Prevalence of vacuolating cytotoxin production and distribution of distinct vacA alleles in Helicobacter pylori from China


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Prevalence of Vacuolating Cytotoxin Production and Distribution of Distinct vacA Alleles in Helicobacter pylori from China

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Studies of Helicobacter pylori from the West have linked production of vacuolating cytotoxin and a particular signal sequence (s1a) allele of the underlying vacA gene to peptic ulcer disease (PUD). Among Chinese H. pylori, most isolates from both PUD and gastritis patients were toxigenic (35/46 and 29/35, respectively). Polymerase chain reaction and DNA sequencing showed that 95 of 96 isolates carried vacA s1a alleles. In the mid-region, 78 of 96 isolates carried m2; 14 were m1-like but only 87% identical (DNA-level) to classical m1 and were designated m1b; the other 4 were unusual hybrids (m1b-type proximal, m2-type distal). Isolates with m1b and m1b-m2 alleles produced higher levels of vacuolating activity than did isolates with m2 alleles ($P < .01$). There was no association between any vacA allele and disease. These results suggest that the composition of H. pylori gene pools varies geographically and that other as-yet-unknown polymorphic H. pylori genes are important in PUD.

Helicobacter pylori is the major cause of gastritis and peptic ulcer disease (PUD) and an early risk factor for gastric cancer [1, 2], even though most infections are asymptomatic. It is extremely diverse as a species [3–5], and two disease-associated genetic loci have been identified to date: the polymorphic gene encoding cytotoxin (vacA) and the cytotoxin-associated gene (cag) pathogenicity island, which is often detected by tests for cagA, one of its component genes [6]. Some 60%–70% of clinical isolates from Western patients with PUD produce a toxin (VacA) that induces vacuole formation in cultured mammalian cells, whereas 30%–50% of isolates from gastritis or non-ulcer dyspepsia patients are toxigenic [7–9]. The purified toxin can also cause gastric epithelial damage and mucosal ulceration in a mouse model [10]. Although about half of H. pylori isolates produce vacuolating cytotoxic activity in vitro, all possess the vacA cytotoxin gene [11]. DNA-level analysis of vacA from multiple H. pylori isolates from the United States indicated that all strains possess one of two types of signal sequence, s1 (which can be subtyped into s1a and s1b) or s2, and one of two types of middle region, m1 or m2 [12]. Vacuolization and more severe clinical manifestations seem to be associated with particular vacA alleles. H. pylori strains with the signal sequence allele s1a are associated with higher levels of vacuolating cytotoxic activity, enhanced gastric inflammation, and duodenal ulceration than are the s1b or s2 alleles. The m1 middle region allele is associated with higher levels of toxin activity and more severe gastric epithelial damage than is the m2 allele [12, 13].

A second disease-associated marker, cagA, encodes a large immunodominant protein (CagA) of unknown function. The cagA gene is one of many genes in a 38-kb pathogenicity island” (PAI), some of whose encoded proteins help elicit a severe inflammatory response that is probably injurious to host tissue [14, 15]. Strains that do not produce the CagA protein generally lack the entire cag PAI. Recently we found that essentially all (82 of 83 tested) H. pylori isolates from patients with PUD or chronic gastritis in Shanghai, China, were cagA” [16]. This remarkable uniformity prompted us to also investigate the toxigenicity and alleles of the vacA gene of 81 of these isolates and 15 other H. pylori isolates from China.

Materials and Methods

Patients and H. pylori isolates. A total of 96 independent H. pylori isolates, each from a different patient, were examined. Eighty-one isolates were from Chinese patients with PUD (46) or with chronic gastritis (35) in two hospitals in Shanghai [16]. An additional 15 strains were from patients with PUD or chronic gastritis in Guangzhou, which is >1000 kilometers from Shanghai and is also separated from it linguistically. DNAs were isolated from these 96 H. pylori strains with the InstaGene Matrix Kit
Vacuolating cytotoxin assay. 

*H. pylori* isolates were assayed for cytotoxin activity by a modification of previously used procedures [17, 8]. Briefly, *H. pylori* colonies from blood agar plates were inoculated with a wire loop in 6 mL of Brucella broth (Difco Laboratories, Detroit) supplemented with 5% (vol/vol) fetal calf serum (FCS; Sigma, St. Louis). The cultures were incubated at 37°C in an atmosphere of 10% CO₂, 5% O₂, and 85% N₂ on a gyratory shaker at 140 rpm for 48 h (late exponential growth). The liquid *H. pylori* cultures were centrifuged at 3000 × g for 20 min, and the supernatants were added directly to the test medium of HeLa cells prepared as follows. Confluent HeLa cells cultured in Dulbecco’s MEM (DMEM) with 10% fetal calf serum (FCS) were trypsinized and adjusted to a density of 2 × 10⁵ cells/mL. One milliliter of this cell suspension was seeded into each well of a 24-well microtiter plate (Becton Dickinson Europe, Meylan, France). After 48 h of incubation at 37°C in 5% O₂ and 95% CO₂, the culture medium was replaced by 0.8 mL of test medium (DMEM with 25 mM HEPES, pH 7.4, without FCS), and 0.8 mL of *H. pylori* culture supernatant was added. The HeLa cells were incubated at 37°C in 5% O₂ and 95% CO₂ and examined periodically by phase-contrast microscopy for the presence of intracellular vacuoles. The supernatant of each *H. pylori* isolate was tested in duplicate and scored as positive if at least 50% of the cells contained vacuoles. To determine the titer of cytotoxin activity, the culture supernatants were serially 2-fold diluted in test medium, and aliquots were applied to cells as above. The titer was defined as the highest final dilution of the sample that showed a positive response after 48 h of incubation. Culture supernatants with no cytotoxin activity at a 1:2 dilution were considered to be cytotoxin-negative. Sterilized Brucella broth was used as a negative control, and a culture supernatant of a cytotoxin-positive Dutch *H. pylori* strain (no. 157 [9]) was used as a positive control.

Polymerase chain reaction (PCR)—based vacA typing. Initial typing of vacA for s (signal) sequence alleles and m (middle) region alleles was carried out with the PCR primers described by Atherton et al. [12]. The s or m regions of the vacA gene from representative *H. pylori* isolates that were not typeable with these original primers were sequenced, and new primers for typing vacA of such isolates were designed (table 1).

DNA sequencing. A 0.7-kb segment carrying the vacA midregion from 4 isolates that, by PCR tests, did not seem to be of the standard m1 or m2 type was PCR-amplified with primers VAm-F and VAm-R (table 1). PCR products from 4 representative strains were analyzed by gel electrophoresis on 1% agarose. Bands containing PCR products were excised from the gel and purified with the QIAprep Spin Miniprep Kit (Qiagen). Manual dideoxynucleotide chain-termination DNA sequencing was done with the T7 Sequenase Quick-Denature Plasmid Sequencing Kit (US Biochemicals, Cleveland) using each of the following primers: M13-F (5'-GTAAAACGACGGCCAGTC-3'), M13-R (5'-CAGGAAACGTCATGAC-3'), M13-87-203 (m2) [11], M13-60190 (m1) [11]. Ligated DNAs were transformed into competent Escherichia coli DH5α with selection for ampicillin resistance. Recombinant plasmids were extracted and purified with the QIAprep Spin Miniprep Kit (Qiagen). Manual dideoxynucleotide chain-termination DNA sequencing was done with the T7 Sequenase Quick-Denature Plasmid Sequencing Kit (US Biochemicals, Cleveland) using each of the following primers: M13-F (5'-GTAAAACGACGGCCAGTC-3'), M13-R (5'-CAGGAAACGTCATGAC-3'), M13-87-203 (m2) [11], M13-60190 (m1) [11].

**Table 1.** *vacA*-specific oligonucleotide primers used for polymerase chain reaction (PCR) typing and sequencing.

<table>
<thead>
<tr>
<th>Region</th>
<th>Primer</th>
<th>Nucleotide sequence</th>
<th>Location (size) of PCR product</th>
</tr>
</thead>
<tbody>
<tr>
<td>m1</td>
<td>VA3-F</td>
<td>5'-GGTCCAAATTACGTCATGG-3'</td>
<td>2741–3030 (290 bp)*</td>
</tr>
<tr>
<td></td>
<td>VA3-R</td>
<td>5'-CCATTTGATCTGAGAACA-3'</td>
<td></td>
</tr>
<tr>
<td>m2</td>
<td>VA4-F</td>
<td>5'-GGAGCCCCAGAAACATTG-3'</td>
<td>976–1327 (352 bp)*</td>
</tr>
<tr>
<td></td>
<td>VA4-R</td>
<td>5'-CTAGACGGCTTGCAC-3'</td>
<td></td>
</tr>
<tr>
<td>s1</td>
<td>VAm1-F</td>
<td>5'-ATGGAAATACAAACAC-3'</td>
<td>797–1055 (259 bp)*</td>
</tr>
<tr>
<td></td>
<td>VAm1-R</td>
<td>5'-CTGCTTGAATGCGCAAC-3'</td>
<td></td>
</tr>
<tr>
<td>s1a</td>
<td>SS1-F</td>
<td>5'-GTCAGCATCACAC-3'</td>
<td>866–1055 (190 bp)*</td>
</tr>
<tr>
<td></td>
<td>SS1-R</td>
<td>5'-CTGCTTGCATGCGCAAC-3'</td>
<td></td>
</tr>
<tr>
<td>s1b</td>
<td>SS3-F</td>
<td>5'-AGCGCCATACCCGGCAAG-3'</td>
<td>(187 bp)*</td>
</tr>
<tr>
<td>s2</td>
<td>VAm1-F</td>
<td>5'-ATGGAAATACAAACAC-3'</td>
<td>284–569 (286 bp)*</td>
</tr>
<tr>
<td></td>
<td>VAm1-R</td>
<td>5'-CTGCTTGAATGCGCAAC-3'</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VAm-R</td>
<td>5'-GGAGATGTGTTCAGAACA-3'</td>
<td></td>
</tr>
</tbody>
</table>

* Nucleotide positions in *vacA* gene of *H. pylori* 60190 (m1) [11].

* Nucleotide positions in *vacA* gene of *H. pylori* 87-203 (m2) [11].

§ Known to be s1b-specific, but no published coordinates for strains of this type [12].

Ⅱ Nucleotide positions in *vacA* gene of *H. pylori* Tx30a (s2) [12].
with primer set SS1-F/VA1-R and sequenced similarly as described for the m region. Representative sequences have been deposited in Genbank under accession numbers AF035609, AF035610, AF035611, AF035612, AF035613, AF035614, AF035615, and AF035616.

Statistical methods. The \( \chi^2 \) test with Yates's continuity correction was used for analysis of categorical data. Data on cytotoxin activity were compared with the Spearman's rank correlation test.

Results

Cytotoxin activity. The culture supernatants from 64 (79\%) of the 81 Shanghai \( H. pylori \) isolates tested induced vacuoles in HeLa cells. With 61 of these 64 isolates, small vacuoles in the perinuclear region were observed by 8 h after addition of supernatants. By 18 h, those vacuoles had increased in number and size and occupied a large part of the cytoplasm. They remained visible for at least 48 h, essentially as had been reported with Western \( H. pylori \) strains \[18\]. Supernatants from 2 of the 64 isolates induced vacuoles within 5 h, but they also elicited HeLa cell detachment and degradation within 18 h, and hence these isolates were not studied further. One of the 64 toxigenic isolates exhibited a very slow vacuolating activity, evident after 48 h but not after 18 h. Two of the 17 nontoxigenic isolates caused an unusual HeLa cell lysis, which was distinct from detachment and degradation above and was not associated with intracellular vacuole formation.

Lack of clinical correlation with \( VacA \) phenotype. Thirty-five (76.1\%) of 46 \( H. pylori \) isolates from patients with PUD and 29 (82.9\%) of 35 \( H. pylori \) isolates from patients with chronic gastritis were toxigenic (difference not statistically significant, \( P > .05 \)). The cytotoxin titer of the culture supernatants of the \( H. pylori \) isolates did not correlate with patient disease status (\( P > .05 \); table 2). There was also no significant correlation between toxigenicity and the activity or degree of inflammation, the degree of atrophy, or the degree of intestinal metaplasia in the patients from whom these \( H. pylori \) were isolated (data not shown).

\( vacA \) alleles. The 81 isolates of patients from Shanghai as well as 15 isolates of patients from Guangzhou were typed by PCR for \( s \) (5' signal sequence) alleles of the \( vacA \) gene, using the standard primers. Each isolate yielded a 259-bp PCR fragment (figure 1, lane 2) characteristic of the \( s1 \) (potentially more virulent) allele, and none yielded the 286-bp fragment characteristic of the \( s2 \) (less virulent) allele. In further characterization by \( s1a \) versus \( s1b \) subtyping, 88 of these 96 isolates yielded a diagnostic 190-bp fragment (figure 1, lane 3), and many also yielded a faint 250-bp fragment (figure 1, lane 4) with the \( s1a \)-specific primer sets. Seven isolates yielded only a 250-bp fragment (figure 1, lane 5) with these primers, instead of the 190-bp fragment that is usually generated with the \( s1a \)-specific primers. None of the isolates yielded the diagnostic 187-bp fragment with the \( s1b \)-specific primers. The \( s \) region of 4 of the 7 anomalous isolates was sequenced and found to be \( s1a \)-like (Genbank accession nos. AF035612, AF035613, AF035614, and AF035615). However, these isolates carried substitutions of 2–3 bp at the standard annealing site of primer SS1-F (most critically, a 1-bp mismatch at the 3’ end and a second mismatch at either 2 or 5 nt from the 3’ end [figure 2]), which accounts for these particular isolates not yielding a standard 190-bp PCR fragment. Finally, 1 isolate, although \( s1 \)

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|c|c|}
\hline
\multicolumn{2}{|l|}{Vaccuolating cytotoxin activity titer} & \multicolumn{4}{|c|}{\textit{Total}} \\
\hline
\textit{Disease} & 0 & 1:2 & 1:4 & 1:8 & 1:16 \\
\hline
Chronic gastritis & 6 & 20 & 5 & 2 & 2 \\nPeptic ulcer disease & 11 & 23 & 4 & 7 & 1 \\
Total & 17 & 43 & 9 & 9 & 3 \\
\hline
\end{tabular}
\caption{Relationship between disease and vaccuolating cytotoxin activity.}
\end{table}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
\textit{strain} & \textit{primer SS1-F} & \textit{GGTCAAGATC} & \textit{CAGGACGTT} & \textit{ACAAGCGCC} & \textit{AACAGTGC} \\
\hline
R34A & \textit{GTC} & \textit{A...A...} & \textit{...T} & \textit{...A...} \\
R50A & \textit{T} & \textit{A...A...} & \textit{...A...} \\
R40A & \textit{...A...} & \textit{...A...} \\
R26A & \textit{A...A...} & \textit{G...A...} & \textit{...} \\
\hline
\end{tabular}
\caption{Sequence divergence at SS1-F primer binding site in certain Chinese \( H. pylori \) strains. Shown is segment of \( s1 \) region sequence of reference strain 60190 (coordinates 855–894 bp) \[12\] and of corresponding region from 4 unusual Chinese strains (represented in figure 1, lane 5). SS1-F primer binding site in strain 60190 DNA is underlined. Dots indicate identical nucleotides.}
\end{table}
in PCR tests, did not yield a product with either the s1a- or s1b-specific primers and was not studied further.

These 96 isolates were also tested for mid-region (m) alleles. Seventy-eight yielded a fragment of 352 bp (figure 1, lane 7) with m2-specific primers, and none yielded a discrete fragment with standard m1 primers. The remaining 18 anomalous isolates were assessed by PCR with new mid-region primers (VAm-F/VAm-R, table 1), and a fragment of the expected size (0.7 kb) was obtained in each case (data not shown). This fragment was sequenced from 4 representative isolates. Two of the isolates (R13A and R59A in table 3) had an m1-like sequence that is distinct from the canonical m1 sequence from US strains; they had 87%–88%, instead of the usual >95%, DNA sequence identity to standard m1 alleles and contained 4 of 19 and 7 or 8 of 20 noncomplementary bases at the annealing sites for standard m1 forward and reverse primers, respectively (figure 3A). We designate these alleles as “m1b” to reflect this divergence from canonical m1 alleles. The other 2 isolates yielded a slightly larger “0.7-kg” PCR fragment and contained a novel hybrid sequence closely matched to m1b at its 5′ end and to m2 at its 3′ end (figure 3B; table 3). These were designated “m1b-m2.” New PCR primers were designed based on m1b and m1b-m2 hybrid sequences and were used to examine the other 14 uncharacterized isolates. Twelve of those isolates yielded a 291-bp PCR product with the new m1b-specific primers VAm-F3 and VAm-R3 (figure 1, lane 8), as expected on the basis of the sequenced alleles. The other 2 isolates yielded a 295-bp fragment with the new m1b-specific primer VAm-F3, plus the standard m2 primer VA4-R (figure 1, lane 9), and thus are also of the m1b-m2 type. Thus, of these 96 Chinese H. pylori strains, 78 were vacA m2, 14 were vacA m1b, and 4 were of a novel m1b-m2 hybrid type. The distribution of vacA alleles among the H. pylori isolates from Shanghai and Guangzhou patients was similar (table 4; P > .5).

Relation of vacA genotype with cytotoxic activity and disease outcome in H. pylori–infected patients. Strains with the m1b and m1b-m2 hybrid alleles of the vacA gene produced higher levels of vacuolating activity than did strains with the m2 alleles (P < .01; table 5). There was no significant difference in the vacuolating activities of strains with m1b versus those with m1b-m2 alleles (P > .05). Similarly, there was no significant correlation between any vacA genotype and clinical outcome (P > .05; table 6).

Discussion

This study shows that the distribution of vacA alleles in Chinese H. pylori isolates differs from that of the H. pylori isolates in Western countries and identifies new types of vacA alleles. In addition, we found that most Chinese isolates produce an active vacuolating cytotoxin, independent of whether they were isolated from patients with PUD (76.1% toxigenic) or with chronic gastritis only (82.9% toxigenic).

Ninety-five of the 96 isolates tested carried an s1a allele at the 5′ end (signal sequence) of vacA. Neither the s1b nor the s2 alleles, which are common in the United States and are associated with low or no toxigenicity [12, 13], were found in Chinese isolates. Seventy-eight (81%) of the 96 Chinese isolates carried middle region alleles that, by standard PCR tests, matched the already known m2 (less toxigenic) type, and no isolate carried the canonical m1 allele that is so common among virulent Western strains [12]. The second most frequent allele (14 [14.6%] of 96 isolates) was related to the standard m1 type but shared only 87% instead of >95% DNA sequence identity with it and was thus designated m1b. A remarkable hybrid allele, with m1b- and m2-type sequences in its 5′ and 3′ region, respectively, was found in 4 independent isolates. This hybrid might be ascribed to a rare recombination event between m1b and m2 alleles in a region of limited homology, such as the 14-bp match shown in figure 3B. Isolates carrying these hybrid alleles were similar to m1b isolates in their high toxigenicity.

Previously, it was shown that all but 1 of the 81 Shanghai H. pylori isolates were cagA+ [16]. The cagA gene was also found

Table 3. DNA sequence relatedness in 0.7 kb from vacA gene mid-region of different H. pylori isolates.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>R13A [m1b]</td>
<td>97</td>
<td>96–74*</td>
<td>97–74*</td>
<td>87</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>R59A [m1b]</td>
<td>96–77*</td>
<td>97–77*</td>
<td>88</td>
<td>75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R10A [m1b–m2]</td>
<td>98–96*</td>
<td>88–81*</td>
<td>73–96*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R34A [m1b–m2]</td>
<td></td>
<td>88–78*</td>
<td>74–97*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60190 [m1]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>87-203 [m2]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Strains R13A, R59A, R10A, and R34A are from Shanghai and are described herein. GenBank accession nos. for strains R13A, R59A, R10A, R34A, 60190, and 87-203 are AF035610, AF035611, AF035609, AF035616, U05676, and U05677, respectively. 0.7-kb vacA middle region corresponds to nt 2392–3097 of H. pylori 60190 vacA [11].

* Proximal (nt 1–389) and distal (nt 390–end) of 0.7-kb middle region of vacA.
Figure 3. Structures of middle region alleles of vacA gene. Shown are middle region segments that correspond to nt 2392–3097 of canonical m1 allele of reference strain 60190 [11]. Dots indicate nucleotides that are identical among alleles characterized.

A, Comparison of typical m1b allele (Chinese strain R13A; upper line) and canonical m1 allele (strain 60190; lower line). Positions of binding sites for standard m1 primers [11] and for m1b-specific primers are underlined (see also table 1).

B, Comparisons of m1b-m2 hybrid allele (Chinese strain R10A; upper line), m1b allele (Chinese strain R13A; middle line), and canonical m2 allele (strain 87-203 [11]; lower line). m1b-m2 hybrid region resembles m1b alleles proximally and m2 alleles distally. Underlined 14 nucleotides at position 390 (upper line) identifies portion of m1b, m2, and m1b-m2 hybrid alleles that are matched in sequence and in which recombination would have generated observed m1b-m2 hybrid alleles. Blank region in m1b sequence identifies 15-bp segment that is absent from these m1b alleles (and also canonical m1 alleles) and present in m2 alleles.

in each of the 15 isolates from Guangzhou (unpublished data), a city that is >1000 km from Shanghai. This contrasts with the 30% cagA frequency among H. pylori from Western countries. The similar distributions of vacA alleles and the presence of cag PAI and in an H. pylori population that is more clonal with respect to vacA alleles and cagA than in the West [19]. Alternatively, it might reflect the distribution of genotypes of H. pylori strains that had colonized the ancestors of the present-day Chinese population, coupled with a tendency of H. pylori to be transmit-
Table 4. Distribution of *vacA* alleles in *H. pylori* isolated from patients in two cities in China.

<table>
<thead>
<tr>
<th>Allele</th>
<th>Shanghai</th>
<th>Guangzhou</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>s1a/m2</td>
<td>66 (81.5)*</td>
<td>12 (80.0)</td>
<td>78</td>
</tr>
<tr>
<td>s1a/m1b</td>
<td>11 (13.6)</td>
<td>3 (20)</td>
<td>14</td>
</tr>
<tr>
<td>s1a/m1b–m2</td>
<td>4 (4.9)</td>
<td>0 (0)</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>81</td>
<td>15</td>
<td>96</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%). There was no significant difference in distribution of *vacA* alleles between cities (*P > .5, χ² test*).

* 1 isolate was s1 by polymerase chain reaction but did not yield product with either s1a- or s1b-specific primers.

H. pylori especially in Asian strains. This might reflect either the back-

Table 5. Relationship between *vacA* type and vacuolating cytotoxin titer.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>0</th>
<th>1:2</th>
<th>1:4</th>
<th>1:8</th>
<th>1:16</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>m2</td>
<td>15</td>
<td>41</td>
<td>7</td>
<td>3</td>
<td>0</td>
<td>66</td>
</tr>
<tr>
<td>m1b</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>m1b–m2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>43</td>
<td>9</td>
<td>9</td>
<td>3</td>
<td>81</td>
</tr>
</tbody>
</table>

NOTE. Data are no. of strains. *vacA* m1b or m1b–m2 strains produce higher levels of cytotoxin activity than do *vacA* m2 strains (*P < .01*, Spearman’s rank correlation test). There was no significant difference between strains with m1b and strains with m1b–m2 in their ability to produce cytotoxin activity (*P > .05*).

Table 6. Relationship between disease and *vacA* mid-region alleles.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Allele</th>
<th>m2</th>
<th>m1b</th>
<th>m1b–m2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic gastritis</td>
<td></td>
<td>26</td>
<td>7</td>
<td>2</td>
<td>35</td>
</tr>
<tr>
<td>Peptic ulcer disease</td>
<td></td>
<td>40</td>
<td>4</td>
<td>2</td>
<td>46</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>66</td>
<td>11</td>
<td>4</td>
<td>81</td>
</tr>
</tbody>
</table>

NOTE. Data are no. of strains. *vacA* type was determined by polymerase chain reaction and DNA sequencing. All except 1 Chinese strain carried s1a allele by *vacA* leader region. There was no significant difference between strains from patients with chronic gastritis and those with peptic ulcer disease in their *vacA* alleles (*P > .05, χ² test*).

Acknowledgments

We thank Philip Roorda for his assistance with vacuolization assay and Catharjine B. Heringa (Yamanouchi Europe) for access to patient data and *H. pylori* isolates from Guangzhou.

References


