Association of Nonsynonymous Substitutions in the Intermediate Region of the vacA Gene of Helicobacter pylori with Gastric Diseases in Taiwan

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The amino acid sequences corresponding to the vacA intermediate region from 39 isolates of Helicobacter pylori were investigated. The substitution of a G for the third S in the i1-type cluster B sequence (QASEGIT SSK) conferred a greater risk of gastric diseases (P < 0.03; Fisher’s exact test). The conserved substitutions of an F for the Y in the cluster A sequence and of an M for the second N in the i1-type cluster C sequence could be a marker of VacA in the Taiwanese strains.

Helicobacter pylori infects more than 50% of the world’s human population and causes peptic ulcers and gastric malignancy (5). One of the virulence determinants is the vacuolating cytotoxin (VacA) encoded by the vacA gene. The polymorphism of vacA occurs in the signal (s) and mid (m) regions (1). Recently, a new polymorphism, in the intermediate (i) region between the s and m regions, was identified and associated with gastric cancer (12). There are two types of the s (s1 and s2), i (i1 and i2), and m (m1 and m2) regions of vacA (1, 12), and subtypes are also continually being identified (2, 11, 15, 17). Previous reports have shown that the s region affects the vacuolating activity of the toxin in vitro and the m region determines the specificity of toxin binding to host cells (6–8, 10). Rhead et al. (12) further demonstrated that i1-type clusters B (10 amino acids; QAS EGITSSK) and C (10 amino acids; ASNSVKLNGN), but not cluster A (14 amino acids; YKDSADRTRTVDFN), play an important role in the vacuolating activity. The correlation between the vacA genotype and gastric diseases varies across geographic regions. In addition, the distinct dominant vacA genotypes differ among clinical strains from the different geographic regions (11, 15, 16, 18). In Taiwan, there are two specific subtypes in the m region (m1T and chimeric m1Tm2) (17). The sequences of m1T are much more homologous to m1 strains than m2 strains, and several mutations were found in the m1 primer regions. Since Taiwanese isolates have the m1T vacA subtype and gastric cancer is the major leading cause of cancer death in Taiwan (4), it is worth investigating the prevalences of i region types in our clinical isolates and their association with gastric diseases.

H. pylori strains were successfully isolated from the biopsy specimens of 107 dyspeptic patients who underwent panendoscopy in the National Cheng-Kung University Hospital, Tainan, Taiwan. All patients gave informed consent. The endoscopic diagnoses of these patients included duodenal ulcer (DU; n = 30), gastric ulcer (GU; n = 30), gastritis (n = 30), and gastric cancer (GCA; n = 17). During panendoscopy for each patient, five bits of gastric biopsy specimen, including two from the antrum, two from the corpus, and one from the cardia were obtained. One each from the antrum, corpus, and cardia were used for the histological examination, and two additional specimens, from the antrum and corpus, were cultured for H. pylori. Both the antrum and corpus biopsy specimens were mixed for the isolation of H. pylori, and sweeps of isolates obtained from the culture plates were stocked until testing. The bacterial culture and histological examination were described previously (13). Bacterial DNA was extracted according the methods of Sheu et al. (14). For vacA, the primer sequences designed by Wang et al. (17) were used to type the s and m regions, and the i region was amplified using the primer designed by Rhead et al. (12). The annealing temperature of PCR depended on the primer sequences and ranged from 52°C to 56°C. All of the 107 H. pylori isolates were uniformly of the s1a subtype in the s region and of the i1 type in the i region. Three polymorphic subtypes, namely m1T (19/107, 17.8%), m1Tm2 (7/107, 6.5%), and m2 (81/107, 75.7%), were found in the m region of these isolates. There were no significant differences among patients with different clinical diseases in each vacA s-i-m genotype (Table 1). For the antra and corpora of the hosts with each of the three vacA s-i-m genotypes of H. pylori, there were similar histological features (acute and chronic inflammation scores, H. pylori density, atrophy, and intestinal metaplasia), but there was a difference in lymphoid follicles. The s1a/i1/m1Tm2 genotype had a higher frequency of lymphoid follicle formation in the corpus than the s1a/i1/m1T and s1a/i1/m2 strains (P value < 0.05; chi-square test).

We randomly selected bacterial DNA from 39 patients (10 with DU, 10 with GU, 10 with gastritis, and 9 with GCA) for

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sequencing of the vacA i region. The sequences of the i region in 39 strains were almost identical to the vacA gene of strain 60190 (GenBank U05676) except for three to six mutations leading to amino acid substitutions. Among these sequences, there were two conserved substitutions of F for Y in cluster A (YKDSADRTTRVDFN) and M for the second N in cluster C (ASNSVKNLNG) in all strains. The third S-to-G mutation in cluster B (QASEGITSK) of the i region was found in strains from patients with DU, GU, and GCA but not in strains from patients with gastritis (Table 2). In strains from gastritis patients, the third S in cluster B remained S (5/10) or was mutated to N (1/10) or D (4/10). The sexes and ages of the patients infected with strains having S-to-G substitutions and those of the patients infected with strains having other substitutions at that position (D, N, or S [i.e., synonymous nucleotide mutation]) were similar (sex, \( P = 0.117 \) [chi-square test]; age, 50.88 ± 11.93 and 52.95 ± 11.57 years, respectively \( [P = 0.589; t \text{ test}] \)). The DU patient strains had the highest rate of S-to-G substitution (7/10, 70%), and the substitution was significantly associated with DU, GU (5/10, 50%), and GCA (5/9, 55.56%) in comparison with gastritis \( (P < 0.03; \text{Fisher’s exact test}) \) (Table 2). In the strains with S-to-G substitution \( (n = 17) \), their m subtypes had no significant differences among the infected patients with DU, GU, and GCA \( (P = 0.532; \text{chi-square test}) \).

Rhead et al. (12) showed that the i1 genotype predicted gastric cancer in the Iranian population, and it was also demonstrated as a risk factor for DU (3). In contrast, our results demonstrated that the same vacA i region genotype was present in all strains and not associated with particular gastric diseases. Ogiwara et al. (9) also showed that i1 was the predominant genotype and concluded that i1, s, and m genotypes were not disease determinants in populations in eastern and southeastern Asia. Therefore, using the vacA i1 genotype as a disease marker has geographic limitations. However, in this study, we found that the conserved substitutions of M for the second N in cluster C and F for the first Y in cluster A could be a potential VacA marker in the Taiwanese strains. The substitution of a G for the third S in cluster B of vacA i region predisposed patients to a greater risk of gastric diseases. Whether the specific amino acid change (S to G) of VacA toxin makes it more virulent remains a subject for further study.

**Nucleotide sequence accession numbers.** The sequence of one strain from a DU patient with an S-to-G substitution was deposited in GenBank (accession no. FJ428579), and three strains from patients with gastritis for which the S residue remained S or mutated to N or D were also deposited in GenBank (accession no. FJ428578, FJ428580, and FJ428581).

### TABLE 1. vacA genotypes of *H. pylori* among different clinical patient groups

<table>
<thead>
<tr>
<th>Genotype</th>
<th>DU (n = 30)</th>
<th>GU (n = 30)</th>
<th>Gastritis (n = 17)</th>
<th>GCA (n = 10)</th>
<th>Total (n = 107)</th>
<th>( P ) value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>s1a1/m1T</td>
<td>5 (16.7)</td>
<td>7 (23.3)</td>
<td>5 (16.7)</td>
<td>2 (11.8)</td>
<td>19 (17.8)</td>
<td>0.773</td>
</tr>
<tr>
<td>s1a1/m1Tm2</td>
<td>2 (6.7)</td>
<td>2 (6.7)</td>
<td>2 (6.7)</td>
<td>1 (5.9)</td>
<td>7 (6.5)</td>
<td>0.999</td>
</tr>
<tr>
<td>s1a1/m2</td>
<td>23 (76.7)</td>
<td>21 (70)</td>
<td>23 (76.7)</td>
<td>14 (82.4)</td>
<td>81 (75.7)</td>
<td>0.804</td>
</tr>
</tbody>
</table>

* The differences of the three genotypes in patients with gastric diseases were assessed by the chi-square test. The \( P \) value is for a comparison of the percentages of patients with different clinical diseases in each s-i-m genotype.

### TABLE 2. Association between the amino acid substitution of the third S in cluster B of vacA and gastric diseases

<table>
<thead>
<tr>
<th>Patients with indicated disease (no.)</th>
<th>No. (%) of patients with <em>H. pylori</em> with indicated amino acid substitution for the third S/total no. of patients</th>
<th>Relative risk (95% confidence interval)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastritis</td>
<td>0/10 (0)</td>
<td>3.497 (1.529–8)</td>
<td>0.011</td>
</tr>
<tr>
<td>DU</td>
<td>7/10 (70)</td>
<td>4.329 (1.605–11.628)</td>
<td>0.003</td>
</tr>
<tr>
<td>GU</td>
<td>5/10 (50)</td>
<td>3.003 (1.466–6.135)</td>
<td>0.033</td>
</tr>
<tr>
<td>GCA</td>
<td>9/9 (55.56)</td>
<td>3.497 (1.529–8)</td>
<td>0.011</td>
</tr>
</tbody>
</table>

* The difference was assessed by Fisher’s exact test, and \( P \) values of \(<0.05\) were taken to indicate a significant difference. For the three \( P \) values given, the amino acid substitution \( (S \text{ to } G) \) was significantly associated with DU, GU, and GCA in comparison with gastritis.

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### REFERENCES


