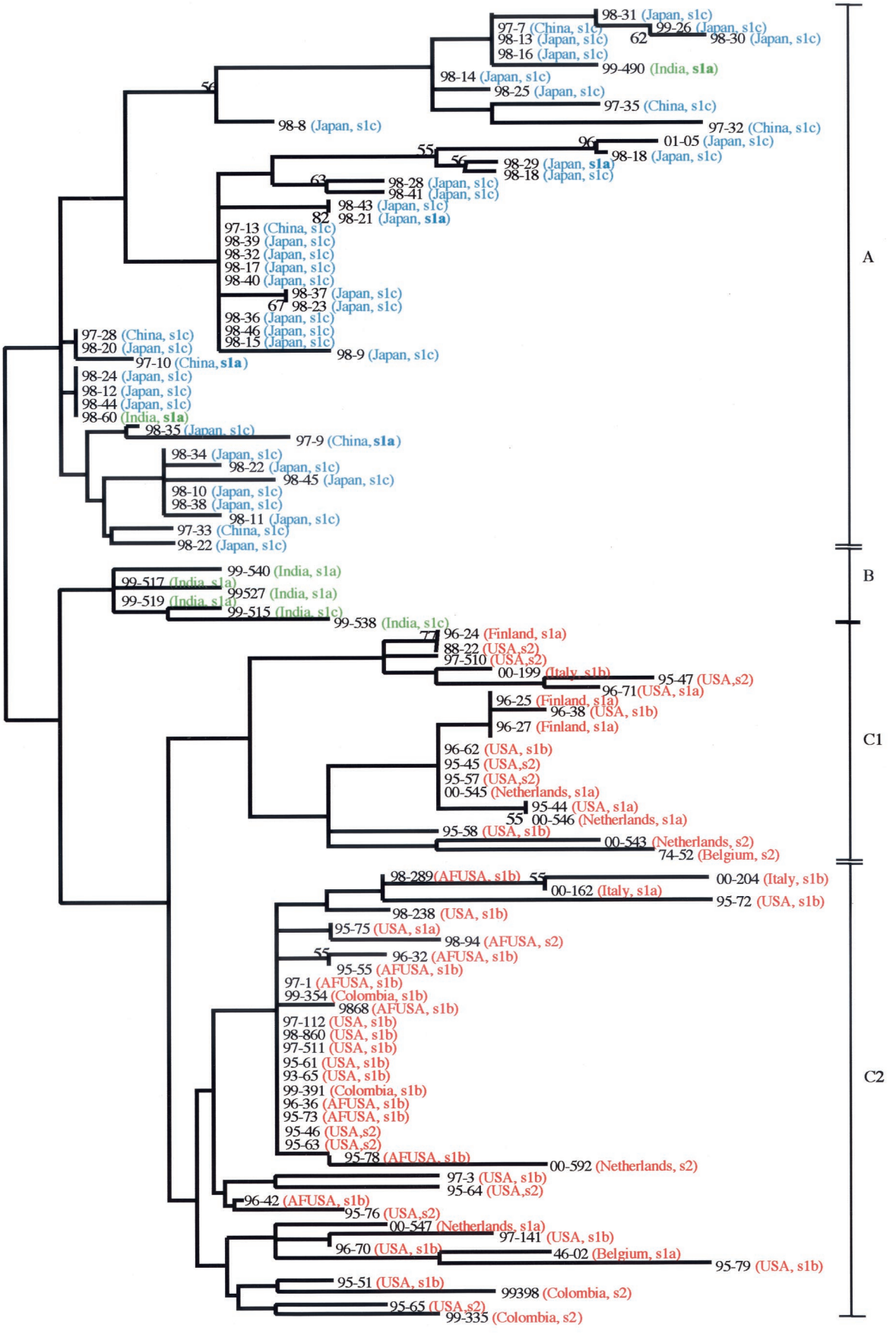
98-31 (Japan, s1c)
97-7 (China, s1c)
99-26 (Japan, s1c)
98-13 (Japan, s1c)
62
98-30 (Japan, s1c)
98-16 (Japan, s1c)
99-490 (India, s1a)
98-14 (Japan, s1c)
98-25 (Japan, s1c)
97-35 (China, s1c)
98-8 (Japan, s1c)
97-32 (China, s1c)
01-05 (Japan, s1c)
98-18 (Japan, s1c)
98-29 (Japan, s1a)
98-18 (Japan, s1c)
98-28 (Japan, s1c)
98-41 (Japan, s1c)
98-43 (Japan, s1c)
98-21 (Japan, s1a)
97-13 (China, s1c)
98-39 (Japan, s1c)
98-32 (Japan, s1c)
98-17 (Japan, s1c)
98-40 (Japan, s1c)
98-37 (Japan, s1c)
98-23 (Japan, s1c)
98-36 (Japan, s1c)
98-46 (Japan, s1c)
98-15 (Japan, s1c)
98-9 (Japan, s1c)
97-28 (China, s1c)
98-20 (Japan, s1c)
97-10 (China, s1a)
98-24 (Japan, s1c)
98-12 (Japan, s1c)
98-44 (Japan, s1c)
98-60 (India, s1a)
98-35 (Japan, s1c)
97-9 (China, s1a)
98-34 (Japan, s1c)
98-22 (Japan, s1c)
98-45 (Japan, s1c)
98-10 (Japan, s1c)
98-38 (Japan, s1c)
98-11 (Japan, s1c)
97-33 (China, s1c)
98-22 (Japan, s1c)
A
99-540 (India, s1a)
99-517 (India, s1a)
99527 (India, s1a)
99-519 (India, s1a)
99-515 (India, s1c)
99-538 (India, s1c)
B
96-24 (Finland, s1a)
88-22 (USA,s2)
97-510 (USA,s2)
00-199 (Italy, s1b)
95-47 (USA,s2)
96-71(USA, s1a)
96-25 (Finland, s1a)
96-38 (USA, s1b)
96-27 (Finland, s1a)
96-62 (USA, s1b)
95-45 (USA,s2)
95-57 (USA,s2)
00-545 (Netherlands, s1a)
95-44 (USA, s1a)
55
00-546 (Netherlands, s1a)
95-58 (USA, s1b)
00-543 (Netherlands, s2)
74-52 (Belgium, s2)
C1
98-289(AFUSA, s1b)
55
00-204 (Italy, s1b)
00-162 (Italy, s1a)
95-72 (USA, s1b)
98-238 (USA, s1b)
95-75 (USA, s1a)
98-94 (AFUSA, s2)
55
96-32 (AFUSA, s1b)
95-55 (AFUSA, s1b)
97-1 (AFUSA, s1b)
99-354 (Colombia, s1b)
9868 (AFUSA, s1b)
97-112 (USA, s1b)
98-860 (USA, s1b)
97-511 (USA, s1b)
95-61 (USA, s1b)
93-65 (USA, s1b)
99-391 (Colombia, s1b)
96-36 (AFUSA, s1b)
95-73 (AFUSA, s1b)
95-46 (USA,s2)
95-63 (USA,s2)
95-78 (AFUSA, s1b)
00-592 (Netherlands, s2)
97-3 (USA, s1b)
95-64 (USA,s2)
96-42 (AFUSA, s1b)
95-76 (USA,s2)
00-547 (Netherlands, s1a)
97-141 (USA, s1b)
96-70 (USA, s1b)
46-02 (Belgium, s1a)
95-79 (USA, s1b)
95-51 (USA, s1b)
99398 (Colombia, s2)
95-65 (USA,s2)
99-335 (Colombia, s2)
C2

TABLE 3. Comparison of variations in HP0638 CT repeat region sequences among the 109 *H. pylori* strains, grouped by HP0638 frame status

HP0638 status	No. (%) of *cagA*-positive strains	No. of CT repeats	No. of strains by geographic region[a]			
			East Asia (n = 46)	India (n = 8)	Western countries (n = 55)	Total (n = 109)
In frame (n = 89)	89 (100)	3 + 1	**31**	2	0	33
		<4 (other than 3 + 1)	**11**	2	0	13
		5	**4**	0	2	6
		6	0	4	**24**	28
		8	0	0	**1**	1
		9	0	0	**7**	7
		12	0	0	**1**	1
Total			46	8	35	89
Out of frame (n = 20)	4 (20)	4	0	0	1	1
		5	0	0	3	3
		7	0	0	7	7
		8	0	0	4	4
		9	0	0	2	2
		10	0	0	2	2
		12	0	0	1	1
Total			0	0	20	20

[a] For values shown in boldface, the distribution of CT repeats was significantly ($P < 0.001$) different between East Asia isolates (all 46 had ≤5 repeats) and Western isolates (33 of 35 had ≥6 repeats).

patterns. There was no correlation between specific CT repeat patterns and clinical outcome (DU, GU, NUD, or GCA) (Table 2). In strains with both in-frame and out-of-frame HP0638, 5-, 8-, 9-, or 12-CT-repeat patterns were found (Tables 2 and 3). In strains in which a TT sequence was present 6 bp upstream of the CT repeats or a CC sequence was present immediately upstream of the CT repeats, HP0638 was in frame, even when five CT repeats were present. For strains with CTAA deleted immediately upstream of the CT repeats, frame status depended on the number of repeats (8, in frame; 9 or 12, out of frame) (Table 2).

HP0638 status of strains from an extended family. Next, we examined 18 strains from an extended Dutch family, including parents and six adult daughters (Table 4). Since the 15 strains from the mother and from her daughters all had been shown to have nearly identical randomly amplified polymorphic DNA patterns, they were believed to be from the same origin, whereas the strains from the father clearly differed (37). Each of the 15 strains from the mother or daughters showed the same unique 5 + 2-CT-repeat pattern, and HP0638 was in frame in all. In contrast, the father's strains showed a six- or seven-CT-repeat pattern, and all had HP0638 out of frame (Table 4).

HP0638 status of paired isolates obtained years apart from the same host. Next, we examined HP0638 frame status for pairs of *H. pylori* isolates obtained an average 8 years apart from each of 11 persons (Table 5). Nine of 11 strain pairs (patients 3 to 13) were highly related to one another, based on *recA* sequence analysis and on randomly amplified polymorphic DNA and amplified fragment length polymorphism analyses (19). In two (22%) of the nine patients, the number of CT repeats differed between the first and second isolates and, in both patients, HP0638 was out of frame in the first isolate. In only one patient (patient 5) did the HP0638 frame status differ between the two isolates; despite this difference, both isolates were *cagA* negative. In patient 9, the numbers of CT repeats were eight in the first isolate and seven in the second, but in both isolates HP0638 was out of frame.

DISCUSSION

Although *H. pylori* has worldwide distribution (39, 41), the important differences in the *vacA* s1 alleles (39) and in the 5′ region of *cagA* (41), among other polymorphisms, strongly indicate that certain strains predominate in specific geographic areas. Despite evidence suggesting such geographic characters for *H. pylori* (1, 11, 23, 45), it remains difficult to clearly stratify *H. pylori* strains into geographic groupings. Therefore, we examined additional strain-specific markers that clearly differentiate *H. pylori* strains. We found clear geographic differences in HP0638 sequences for the first 150 nucleotides (Fig. 1), and we found geographic characters even for the CT repeat patterns in the signal sequence region (Table 3). HP0638 frame status correlated strongly with *cagA* and *vacA* status; all of the strains

FIG. 1. Phylogram of *H. pylori* strains based on the first 150 bp of the HP0638 ORF. Regions from 109 strains were aligned by using GCG Pileup, subjected to phylogenetic analysis by using Paup 4.0b2, and subjected to neighbor-joining analysis based on Kimura's two-parameter model distance matrices (25). Isolates from East Asia (blue) and western countries (red) segregate into two major branches (A and C). Branch C can be divided into two subbranches (C1 and C2); all strains from African-Americans (AFUSA) are on subbranch C2. *vacA* s-region allelic types also are shown. Branch B, which is paraphyletic to the other two major branches, is composed of strains from the Indian subcontinent (green).

TABLE 4. Variations in HP0638 CT repeat region sequences among members of an extended family in The Netherlands[a]

Strain designation	Patient age (yr)[b]	Family relationship	*cagA* status	No. of CT repeats	HP0638 CT repeat region sequence	Frame status
1a	60	Father	−	6	CTCTTA$(CT)_6$CGTTTTG	Out
1b			−	7	CTCTTA$(CT)_7$CGTTTTG	Out
1c			−	7	CTCTTA$(CT)_7$CGTTTTG	Out
2a	55	Mother	+	5 + 2	CTTTTA$(CT)_5$TT$(CT)_2$CGTT	In
2b			−	5 + 2	CTTTTA$(CT)_5$TT$(CT)_2$CGTT	In
3a	35	Daughter 1	+	5 + 2	CTTTTA$(CT)_5$TT$(CT)_2$CGTT	In
4a	32	Daughter 2	+	5 + 2	CTTTTA$(CT)_5$TT$(CT)_2$CGTT	In
4b			−	5 + 2	CTTTTA$(CT)_5$TT$(CT)_2$CGTT	In
5a	29	Daughter 3	+	5 + 2	CTTTTA$(CT)_5$TT$(CT)_2$CGTT	In
5d			−	5 + 2	CTTTTA$(CT)_5$TT$(CT)_2$CGTT	In
6a	26	Daughter 4	+	5 + 2	CTTTTA$(CT)_5$TT$(CT)_2$CGTT	In
6b			+	5 + 2	CTTTTA$(CT)_5$TT$(CT)_2$CGTT	In
6c			+	5 + 2	CTTTTA$(CT)_5$TT$(CT)_2$CGTT	In
7a	25	Daughter 5	+	5 + 2	CTTTTA$(CT)_5$TT$(CT)_2$CGTT	In
7b			+	5 + 2	CTTTTA$(CT)_5$TT$(CT)_2$CGTT	In
8a	24	Daughter 6	+	5 + 2	CTTTTA$(CT)_5$TT$(CT)_2$CGTT	In
8b			+	5 + 2	CTTTTA$(CT)_5$TT$(CT)_2$CGTT	In
8c			+	5 + 2	CTTTTA$(CT)_5$TT$(CT)_2$CGTT	In

[a] Strain designations, family relationships, and *cagA* status (−, negative; +, positive) were as previously described (37).
[b] Age on date when *H. pylori* culture was obtained.

in which HP0638 was in frame were *cagA* positive and *vacA* s1, whereas most of the strains in which HP0638 was out of frame were *cagA* negative (80%) and *vacA* s2 (70%). In our study, HP0638 frame status was most closely correlated with *cagA* status, suggesting that *cagA* positivity could affect selection for in-frame status. Further studies are needed to assess the correlation between HP0638 status and *cagA* positivity.

That the isolates from all the daughters in the extended Dutch family shared the same 5 + 2-CT-repeat pattern and HP0638 in-frame status with the isolates from the mother but not the father further supports the presumption that the daughters acquired their *H. pylori* isolates from their mother, confirming and extending previous findings (37). These findings also suggest the stability of the HP0638 frame status and CT repeat pattern under in vivo conditions over the presumed combined 150 person-years of colonization. The stability of HP0638 sequences in this setting encourages us to examine other epidemiologically related strains to assess variability and to determine the underlying causes of variability. The CT repeat differences observed in the HP0638 signal sequence regions in two of nine paired strains from the same hosts (Table 4) are consistent with the presumed high frequency of slipped-strand mispairing associated with dinucleotide repeats (27) and further support the concept that the *H. pylori* population in an individual host represents a mixture of closely related clonal variants, or "quasispecies" (19). That the 206-nucleotide sequence within *recA* was identical within each pair (19) indicates that the strains from each patient had a similar origin and suggests that the CT repeat region may be hypervariable. Alternatively, it is possible that the numbers of HP0638 CT repeats in the paired strains changed as a result of in vitro passage. Arguing against that hypothesis is the finding that the 15 related isolates obtained from the extended family were all identical for frame status and number of CT repeats (Table 5). Further long-term in vitro passages and animal models will help elucidate whether in vivo variation or in vitro passage accounts for changes in HP0638 frame status in *H. pylori*.

Of the 109 patients studied, those carrying strains in which HP0638 was in frame had more severe diseases than did those with strains in which HP0638 was out of frame (NUD, [70%]) (Table 3). Among the strains of each group, with HP0638 either in frame or out of frame, there was no correlation between specific CT repeat patterns and clinical outcome. Thus, among HP0638 polymorphisms, only HP0638 frame status affected clinical status. However, the correlation of HP0638 frame status with *cagA* status was so strong (Table 2) that it was not possible to establish the relationship of HP0638 status to pathogenicity. Yamaoka et al. (44) reported that HP0638 mutants induced significantly lower IL-8 levels from AGS gastric epithelial cells than did their wild-type parent strains. However, in a recent study (5a), HP0638 disruption did not affect IL-8 production or CagA tyrosine phosphorylation status in AGS gastric epithelial cells. Akanuma et al. [M. Akanuma, K. Ogura, S. Maeda, Y. Mitsuno, Y. Hirata, H. Yoshida, Y. Shiratori, and M. Omata, Abstr. Gastroenterol. **120**(Suppl. 1)**:**A-100, 2001] also reported that HP0638 mutants induced IL-8 levels similar to those induced by their wild-type parent strains but that HP0638 is important for gastric *H. pylori* infection of gerbils. In vitro experiments indicate that IL-8 is induced in epithelial cells after viable *H. pylori* organisms attach (30); outer membrane proteins would be good candidates for the unknown proinflammatory virulence factors. Further study of

TABLE 5. Variations in HP0638 CT repeat region sequences in paired *H. pylori* strains obtained 7 to 10 years apart from the same host[a]

Strain designation	Yr apart	*H. pylori* genotype					No. of CT repeats	HP0638 CT repeats region sequence	Frame status
		cagA	*vacA*		*iceA*	IS*605*			
			s type	m type					
1A	8.8	+	s1a	m1	*2*	−	6	$CTAA(CT)_6CGTT$	In
1B		+	s1a	m1	*1*	+	6	$CTAA(CT)_6CGTT$	In
2A	7.2	−	s2	m2	*2*	−	6	$CTAA(CT)_6CGTT$	In
2B		+	s1a	m1	*2*	−	6	$CTAA(CT)_6CGTT$	In
3A	7.9	−	s2	m2	*2*	+	10	$CTAA(CT)_{10}CGTT$	Out
3B		−	s2	m2	*2*	+	10	$CTAA(CT)_{10}CGTT$	Out
4A	8.7	+	s1a	m1	*1*	+	6	$CTAA(CT)_6CGTT$	In
4B		+	s1a	m1	*1*	+	6	$CTAA(CT)_6CGTT$	In
5A	7.0	−	s2	m2	*2*	−	7	$CTAA(CT)_7CGTT$	Out
5B		−	s2	m2	*2*	−	6	$CTAA(CT)_6CGTT$	In
7A	7.0	+	s1a	m1	*2*	−	6	$CTAA(CT)_6CGTT$	In
7B		+	s1a	m1	*2*	−	6	$CTAA(CT)_6CGTT$	In
8A	9.8	+	s1a	m1	*1*	+	6	$CTAA(CT)_6CGTT$	In
8B		+	s1a	m1	*1*	+	6	$CTAA(CT)_6CGTT$	In
9A	8.8	−	s2	m2	*2*	−	8	$CTAA(CT)_8CGTT$	Out
9B		−	s2	m2	*2*	−	7	$CTAA(CT)_7CGTT$	Out
10A	10.2	+	s1b	m1	*2*	−	6	$CTAA(CT)_6CGTT$	In
10B		+	s1b	m1	*2*	−	6	$CTAA(CT)_6CGTT$	In
11A	7.8	+	s1a	m1	*2*	−	6	$CTAA(CT)_6CGTT$	In
11B		+	s1a	m1	*2*	−	6	$CTAA(CT)_6CGTT$	In
13A	7.4	+	s1a	m1	*1*	+	6	$CTAA(CT)_6CGTT$	In
13B		+	s1a	m1	*1*	+	6	$CTAA(CT)_6CGTT$	In

[a] Strain designations and genotypes (+, positive; −, negative) were as described previously (19).

the role of HP0638 as well as other outer membrane proteins in *H. pylori* pathogenesis is needed.

In summary, the phylogeny of HP0638 has geographic characters, and its frame status is correlated with *cagA*, *vacA*, and *iceA* genotypes. The CT dinucleotide repeat pattern in the putative signal sequence of HP0638 also has geographic characters and appears relatively stable in individual patients and families over periods of years. Nevertheless, strains obtained from the same host and appearing otherwise identical can vary in frame status. HP0638 frame status but not the number or pattern of CT repeats is correlated with clinical outcome. If confirmed in independent studies, analysis of HP0638 polymorphisms may be useful as an *H. pylori* typing system for epidemiological purposes.

ACKNOWLEDGMENTS

This work was supported in part by grants R01GM63270, R01DK53707, K08DK02381, and R29CA77955 from the National Institutes of Health and by the Medical Research Service of the Department of Veterans Affairs.

REFERENCES

1. **Achtman, M., T. Azuma, D. E. Berg, Y. Ito, G. Morelli, Z. J. Pan, S. Suerbaum, S. A. Thompson, A. van der Ende, and L. J. van Doorn.** 1999. Recombination and clonal groupings within *Helicobacter pylori* from different geographical regions. Mol. Microbiol. **32:**459–470.
2. **Akopyants, N. S., A. Fradkov, L. Diatchenko, J. E. Hill, P. D, Siebert, S. A. Lukyanov, E. D. Sverdlov, and D. E. Berg.** 1998. PCR-based subtractive hybridization and differences in gene content among strains of *Helicobacter pylori*. Proc. Natl. Acad. Sci. USA **95:**13108–13113.
3. **Akopyanz, N. S., N. O. Bukanov, T. U. Westblom, S. Kresovich, and D. E. Berg.** 1992. DNA diversity among clinical isolates of *Helicobacter pylori* detected by PCR-based RAPD fingerprinting. Nucleic Acids Res. **20:**5137–5142.
4. **Alm, R. A., L. S. Ling, D. T. Moir, B. L. King, E. D. Brown, P. C. Doig, D. R. Smith, B. Noonan, B. C. Guild, B. L deJonge, G. Carmel, P. J. Tummino, A. Caruso, M. Uria-Nickelsen, D. M. Mills, C. Ives, R. Gibson, D. Merberg, S. D. Mills, Q. Jiang, D. E. Taylor, G. F. Vovis, and T. J. Trust.** 1999. Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. Nature **397:**176–180.
5. **Ando, T., G. I. Perez-Perez, K. Kusugami, M. Ohsuga, K. C. Bloch, and M. J. Blaser.** 2000. Anti-CagA immunoglobulin G responses correlate with interleukin-8 induction in human gastric mucosal biopsy culture. Clin. Diagn. Lab. Immunol. **7:**803–809.

5a. **Ando, T., R. M. Peek, Y.-C. Lee, U. Krishna, K. Kusugami, and M. J. Blaser.** Host cell responses to genotypically similar *Helicobacter pylori* isolates from United States and Japan. Clin. Diagn. Lab. Immunol., in press.

6. **Atherton, J. C., P. Cao, R. M. Peek, Jr., 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.
7. **Atherton, J. C., P. M. Sharp, T. L. Cover, G. Gonzalez-Valencia, R. M. Peek, S. A. Thompson, C. J. Hawkey, M. J. Blaser.** 1999. Vacuolating cytotoxin (*vacA*) alleles of *Helicobacter pylori* comprise two geographically widespread types, m1 and m2, and have evolved through limited recombination. Curr. Microbiol. **39:**211–218.
8. **Blaser, M. J.** 1999. Hypothesis: the changing relationships of *Helicobacter pylori* and humans: implications for health and disease. J. Infect. Dis. **179:**1523–1530.
9. **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.
10. **Bukanov, N. O., and D. E. Berg.** 1994. Ordered cosmid library and high-resolution physical-genetic map of *Helicobacter pylori* strain NCTC11638. Mol. Microbiol. **11:**509–523.
11. **Campbell, S., A. Fraser, B. Holliss, J. Schmid, and P. W. O'Toole.** 1997. Evidence for ethnic tropism of *Helicobacter pylori*. Infect. Immun. **65:**3708–3712.
12. **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.
13. **Covacci, A., S. Censini, M. Bugnoli, R. Petracca, D. Burroni, G. Macchia, A. Massone, E. Papini, Z. Xiang, N. Figura, and R. Rappuoli.** 1993. Molecular characterization of the 128-kDa immunodominant antigen of *Helicobacter pylori* associated with cytotoxicity and duodenal ulcer. Proc. Natl. Acad. Sci. USA **90:**5791–5795.
14. **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.
15. **Cover, T. L., Y. Glupczynski, A. P. Lage, A. Burette, M. K. Tummuru, G. I. Perez-Perez, and M. J. Blaser.** 1995. Serologic detection of infection with *cagA*$^+$ *Helicobacter pylori* strains. J. Clin. Microbiol. **33:**1496–1500.
16. **Garner, J. A., and T. L. Cover.** 1995. Analysis of genetic diversity in cytotoxin-producing and non-cytotoxin-producing *Helicobacter pylori* strains. J. Infect. Dis. **172:**290–293.
17. **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.
18. **Kersulyte, D., H. Chalkauskas, and D. E. Berg.** 1999. Emergence of recombinant strains of *Helicobacter pylori* during human infection. Mol. Microbiol. **31:**31–43.
19. **Kuipers, E. J., D. A. Israel, J. G. Kusters, M. M. Gerrits, J. Weel, A. van Der Ende, R. W. van Der Hulst, H. P. Wirth, J. Hook-Nikanne, S. A. Thompson, and M. J. Blaser.** 2000. Quasispecies development of *Helicobacter pylori* observed in paired isolates obtained years apart from the same host. J. Infect. Dis. **181:**273–282.
20. **Kuipers, E. J., G. I. Pérez-Pérez, S. G. M. Meuwissen, and M. J. Blaser.** 1995. *Helicobacter pylori* and atrophic gastritis; importance of the *cagA* status. J. Natl. Cancer Inst. **87:**1777–1780.
21. **Leunk. R. D., P. T. Johnson, B. C. David, W. G. Kraft, and D. R. Morgan.** 1988. Cytotoxic activity in broth-culture filtrates of *Campylobacter pylori*. J. Med. Microbiol. **26:**93–99.
22. **Mobley, H. L.** 1996. Defining *Helicobacter pylori* as a pathogen: strain heterogeneity and virulence. Am. J. Med. **100:**2S–9S.
23. **Mukhopadhyay, A. K., D. Kersulyte, J. Y. Jeong, S. Datta, Y. Ito, A. Chowdhury, S. Chowdhury, A. Santra, S. K. Bhattacharya, T. Azuma, G. B. Nair, and D. E. Berg.** 2000. Distinctiveness of genotypes of *Helicobacter pylori* in Calcutta, India. J. Bacteriol. **182:**3219–3227.
24. **Odenbreit, S., J. Puls, B. Sedlmaier, E. Gerland, W. Fischer, and R. Haas.** 2000. Translocation of *Helicobacter pylori* CagA into gastric epithelial cells by type IV secretion. Science **287:**1497–1500.
25. **Page, R. D.** 1996. TreeView: an application to display phylogenetic trees on personal computers. Comput. Appl. Biosci. **12:**357–358.
26. **Pan, Z. J., D. E. Berg, R. W. van der Hulst, W. W. Su, A. Raudonikiene, S. D. Xiao, J. Dankert, G. N. 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.
27. **Peck, B., M. Ortkamp, K. D. Diehl, E. Hundt, and B. Knapp.** 1999. Conservation, localization and expression of HopZ, a protein involved in adhesion of *Helicobacter pylori*. Nucleic Acids Res. **27:**3325–3333.
28. **Pride, D. T., R. J. Meinersmann, and M. J. Blaser.** 2001. Allelic variation within *Helicobacter pylori babA* and *babB*. Infect. Immun. **69:**1160–1171.
29. **Schmitt, W., and R. Haas.** 1994. Genetic analysis of the *Helicobacter pylori* vacuolating cytotoxin: structural similarities with the IgA protease type of exported protein. Mol. Microbiol. **12:**307–319.
30. **Sharma, S. A., M. K. Tummuru, G. G. Miller, and M. J. Blaser.** 1995. Interleukin-8 response of gastric epithelial cell lines to *Helicobacter pylori* stimulation in vitro. Infect. Immun. **63:**1681–1687.
31. **Suerbaum, S., J. M. Smith, K. Bapumia, G. Morelli, N. H. Smith, E. Kunstmann, I. Dyrek, and M. Achtman.** 1998. Free recombination within *Helicobacter pylori*. Proc. Natl. Acad. Sci. USA **95:**12619–12624.
32. **Tee, W., J. Lambert, R. Smallwood, M. Schembri, B. C. Ross, and B. Dwyer.** 1992. Ribotyping of *Helicobacter pylori* from clinical specimens. J. Clin. Microbiol. **6:**1562–1567.
33. **Telford, J. L., P. Ghiara, M. Dell'Orco, M. Comanducci, D. Burroni, M. Bugnoli, M. F. Tecce, S. Censini, A. Covacci, and Z. Xiang.** 1994. Gene structure of the *Helicobacter pylori* cytotoxin and evidence of its key role in gastric disease. J. Exp. Med. **179:**1653–1658.
34. **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, E. K. Hickey, D. E. Berg, 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, and J. C. Venter.** 1997. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature **388:**539–547.
35. **Tummuru, M. K., S. A. Sharma, and M. J. Blaser.** 1995. *Helicobacter pylori picB*, a homologue of the *Bordetella pertussis* toxin secretion protein, is required for induction of IL-8 in gastric epithelial cells. Mol. Microbiol. **18:**867–876.
36. **Tummuru, M. K. R., T. L. Cover, and M. J. Blaser.** 1993. Cloning and expression of a high-molecular-mass major antigen of *Helicobacter pylori*: evidence of linkage to cytotoxin production. Infect. Immun. **61:**1799–1809.
37. **van der Ende, A., E. A. J. Rauws, M. Feller, C. J. J. Mulder, G. N. J. Tytgat, and J. Dankert.** 1996. Heterogeneous *Helicobacter pylori* isolates from members of a family with a history of peptic ulcer disease. Gastroenterology **111:**638–647.
38. **van der Ende, A., Z. J. Pan, A. Bart, R. W. van der Hulst, M. Feller, S. D. Xiao, G. N. Tytgat, and J. Dankert.** 1998. *cagA*-positive *Helicobacter pylori* populations in China and The Netherlands are distinct. Infect. Immun. **66:**1822–1826.
39. **van Doorn, L. J., C. Figueiredo, F. Megraud, S. Pena, P. Midolo, D. M. Queiroz, F. Carneiro, B. Vanderborght, M. D. Pegado, R. Sanna, W. De Boer, P. M. Schneeberger, P. Correa, E. K. Ng, J. Atherton, M. J. Blaser, and W. G. Quint.** 1999. Geographic distribution of *vacA* allelic types of *Helicobacter pylori*. Gastroenterology **116:**823–830.
40. **van Doorn, L. J., C. Figueiredo, R. Rossau, G. Jannes, M. van Asbroek, J. C. Sousa, F. Carneiro, and W. G. Quint.** 1998. Typing of *Helicobacter pylori vacA* gene and detection of *cagA* gene by PCR and reverse hybridization. J. Clin. Microbiol. **36:**1271–1276.
41. **van Doorn, L. J., C. Figueiredo, R. Sanna, M. J. Blaser, and W. G. Quint.** 1999. Distinct variants of *Helicobacter pylori cagA* are associated with *vacA* subtypes. J. Clin. Microbiol. **37:**2306–2311.
42. **van Doorn, L. J., Y. Henskens, N. Nouhan, A. Verschuuren, R. Vreede, P. Herbink, G. Ponjee, K. van Krimpen, R. Blankenburg, J. Scherpenisse, and W. Quint.** 2000. The efficacy of laboratory diagnosis of *Helicobacter pylori* infections in gastric biopsy specimens is related to bacterial density and *vacA*, *cagA*, and *iceA* genotypes. J. Clin. Microbiol. **38:**13–17.
43. **Wilson, K.** 1995. Preparation of genomic DNA from bacteria, p. 2.4.1–2.4.5. *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.), Current protocols in molecular biology, vol. 1. John Wiley & Sons, Inc., New York, N.Y.
44. **Yamaoka, Y., D. H. Kwon, and D. Y. Graham.** 2000. A M(r) 34,000 proinflammatory outer membrane protein (*oipA*) of *Helicobacter pylori*. Proc. Natl. Acad. Sci. USA **97:**7533–7538.
45. **Yamaoka, Y., H. M. Malaty, M. S. Osato, and D. Y. Graham.** 2000. Conservation of *Helicobacter pylori* genotypes in different ethnic groups in Houston, Texas. J. Infect. Dis. **181:**2083–2086.
46. **Yamaoka, Y., T. Kodama, K. Kashima, D. Y. Graham, and A. R. Sepulveda.** 1998. Variants of the 3′ region of the *cagA* gene in *Helicobacter pylori* isolates from patients with different *H. pylori*-associated diseases. J. Clin. Microbiol. **36:**2258–2263.