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Two Different Families of *hopQ* Alleles in *Helicobacter pylori*

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***Helicobacter pylori* genomes contain about 30 different *hop* genes, which encode outer membrane proteins. In this study, we analyzed genetic diversity in the *H. pylori hopQ* (*omp27*) locus, which corresponds to HP1177 in the genome of *H. pylori* reference strain 26695. *hopQ* and its flanking genes were PCR amplified from multiple *H. pylori* strains, and the nucleotide sequences were determined. This analysis revealed the existence of two different families of *hopQ* alleles. Type I *hopQ* alleles are present in the genomes of two fully sequenced *H. pylori* strains, whereas the existence of type II *hopQ* alleles has not previously been recognized. Type I and type II *hopQ* alleles are 75 to 80% identical in nucleotide sequences and encode predicted outer membrane proteins that are 68 to 72% identical in amino acid sequences. PCR-based methods were developed to enable rapid differentiation between type I and type II *hopQ* alleles. Type I *hopQ* alleles were found significantly more commonly in *cag*$^+$/type s1-*vacA* strains from patients with peptic ulcer disease than in *cag*-negative/s2-*vacA* strains from patients without ulcer disease ($P < 0.001$). Determination of *hopQ* allelic types provides a new method for classification of *H. pylori* strains. Further studies in multiple populations of patients are indicated to evaluate the usefulness of this approach for distinguishing potentially ulcerogenic *H. pylori* strains from less virulent strains.**

Helicobacter pylori is a gram-negative bacterium that persistently colonizes the stomachs of more than half of the world's human population. Colonization of the stomach by *H. pylori* consistently induces gastric inflammation, known as superficial chronic gastritis, and is a risk factor for development of peptic ulcer disease and gastric malignancies (16, 20).

H. pylori exhibits a very high level of intraspecies genetic diversity (11, 31, 55). When *H. pylori* strains isolated from unrelated humans are compared, each isolate is genetically unique, with a level of relatedness among most orthologous sequences ranging from about 90 to 99% nucleotide identity (1, 4, 21, 24, 42, 49). This diversity is the consequence of numerous point mutations, combined with a very high rate of intraspecies genetic recombination (11, 24, 49, 55). In addition, certain genes are present in some *H. pylori* strains but absent from others (3, 6, 13, 45). Insight into genetic variation among *H. pylori* strains has been gained by comparing the complete genome sequences of two different *H. pylori* strains (26695 and J99) (6, 51). Overall, the nucleotide sequences of these two genomes are about 94% identical. However, 206 different genes have been identified that are present in only one strain but not the other (117 genes present only in strain 26695 and 89 present only in strain J99) (6).

The majority of *H. pylori*-infected persons remain asymptomatic for decades, but a subset develop peptic ulcer disease, distal gastric adenocarcinoma, or gastric lymphomas (16, 20). Genetic variation among *H. pylori* strains could be a factor that helps to explain these diverse clinical outcomes of *H. pylori* infection. Thus far, two candidate markers for distinguishing virulent *H. pylori* strains (i.e., those associated with peptic ulceration or gastric carcinoma) from less virulent strains have been studied extensively: presence or absence of the *cag* pathogenicity island and polymorphisms in *vacA* alleles.

The *cag* pathogenicity island is a ~40-kb DNA segment that encodes a highly antigenic protein (CagA) and a type IV secretion pathway utilized for translocating CagA into host cells (2, 7, 14, 15, 35, 46, 47, 52). Upon entry into eukaryotic cells, CagA undergoes phosphorylation of tyrosine residues and induces cytoskeletal alterations in cells (7, 15, 35, 46, 47). The *cag* pathogenicity island also encodes factors that activate proinflammatory signal transduction pathways in epithelial cells and that probably contribute to an enhanced inflammatory response in the gastric mucosa (2, 14, 52).

The *vacA* gene encodes a secreted "vacuolating toxin" (9, 34, 39). Effects of VacA on cells include the formation of intracellular vacuoles, formation of anion-selective channels in the plasma membrane, induction of apoptosis, and alterations in the permeability of epithelial monolayers. In contrast to the *cag* island, which is present in some *H. pylori* strains but absent from others, all *H. pylori* strains contain a *vacA* allele. Two families of *vacA* alleles (designated type s1 and type s2) can be differentiated by analysis of the 5′ end of *vacA* (8). Type s1 and type s2-VacA protoxins undergo cleavage of amino-terminal signal sequences at two different sites, and as a result, secreted type s2-VacA toxins contain a 12-amino-acid amino-terminal hydrophilic segment that is absent from secreted type s1 toxins (8, 30, 32). The presence of this segment renders type s2-VacA toxins defective in vacuolating toxin activity.

Type s1-*vacA* alleles are found predominantly in *H. pylori* strains that contain the *cag* pathogenicity island, and type s2-*vacA* alleles are found predominantly in strains that lack the *cag* pathogenicity island (8, 22, 44, 54). In many different studies, infection with *cag*$^+$/s1-*vacA* strains has been associated with a higher risk of peptic ulceration than has infection with

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cag-negative/s2-*vacA* strains (8, 18, 22, 25, 29, 43, 44, 48, 53, 54). A role for the *cag* pathogenicity island and VacA in the pathogenesis of peptic ulcer disease also has been demonstrated in animal models (36, 50). In several studies, strains classified as cag^+/s1-*vacA* have been associated with an increased risk of distal gastric carcinoma (12, 33). Notably, associations between cag^+/s1-*vacA* strains and severe clinical illness have not been detected as readily in Asian populations as in Western populations (38).

In addition to the *cag* pathogenicity island and *vacA*, several other candidate markers for distinguishing virulent *H. pylori* strains from less virulent strains have been proposed. These include *iceA*, *babA2*, and *oipA* (22, 41, 54, 56). In an effort to identify new markers of virulence, a recent study used whole-genome DNA microarray methodology to analyze gene content in 15 different *H. pylori* strains (45). The *cag* pathogenicity island was present in 10 strains and was absent or present in an incomplete form in 5 strains. Ten genes located elsewhere in the *H. pylori* genome were reported to be "coinherited" with the *cag* island (i.e., present more frequently in cag^+ strains than in *cag*-negative strains). This group included six genes predicted to encode proteins of unknown function (hypothetical proteins), two genes predicted to encode type II DNA methyltransferases, and two genes predicted to encode outer membrane proteins (*babA* and *omp27*). It was suggested that these genes coinherited with the *cag* island might contribute to the virulence of cag^+ strains (45).

In the present study, we sought to examine in further detail the diversity that is reported to exist in the *omp27* locus. *H. pylori omp27*, corresponding to HP1177 in the genome of *H. pylori* 26695 and JHP1103 in the genome of *H. pylori* J99 (6, 51), belongs to the *hop* family of paralogous genes and has recently been renamed *hopQ* (5). We report here that *hopQ* alleles can be classified into two highly divergent families and report the use of PCR-based methodology for classifying *H. pylori* strains based on *hopQ* genotypes. We report that type I *hopQ* alleles are found significantly more commonly in cag^+/s1-*vacA* strains from patients with peptic ulcer disease than in *cag*-negative/s2-*vacA* strains from patients without ulcer disease. These data confirm and extend the microarray results reported by Salama et al. (45) and suggest that variation in *hopQ* genotypes among *H. pylori* strains may be a factor that influences the clinical outcome of *H. pylori* infections.

MATERIALS AND METHODS

Bacterial strains. *H. pylori* strains 26695 and J99 are reference strains for which the entire genome sequences are known (6, 51), and *H. pylori* Tx30a is an additional reference strain (ATCC 51932). The other 30 *H. pylori* strains utilized in this study were isolated from patients in Denver, Colorado, or Nashville, Tennessee, who underwent routine upper gastrointestinal endoscopy for a variety of indications. Fifteen of these patients had peptic ulcer disease; the other 15 patients had no ulcer detected at the time of endoscopy and no previous history of peptic ulceration. The *cagA* and *vacA* genotypes of these strains have been reported previously (8, 17, 19). *H. pylori* strains were cultured at 37°C on trypticase soy agar plates containing 5% sheep blood in ambient air containing 5% CO_2.

Nucleotide sequence analysis of *hopQ* alleles. DNA was extracted from *H. pylori* as described previously (8). In initial experiments, 3-kb amplicons containing *hopQ* (corresponding to HP1177 in the genome of *H. pylori* 26695) were PCR amplified by using primers BA7676 and BA7674, derived from the sequences of two flanking genes (HP1178 and HP1175, respectively) (Table 1). The nucleotide sequences of 3-kb amplicons were then determined in the Vanderbilt University Core Nucleotide Sequencing Laboratory, using the primer-walking method. The relatedness of *hopQ* alleles or deduced *hopQ* products from different strains was analyzed by aligning sequences using the ClustalW algorithm in a Macvector software package.

The *hopQ* (*omp27* or HP1177) sequence from *H. pylori* strain 26695 corresponds to GenBank accession number AE000623, and the *hopQ* (JHP1103) sequence from *H. pylori* strain J99 corresponds to GenBank accession number AE001538 (6, 51).

PCR-based methodology for typing *hopQ* alleles. Primers used for PCR-based typing of *hopQ* alleles are shown in Table 1. Primers OP5136 and OP4829 were designed to specifically amplify type I *hopQ* fragments, and primers BA8363 and BA8364 were designed to specifically amplify type II *hopQ* fragments. Thermal cycling parameters for each pair of primers were set at 95°C for 1 min, 50 or 55°C for 1 min, and 72°C for 2 min, for a total of 30 cycles.

Nucleotide sequence accession number. The *hopQ* nucleotide sequences described in this study have been assigned GenBank accession numbers AY147193 to AY147201.

TABLE 1. Oligonucleotide primers used for analysis of *H. pylori hopQ* alleles

Region amplified	Primer designation	Primer sequence
hopQ and flanking genes	BA7676[a]	5′AACGCTAAGGCTTTATGCTTATGCTC
	BA7674[b]	5′GTCGTTAAACCCGCTCTAAACTCGG
Type I *hopQ*	OP5136[c]	5′CAACGATAATGGCACAAACT
	OP4829[d]	5′GTCGTATCAATAACAGAAGTTG
Type II *hopQ*	BA8363[e]	5′TCCAATCCAGAAGCGATTAA
	BA8364[f]	5′GTTTTAATGGTTACTTCCACC

[a] Primer derived from HP1178 (*deoD*).
[b] Primer derived from HP1175.
[c] Forward primer corresponding to nucleotides 186 to 205 in *hopQ* from *H. pylori* 26695.
[d] Reverse primer corresponding to nucleotides 689 to 710 in *hopQ* from *H. pylori* 26695.
[e] Forward primer corresponding to nucleotides 199 to 218 in *hopQ* from *H. pylori* J262.
[f] Reverse primer corresponding to nucleotides 609 to 629 in *hopQ* from *H. pylori* J262.

RESULTS

Genetic diversity in the *hopQ* locus. As a first step in investigating genetic diversity present in the *hopQ* locus (corresponding to HP1177 in the genome of *H. pylori* 26695), we designed PCR primers based on the nucleotide sequences of two flanking genes (HP1175 [predicted to encode a conserved hypothetical integral membrane protein] and HP1178 [designated *deoD*, predicted to encode purine-nucleoside phosphorylase]) (Table 1). After use of these primers (designated BA7674 and BA7676, respectively) in PCR, a 3-kb product was successfully amplified from two strains for which the entire genome sequence is known (strains 26695 and J99), as well as from nine additional *H. pylori* strains (J104, J258, J262, Tx30a, J154, J190, J63, 86-313, and 87-230). Thus, we did not detect any evidence of large deletions in the *hopQ* loci of any of these strains. As a next step, we analyzed the nucleotide sequences of these 3-kb PCR products. Each PCR product contained a *hopQ* orthologue (corresponding to HP1177 in the genome of *H. pylori* 26695), flanked by sequences orthologous to HP1175 and HP1178 from *H. pylori* strain 26695.

The *hopQ* alleles in these strains ranged from 1,890 to 1,926 nucleotides in length. The nucleotide sequences of *hopQ* alleles amplified from *H. pylori* strains J104 and J258 and the *hopQ* sequences from reference strains 26695 and J99 were all

TABLE 2. Analysis of relatedness among *hopQ* alleles and deduced *hopQ* products from different *H. pylori* strains[a]

Strain	Nucleotide or amino acid identity (%)[b]										
	26695	J99	J104	J258	J262	Tx30a	J154	J190	J63	86–313	87–230
26695		89	95	95	**76**	**79**	**79**	**77**	**77**	**79**	**75**
J99	87		91	90	**77**	**79**	**79**	**78**	**78**	**77**	**77**
J104	95	89		93	**75**	**77**	**77**	**76**	**77**	**77**	**75**
J258	94	88	93		**78**	**80**	**80**	**80**	**78**	**79**	**78**
J262	**70**	**70**	**68**	**70**		94	94	95	95	94	93
Tx30a	**70**	**70**	**69**	**71**	94		99	97	96	94	93
J154	**71**	**70**	**69**	**71**	94	99		97	96	94	93
J190	**71**	**71**	**69**	**72**	95	96	96		95	94	93
J63	**71**	**71**	**70**	**72**	94	96	96	95		93	93
86–313	**72**	**70**	**70**	**70**	94	93	93	93	92		93
87–230	**69**	**70**	**68**	**70**	93	93	93	94	93	93	

[a] The *hopQ* alleles in strains 26695, J99, J104, and J258 are classified as type I, and the *hopQ* alleles in strains J262, Tx30a, J154, J190, J63, 86–313, and 87–230 are classified as type II. Levels of relatedness shown in boldface indicate the considerable divergence between type I and type II *hopQ* alleles.

[b] Values in the top half of the table represent nucleotide identity, and values in the bottom half represent amino acid identity.

relatively closely related to each other (ranging from 89 to 95% nucleotide identity) (Table 2). Similarly, the nucleotide sequences of *hopQ* alleles amplified from *H. pylori* strains J262, Tx30a, J154, J190, J63, 86-313, and 87-230 were all closely related to each other (93 to 99% nucleotide identity). In contrast, the sequences of *hopQ* alleles amplified from strains J262, Tx30a, J154, J190, J63, 86-313, and 87-230 were only 75 to 80% identical to *hopQ* sequences from strains 26695, J99, J104, and J258. Thus, phylogenetic analysis indicated the existence of two separate lineages of *hopQ* alleles (Table 2). Hereafter, we define the family of *hopQ* alleles similar to those of the fully sequenced reference strains (26695 and J99) as type I *hopQ* alleles and define the newly recognized family of *hopQ* alleles present in strains J262, Tx30a, J154, J190, J63, 86-313, and 87-230 as type II *hopQ* alleles.

All of the *hopQ* alleles analyzed encoded predicted proteins with molecular masses ranging from 68 to 70 kDa. The deduced type I HopQ protein sequences encoded by strains 26695, J99, J104, and J258 were all relatively closely related to each other (ranging from 88 to 95% amino acid identity) (Table 2). Similarly, the deduced type II HopQ protein sequences encoded by strains J262, Tx30a, J154, J190, J63, 86-313, and 87-230 were closely related to each other (92 to 99% amino acid identity). In contrast, the deduced type II HopQ sequences encoded by strains J262, Tx30a, J154, J190, J63, 86-313, and 87-230 were highly divergent (68 to 71% amino acid identity) when compared with HopQ sequences from strains 26695, J99, J104, and J258 (Table 2). Type I and type II HopQ protein sequences were closely related within a region comprising 55 amino acids at the amino terminus (which includes a predicted 21-amino-acid amino-terminal signal sequence) and within a region comprising about 183 amino acids at the carboxy terminus (Fig. 1). In contrast, the midregions of type I and type II HopQ proteins were highly divergent (Fig. 1).

As reported previously (5), the paralogous *H. pylori* genes to which type I *hopQ* alleles are most closely related (based on phylogenic analysis of the deduced protein sequences) include *hopA*, *hopZ*, *hopD*, *hopS* (*babA*), *hopU*, *hopT* (*babB*), *hopM*, *hopN*, *hopO*, and *hopP*. Analysis of type II *hopQ* alleles indicated that they were related to the same group of paralogous genes. When type II *hopQ* alleles were compared with the known genome sequences of *H. pylori* strains 26695 and J99, using the BLAST network service of the National Center for Biotechnology Information, the highest level of relatedness was with the type I *hopQ* alleles (HP1177 and JHP1103) in these two genomes (76 to 79% nucleotide identity). Sequences closely related (>80% nucleotide identity) to type II *hopQ* alleles were not identified in either of the two fully sequenced *H. pylori* genomes. Thus, each of the fully sequenced *H. pylori* genomes contains a single type I *hopQ* allele.

Distribution of type I and type II *hopQ* alleles among *H. pylori* strains. To investigate further the prevalence and distribution of type I and type II *hopQ* alleles among *H. pylori* strains, we next examined a larger panel of well-characterized strains. We selected 15 *H. pylori* strains (each of which possessed the *cag* pathogenicity island and type s1-*vacA* alleles) that had been isolated from patients with peptic ulcer disease and 15 strains (each of which lacked the *cag* pathogenicity island and possessed type s2-*vacA* alleles) that had been isolated from patients with no history of peptic ulcer disease (Table 3). We reasoned that these two groups of strains should represent two ends of a spectrum of virulence. DNA was extracted from the 30 different strains, and, when used as a template for PCR amplification of a fragment of the 16S rRNA gene (40), each of the DNA preparations yielded a product of the expected size (data not shown). Using primers BA7676 and BA7674, we attempted to amplify 3-kb products containing *hopQ* sequences from each of the 30 DNA preparations, as described above. Amplicons of the expected size (3 to 3.5 kb) were obtained from 28 of the 30 strains, a 5-kb amplicon was obtained from one strain (*H. pylori* strain J166), and no amplicon was obtained from one strain (*H. pylori* strain 87-75). The complete *hopQ* sequences of eight of these strains were determined as described above (Table 2). Partial nucleotide sequences of *hopQ* alleles from the remaining strains then were determined by sequencing the PCR products, using a single primer (OP4702, 5′ATGAAAAAAACGAAAAAAAC), derived from the nucleotide sequence of the 5′ end of *hopQ*. This approach yielded about 400 nucleotides of *hopQ* sequence from 20 different strains. Each of these 20 *hopQ* sequences could be classified into either the type I or type II family, based on alignments with the two families of *hopQ* alleles shown in Table 2. Thus, among the 28 *H. pylori* strains from which *hopQ* sequence data were successfully obtained, 14 strains contained type I *hopQ* alleles and 14 strains contained type II *hopQ* alleles (Table 3). Thirteen (87%) of 15 cag^{+} *H. pylori* strains from patients with peptic ulcer disease contained type I *hopQ* alleles, and 1 (8%) of 13 evaluable *cag*-negative *H. pylori* strains from patients with gastritis alone contained type I *hopQ* alleles ($P < 0.001$, Fisher's exact test). Conversely, two (13%) of 15 cag^{+} *H. pylori* strains from patients with peptic ulcer disease contained type II *hopQ* alleles, and 12 (92%) of 13 evaluable *cag*-negative *H. pylori* strains from patients with gastritis alone contained type II *hopQ* alleles ($P < 0.001$).

PCR-based methodology for detecting type I and type II *hopQ* alleles. Based on analysis of the *hopQ* nucleotide sequences described above, we designed PCR primers that could be used to selectively amplify type I *hopQ* sequences but not type II *hopQ* sequences (Table 1). When tested on a panel of

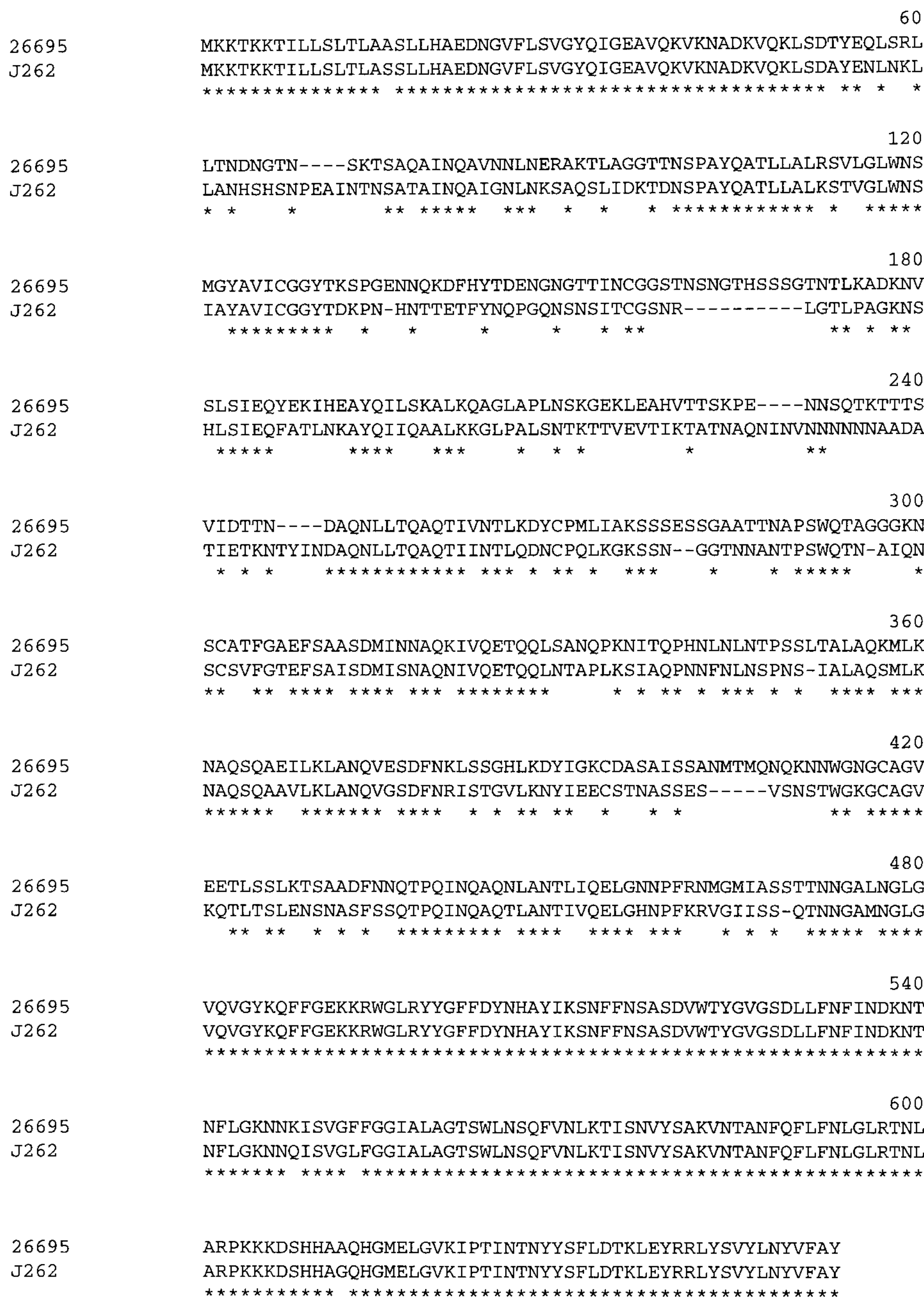

FIG. 1. Alignment of the deduced HopQ amino acid sequence of *H. pylori* strain 26695 (the prototype type I HopQ sequence) with that of HopQ from strain J262 (the prototype type II HopQ sequence). Stars indicate identical amino acid residues. The amino-terminal and carboxy-terminal regions of the two proteins are closely related, but the midregions are highly divergent.

11 strains for which the entire *hopQ* nucleotide sequences had been determined (Table 2), these primers functioned effectively to amplify products of the expected size from strains known to contain type I *hopQ* alleles but not from strains known to contain type II *hopQ* alleles (Fig. 2). We also designed primers that could be used to selectively amplify only type II *hopQ* sequences but not type I *hopQ* sequences (Table 1). When tested on the same group of strains, these primers functioned as predicted and amplified products of the expected size only from strains known to contain type II *hopQ* alleles (Fig. 2).

We then used these primers to detect the presence of type I and type II *hopQ* alleles in the panel of 30 different well-characterized *H. pylori* strains. By use of PCR primers specific for the amplification of type I *hopQ* fragments, PCR products of the expected size were successfully amplified from 16 of the 30 strains (Tables 3 and 4). By use of PCR primers specific for amplifying type II *hopQ* fragments, PCR products of the ex-

TABLE 3. Genotypes of *H. pylori* strains analyzed in this study[a]

Strain	*cagA* type	*vacA* type	Type of *hopQ* allele in HP1177 locus based on nucleotide sequence analysis[b]	Type of *hopQ* allele detected using PCR-based typing methods[c]
J104	+	sla/m1	I*	I
J166	+	slb/m1	I	I, II
J258	+	sla/m1	I*	I
J116	+	sla/m2	I	I, II
J123	+	sla/m2	I	I
J87	+	sla/m2	I	I, II
J223	+	sla/m2	I	I, II
87-29	+	slb/m1	I	I
87-199	+	sla/m1	I	I
87-33	+	slb/m1	I	I
92-21	+	sla/m1	II	I, II
J128	+	sla/m2	I	I
92-25	+	slb/m1	I	I
J178	+	sla/m1	I	I
J133	+	slb/m2	II	II
J262	−	s2/m2	II*	II
J154	−	s2/m2	II*	II
J190	−	s2/m2	II*	II
J195	−	s2/m2	II	II
87-203	−	s2/m2	I	I, II
87-75	−	s2/m2	NA[d]	I, II
86-313	−	s2/m2	II*	II
87-225	−	s2/m2	II	II
87-90	−	s2/m2	NA[d]	None
87-230	−	s2/m2	II*	II
92-20	−	s2/m2	II	II
92-23	−	s2/m2	II	II
J63	−	s2/m2	II*	II
86-338	−	s2/m2	II	II
92-28	−	s2/m2	II	II

[a] All 15 $cagA^+$ strains were isolated from patients with peptic ulcer disease, whereas all 15 *cagA*-negative strains were isolated from patients without peptic ulcer disease.
[b] The HP1177 locus was PCR amplified using primers BA7676 and BA7674 (derived from flanking genes). Subsequently, either complete nucleotide sequence analysis of the *hopQ* allele was performed (indicated by asterisks), or sequence analysis of the 5′ end of *hopQ* was performed using primer OP4702.
[c] Data are based on results of PCRs using primers that selectively amplified type I *hopQ* alleles or primers that selectively amplified type II *hopQ* alleles.
[d] NA, not applicable, due to failure to obtain the desired PCR product (strain 87-75) or failure to obtain *hopQ* sequence data (strain 87-90).

pected size were successfully amplified from 20 of the 30 strains (Tables 3 and 4). As described above, based on partial nucleotide sequence analysis of 3-kb amplicons, 14 strains were known to contain type I *hopQ* alleles and 14 strains were known to contain type II *hopQ* alleles. These allelic types were successfully identified in all 28 strains by using PCR with type-specific *hopQ* primers (Table 3). Thus, there was a high level of agreement between results obtained using two different experimental approaches for analyzing *hopQ* genotypes. The only apparent discrepancy involved the amplification of both type I and type II products from seven different *H. pylori* strains, whereas sequence analysis of 3-kb amplicons from these strains revealed the presence of only one type of *hopQ* allele in each strain (Table 3).

To investigate the basis for amplification of both type I and type II *hopQ* fragments from seven strains, two strains (J116 and J166) were selected for more detailed analysis. Nucleotide sequence analysis of the ~0.5-kb PCR products amplified from these strains using type I-specific and type II-specific *hopQ* primers confirmed that both type I and type II *hopQ* products had been amplified from each strain. To rule out the possibility that this phenomenon reflected the presence of a mixed population of bacteria, single colonies of strain J116 and strain J166 were isolated and then reanalyzed by PCR. By using type I-specific and type II-specific *hopQ* primers, both type I and type II *hopQ* fragments were amplified from single colonies of each strain. Therefore, it seems likely that these strains contain two different *hopQ* alleles (one in a locus corresponding to HP1177 and one in a different chromosomal locus). The amplification of only one *hopQ* allelic type from these strains using primers derived from HP1178 and HP1175 suggests that the second *hopQ* locus is flanked by genes unrelated to HP1178 and HP1175.

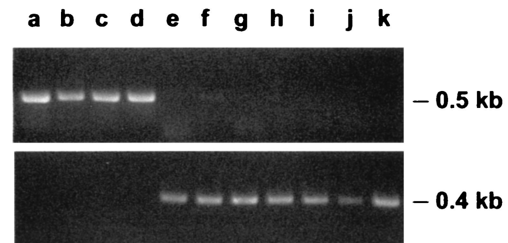

FIG. 2. PCR-based methodology for identifying two families of *hopQ* alleles. Top panel: primers OP5136 and OP4829 amplified a product of the expected size from strains J99, 26695, J104, and J258 (lanes a to d, respectively) but not from the other strains tested (lanes e to k). Bottom panel: primers BA8363 and BA8364 amplified a product of the expected size from strains J262, Tx30a, J154, J190, J63, 86-313, and 87-230 (lanes e to k, respectively) but not from the other strains tested (lanes a to d).

Based on PCR-based detection of *hopQ* alleles, type I *hopQ* alleles were detected in 14 (93%) of 15 cag^+ *H. pylori* strains cultured from patients with peptic ulcer disease. In contrast, type I *hopQ* alleles were detected in only 2 (13%) of 15 *cag*-negative *H. pylori* strains cultured from patients with no history of peptic ulcer disease ($P < 0.001$). By use of PCR primers specific for the amplification of type II *hopQ* fragments, PCR products of the expected size were successfully amplified from 6 (40%) of 15 cag^+ *H. pylori* strains cultured from patients with peptic ulcer disease and from 14 (93%) of 15 *cag*-negative *H. pylori* strains cultured from patients with no history of peptic ulcer disease ($P = 0.005$) (Table 4). These data indicate that type I *hopQ* sequences are found significantly more frequently in cag^+ *H. pylori* strains from patients with peptic ulcer disease (putative "ulcerogenic" strains) than in *cag*-negative strains

TABLE 4. Detection of *hopQ* alleles in *H. pylori* strains using PCR-based methodology[a]

Type of *H. pylori* strain and associated clinical condition (*n*)	No. of strains from which the indicated *hopQ* product was amplified			
	Type I only	Type II only	Type I and type II	Neither type
cag^+/s1-*vacA*, peptic ulcer (15)	9	1	5	0
cag-negative/s2-*vacA*, no peptic ulcer (15)	0	12	2	1

[a] Type I *hopQ* fragments were amplified from cag^+ strains significantly more frequently than from *cag*-negative strains ($P < 0.001$). Type II *hopQ* fragments were amplified significantly more frequently from *cag* mutant strains than from *cag*+ strains ($P = 0.005$).

from patients with no history of ulcer disease (putative nonulcerogenic strains).

DISCUSSION

A comparison of the complete genome sequences of two different *H. pylori* strains has provided considerable insight into intraspecies genetic diversity in *H. pylori* (6). Most orthologous sequences in these two genomes show levels of relatedness ranging from 90 to 99% nucleotide identity. Analyses of larger collections of strains have reported similar levels of relatedness for most *H. pylori* genes analyzed thus far, including *flaA*, *ureA*, *babA*, and *cysS* (1, 21, 42, 49).

In the present study, we analyzed genetic diversity among *hopQ* alleles of *H. pylori*. The two previously known *hopQ* alleles (from the two fully sequenced reference strains, 26695 and J99) are 89% identical in nucleotide sequences. Among the nine completely sequenced *hopQ* alleles described in the present study, we report that *hopQ* alleles from two different *H. pylori* strains (J104 and J258) are closely related to these previously characterized *hopQ* alleles. In contrast, *hopQ* sequences from seven strains are considerably different from the previously characterized *hopQ* alleles. Based on phylogenetic analysis (Table 2), it seems appropriate to classify *H. pylori* *hopQ* alleles into two different families, designated type I and type II. The *hopQ* alleles in the two fully sequenced *H. pylori* genomes (from strains 26695 and J99) represent prototypes for the type I family of *hopQ* alleles. In contrast, type II *hopQ* alleles have not been previously recognized. These data indicate that, despite the availability of two complete *H. pylori* genome sequences, the full range of genetic diversity among *H. pylori* strains is by no means completely characterized.

Each of the two fully sequenced *H. pylori* genomes contains a single copy of a type I *hopQ* allele and no sequences closely related (i.e., >80% nucleotide identity) to type II *hopQ* alleles. Interestingly, in the present study, we identified several strains that apparently contain both a type I *hopQ* allele and a type II *hopQ* allele. Nucleotide sequence analysis indicates that one allele is located in a locus corresponding to HP1177 in the genome of *H. pylori* 26695, and the second *hopQ* allele is presumed to be in a different, not-yet-identified locus. Duplication of *H. pylori* genes encoding outer membrane proteins has been reported previously for *babA*, *HopJ/K*, and *hopM/N*, and in the case of *babA*, such duplication can be strain specific (5, 26). Our data indicating the presence of strain-specific variation in the number of *hopQ* alleles per genome are therefore not entirely surprising.

Intraspecies genetic recombination occurs commonly in *H. pylori*, and evidence of such recombination has been readily detected by analysis of several different *H. pylori* genes (1, 8, 10, 28, 49). It seems likely that evidence of recombination between type I and type II *hopQ* alleles could be detected if further analysis were undertaken. However, the striking finding presented here is that the phylogenetic structure of these two families of *hopQ* alleles seems to have remained relatively intact despite frequent intraspecies genetic recombination. Based on the substantial amino acid sequence diversity that exists between type I and type II HopQ proteins, we think that it is likely that these two families of proteins possess differing functional properties. For example, if HopQ proteins function as adhesins, there might be differences in the binding specificities of type I versus type II HopQ proteins. Similar to the classification of *hopQ* alleles into two highly divergent families, *H. pylori vacA* alleles (encoding secreted vacuolating toxins) have been classified into highly divergent families, designated type m1 and type m2 (8, 10). The nucleotide sequences of type m1- and type m2-*vacA* alleles are only about 70% identical within a 0.7-kb midregion of the gene. As predicted, based on the existence of two families of *vacA* allelic types, the products of type m1- and type m2-*vacA* alleles have indeed been reported to differ in functional properties (27, 37).

In a recent study, the gene content of 15 different *H. pylori* strains was analyzed by using DNA microarray methodology (45). A group of 1,281 different genes was detected in all 15 strains, whereas 362 genes were nonconserved (and hence presumed to be nonessential). Based on the detection of *hopQ* sequences in 80% of the 15 strains studied, *hopQ* (*omp27*) was classified as a nonconserved gene. The region of *hopQ* utilized on these microarray chips corresponded to a 1,308-nucleotide fragment (nucleotides 70 to 1377 of *hopQ* from strain 26695) (N. Salama, personal communication), i.e., a type I *hopQ* allele. It seems likely that type II *hopQ* sequences would not hybridize efficiently to this DNA under high-stringency conditions, and we speculate that type II *hopQ* alleles were not detected using the previously described microarray methodology. In the present study, by using two different sets of PCR primers designed to specifically amplify only type I and type II *hopQ* alleles, we were able to detect the presence of *hopQ* sequences in 29 of 30 different *H. pylori* strains (Table 3). These results suggest that *hopQ* alleles are present in all or nearly all *H. pylori* strains.

Interestingly, the microarray study by Salama et al. reported that *hopQ* alleles were detected in cag^+ strains more commonly than in *cag*-negative strains (10 of 10 cag^+ strains versus 2 of 5 *cag*-negative strains) (45). In the present study, we found that type I *hopQ* alleles were present in cag^+ strains significantly more commonly than in *cag*-negative strains. Based on the presumption that the previous microarray methodology only could detect type I *hopQ* alleles, our present results seem consistent with the previous microarray results (45).

Previous studies have concluded that type s1-*vacA* alleles are found predominantly in *H. pylori* strains that contain the *cag* pathogenicity island, whereas type s2-*vacA* alleles are found predominantly in *H. pylori* strains that lack the *cag* pathogenicity island (8, 22, 44, 54). Other genes found more commonly in cag^+ strains than in *cag*-negative strains include *babA2* alleles (encoding a functional Lewis b binding adhesin) (22, 26), several additional genes identified in a microarray analysis (45), and type I *hopQ* alleles. Because *H. pylori* has a recombinational population structure (23, 49), these groups of strains probably do not represent distinct phylogenetic lineages. Instead, these examples of linkage disequilibrium presumably indicate that a selective advantage is conferred to strains with certain genotypes.

A genotype-based classification of *H. pylori* strains is potentially useful for predicting the clinical outcome of infections. For example, previous studies have concluded that *H. pylori* strains carrying the *cag* pathogenicity island and type s1-*vacA* alleles are associated with a higher risk of peptic ulcer disease than are strains that lack these genetic elements (8, 18, 22, 25,

29, 43, 44, 48, 53, 54). One recent study concluded that "triple-positive" strains (i.e., possessing the *cag* pathogenicity island, type s1-*vacA* alleles, and *babA2* alleles) are associated with a particularly high risk of peptic ulceration (22). The results of the present study provide further evidence indicating that putative ulcerogenic strains may contain genetic elements different from those found in nonulcerogenic strains. In future studies, it will be important to analyze *H. pylori* strains from multiple populations of patients and from various parts of the world in order to gain further insight into the patterns of linkage disequilibrium that exist in this species. In addition, multivariate analysis of large collections of *H. pylori* strains from humans with well-defined clinical diagnoses may permit identification of the *H. pylori* factors that are most relevant for predicting clinical outcome.

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