Clinical Relevance of the vacA, iceA, cagA, and flaA Genes of Helicobacter pylori Strains Isolated in Eastern Taiwan

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The genotypes of Helicobacter pylori flaA, cagA, vacA, and iceA were determined for DNA isolated from patients with chronic gastritis or peptic ulcer in eastern Taiwan. The vacA gene encoding the s1a subtype was found to predominate in peptic ulcer patients, and the iceA1 genotype was associated with chronic gastritis. cagA and flaA genes were not found to be associated with these types of disease.

Helicobacter pylori is a microaerophilic, gram-negative bacterium that infects human gastric mucosa and is involved in the pathogenesis of peptic ulcer (PU), chronic gastritis (CG), and gastric carcinoma (7). This organism has a worldwide distribution, and its prevalence ranges from 25% in developed countries to over 90% in developing areas. Depending on various factors such as the environment, host genetics, and bacterial virulence, some of the infected individuals may not develop diseases (4, 21).

H. pylori isolates are highly diverse genetically and extremely heterogeneous. Therefore, genotyping is useful in molecular epidemiological studies and identification of the predominant H. pylori strains that are circulating in a given geographic area. Several H. pylori genes related to the risk of disease have been identified. For instance, vacA, present in all H. pylori isolates tested, encodes the major secreted vacuolating toxin that causes damage to epithelial cells in vitro (6, 22). The vacA gene contains at least two variable parts, the s region (encoding the signal peptide, typed s1a, s1b, s1c, or s2) and the m region (middle, typed m1 or m2). Strains with the vacA s1-m1 genotype are associated with PU (2). The cagA gene, considered a marker for the presence of the chromosomal pathogenicity (cag) island (ca. 40 kb), is associated with a more severe clinical outcome, such as PU, atrophic gastritis, and gastric adenocarcinoma (3, 5, 13). FlaA, flagellin encoded by flaA, is required for (i) bacterial colonization, (ii) bacterial survival in the stomach mucus, and (iii) display of active motility in viscous environments inhibitory to the motility of other bacteria (12). Two distinct iceA alleles, iceA1 and iceA2, are known. iceA1 is homologous to the Neisseria lactamica NlaIII gene encoding restriction endonuclease NlaIII, but iceA2 shows no homology to any known gene (8). Patients in the United States and The Netherlands carrying iceA1 strains produced higher levels of the cytokine interleukin 8 in the gastric mucosa and had higher rates of PU than those with iceA2 strains (19, 23), with the mechanisms involved being unclear.

In western Taiwan, 50 to 55% of adults are infected by H. pylori, and the genotypes of several virulence-related genes in the isolated strains have been studied (14, 20, 26). The prevalence rate is significantly higher in duodenal ulcer (>95%) and gastric ulcer (80%) patients than in asymptomatic healthy volunteers (50%) (15). In contrast, similar studies are still scanty in eastern Taiwan, a region separated from western Taiwan by the Central Mountains, where the lifestyles of the people and their ethnic groups are very different from those in western Taiwan. Studies on the diversity of H. pylori genes may be important not only for predicting the clinical outcomes of the infection but also for better understanding the worldwide distribution of the microorganism and its evolutionary origins. In the present study, we used PCR to detect the presence of flaA and cagA and the genotypes of vacA and iceA among 167 H. pylori-infected gastric biopsy specimens obtained from the Buddhist Tzu Chi General Hospital in Hualien, a county in eastern Taiwan. The association between the genotypes of the isolates and the clinical features of the patients was also analyzed. This is the first report about the relationships between the genotypes of several virulence-related H. pylori genes and the clinical diagnoses of patients in eastern Taiwan.

All of the 167 specimens were positive by Campylobacter-like organism testing (Delta West, Bentley, Western Australia, Australia) and PCR amplification of the H. pylori 16S rRNA genes. Fifty-five subjects (22 males and 33 females; mean age, 52.4 ± 12.9 years) had PU (32.9%), and 112 (47 males and 65 females; mean age, 56.0 ± 11.2 years) had CG (67.1%). These prevalences are different from those in western Taiwan, where the prevalence of PU was found to be higher than that of CG (14, 20).

Throughout the experiments, H. pylori NCTC11637 was used as the reference and control strain. For genotyping, the primers were designed according to the published sequences (1, 10, 23). PCR was performed in a thermal cycler (MJ Research, Waltham, Mass.) with a reaction mixture of 50 µl containing 100 ng of isolated DNA. The PCR consisted of a 5-min preincubation at 94°C, followed by 35 cycles of 1 min at 94°C, 30 s at either 49°C (for flaA genes), 55°C (for cagA genes), 50°C (for vacA and iceA alleles), or 52°C (for 16S
The PCR results showed high proportions of flaA-positive (149/167 specimens; 89.2%), cagA-positive (152/167 specimens; 91%), and iceAI-positive (127/167 specimens; 76%) genotypes, which are similar to those reported in Asia (20, 25) but higher than those in western Europe (9). All biopsy specimens carried the potentially toxigenic vacA s1 allele, with the majority being the s1a subtype (78/167 specimens; 46.7%), majoritly being the s1a subtype (78/167 specimens; 46.7%), and the s1c subtype found in Hong Kong, Korea, Japan, and western Taiwan (46 to 64%) (11, 20, 24, 25). Among the strains, 54.5% (91/167) were of the s1a subtype found in Hong Kong, Korea, Japan, and western Taiwan (30.7%) (17) but higher than those from Colombia (4.5%) (11). These data confirm the geographic differences in vacA genotypes. Multiple vacA genotypes were found in 29.3% (49/167) of the specimens, which is similar to the results from Chile (32%) (16) and Portugal (30.7%) (17) but higher than those from Colombia (4.5%) (17). Moreover, the vacA s1a-s1c mixed form was the major subtype (46/49 specimens; 93.9%) and was more prevalent in patients with CG.

The iceAI genotype was the predominant subtype (76%), similar to the situation found in east Asia (25) but different from that in Portugal and Colombia (17), where iceAI is predominant. In The Netherlands, the iceAI genotype was found to be associated with PU (23); however, we found that iceAI-positive infections were significantly associated with CG in this study (P = 0.028).

Regarding associations among the genotypes, cagA was present in 96.9% of 127 iceAI-positive specimens and 80% of 25 iceA2-positive specimens, and flaA was present in 79.9% of 149 iceAI-positive specimens and 14.1% of iceA2-positive specimens (Table 1). Finally, the presence of cagA and flaA genes is not correlated with certain types of disease, whereas the vacA gene encoding the s1a subtype is predominant in patients with PU (P = 0.03) and the iceAI genotype is associated with CG in eastern Taiwan (P = 0.028).

<table>
<thead>
<tr>
<th>TABLE 1. Association of vacA with cagA and iceA genotypes</th>
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<tbody>
<tr>
<td>vacA subtype</td>
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<tr>
<td>S region</td>
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<tr>
<td>s1a (n = 75)</td>
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<td>s1c (n = 32)</td>
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* Specimens from 53 patients with mixed infection were excluded.

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REFERENCES


