Prevalence of *Helicobacter pylori* vacA, cagA, cagE, iceA and babA2 genotypes in Thai dyspeptic patients

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**KEYWORDS**
*Helicobacter pylori*; Prevalence; vacA; cagA; cagE; iceA; babA2; Thai dyspeptic patients

**Summary**

**Objectives:** To investigate the prevalence of the vacA, cagA, cagE, iceA, and babA2 genotypes in *Helicobacter pylori* strains isolated from Thai dyspeptic patients, and to determine whether any correlation exists between these genotypes and clinical manifestations.

**Methods:** *Helicobacter pylori* was examined in 112 patients (62 with non-ulcer dyspepsia (gastritis), 34 with peptic ulcer disease, and 16 with gastric cancer (GCA)), detected by culture or direct detection from gastric biopsies. Allelic variants of the vacA, cagA, cagE, iceA, and babA2 genotypes were identified by using the polymerase chain reaction.

**Results:** The positive rates for the vacAs1, vacAs2, cagA, cagE, iceA1, iceA2, and babA2 genes in *H. pylori* of dyspeptic patients were 100%, 0%, 98.2%, 88.4%, 45.5%, 33.1%, and 92%, respectively. The allelic variant vacAs1m1 was more prevalent (58%) than vacAs1m2 (42%). The cagA and cagE genes were commonly found together (87.5%). The most predominant genotypes were vacAs1m1, cagA, cagE, iceA1, and babA2. The various genes alone or in combination had no statistically significant association with the clinical outcomes ($p > 0.05$).

**Conclusion:** Neither single gene nor combination of vacA, cagA, cagE, iceA, and babA2 genes was significantly helpful in predicting the clinical outcome of *H. pylori* infection in Thai patients. The high prevalence of these genes in *H. pylori* isolated from Thai patient groups suggests that *H. pylori* strains are geographically dependent.

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Introduction

*Helicobacter pylori* is considered an important etiological agent in the development of gastritis, peptic ulcers and gastric carcinoma.\(^1\)\(^2\) The occurrence of such diverse diseases with *H. pylori* may depend on specific properties of the organism, host genetic factors, and environmental factors.\(^3\) Although more than 50% of the world’s population is infected with *H. pylori*, a minority of carriers develop serious gastrointestinal diseases;\(^3\)-\(^5\) however, increasing evidence suggests that the genetic variability of *H. pylori* may itself be of clinical importance.\(^3\)-\(^7\) Several putative genes, such as *vacA*, *cagA*, *cagE*, *iceA* and *babA*, have been identified and may play important roles in the pathogenesis of *H. pylori* infection.\(^8\)-\(^10\)

The vacuolating cytotoxin gene (*vacA*) is present in all *H. pylori* strains.\(^4\) The *vacA* genotype comprises a hypervariable signal sequence and a middle region allele. The *vacA* subtypes are determined by the combination of s1a, s1b, s1c and s2, and m1, m2a and m2b.\(^4,11\) Although all strains of *H. pylori* contain the *vacA* gene, they vary in terms of their ability to produce cytotoxin. Type m1 strains demonstrate more toxic activity than m2, type s1a is more active than s1b, and type s2 is less active than s1.\(^6\)\(^12\)

The cytotoxic associated gene A (*cagA*) has been proposed as a marker for a genomically pathogenic island (*cag-PAI*) of approximately 40 kbp whose presence is associated with more severe clinical outcomes.\(^13\)\(^14\) The *cagA*-positive *H. pylori* strains are known to induce interleukin-8 (IL-8) production and mucosal inflammation.\(^13\)\(^14\) Other members of *cag-PAI* have also been evaluated for their involvement in virulence, and *cagE* is one of the marker genes in *cag* of the *cag-PAI*. It is essential for *cagA* translocation and phosphorylation.\(^15\)\(^16\) The presence of the *cagE* gene has also been associated with a more severe clinical outcome.\(^17\)

The induced by contact with epithelium (*iceA*) gene has recently been discovered. The two main allelic variants of the gene are *iceA1* and *iceA2*. The expression of *iceA1* is upregulated on contact between *H. pylori* and human epithelial cells, and may be associated with peptic ulcer disease.\(^7\)\(^18\)\(^19\)

The blood group antigen-binding adhesin gene (*babA*) is involved in the binding activity between bacterial adhesin and human Lewis-b blood group antigens on gastric epithelial cells.\(^20\) Although three *bab* alleles have been identified (*babA1, babA2, babB*), only the *babA2* gene product is necessary for Lewis-b binding activity. Several researchers suggest that the presence of *babA2* is related to the occurrence of peptic ulcers and gastric cancer.\(^20\)\(^21\)

In Thailand, an average 48% of dyspeptic patients are infected with *H. pylori*, but a higher prevalence has been found in gastric ulcer patients and peptic ulcer patients than in gastritis patients.\(^22\)-\(^24\) Some virulence-related gene products such as VacA and CagA in the isolated strains have been studied;\(^25\)\(^26\) however, the involvement of *H. pylori* genotypes in specific diseases remains controversial.\(^8\)\(^9\)\(^21\)\(^27\) A mainstream challenge for researchers to identify the particular *H. pylori* genes, including *vacA, cagA, cagE, iceA*, and *babA2*, has been elucidated;\(^9\) however, no study in Thailand has simultaneously investigated the prevalence and relationship to clinical outcomes of these putative genes. To understand the clinical relevance of *H. pylori* genotyping in predicting infection outcomes, and *H. pylori* genes in different geographical regions for the basic knowledge of Thai dyspeptic patients, we investigated the prevalence of *vacA, cagA, cagE, iceA*, and *babA2* genes of *H. pylori* obtained from 112 Thai patients with gastritis, peptic ulcers, and gastric cancer. The correlation between the genetic status of the isolates and the occurrence of gastrointestinal diseases was assessed.

Materials and methods

Patients

Gastric biopsies and *H. pylori* isolates were obtained from 112 patients who had undergone routine endoscopy for symptoms of dyspepsia at the hospitals in central and northeast Thailand. We included 34 patients with peptic ulcer disease (PUD; 20 with gastric ulcers (GU), 14 with duodenal ulcers (DU)), 62 with non-ulcer dyspepsia or gastritis (GT), and 16 with gastric cancer (GCA). The patients were 55 males and 57 females with an age range of 18 to 88 years (mean 49.5 years).

The study was approved by the ethics committee of Khon Kaen University and performed in accordance with the Declaration of Helsinki. Informed consent was obtained from each patient prior to entering the study.

Clinical samples and culture

Three gastric mucosal biopsy specimens from the antrum and corpus were obtained from each patient and divided into three parts. Both antral and corpus specimens were used for culture, the rapid urease test (RUT), and histological examination.

Culture was performed according to the method of Hazell,\(^26\) with modifications. Briefly, each antral and corpus specimen was immediately placed into Stuart’s transport medium and brought to the laboratory within 2 h at 4 °C. Each of the biopsy specimens was homogenized separately in 200 μl of normal saline and cultured on 7% human blood agar (Difco, Detroit, MI, USA) and brain heart infusion agar (Difco) containing the supplement SR147 (5 mg/l trimethoprim, 10 mg/l vancomycin, 5 mg/l amphotericin B, 5 mg/l cefsulodin (SR147, OXOID, Unipath Ltd, Basingstoke, UK)). The plates were incubated at 37 °C under microaerophilic conditions (5% O2, 10% CO2, 85% N2) and were examined after 4 and 7 days of incubation. Characteristic colonies of *H. pylori* were confirmed by Gram staining, oxidase, catalase and urease tests. The *H. pylori* colonies were further used for DNA extraction.

Commercial rapid urease test (RUT, Pronto Dry test)

The RUT was performed according to the manufacturer’s instructions (Medical Instruments Corp., Solothurn, Switzerland). Briefly, one antral and one corpus specimen together were directly inoculated onto the commercial RUT agar gel. The results were observed and recorded within 24 hours. A positive RUT was indicated when the color changed from yellow to pink. The positive RUTs were used for chromosomal DNA extraction if the culture was negative.
Genomic DNA extraction

DNA from 30 H. pylori isolates and 82 of each antrum and corpus gastric biopsy positive by the RUT, were extracted using the genomic DNA purification kit (Puregene, Gentra Systems, USA), according to the manufacturer’s instructions. Briefly, a loop full of cell culture or the gastric biopsy samples (obtained from the urease test agar assay homogenized with 200 µl of normal saline) were incubated with 450 µl cell lysis solution and 2.5 µl proteinase K solution for 3 h at 55 °C. The lysate was incubated at 98 °C for 10 min and then 2.5 µl RNase A solution was added to the cell lysate and incubated at 37 °C for 60 min. Then, 200 µl protein precipitation solution was added and centrifuged at 13 000 g for 3 min. The supernatant was collected and 400 µl of 100% isopropanol was added and centrifuged at 13 000 g for 5 min. The supernatant was carefully discarded. Then, 300 µl of 70% ethanol was added to the pellet and centrifuged. The ethanol was poured off and left to dry for 3 h. Then, 50 µl DNA hydration solution was added and incubated for 1 h at 65 °C. DNA was stored at –20 °C until used.

PCR assays for glmM gene and virulence genes (vacA, cagA, cagE, iceA, babA2)

Primer sequences, sizes, and conditions of PCR amplifications of the glmM gene29 — for detection and confirmation of H. pylori — and the virulence genes (i.e., vacA,30,31 cagA,12 cagE,16 iceA2, and babA213), were designed based on published papers with a modification of PCR mixtures and PCR conditions (Table 1).

Each PCR of glmM, vacA, cagA, cagE, iceA, and babA2 was performed in a total volume of 50 µl containing 100 ng genomic DNA from H. pylori culture or 400 ng genomic DNA from gastric biopsies in which the RUT was positive, 200 µM each of dNTP (Gibco BRL, USA), 1 × PCR buffer (20 mM Tris-HCl, pH 8.4), 50 mM KCl, 1.5 mM MgCl2 (2 mM MgCl2 for cagA), 0.5 µM of each primer (0.2 µM for babA2 and 0.3 µM for cagA), and 1.5 units of Taq polymerase (Gibco BRL, USA). For each batch of PCR assay, distilled water instead of the genomic DNA templates was used as a negative control.

The reaction mixtures were cycled in an automated thermal cycler (GeneAmp, PCR 2400, Perkin-Elmer, USA) under the conditions shown in Table 1. After amplification, 10 µl of PCR product was electrophoresed on 1.5—2% agarose gel, stained with ethidium bromide, and examined under UV illuminator.

Data analysis

Fisher’s exact test or the Chi-square test was used for analysis of categorical data. A p-value of <0.05 was considered statistically significant.

Results

Helicobacter pylori-infected patients were evaluated for the relation of age, gender, and ethnic group with the severity of disease as shown in Table 2. The severity of disease was diagnosed by endoscopic findings and a pathologist. The results show that there was no significant difference among these parameters with regard to the gastroduodenal patient

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Primer sequence and PCR conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genes</td>
<td>Primer sequence (5’→3’)</td>
</tr>
<tr>
<td>glmM</td>
<td>AAGCTTTTACGGGTTAGGGGTTTTAAGCTTACTTTTCTA</td>
</tr>
<tr>
<td>vacA</td>
<td>ATGGAAATACTACACAAACACACCTGGTTAACCTA</td>
</tr>
<tr>
<td>s1/s2</td>
<td>GTCGACCATACACCGCAACCTGGTTAACCTA</td>
</tr>
<tr>
<td>s1a</td>
<td>AGCACCACATGGCAAGGCTGGTTAACCTA</td>
</tr>
<tr>
<td>s1b</td>
<td>CTCGACCATACACCGCAACCTGGTTAACCTA</td>
</tr>
<tr>
<td>s1c</td>
<td>CTGCACCATACACCGCAACCTGGTTAACCTA</td>
</tr>
<tr>
<td>m1/m2</td>
<td>CAATCTGGTTTACGGGTTTTAAGCTTACTTTTCTA</td>
</tr>
<tr>
<td>cagA</td>
<td>TATTAGTACAAATAGCAACTTTGAGGCACTTAGTAACTAC</td>
</tr>
<tr>
<td>cagE</td>
<td>TTAGAAATCAGAGGTAGGAGTAGGAACGTGCTAG</td>
</tr>
<tr>
<td>iceA1</td>
<td>CTTGTGTGATAGCAACATTTTAATTTTCTTGTGCTGA</td>
</tr>
<tr>
<td>iceA2</td>
<td>CAAACGAAAACAAAAAGCGTGCCTGTTGTA</td>
</tr>
</tbody>
</table>
patients, only two (1.8%) showed an absence of cag
(data not shown).

difference between the gastroduodenal diseases groups
minant genotype in our study albeit there was no significant
subtypes. The results showed that vac
H. pylori
112
samples (38 gastric biopsies and 30
used for detecting several of the genes; we only checked 68
cient template DNA extracted from all of the gastric biopsies
p
(35.7%) in the duodenal ulcer patients (Table 2).

As1 subtypes, we did not have suffi-
ences of the combination genotypes
H. pylori
A gene was commonly
in the duodenal ulcer patients (57.1%; 8/14), whereas
ice
A was most commonly found in the gastric ulcer patients
(40%; 8/20). The ice
A-negative strain, in which neither ice
A nor ice
E was detected, was found in 21.4% of the 112
H. pylori
strains. This finding is similar to that reported by Han
et al. 34
The bab
A2 gene was detected in 92% (103/112) of the
H. pylori
infected patients. The bab
A2 gene was commonly found in all patient groups; however, there was no statistically
different in each of the individual genes
among the patient groups (p > 0.05) (Table 3).

For detection of vacAs1 subtypes, we did not have suffi-
template DNA extracted from all of the gastric biopsies
for detecting several of the genes; we only checked 68
samples (38 gastric biopsies and 30 H. pylori isolates) of the
112
H. pylori-infected patients for the vacAs1 (a, b, c)
subtypes. The results showed that vacAs1c was the predo-
minant genotype in our study albeit there was no significant
difference between the gastroduodenal diseases groups
data not shown).

The cag
A gene was detected in 98.2% (110/112) of the
H. pylori-infected patients. Of the 112 H. pylori-infected
patients, ice
A1 and ice
A2 were detected in 45.5% (51/112) and
33.1% (37/112), respectively. ice
A1 was most commonly
in the duodenal ulcer patients (57.1%; 8/14), whereas
ice
A2 was most commonly found in the gastric ulcer patients
(40%; 8/20). The ice
A-negative strain, in which neither ice
A nor ice
E was detected, was found in 21.4% of the 112
H. pylori
strains. This finding is similar to that reported by Han
et al. 34
The bab
A2 gene was detected in 92% (103/112) of the
H. pylori
infected patients. The bab
A2 gene was commonly found in all patient groups; however, there was no statistically
different in each of the individual genes
among the patient groups (p > 0.05) (Table 3).

The frequency distributions of the combination genotypes
H. pylori
are presented in Table 4. The four major geno-
types found were: (1) vacAs1m1, cag
A, ice
A1, and
bab
A2 (22.3%); (2) vacAs1m2, cag
A, ice
A1, and
bab
A2 (11.6%); (3) vacAs1m1, cag
A, ice
A1, and
bab
A2 (15.2%); and (4) vacAs1m2, cag
A, ice
A1, and
bab
A2 (11.6%). No significant difference was found among the patient groups (p > 0.05) (Table 4).

Discussion

The clinical relevance of the putative virulence-associated
genes of H. pylori and geographical region is still a matter of
controversy. The present study reported the relationship
between some virulence genes (vac
A, cag
A, ice
A, bab
A) of
H. pylori and the clinical status among Thai patients.

All strains of H. pylori contain the vac
A gene, but they vary
in terms of their ability to produce cytotoxin.9 Type s1 and m1
strains demonstrate more toxic activity than s2 and m2
strains.1,12,26,35 In Western studies, the presence of vacAs1

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Non-ulcer GTab (%) (N = 62)</th>
<th>Peptic ulcer</th>
<th>GCAa (%) (N = 16)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GUc (%) (N = 20)</td>
<td>DUd (%) (N = 14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (14.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>21–40</td>
<td>16 (25.8)</td>
<td>4 (20)</td>
<td>3 (21.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>41–60</td>
<td>36 (58.1)</td>
<td>11 (55)</td>
<td>6 (42.9)</td>
<td>7 (43.7)</td>
</tr>
<tr>
<td>&gt;60</td>
<td>10 (16.1)</td>
<td>5 (25)</td>
<td>3 (21.4)</td>
<td>9 (56.3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gender</th>
<th>Non-ulcer GT</th>
<th>Peptic ulcer</th>
<th>GCA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (M)</td>
<td>28 (45.2)</td>
<td>11 (55)</td>
<td>9 (64.3)</td>
<td>7 (43.7)</td>
</tr>
<tr>
<td>Female (F)</td>
<td>34 (54.8)</td>
<td>9 (45)</td>
<td>5 (35.7)</td>
<td>9 (56.3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ethnic group</th>
<th>Non-ulcer GT</th>
<th>Peptic ulcer</th>
<th>GCA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thai</td>
<td>41 (66.1)</td>
<td>12 (60)</td>
<td>7 (50)</td>
<td>8 (50)</td>
</tr>
<tr>
<td>Thai-Chinese</td>
<td>15 (24.2)</td>
<td>6 (30)</td>
<td>5 (53.7)</td>
<td>6 (37.5)</td>
</tr>
<tr>
<td>Chinese</td>
<td>5 (8.1)</td>
<td>2 (10)</td>
<td>2 (14.3)</td>
<td>2 (12.5)</td>
</tr>
<tr>
<td>Loa-Loa</td>
<td>1 (1.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

a Helicobacter pylori-infected gastritis patients.
b Helicobacter pylori-infected gastric ulcer patients.
c Helicobacter pylori-infected duodenal ulcer patients.
d Helicobacter pylori-infected gastric cancer patients.

Table 2 Distribution of 112 patients with different clinical outcomes, according to age, gender and ethnic group.
and cagA has been shown to be significantly associated with peptic ulcers.1,10 However, several studies in Asian populations have not confirmed this relationship, indicating that there are important geographic differences.19,27,36 All vacA genotypes from our 112 H. pylori-infected dyspeptic patients contained the s1 signal region while 58% and 42% of H. pylori strains possessed the m1 and m2 middle region, respectively. Our results are in agreement with previous reports that show a predominance of s1 in Asian populations; the s1 and m1 genotypes from our 112 countries, and no association with severity of disease.37,39 Qiao et al.30 reported that type s1 of H. pylori was more severe and gastric cancer.9,32,38 In this study, the cagA-positive and cagE-positive and cagA-negative strains.41 Peek et al.18 demonstrated that iceA1 expression is significantly related to the host mucosal response, which led to the

diseases. They have indicated that the diversity of the tyrosine CagA phosphorylation occurs at the unique Glu—Prol—ile—Ala (EP1YA) motifs present in the C-terminal region, affected by protein-tyrosine phosphatase (SHP-2), and actively involved in the regulation of the spreading, migration, and adhesion of cells. It may induce abnormal proliferation and movement of gastric epithelial cells and be associated with the mortality rate of gastric cancer in Asia.40 These findings should be further studied in H. pylori cagA-positive strains isolated from Thai dyspeptic patients in the future.

The cagE gene, also within the pathogenicity island and shown to stimulate production of several cytokines from infected epithelial cells, was found in 88.4% of H. pylori and 87.5% of cagA-positive H. pylori in this study. This result corresponds to those found in a previous report on children in the USA.9, however, we found that when cagA was positive, cagE was negative in 10.7% of samples (12/112), whereas only one sample was cagE-positive and cagA-negative.

The iceA gene may be associated with peptic ulcer disease;1,10 however, some studies have failed to confirm this correlation, and some groups have suggested a reverse relationship.19 There are two distinct allelic variants of iceA, namely iceA1 and iceA2.7,19,37 One study has suggested that iceA1 is associated with the development of peptic ulcers, and that iceA1-positive strains produce more of the proinflammatory factor IL-8 than iceA1-negative strains.41

### Table 3 The vacA, cagA, cagE, iceA, babA2 status of H. pylori strains obtained from 112 patients with different clinical outcomes

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Non-ulcer GTa (%) (N = 62)</th>
<th>Peptic ulcer</th>
<th>GCAd (%) (N = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GUb (%) (N = 20)</td>
<td>DUc (%) (N = 14)</td>
<td></td>
</tr>
<tr>
<td>vacA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>s1m1</td>
<td>36 (58.1)</td>
<td>12 (60)</td>
<td>9 (64.3)</td>
</tr>
<tr>
<td>s1m2</td>
<td>26 (41.9)</td>
<td>8 (40)</td>
<td>5 (35.7)</td>
</tr>
<tr>
<td>cagA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>60 (96.8)</td>
<td>20 (100)</td>
<td>14 (100)</td>
</tr>
<tr>
<td>Negative</td>
<td>2 (3.2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>cagE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>54 (87.1)</td>
<td>18 (90)</td>
<td>12 (85.7)</td>
</tr>
<tr>
<td>Negative</td>
<td>8 (12.9)</td>
<td>2 (10)</td>
<td>2 (14.3)</td>
</tr>
<tr>
<td>iceA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iceA1</td>
<td>29 (46.8)</td>
<td>7 (35)</td>
<td>8 (57.1)</td>
</tr>
<tr>
<td>iceA2</td>
<td>19 (30.6)</td>
<td>8 (40)</td>
<td>4 (28.6)</td>
</tr>
<tr>
<td>iceA--</td>
<td>14 (22.6)</td>
<td>5 (25)</td>
<td>2 (14.3)</td>
</tr>
<tr>
<td>babA2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>57 (91.9)</td>
<td>17 (85)</td>
<td>14 (100)</td>
</tr>
<tr>
<td>Negative</td>
<td>5 (8.1)</td>
<td>3 (15)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>cagA, cagE</td>
<td>53 (85.5)</td>
<td>18 (90)</td>
<td>12 (85.7)</td>
</tr>
</tbody>
</table>

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a Helicobacter pylori-infected gastritis patients.  
b Helicobacter pylori-infected gastric ulcer patients.  
c Helicobacter pylori-infected duodenal ulcer patients.  
d Helicobacter pylori-infected gastric cancer patients.
found a high prevalence of Helicobacter pylori strains carrying iceA and vacA with H. pylori were associated with the highest risk of developing intestinal complications (45.5%) was the most frequent genotype detected in our population. This finding agrees with previous reports that have shown the iceA allele more frequently found than the iceA allele in Chinese, Japanese, Korean and Dutch populations. 4,9 iceA has been found to be predominant among Brazilian, European and American patients. 3,9,42

The babA2 gene has been shown to be associated with a higher risk of ulcer or adenocarcinoma development, and has been strongly associated with vacAs1 (79% babA2-positive) and cagA genotypes (80% babA2-positive) in German adults. 21 H. pylori with triple positive genotypes, vacAs1, cagA and babA2, has been reported in only 31% of dyspeptic children with H. pylori infection. 10 A previous study showed that H. pylori strains carrying babA2, cagA, and vacAs1m1 genotype were associated with the highest risk of developing intestinal metaplasia. 27 We, however, did not find any specific disease association between H. pylori genotype and the clinical outcome of infection, even though all of our H. pylori had vacAs1, 92% babA2 and 98% cagA. Our results agree with Kim et al., who did not find any association with cagA, vacA subtype, iceA1 and babA of H. pylori isolates from Korean patients, and Han et al. who also did not find any association with iceA1 and babA2 of H. pylori isolates from Shanghai patients. Several factors involved in the pathogenesis of H. pylori have been studied, including bacterial genotypes. 1,7,9,10 We found a high prevalence of vacAs1, cagA, cagE, iceA and babA2 in our H. pylori-infected dyspeptic patients in accord with research in other East Asian countries, 19,30,37 and no significant difference was found between the various genes and severity of diseases. Past studies have reported high incidences of gastric cancer among Japanese and Chinese people and very low incidences in the Thai and Vietnamese populations. 43 In fact, we found no significant difference between the various genes and severity of diseases, indicating that the presence of cagA or subtypes of vacA cannot serve as real virulence markers for the development of gastric cancer, as was found in Japan and Korea.

In conclusion, this is the first report of the high prevalence of H. pylori virulence genes in Thai dyspeptic patients. There was no significant difference in any one specific bacterial gene and the gene pattern being associated with a particular clinical outcome. It is therefore necessary to define both the environmental and host factors in association with the bacterial characteristics for use in the prediction of the severity of disease.

Acknowledgements

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Conflict of interest: No conflict of interest to declare.

References


Table 4 Frequency distribution of combination genotypes of 112 H. pylori in the gastrointestinal dyspeptic patients

<table>
<thead>
<tr>
<th>Combination genotypes</th>
<th>Non-ulcer GTa (%) (N = 62)</th>
<th>Peptic ulcer</th>
<th>GCAc (%) (N = 16)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Clinical status</td>
<td>GUd (%) (N = 20)</td>
<td>DUf (%) (N = 14)</td>
<td>Total (%)</td>
</tr>
<tr>
<td>vacAs1m1, cagA, cagE, iceA1, babA2</td>
<td>13 (21)</td>
<td>4 (20)</td>
<td>5 (35.7)</td>
<td>3 (18.8)</td>
</tr>
<tr>
<td>vacAs1m2, cagA, cagE, iceA1, babA2</td>
<td>11 (17.7)</td>
<td>2 (10)</td>
<td>2 (14.3)</td>
<td>3 (18.8)</td>
</tr>
<tr>
<td>vacAs1m1, cagA, cagE, iceA2, babA2</td>
<td>11 (17.7)</td>
<td>1 (5)</td>
<td>1 (7.1)</td>
<td>4 (25)</td>
</tr>
<tr>
<td>vacAs1m2, cagA, cagE, iceA2, babA2</td>
<td>4 (6.5)</td>
<td>5 (25)</td>
<td>2 (14.3)</td>
<td>2 (12.5)</td>
</tr>
<tr>
<td>Others</td>
<td>23 (37.1)</td>
<td>8 (40)</td>
<td>4 (28.6)</td>
<td>4 (25)</td>
</tr>
<tr>
<td>Total</td>
<td>62 (100)</td>
<td>20 (100)</td>
<td>14 (100)</td>
<td>16 (100)</td>
</tr>
</tbody>
</table>

a Helicobacter pylori-infected gastritis patients.
b Helicobacter pylori-infected gastric ulcer patients.
c Helicobacter pylori-infected duodenal ulcer patients.
d Helicobacter pylori-infected gastric cancer patients.

Hypothesis that the levels of transcription within the host environment may contribute to disease development. In contrast, iceA2 expression may be more influenced by gene structure, which has a repeated protein structure but it does not have homology with known proteins. Indeed, our H. pylori study of the iceA allele demonstrated that iceA1 (45.5%) was the most frequent genotype detected in our population. This finding agrees with previous reports that have shown the iceA1 allele more frequently found than the iceA2 allele in Chinese, Japanese, Korean and Dutch patients; 7,19,34,37 iceA2 has been found to be predominant among Brazilian, European and American patients. 9,19,42

The babA2 gene has been shown to be associated with a higher risk of ulcer or adenocarcinoma development, and has been strongly associated with vacAs1 (79% babA2-positive) and cagA genotypes (80% babA2-positive) in German adults. 21 H. pylori with triple positive genotypes, vacAs1, cagA and babA2, has been reported in only 31% of dyspeptic children with H. pylori infection. 10 A previous study showed that H. pylori strains carrying babA2, cagA, and vacAs1m1 genotype were associated with the highest risk of developing intestinal metaplasia. 27 We, however, did not find any specific disease association between H. pylori genotype and the clinical outcome of infection, even though all of our H. pylori had vacAs1, 92% babA2 and 98% cagA. Our results agree with Kim et al., who did not find any association with cagA, vacA subtype, iceA1 and babA of H. pylori isolates from Korean patients, and Han et al. who also did not find any association with iceA1 and babA2 of H. pylori isolates from Shanghai patients.

Several factors involved in the pathogenesis of H. pylori have been studied, including bacterial genotypes. 1,7,9,10 We found a high prevalence of vacAs1, cagA, cagE, iceA and babA2 in our H. pylori-infected dyspeptic patients in accord with research in other East Asian countries, 19,30,37 and no significant difference was found between the various genes and severity of diseases. Past studies have reported high incidences of gastric cancer among Japanese and Chinese people and very low incidences in the Thai and Vietnamese populations. 43 In fact, we found no significant difference between the various genes and severity of diseases, indicating that the presence of cagA or subtypes of vacA cannot serve as real virulence markers for


