JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2003, p. 5755–5759 Vol. 41, No. 12
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.12.5755–5759.2003

Fine-Structure Molecular Typing of Irish *Helicobacter pylori* Isolates and Their Genetic Relatedness to Strains from Four Different Continents

Ian M. Carroll,[1]* Niyaz Ahmed,[2] Sarah M. Beesley,[1] Aleem A. Khan,[3] Sheikh Ghousunnissa,[2] Colm A. O Moráin,[4] and Cyril J. Smyth[1]

Department of Microbiology, Moyne Institute of Preventive Medicine, Trinity College, Dublin 2,[1] and Department of Gastroenterology, The Adelaide and Meath Hospital, Tallaght, Dublin 24,[4] Ireland, and Centre for DNA Fingerprinting and Diagnostics (CDFD), Nacharam,[2] and Deccan College of Medical Sciences and Allied Hospitals,[3] Hyderabad, India

Received 4 March 2003/Returned for modification 29 May 2003/Accepted 1 August 2003

Genotyping of 74 Irish *Helicobacter pylori* isolates was performed at four different loci (*vacA* signal sequence and mid-region, insertion-deletion polymorphisms at the 3′ end of the *cag* pathogenicity island, and *cagA*). The predominant *vacA* alleles and insertion-deletion motifs suggest an ancestral relationship between Irish isolates and either specific East Asian or Northern European strains. In addition, fluorescent amplified fragment length polymorphism-PCR genotyping and phylogenetic analysis of 32 representative Irish *H. pylori* isolates and 22 isolates from four different continents demonstrated that the Irish *H. pylori* isolates examined were weakly clonal and showed some association with both European and Asian isolates. These three genotyping techniques show that Irish *H. pylori* isolates have distinctive features that may have evolved in this insular European population.

Helicobacter pylori displays genetic diversity between strains at a very high level (17). This diversity has been shown to occur at specific loci within *H. pylori* isolates (3, 4, 21, 23). The *vacA* gene, which encodes the vacuolating cytotoxin, is a polymorphic locus present in all *H. pylori* strains. While this gene contains conserved regions, sequence variations occur in the signal sequence and the mid-region of the gene as a mosaic structure (3). The signal sequence can be one of four allelic subtypes (s1a, s1b, s1c, and s2) (25) and the mid-region can be one of two allelic subtypes (m1 and m2) (3). There have been numerous reports correlating these *vacA* allelic types with particular gastric diseases (5, 20), e.g., the *vacA* s1 m1 genotype has been associated with gastric carcinoma in Germany (18).

The *cagA* gene (cytotoxin-associated gene) is another polymorphic locus that is thought to be a marker for virulence and is associated with increased severity of disease in some geographic regions (6). The 3′ end of the *cag* pathogenicity island (*cag* PAI) (the region between the 3′ end of the *cagA* gene and the 3′ end of the glutamate racemase gene [*glr*]) contains different motifs that have previously been used for studying relationships between *H. pylori* isolates from different geographic regions (14). To demonstrate geographic partitioning of *H. pylori* isolates, other studies have investigated sequences of putative virulence and housekeeping genes (1).

Although *H. pylori* is a panmictic organism (10), a study encompassing isolates from different regions of the world identified weakly clonal groupings for this bacterium (1). There have also been indications of nonrandom geographic distributions of *H. pylori* genotypes that may aid in the understanding of the evolution of *H. pylori* and its clinical consequences in different parts of the world (14, 24). The predominant *vacA* alleles have been shown to distinguish *H. pylori* isolates in different geographic regions (13, 15, 16, 19, 24).

Fluorescent amplified fragment length polymorphism (FAFLP) analysis is a genotyping technique that has been used to investigate outbreaks of infection by *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Neisseria meningitidis* (7, 11, 12). FAFLP analysis generates a specific profile for each strain under examination. These profiles can be grouped together, resulting in generalized patterns. Recently, FAFLP was used to examine the geographic partitioning of *H. pylori* isolates [N. Ahmed, A. A. Khan, D. E. Berg, and C. M. Habibullah, abstract from the Int. Workshop on *Campylobacter*, *Helicobacter* and Related Organisms (CHRO), 2001, Freiburg, Germany, Int. J. Med. Microbiol. **291**(Suppl. 31)**:**D-11, 2001].

For the present study, Irish *H. pylori* isolates were characterized by FAFLP and insertion-deletion analyses, along with *vacA* genotyping and *cag* gene status, and compared to isolates from other geographic regions, namely India, United Kingdom, Peru, Spain, Japan, and Africa.

Seventy-four clinical *H. pylori* isolates were examined that had been obtained from single antral gastric biopsies from patients experiencing peptic ulcer disease at the Meath-Adelaide and St. James's Hospitals in Dublin. Culture and storage of isolates were performed as described previously (8). Twenty-two *H. pylori* strains from six different geographic regions were also analyzed (six from India, five from Peru, five from the United Kingdom, four from Spain, one from Africa, and one from Japan). Genomic DNA preparations were provided by Douglas E. Berg and Asish Mukhopadhyay (Washington University Medical School, St. Louis, Mo.) from 16 non-Irish

* Corresponding author. Mailing address: Department of Microbiology, Moyne Institute of Preventive Medicine, Trinity College, Dublin 2, Ireland. Phone: 353 1 6081728. Fax: 353 1 6799294. E-mail: carrolli@tcd.ie.

TABLE 1. Oligonucleotide sequences of primers and PCR product sizes

Amplified gene or DNA region	Primer	Primer sequence (5′-3′)	PCR product size (bp [type])
cagA	CAGAF[a]	GATAACAGGCAAGCTTTTGAGG	349
	CAGAR[a]	CTGCAAAAGATTGTTTGGCAGA	
Signal sequence of *vacA*	VA1-F[b]	ATGGAAATACAACAAACACAC	259 (s1)
	VA1-R[b]	CTGCTTGAATGCGCCAAAC	286 (s2)
Signal sequence sla of *vacA*	SS1-F[b]	GTCAGCATCACACCGCAAC	190
	VA1-R[b]	CTGCTTGAATGCGCCAAAC	
Mid-region of *vacA*	VAG-F[a]	CAATCTGTCCAATCAAGCGAG	570 (m1)
	VAG-R[a]	GCGTCTAAATAATTCCAAGG	645 (m2)
Extreme right end of the *cag* PAI	cagF4584[c]	GTTATTACAAAAGGTGGTTTCCAAAAATC	1,200 (IV)
	cagR5280[c]	GGTTGCACGCATTTTCCCTTAATC	1,000 (IIIa)
			1,000 (Ia)
			700 (IIIa)
			700 (II)
Type IV insertion-deletion motif specific	Xins.R[c]	CGCTCTCTAATTGTTCTAGGA	520
	cagF4584[c]	Same as above	
Type II insertion-deletion motif specific	IS606-1692[c]	CTAACAATTTGCCATTATGCTGT	400
	cagR5280[c]	Same as above	
Type III insertion-deletion motif specific	fcn unk[c]	TGGATTAAATCTTAATGAATTATCG	320
	cagR5280[c]	Same as above	
Type I and IV insertion-deletion motif specific	cagF4856[c]	GCGATGAGAAGAATATCTTTAGCG	350 (I)
	cagR5280[c]	Same as above	400 (IV)

[a] Primer sequences from the work of Yamaoka et al. (25).
[b] Primer sequences from the work of Atherton et al. (3).
[c] Primer sequences from the work of Kersulyte et al. (13).

and non-Indian *H. pylori* strains originally obtained by Robert H. Gilman, Teresa Alarcon, Manuel Lopez Brea, and John Atherton. The Indian preparations were from the Centre for DNA Fingerprinting and Diagnostics (Hyderabad, India).

Genomic DNA was isolated as described previously (8). The sequences of the oligonucleotides used as PCR primers and their corresponding PCR product sizes are listed in Table 1. The signal sequences and mid-regions of the *vacA* gene, the *cagA* gene, and the insertion-deletion motifs at the 3′ end of the *cag* PAI were amplified in separate reactions as reported previously (3, 14, 25). Negative controls for PCR included sterile distilled water and *Escherichia coli* DNA from strain XL1-Blue. The nucleotide sequences of representative *vacA* and *cagA* PCR products were determined to confirm their identities.

Of the 74 isolates examined, 69% were of *vacA* type s1a m2, 27% were of *vacA* type s1a m1, and 4% were of *vacA* type s2 m2 (Table 2). The most common signal sequence and mid-region alleles were s1a and m2, respectively, which is in accordance with previous studies of Irish *H. pylori* isolates (22). The frequency of the predominant *vacA* allele found in this study is similar to those found in Taiwan (82%) (16) and China (85%) (19). However, *vacA* alleles have been shown to differ in frequency and type among East Asian isolates (13, 16, 19), e.g., s1c is the predominant signal sequence allele among East Asian isolates (24). Thus, Irish *H. pylori* isolates share *vacA* similarities with isolates from specific countries within Eastern Asia. This may reflect particular adaptations of *H. pylori* to specific host populations (9, 24) rather than indicating geographic relationships.

All of the 74 Irish *H. pylori* isolates were found to contain the *cagA* gene. This does not agree with a previous Irish study (8), but as no asymptomatic patients were included in the present study, this may have influenced the present finding. Moreover, the *cagA* status of this collection was not known before the study commenced. Characterization of the 3′ end of the *cag* PAI has shown that eight different insertion-deletion motifs can be present (Ia, Ib, Ic, II, IIIa, IIIb, IV, and V) (14). These insertion-deletion motifs have been shown to be well suited to population-level surveys of *H. pylori* genotypes (14). Type I motifs predominate in isolates from Spanish and Peruvian populations but are less common in strains from Northern European populations. Type II motifs are mostly found among Japanese and Chinese strains, but are also common in Northern European strains. Type III motifs predominate among isolates from Indian populations. When insertion-deletion analysis was performed on the isolates, 63 (85%) yielded useable PCR products, 4 (5%) produced multiple PCR products (which made it impossible to determine the different motifs at

TABLE 2. *vacA* typing and *cagA* insertion-deletion motif analysis of 74 Irish *H. pylori* isolates[a]

vacA or insertion-deletion type	No (%) of strains
vacA types	
s1a	71 (95.9)
s2	3 (4.1)
m1	20 (27)
m2	54 (73)
s1a m2	51 (68.9)
s1a m1	20 (27)
s2 m2	3 (4.1)
Insertion-deletion types	
Ia	6 (9.5)
II	31 (49.2)
IIIa	3 (4.8)
IIIb	1 (1.6)
IV	22 (34.9)

[a] For insertion-deletion motif analysis, only 63 isolates were typeable.

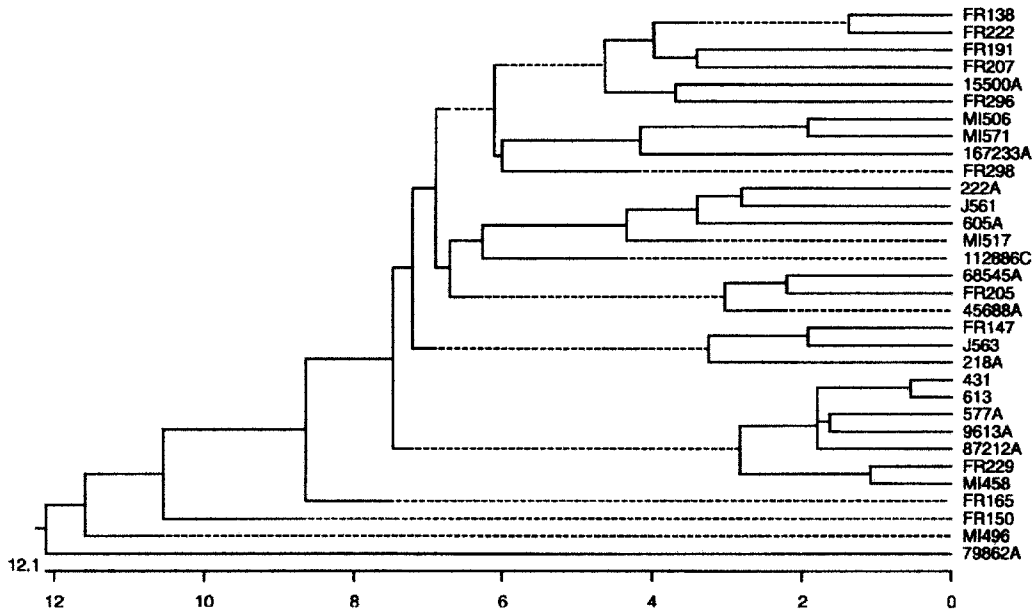

FIG. 1. Phylogenetic tree of 32 Irish *H. pylori* isolates. The neighbor-joining network was developed from the binary data obtained by the Genotyper macro. The scale at the bottom shows percentage heterogeneity among the isolates based on sharing of FAFLP fragments.

this locus), and 7 (10%) did not yield PCR products under the test conditions used, despite repeated attempts. Five insertion-deletion motifs were detected. Of the 63 characterizable isolates, 31 (49%) were found to have type II motifs, 22 (35%) had type IV motifs, 6 (9%) had type Ia motifs, 3 (5%) had type IIIa motifs, and 1 (2%) had a type IIIb motif (Table 2). These findings suggest that Irish *H. pylori* isolates are similar to Northern European strains, as type II motifs are common in this region, but not to East Asian isolates, as the frequencies of occurrence are much higher (95%) (14). However, although type II motifs are common in Irish and Northern European isolates, type I motifs also have a high prevalence among Northern European strains, whereas Irish isolates show a high prevalence of type IV motifs. Interestingly, the only isolate yielding a type IV motif in a study of 500 strains was from England (14). A more extensive study of English strains by insertion-deletion analysis is required to clarify the frequency of occurrence of type IV motifs among isolates from different ethnic groups, including the Irish diaspora. Thus, this collection of Irish *H. pylori* isolates displays a rare insertion-deletion genotype that may be representative of an insular European population.

FAFLP-PCR was carried out as previously detailed (2). The fingerprints for each Irish isolate were generated by using three selective primers (*Eco*RI plus A, G, and C), and the fingerprints for each non-Irish isolate were generated by using one selective primer (*Eco*RI plus G). Amplified products were sized in base pairs within the user-defined categories of marker sizes. The presence or absence of amplicons within the categories was scored by a user-defined Genotyper macro. Allele scores (presence or absence of amplicons) were converted into a binary format (1 or 0, respectively). This binary format was converted to a nucleotide sequence (1 = G and 0 = A); therefore, fingerprint profiles could be aligned and a neighbor-joining tree could be constructed based on these profiles using Genotyper (Fig. 1) or ClustalX and Treeview (Fig. 2).

As *vacA* and insertion-deletion genotyping suggested potential geographic relationships between Irish *H. pylori* isolates and isolates from specific countries in East Asia and from Northern Europe, respectively, FAFLP-PCR analysis was applied. This technique encompasses the whole genome rather than specific loci and has been previously used to determine the geographic relationship of *H. pylori* isolates [Ahmed et al., Int. J. Med. Microbiol. **291**(Suppl. 31)**:**D-11, 2001]. FAFLP-PCR analysis was used to examine 45 of the 74 Irish *H. pylori* isolates. Of these 45 isolates, 32 (71%) yielded informative profiles. The remaining 13 isolates either did not yield profiles with all three selective primers or did not yield profiles at all. However, the 32 characterizable isolates were still genotypically representative of the larger set of isolates. Twenty-five of these isolates (78%) yielded insertion-deletion motifs (type II, 13 [52%]; type IV, 9 [36%]; type Ia, 2 [8%]; type IIIb, 1 [4%]), while the remaining isolates were not typeable by insertion-deletion analysis (7 [22%] of the 32 isolates; cf. with 11 of 74 [15%]). The distribution of the *vacA* alleles in this selected group of isolates was s1a m2 (19 [60%]), s1a m1 (10 [31%]), and s2 m2 (3 [9%]). The amplification reactions yielded unique profiles for each isolate, and a neighbor-joining phylogenetic tree was constructed based on these profiles (Fig. 1). Analysis of this tree showed that the majority (28 [87.5%]) of isolates in this group differ in percentage heterogeneity by approximately 7.8% with respect to their FAFLP profiles. This supports earlier observations by which there appears to be a relative genetic restriction of *vacA* alleles and insertion-deletion motifs, indicating that Irish *H. pylori* isolates are weakly clonal.

Seven FAFLP-PCR profiles randomly chosen from among those obtained for Irish isolates plus 22 profiles previously generated in the same way at the Centre for DNA Fingerprint-

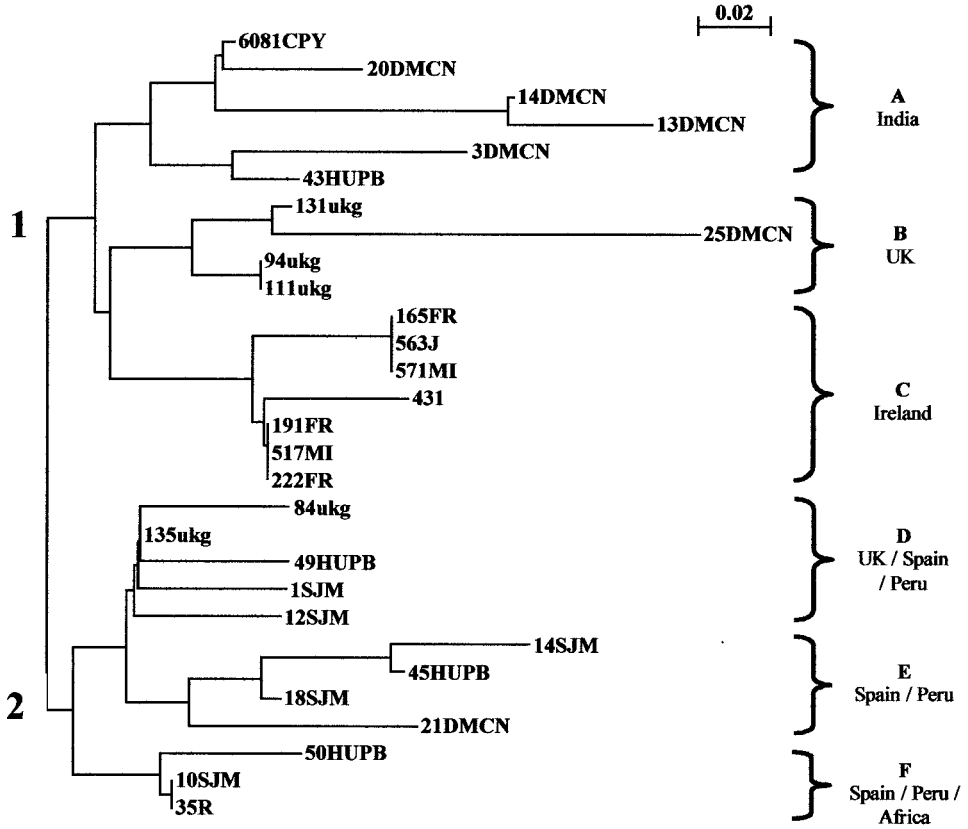

FIG. 2. Phylogenetic tree of various *H. pylori* isolates from different geographic regions. The neighbor-joining network was developed from the binary data obtained by the Genotyper macro. Clustering of isolates is based on sharing of FAFLP fragments. Clustering resulted in two main clusters (1 and 2) and six distinct subgroups (A to F).

ing and Diagnostics for strains encompassing six different geographic regions [Ahmed et al., Int. J. Med. Microbiol. **291**(Suppl. 31)**:**D-11, 2001] were phylogenetically analyzed. The binary data obtained from FAFLP-PCR analysis were used to construct a neighbor-joining phylogenetic tree (Fig. 2). This tree shows two main clusters (1 and 2) and six subgroups (A to F). The fingerprints used to construct this tree were previously generated by using only one selective primer, whereas fingerprints generated by three selective primers were used to construct Fig. 1. Using only one selective primer reduces the sensitivity of the technique. This explains why isolate FR165 shows about 9% heterogeneity with respect to isolates J563 and MI571 in Fig. 1, while these isolates show little difference in Fig. 2. However, each fingerprint is still unique to each isolate and the geographic separation of isolates can clearly be seen in Fig. 2. Subgroups A, B, and C are in cluster 1 and subgroups D, E, and F are in cluster 2. Each subgroup contained either isolates from one region or a combination of isolates from different geographic locations (A, mainly India; B, mainly United Kingdom; C, Ireland; D, mainly United Kingdom and Spain; E, mainly Spain and Peru; and F, Spain, Peru, and Africa). The fact that Irish *H. pylori* isolates do not cluster with isolates from any other region suggests that they may have a unique genotype and are distinct from those from other regions. However, the Irish isolates do reside in cluster 1 with Asian and United Kingdom isolates, demonstrating that they are more like isolates from these regions than like Spanish, Peruvian, and African isolates. These findings are in accordance with a recent study that demonstrated that Indian and European *H. pylori* isolates grouped in the same subpopulation and that East Asian and a subset of European isolates share an ancestral relationship and diverged from each other recently (9).

As indicated by the present genotyping study and by other studies (22), Irish *H. pylori* isolates appear to exhibit restricted diversity. The clustering of the Irish isolates into one subgroup (C) by FAFLP-PCR confirms that Irish isolates are weakly clonal. This weakly clonal behavior was not observed within strains from other geographic regions, as some isolates from specific regions clustered in two or three different subgroups (Indian isolates clustered in subgroups A, B, and E, United Kingdom isolates clustered in subgroups B and D, Peruvian isolates clustered in subgroups D, E, and F, and Spanish isolates clustered in subgroups A, D, E, and F). The clustering of Peruvian and Spanish isolates (Fig. 2) is in accordance with previous studies (14).

In summary, the results obtained for this study suggest that Irish *H. pylori* isolates have associations with Northern European isolates, as revealed by insertion-deletion analysis. Yet the *vacA* allelic predominance among these Irish isolates displays similarities with isolates from China and Taiwan. Further, FAFLP-PCR genotyping confirmed that Irish isolates are

weakly clonal, display more homogeneous fingerprint profiles than do isolates from other geographic locations, and have some associations with both European and Asian isolates. These three genotyping techniques show that Irish *H. pylori* isolates have distinctive features that may have evolved in this insular European population.

Ian Carroll was the recipient of a postgraduate studentship from the Health Research Board (HRB) of Ireland, whose support is gratefully acknowledged. Niyaz Ahmed acknowledges with thanks a research grant from the Department of Biotechnology, Government of India.

We thank Douglas E. Berg and Asish Mukhopadhyay for the supply of genomic DNA from *H. pylori* isolates from non-Irish and non-Indian origins, originally obtained by Robert H. Gilman, Teresa Alarcon, Manuel Lopez Brea, and John Atherton, and Seyed E. Hasnain for his suggestions and encouragement. We also thank Ross Fitzgerald for his helpful suggestions.

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. **Ahmed, N., A. Bal, A. A. Khan, M. Alam, A. Kagal, V. Arjunwadkar, A. Rajput, A. A. Majeed, S. A. Rahman, S. Banerjee, S. Joshi, and R. Bharadwaj.** 2002. Whole genome fingerprinting and genotyping of multiple drug resistant (MDR) isolates of *Pseudomonas aeruginosa* from endophthalmitis patients in India. Infect. Genet. Evol. **1:**237–242.
3. **Atherton, J. C., P. Cao, R. M. Peek, Jr., M. K. R. Tummuru, M. J. Blaser, and T. L. Cover.** 1995. Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori.* J. Biol. Chem. **270:**17771–17777.
4. **Bereswill, S., R. Schonenberger, C. Thies, F. Stahler, S. Strobel, P. Pfefferle, L. Wille, and M. Kist.** 2000. New approaches for genotyping of *Helicobacter pylori* based on amplification of polymorphisms in intergenic DNA regions at the insertion site of the *cag* pathogenicity island. Med. Microbiol. Immunol. **189:**105–113.
5. **Blaser, M. J.** 1992. *Helicobacter pylori*: its role in disease. Clin. Infect. Dis. **15:**386–391.
6. **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.
7. **Desai, M., A. Tanna, R. Wall, A. Efstratiou, R. George, and J. Stanley.** 1998. Fluorescent amplified-fragment length polymorphism analysis of an outbreak of group A streptococcal invasive disease. J. Clin. Microbiol. **36:**3133–3137.
8. **Dundon, W. G., D. G. Marshall, C. A. O'Morain, and C. J. Smyth.** 2000. Population characteristics of Irish *Helicobacter pylori* isolates: a tRNA-associated locus. Ir. J. Med. Sci. **169:**137–140.
9. **Falush, D., T. Wirth, B. Linz, J. K. Pritchard, M. Stephens, M. Kidd, M. J. Blaser, D. Y. Graham, S. Vacher, G. I. Perez-Perez, Y. Yamaoka, F. Megraud, K. Otto, U. Reichard, E. Katzowitsch, X. Wang, M. Achtman, and S. Suerbaum.** 2003. Traces of human migrations in *Helicobacter pylori* populations. Science **7:**1582–1585.
10. **Go, M. F., V. Kapur, D. Y. Graham, and J. M. Musser.** 1996. Population genetic analysis of *Helicobacter pylori* by multilocus enzyme electrophoresis: extensive allelic diversity and recombinational population structure. J. Bacteriol. **178:**3934–3938.
11. **Goulding, J. N., J. V. Hookey, J. Stanley, W. Olver, K. R. Neal, D. A. A. Ala'Aldeen, and C. Arnold.** 2000. Fluorescent amplified-fragment length polymorphism genotyping of *Neisseria meningitidis* identifies clones associated with invasive disease. J. Clin. Microbiol. **38**:4580–4585.
12. **Grady, R., G. O'Neill, B. Cookson, and J. Stanley.** 2000. Fluorescent amplified-fragment length polymorphism analysis of the MRSA epidemic. FEMS Microbiol. Lett. **187:**27–30.
13. **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.
14. **Kersulyte, D., A. K. Mukhopadhyay, B. Velapatiño, W. Su, Z. Pan, C. Garcia, V. Hernandez, Y. Valdez, R. S. Mistry, R. H. Gilman, Y. Yuan, H. Gao, T. Alarcón, M. López-Brea, G. Balakrish Nair, A. Chowdhury, S. Datta, M. Shirai, T. Nakazawa, R. Ally, I. Segal, B. C. Wong, S. K. Lam, F. O. Olfat, T. Borén, L. Engstrand, O. Torres, R. Schneider, J. E. Thomas, S. Czinn, and D. E. Berg.** 2000. Differences in genotypes of *Helicobacter pylori* from different human populations. J. Bacteriol. **182:**3210–3218.
15. **Kim, S. Y., C. W. Woo, Y. M. Lee, B. R. Son, J. W. Kim, H. B. Chae, S. J. Youn, and S. M. Park.** 2001. Genotyping *cagA*, *vacA* subtype, *iceA1*, and *babA* of *Helicobacter pylori* isolates from Korean patients, and their association with gastroduodenal diseases. J. Korean Med. Sci. **16:**579–584.
16. **Lin, C.-W., S.-C. Wu, S.-C. Lee, and K.-S. Cheng.** 2000. Genetic analysis and clinical evaluation of vacuolating cytotoxin gene A and cytotoxin-associated gene A in Taiwanese *Helicobacter pylori* isolates from peptic ulcer patients. Scand. J. Infect. Dis. **37:**51–57.
17. **Marshall, D. G., W. G. Dundon, S. M. Beesley, and C. J. Smyth.** 1998. *Helicobacter pylori*—a conundrum of genetic diversity. Microbiology **144:**2925–2939.
18. **Miehlke, S., C. Kirsch, K. Agha-Amiri, T. Günther, N. Lehn, P. Malfertheiner, M. Stolte, G. Ehninger, and E. Bayerdörffer.** 2000. The *Helicobacter pylori vacA* s1, m1 genotype and *cagA* are associated with gastric carcinoma in Germany. Int. J. Cancer **1:**322–327.
19. **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.
20. **Parsonnet, J., G. D. Friedman, D. P. Vandersteen, Y. Chang, J. H. Vogelman, N. Orentreich, and R. K. Sibley.** 1991. *Helicobacter pylori* infection and the risk of gastric carcinoma. N. Engl. J. Med. **325:**1127–1131.
21. **Pride, D. T., R. J. Meinersmann, and M. J. Blaser.** 2001. Allelic variation within *Helicobacter pylori babA* and *babB.* Infect. Immun. **69:**1160–1171.
22. **Ryan, K. A., A. P. Moran, S. O. Hynes, T. Smith, D. Hyde, C. A. O'Morain, and M. Maher.** 2000. Genotyping of *cagA* and *vacA*, Lewis antigen status, and analysis of the poly(C) tract in the alpha(1,3)-fucosyltransferase gene of Irish *Helicobacter pylori* isolates. FEMS Immunol. Med. Microbiol. **28:**113–120.
23. **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 patient groups in Germany. J. Clin. Microbiol. **36:**1285–1289.
24. **Van Doorn, L.-J., C. Figueiredo, F. Mégraud, S. Pena, P. Midolo, D. M. De Magalhães Queiroz, F. Carneiro, B. Vanderborght, M. D. 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.
25. **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 outcome: studies in four different countries. J. Clin. Microbiol. **37:**2274–2279.