FEBS Letters 517 (2002) 180–184 FEBS 26007

Helicobacter pylori in North and South America before Columbus

Yoshio Yamaoka[a,*], Etsuro Orito[b], Masashi Mizokami[c], Oscar Gutierrez[d], Naruya Saitou[e], Tadashi Kodama[f], Michael S. Osato[a], Jong G. Kim[g], Francisco C. Ramirez[h], Varocha Mahachai[i], David Y. Graham[a]

[a] *Department of Medicine, Veterans Affairs Medical Center and Baylor College of Medicine, Houston, TX 77030, USA*
[b] *Department of Internal Medicine II, Nagoya City University Medical School, Nagoya 467-8601, Japan*
[c] *Department of Laboratory Medicine, Nagoya City University Medical School, Nagoya 467-8601, Japan*
[d] *Universidad Nacional de Colombia, Bogota, Colombia*
[e] *Laboratory of Evolutionary Genetics, National Institute of Genetics, Mishima 411-8540, Japan*
[f] *Third Department of Internal Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan*
[g] *Guro Hospital, Korea University College of Medicine, Seoul 152-050, South Korea*
[h] *Carl T. Hayden Veterans Affairs Medical Center, Phoenix, AZ 85012, USA*
[i] *Gastroenterology Unit, Chulalongkorn Hospital, Bangkok 10330, Thailand*

Received 12 February 2002; revised 17 March 2002; accepted 18 March 2002

First published online 28 March 2002

Edited by Takashi Gojobori

Abstract We present a molecular epidemiologic study, based on an analysis of *vacA*, *cagA* and *cag* right end junction genotypes from 1042 *Helicobacter pylori* isolates, suggesting that *H. pylori* was present in the New World before Columbus. Eight Native Colombian and Alaskan strains possessed novel *vacA* and/or *cagA* gene structures and were more closely related to East Asian than to non-Asian *H. pylori*. Some Native Alaskan strains appear to have originated in Central Asia and to have arrived after strains found in South America suggesting that *H. pylori* crossed the Bering Strait from Asia to the New World at different times. © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: *cagA* gene; *vacA* gene; Native American; *Helicobacter pylori*

1. Introduction

Helicobacter pylori is a major pathogen etiologically involved in peptic ulcer disease and gastric cancer [1]. Although *H. pylori* may have become established in the human stomach thousands of years ago [2,3], it has remained virulent, possibly because (1) the short life span of our ancestors may not have provided a survival advantage to lesser virulent *H. pylori*, and (2) the clinical manifestations of infection typically do not appear until adulthood.

Nearly 500 years ago, Europeans brought many deadly infectious diseases to the New World. Based on genotypic analyses of *H. pylori* strains isolated from different ethnic populations, it has been suggested that *H. pylori* was first brought to the New World from Europe [4]. The genes most commonly used as markers for genomic diversity among populations are *cagA* and *vacA*. *cagA* is part of the *cag* pathogenicity island, an approximately 40 kbp region in the *H. pylori* genome [5,6]. Recently, Kersulyte et al. [4] classified the right end of the *cag* pathogenicity island into five subtypes according to deletion, insertion and substitution motifs. Type I strains were predominant among isolates from Spanish-speaking patients including Peruvian Indians. Type II strains were predominant in China and Japan and type III strains were most common in India. Theoretically, if *H. pylori* were present in the stomachs of the ancestors of Native Peruvian Indians, it should have been genotype II or III. Instead, investigators found Peruvian strains to be similar to *H. pylori* typically found among inhabitants from the Iberian Peninsula.

Molecular epidemiology studies have also used the *vacA* gene, which encodes a vacuolating cytotoxin to assess transmission pathways. The studies have been based on different mosaic combinations of the signal (s) region (s1a, s1b, s1c and s2) and middle (m) region (m1 and m2) allelic types [7,8]. For example, we found that the predominant *vacA* genotype among Hispanics in Houston, TX, USA, was the s1b-m1 subtype, similar to strains from Hispanic America and the Iberian Peninsula [9]. These data are consistent with the notion that transmission of specific genotypes remains conserved within ethnic groups for at least several generations.

This study tests the hypothesis that *H. pylori* existed in the New World before Columbus. To test the hypothesis, *H. pylori* isolated from residual populations of infected patients in the New World were analyzed to determine whether they possessed the Asian-like *cagA* and/or *vacA* structures.

2. Materials and methods

2.1. H. pylori studied

A total of 1042 *H. pylori* isolates from different regions in the world were studied, including 41 strains from native New World populations (Tables 1 and 2). All samples were obtained with informed consent under protocols approved by the local human studies committees. The Alaskan strains originated from 16 'Eskimo' and four 'Aleut' patients. The three strains from Native North Americans were from Navahos

*Corresponding author. Fax: (1)-713-790 1040.
E-mail addresses: yyamaoka@bcm.tmc.edu (Y. Yamaoka), orito@med.nagoya-cu.ac.jp (E. Orito), mizokami@med.nagoya-cu.ac.jp (M. Mizokami), o_gutierrez0@yahoo.com (O. Gutierrez), nsaitou@genes.nig.ac.jp (N. Saitou), saigon@mint.ocn.ne.jp (T. Kodama), mosato@bcm.tmc.edu (M.S. Osato), franmi36715@aol.com (F.C. Ramirez), fmedvmc@md2.md.chula.ac.th (V. Mahachai), dgraham@bcm.tmc.edu (D.Y. Graham).

0014-5793/02/$22.00 © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.
PII: S0014-5793(02)02617-0

living in Arizona. Eighteen strains were from Huitotos, a primitive, isolated group living in the Amazonian jungles in Colombia.

2.2. PCR-based genotyping and sequencing of PCR fragments

Chromosomal DNA was isolated from confluent plate cultures expanded from a single colony. PCR-based genotyping and sequencing for the *vacA*, *cagA* and the *cag* right end motifs were performed as previously described, with modification [4,7,10–12]. We designed specific primer sets which could distinguish different subtypes among the genes. All sequences were reduced to common lengths consisting of nucleotides 6007–6229 (*vacA* s region, GenBank accession no. AE000598), 7805–8260 (*vacA* m region, AE000598), 1472–1691 and 2877–3505 (*cagA*, AE000569), and 3987–5339 (*cag* right-junction motif, AE000569). Sequences were aligned by the CLUSTAL W software and confirmed by visual inspection [13]. Genetic distances were estimated by the six-parameter method and phylogenetic trees were constructed by the neighbor-joining method [14]. The proportion of the nucleotide difference was used for distance calculation and 1000 bootstrap samplings were made to obtain bootstrap probabilities. These analyses were carried out using the ODEN program of the National Institute of Genetics (Mishima, Japan) [15].

3. Results and discussion

3.1. Genotypes from non-Native American strains

Strains from East Asian and non-Asian countries were readily separated by differences in the *vacA* s and m regions (Table 1). The *vacA* s1c genotype was predominant in strains from East Asia (94.7%) whereas the *vacA* s2 genotype was not found in strains from East Asia. Strains from East Asia were predominantly of the m1b genotype (96.8% of m1 strains) whereas strains from non-Asian countries were predominantly of the m1a genotype (99% of m1 strains). The *vacA* m1c genotype was predominant in strains from Calcutta, India [16], and was also found in strains from ethnic Kazakhs and from Pakistanis.

As with the *vacA* gene, strains from East Asian and non-Asian countries could be almost completely distinguished by PCR-based *cagA* 5′ and 3′ region genotyping, which is in agreement with previous studies [10,12,16,17]. As genotypic results from the 5′ region and the 3′ repeat region were almost identical, we used the same notation for typing (which we denoted the East Asian type as *cagA* 1a type and the non-Asian type as *cagA* 2a type) (Table 1). All *cagA* positive Kazakh and Pakistani strains possessed the non-Asian type structure.

We confirmed the findings of Kersulyte et al. [4] that the type I *cag* right-junction motif was most common among strains from ethnic European groups and type II was predominant in strains from East Asia (Table 1). Type II was also found in Kazakhstan (three of four *cag* positive strains). Interestingly, *H. pylori* ATCC51407 that was isolated from a rhesus monkey [18] possessed the type III *cag* right-junction motif as well as *vacA* m1c/*cagA* 2a, which was typical of strains from India. Rhesus monkeys are widely distributed throughout the Indian subcontinent and suggest that the ATCC51407 strain may have been transmitted to monkeys from humans.

3.2. Genotypes from Native American strains

Sequence analysis of the native populations in the New World showed that 29 of the 41 strains (71%) (100% for Arizona, 78% for Colombia and 60% for Alaska) had genotypes characteristic of non-Asian strains (combinations of *vacA* (s1a, s1b or s2 and m1a or m2), *cagA* (2a or negative) and *cag* right junction (type I or negative)).

We found that four (22%) Native Colombian strains possessed novel structures in *vacA* m and *cagA* and four (20%) Native Alaskan strains also possessed novel *vacA* m structures (Table 2). Phylogenetic analysis of the *vacA* s region indicated that the nucleotide differences and the bootstrap values were

Table 1
Distribution of the *vacA*, *cagA* and *cag* right-junction genotypes of *H. pylori* strains from non-Native American populations

Sources of isolates	Number analyzed	Number with indicated genotype														
		vacA s				*vacA* m				*cagA*		*cag* right junction				*cag* island
		s1a	s1b	s1c	s2	m1a	m1b	m1c	m2	1a	2a	I	II	III	others	negative
East Asia																
Japan	210	9	1	200	0	6	197	0	7[a]	206	0	0	192	12	2	4
Korea	178	13	1	164	0	6	149	0	23	174	0	0	158	14	2	4
Taiwan	34	0	0	34	0	0	14	0	20	34	0	0	32	2	0	0
Hong Kong	27	0	0	27	0	0	18	0	9	27	0	0	25	2	0	0
Vietnam	25	1	0	24	0	1	13	0	11	24	0	1	21	3	0	1
Southeast Asia																
Thailand	8	3	0	5	0	3	3	0	2	4	4	0	4	4	0	0
South Asia																
Pakistan	26	22	4	0	0	0	0	9	17	0	26	2	0	24	0	0
Central Asia																
Kazakhstan	5	4	0	0	1	0	0	3	2	0	4	1	3	0	0	1
Europe																
Italy	34	13	11	0	10	13	0	0	21	0	24	24	0	0	0	10
France	73	29	33	0	11	32	0	0	41	0	63	61	0	0	2	10
Africa																
South Africa	13	0	11	0	2	11	0	0	2	0	12	12	0	0	0	1
North America																
Canada	20	16	3	0	1	12	0	0	8	0	20	20	0	0	0	0
USA	183	55	102	0	26	17	2	0	64	0	163	157	0	1	5	20
South America																
Colombia	149	60	62	0	27	104	0	0	45	0	133	130	0	2	1	16
Brazil	16	0	8	0	8	6	0	0	10	0	10	10	0	0	0	6

[a]One Japanese strain has the *vacA* gene with recombination of m1b and m2 genotypes.

Table 2
The *vacA*, *cagA* and *cag* right-junction genotypes of *H. pylori* strains from Native American populations that were different from the non-Asian type

Strain	*vacA* s	*vacA* m	*cagA*	*cag* right junction
Original Native American type				
Alaska-2	s1	m1d	negative	negative
Alaska-10, -11	s1	m1e	negative	negative
Colombia-NA1692	s1	m2c	1c	III
Colombia-NA1764, -NA1766, -NA1768	s1	m1d	1b	III
Recombination of the original Native American and Central Asian type (?)				
Alaska-7	s1	m1d	2b	II
Central Asian type				
Alaska-6*, -13*, -19	s1a	m1c	2a	II
Recombination of East Asian and Central Asian type (?)				
Alaska-18	s1a	m1b	2a	II

*: Aleut. s1: Classified as s1 genotype, but not identical to s1a, s1b or s1c genotypes.

very low even among the established genotypes (e.g. bootstrap value of 44% between s1c and s2) (data not shown). These eight novel Native American strains clustered differently from the established *vacA* s genotypes (e.g. Colombia-NA1692, Alaska-2 and -7 had a cluster that was closely related to s1c: bootstrap value of 71%), however we did not classify this cluster as a new genotype because of the low bootstrap value. This might result from the short sequence sizes and by the fact that *vacA* exhibited free recombination whereas recombinants involving the 5′ and 3′ region of *cagA* were the rarest of any bacterial species [17].

Phylogenetic analysis of the *vacA* m region showed that five Native American strains (Colombia-NA1764, -NA1766, -NA1768, Alaska-2 and -7) formed one cluster with a bootstrap value of 98% that was closely related to the East Asian type m1b (which we denoted as m1d) (Fig. 1A). It has been suggested that the *vacA* m2 genotype can be divided into m2a (both East Asia and non-Asia) and m2b (East Asia) [7], however Colombia-NA1692 was completely different from m2a and m2b with a bootstrap value of 100% (we denoted as m2c). Phylogenetic analysis of the *cagA* gene also showed that four Native Colombian strains (Colombia-NA1692, -NA1764, -NA1766 and -NA1768) formed clusters that were similar but not identical to the East Asian cluster (which we denoted as *cagA* 1b for Colombia-NA1764, -NA1766 and -NA1768 and *cagA* 1c for Colombia-NA1692) (Fig. 1B).

A Native Alaskan strain (Alaska-7) formed a separate cluster and a search of GenBank revealed that two Dutch strains and one U.S. strain (J166) had similar sequences to Alaska-7 in the *cagA* 5′ region. Achtman et al. reported that the *cagA* 5′ region of strain J166 formed a cluster that differed from both the East Asian and non-Asian clusters which they termed 'clone 2' [17]. However, our analyses indicated that these strains could be classified as a subgroup of the non-Asian cluster (which we denoted as *cagA* 2b).

Overall, four Native Colombian strains (Colombia-NA1764, -NA1766, -NA1768 and -NA1692) had similar (i.e. related) but not identical structures of *vacA* m and *cagA* of isolates from East Asia. A *cagA* negative Native Alaskan strain (Alaska-2) also possessed specific *vacA* m structures that were closer to structures from East Asia than to those from non-Asian countries. These findings suggest that these five strains did not originate from modern East Asian people and suggest that they are more ancient or they were not recently transmitted from modern East Asian people. This is also consistent with the hypothesis that *H. pylori* accompanied humans when they crossed the Bering Strait from Asia to the New World.

There was lack of uniformity among the genotypes of the Native Alaskan strains and this may have bearing on the controversy concerning the timing, place(s) of origin, and number of 'waves' of migration into the New World. A recent model for peopling of the New World is based on the 'three wave' hypothesis, which states that there were three separate migrations of people into the New World from Asia, corresponding to the three different language groups [19]. Other studies suggest that only a single major migration occurred [20] or that as many as four major migration waves occurred [21]. Comparison of the *vacA* genotypes of three Native Colombian strains and two Native Alaskan strains showed them to be very similar with respect to the *vacA* m region structure (m1d) suggesting a relationship between these two populations. These strains were similar, but not identical, to the strains from East Asia suggesting that they separated from the original Asian strain long ago. In contrast, three Native Alaskan strains (Alaska-6, -13 and -19) and the Kazakh (Central Asian) strains fell within the same cluster in the *vacA*, *cagA* and *cag* right-junction motifs (*vacA* s1a-m1c, *cagA* 2a, *cag* right-junction type II). Although we found the *vacA* m1c type strains in individuals from Pakistan, all Pakistani *vacA* m1c strains had type III *cag* right-junction motifs. Recent reports also showed that most strains in Calcutta, India, had the *vacA* m1c genotype, however the type II *cag* right-junction motifs were very rare (1%) [4,16]. These findings suggest that these Native Alaskan strains may have originated in Central Asia. Some Native Alaskan strains and Central Asian strains fell within the same cluster suggesting that the Central Asian type Native Alaskan strains crossed through Beringia after they had evolved into the present types (i.e. more recently than the original Native Colombian strains). Testing this hypothesis will require evaluation of additional strains from Central Asia. Two Native Alaskan strains (Alaska-10 and -11) had unique *vacA* genotypes that were considerably different from others and these genotypes were not found in strains from any other region we studied. The origin of this genotype may be different from the other two types and may support the multi-wave models for peopling of the New World.

It remains unclear why some Native Alaskan and Kazakh strains possessed the non-Asian type *cagA* structure although these strains have Asian or Asian-like *vacA* genotypes. There are recent data [22] showing evidence of genetic exchange

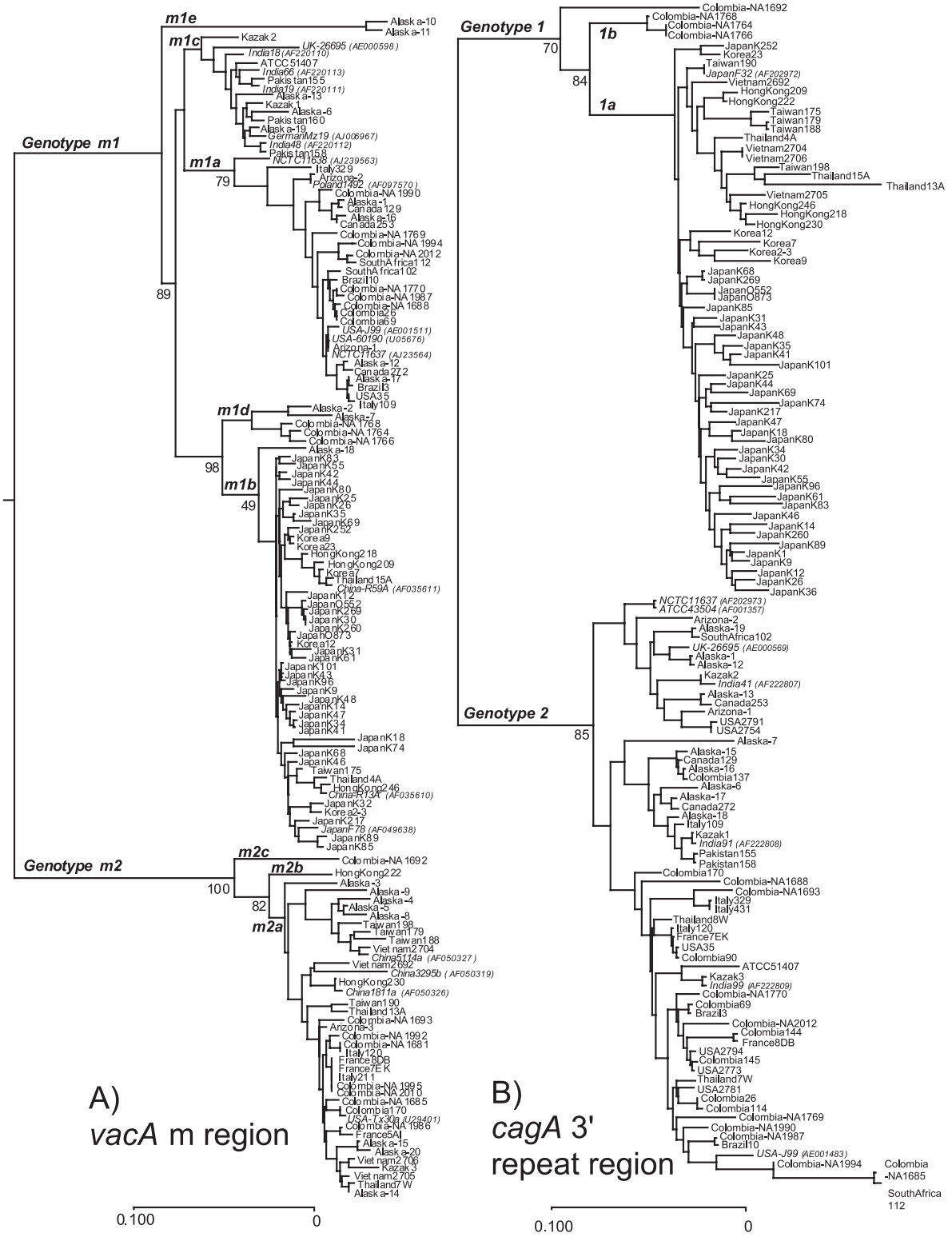

Fig. 1. Phylogenetic tree analysis of the *vacA* m region (A) and *cagA* 3′ repeat region (B) nucleotide sequences of *H. pylori*. Genetic distances were estimated by the six-parameter method and phylogenetic trees were constructed by the neighbor-joining method. The strain name with GenBank accession numbers show the reference strains. Bootstrap values are shown along each main branch. The lengths of the horizontal bars indicate the number of nucleotide substitutions per site.

between two different *H. pylori* strains during a natural mixed infection. When recombination of the *cag* pathogenicity island occurred, the *cag* pathogenicity island including the *cag* left and right junctions was thought to transfer together as a group [23]. If these Native Alaskan and Kazakh strains originated from post-Colombian recombination events between Asian and non-Asian type strains, the strains would be expected to have the type I *cag* right-junction motif typical of

strains from non-Asian countries. These strains had a type II *cag* right-junction motif suggesting that these strains originally possessed the non-Asian type *cagA* structure. Two Native strains (Alaska-7 and -18) also possessed the type II *cag* right-junction motif with the non-Asian-like structure of *cagA*. These two strains had an East Asian-like or East Asian structure of *vacA* suggesting that recombination of the original Native American type or East Asian type and Central Asian type strains might have occurred.

Finally, it remains unclear why most *H. pylori* from Native Americans have genotypes typical of strains from non-Asian countries even in sites where Western influence appears to have been minimal. While further study will be necessary to define the mechanism(s) responsible for the in vivo loss of the Native American strains when in competition with Old World strains, preliminary data suggest that they may differ with respect to colonization. For example, in a preliminary study, we found that both Native Colombian and Western strains colonized mice, but only Western strains could be recovered following inoculation of both strains into mouse stomachs (Yamaoka et al., unpublished data). These findings are consistent with the notion that strains from the Old World may be hardier or more efficient colonizers than the original Native American strains.

3.3. Data deposition

The sequences have been deposited in GenBank with accession numbers: AB057106–AB057226 (*vacA* s region), AB057227–AB057341 (*vacA* m region), AB056898–AB057002 (*cagA* 5′ region), AB057003–AB057105 (*cagA* 3′ region) and AB057432–AB057349 (*cag* right-junction motif).

Acknowledgements: We thank J. Parkinson (Alaska), C.L. Perng (Taiwan), J.Y. Sung (Hong Kong), R.N. Agnes Walsh (Thailand), Z. Nurgalieva (Kazakhstan), G. Realdi (Italy), A. Simjee (South Africa), J.J. Pedrazzoli (Brazil), and S. Mendonca (Brazil) for *H. pylori* strains. We thank M. Gomez (Colombia) for assistance in obtaining samples from Native Colombians. This material is based upon work supported in part by the Office of Research and Development Medical Research Service Department of Veterans Affairs and by Public Health Service Grant DK-53659. We also acknowledge the generous support of Hilda Schwartz.

References

[1] Graham, D.Y. (1997) Gastroenterology 113, 1983–1991.
[2] Covacci, A., Telford, J.L., Del Giudice, G., Parsonnet, J. and Rappuoli, R. (1999) Science 284, 1328–1333.
[3] Blaser, M.J. (1997) J. Physiol. Pharmacol. 48, 307–314.
[4] Kersulyte, D., Mukhopadhyay, A.K., Velapatino, B., Su, W., Pan, Z., Garcia, C., Hernandez, V., Valdez, Y., Mistry, R.S., Gilman, R.H., Yuan, Y., Gao, H., Alarcon, T., Lopez-Brea, M., Balakrish Nair, G., Chowdhury, A., Datta, S., Shirai, M., Nakazawa, T., Ally, R., Segal, I., Wong, B.C., Lam, S.K., Olfat, F.O., Borén, T., Engstrand, L., Torres, O., Schneider, R., Thomas, J.E., Czinn, S. and Berg, D.E. (2000) J. Bacteriol. 182, 3210–3218.
[5] Alm, R.A., Ling, L.S., Moir, D.T., King, B.L., Brown, E.D., Doig, P.C., Smith, D.R., Noonan, B., Guild, B.C., deJonge, B.L., Carmel, G., Tummino, P.J., Caruso, A., Uria-Nickelsen, M., Mills, D.M., Ives, C., Gibson, R., Merberg, D., Mills, S.D., Jiang, Q., Taylor, D.E., Vovis, G.F. and Trust, T.J. (1999) Nature 397, 176–180.
[6] Tomb, J.F., White, O., Kerlavage, A.R., Clayton, R.A., Sutton, G.G., Fleischmann, R.D., Ketchum, K.A., Klenk, H.P., Gill, S., Dougherty, B.A., Nelson, K., Quackenbush, J., Zhou, L., Kirkness, E.F., Peterson, S., Loftus, B., Richardson, D., Dodson, R., Khalak, H.G., Glodek, A., McKenney, K., Fitzegerald, L.M., Lee, N., Adams, M.D., Hickey, E.K., Berg, D.E., Gocayne, J.D., Utterback, T.R., Peterson, J.D., Kelley, J.M., Cotton, M.D., Weidman, J.M., Fujii, C., Bowman, C., Watthey, L., Wallin, E., Hayes, W.S., Borodovsky, M., Karp, P.D., Smith, H.O., Fraser, C.M. and Venter, J.C. (1997) Nature 388, 539–549.
[7] Atherton, J.C., Cao, P., Peek, R.M., Tummuru, M.K.R., Blaser, M.J. and Cover, T.L. (1995) J. Biol. Chem. 270, 17771–17777.
[8] van Doorn, L.J., Figueiredo, C., Mégraud, F., Pena, S., Midolo, P., Maria de Magalhaes Queiroz, D., Carneiro, F., Vanderborght, B., Pegado, M.D., Sanna, R., De Boer, W., Schneeberger, P.M., Correa, P., Ng, E.K., Atherton, J.C., Blaser, M.J. and Quint, W.G. (1999) Gastroenterology 116, 823–830.
[9] Yamaoka, Y., Malaty, H.M., Osato, M.S. and Graham, D.Y. (2000) J. Infect. Dis. 181, 2083–2086.
[10] Yamaoka, Y., Kodama, T., Kashima, K., Graham, D.Y. and Sepulveda, A.R. (1998) J. Clin. Microbiol. 36, 2258–2263.
[11] Yamaoka, Y., Kodama, T., Kita, M., Imanishi, J., Kashima, K. and Graham, D.Y. (1998) Helicobacter 4, 241–253.
[12] Yamaoka, Y., El-Zimaity, H.M.T., Gutierrez, O., Figura, N., Kim, J.G., Kodama, T., Kashima, K. and Graham, D.Y. (1999) Gastroenterology 117, 342–349.
[13] Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994) Nucleic Acids Res. 22, 4673–4680.
[14] Saitou, N. and Nei, M. (1987) Mol. Biol. Evol. 4, 406–425.
[15] Ina, Y. (1994) Comput. Appl. Biosci. 10, 11–12.
[16] Mukhopadhyay, A.K., Kersulyte, D., Jeong, J.Y., Datta, S., Ito, Y., Chowdhury, A., Chowdhury, S., Santra, A., Bhattacharya, S.K., Azuma, T., Nair, G.B. and Berg, D.E. (2000) J. Bacteriol. 182, 3219–3227.
[17] Achtman, M., Azuma, T., Berg, D.E., Ito, Y., Morelli, G., Pan, Z.J., Suerbaum, S., Thompson, S.A., van der Ende, A. and van Doorn, L.J. (1999) Mol. Microbiol. 32, 459–470.
[18] Drazek, E.S., Dubois, A. and Holmes, R.K. (1994) J. Clin. Microbiol. 32, 1799–1804.
[19] Greenberg, J.H., Turner, C.G. and Zegura, S.L. (1986) Curr. Anthropol. 27, 477–497.
[20] Underhill, P.A., Jin, L., Zemans, R., Oefner, P.J. and Cavalli-Sforza, L.L. (1996) Proc. Natl. Acad. Sci. USA 93, 196–200.
[21] Torroni, A., Neel, J.V., Barrantes, R., Schurr, T.G. and Wallace, D.C. (1994) Proc. Natl. Acad. Sci. USA 91, 1158–1162.
[22] Kersulyte, D., Chalkauskas, H. and Berg, D.E. (1999) Mol. Microbiol. 31, 31–43.
[23] Akopyants, N.S., Clifton, S.W., Kersulyte, D., Crabtree, J.E., Youree, B.E., Reece, C.A., Bukanov, N.O., Drazek, E.S., Roe, B.A. and Berg, D.E. (1998) Mol. Microbiol. 28, 37–53.