

Genotypic Characterization of *Helicobacter pylori* *cagA* and *vacA* from Biopsy Specimens of Patients with Gastroduodenal Diseases

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Abstract

Background: *Helicobacter pylori* infection is closely associated with gastroduodenal diseases. *H. pylori* infection with different *vacA* and *cagA* genotypes may result in divergent consequences. The aim of the present study was to investigate the prevalence of *H. pylori* infection and the correlation between *cagA* and *vacA* genotypes with the consequences of *H. pylori* infection in Taiwan.

Methods: Genomic DNA from 97 gastric biopsies of patients with various gastroduodenal diseases was collected, and the prevalence of *H. pylori* infection, *cagA* genotypes and *vacA* genotypes, was analyzed by polymerase chain reaction. In addition, the correlations between *cagA* and *vacA* genotypes and the consequences of *H. pylori*-infection were statistically examined.

Results: Our results indicated that 57.7% of this sample of patients with gastroduodenal diseases were infected with *H. pylori*. Prevalence of *cagA*⁺ strain in *H. pylori*-infected patients was 71.4%. All of the genotypes of the *cagA*⁺ *H. pylori* strains among our patients were type A. Prevalence of *vacA* signal region s1 and middle region m2 genotype in *H. pylori*-infected patients was 98.2% and 53.6%, respectively.

Conclusions: Our study demonstrated that individuals infected with *H. pylori* strains that carried *cagA* gene and *vacA* s1/m2 genotypes were associated with the development of gastroduodenal diseases, compared to those infected with *cagA*⁻ gene and *vacA*⁻ *H. pylori* strains.

Key Words: *Helicobacter pylori*, *cagA*, *vacA*, gastroduodenal diseases.

Introduction

HELICOBACTER PYLORI is a curved, microaerophilic Gram-negative bacterium. Much evidence in epidemiological, pathological and animal experiments indicates that *H. pylori* infection can result in gastritis or gastric cancer. Approximately 50% of people worldwide are infected with *H. pylori* (1, 2). The prevalence of *H. pylori* infection in developing countries is as high as 70–90% (3), whereas

that in Europe and the U.S. is usually less than 10% (4). These surveys demonstrate that environmental and racial differences can result in different rates of *H. pylori* infection.

Vacuolating cytotoxin gene A (*vacA*) and cytotoxin-associated gene A (*cagA*) are two of the most important *H. pylori* pathogenic factors (5); *cagA* can be a marker gene to detect peptic ulcer (6). About 60–70% of *H. pylori* isolates in Western countries are *cagA*⁺ strains (7); however, more than 90% of *H. pylori* isolates in Asian areas are *cagA*⁺ strains (8–11). The *cagA* gene can be classified into type A, B, C and D, according to the appearance of specific types and numbers of its 3'-terminal repetitive sequences designated as R1 (repeat sequence 1, 15 bp), R2 (42 bp) and R3 (147 bp) (11). Ninety-five percent of *cagA* from isolates of Japanese patients with gastric ulcer and duodenal ulcer are type A genotype (11). However, only 50% and 76% of *H. pylori* from patients with gastric ulcer and duodenal ulcer among Western countries, respectively, carry type A genotype (11).

VacA can induce host cell vacuolation and eventually cell death (12). A high degree of sequence variability also exists in the *vacA* gene. The signal region of *vacA* gene can be classified as s1, which can be further classified into s1a and s1b, as

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*This work was supported by grants HK-93-A-30 from Hung Kuang University, 92-OM-B-037 from Chung Shan Medical University, and TCVGH-HK948004 from Taichung Veterans General Hospital and Hung Kuang University, Taiwan, Republic of China.

**Protocol for this study was approved by the Ethics Committee of Hung Kuang University.

Accepted for publication June 2005.

well as s2 subtypes. The middle region of *vacA* gene can be divided into m1 and m2 subtypes. Different subtypes of these 2 regions combine in different strains to form mosaic genotypes (13, 14). Genotypes of s1/m1, s1/m2 and s2/m2 are more prevalent in Western countries (13, 15–17). The frequency of s1/m1 and s1/m2 genotype is approximately half, respectively, in isolates from Central America, North America and Europe, while s1/m1 genotype is more prevalent in South America and Spain (13, 15, 18). In Asian areas, s1a/m1 genotype is most prevalent in Japan (19), but s1a/m2 genotype is more frequent in mainland China (20).

As described above, ample evidence demonstrates that differential *vacA* and *cagA* genetic characteristics exist in *H. pylori* strains isolated from different geographical regions. Therefore, using molecular techniques to study the correlation of *H. pylori* genotypes or strains with gastroduodenal diseases has become an important study subject. The current study attempted to identify the frequency of *H. pylori vacA* and *cagA* genes in Taiwanese patients with gastroduodenal diseases, using gastric biopsy for analytic target samples. The putative association of *vacA* and *cagA* genotypes with the consequences of *H. pylori* infection was also examined.

Materials and Methods

Study Subjects and Samples

Gastric biopsies from 97 patients referred for upper gastrointestinal tract endoscopy with gastritis, gastric ulcer and duodenal ulcer in the Department of Gastroenterology in Taichung Veterans General Hospital were collected. Written consent was obtained from all the study subjects after the nature of the procedure was explained. The protocol for the research was approved by the Ethics Committee of Hung Kuang University. Basic information on the study subjects is given in Table 1.

Tissue Preparation and PCR Reactions

Gastric biopsies from all the study subjects were stored at temperatures under -70°C until use. Each biopsy was digested with tissue extraction buffer (50 mL Tris-HCl, pH 8.0, 1 mM EDTA, 0.45% Tween 20, 0.45% Nonidet P-40 and 100 $\mu\text{g}/\text{mL}$ proteinase K) at 55°C for 3 hours. Then 200 μL of phenol was added to tissue lysate to extract genomic DNA. Genomic DNA was subsequently quantified for polymerase chain reaction (PCR) reactions. PCR amplifications were carried out in 15 μL PCR reaction mixture containing 50 ng of genomic DNA, 1 μM of specific primers for each target gene amplified (Table 2), 1.5 μL 10x Failsafe™ PCR buffer (Epicentre Tech, Taiwan) and 1 unit of Taq DNA polymerase, using PCR thermocycler (PCR system 9700, Perkin Elmer). Amplified fragments were separated in 2% agarose gel and visualized under ultraviolet transilluminator. PCR genotyping of each biopsy was carried out at least twice to confirm the credibility and reproducibility of genotyping results.

Statistical Analysis

Results were analyzed by using SPSS software. The association between different groups of patients and *H. pylori* genotypes was analyzed by Pearson Chi-Square test with Yates's correction. The Mann-Whitney test was used for assessing correlation between various *H. pylori*-related diseases and concurrent presence of *cagA* and *vacA* genotypes. Significance was indicated by a two-tailed *p* value of less than 0.05.

Results

Prevalence of *H. pylori* infection was detected by PCR techniques using *H. pylori* 16S rRNA-specific primers (16S-1 and 16S-2, Table 2) as described by Warburton et al. (17). The prevalence of *H. pylori* infection in our patients was 57.7%

TABLE 1
Clinical Diagnosis and Frequency of H. pylori Infection (16S rRNA) among Subjects in This Study

Diagnosis	Sample n	Age Range	Mean \pm SD	Sex		<i>H. pylori</i> (16S rRNA) Positivity	
				Male	Female	n (%)	n (%)
Gastritis	11	21–66	41 \pm 17.20	7	4	4	(36.4)
Gastric ulcer	66	17–88	57 \pm 14.32	46	20	37	(56.1)
Duodenal ulcer	20	21–88	58 \pm 15.30	12	8	15	(75.0)
Total	97	17–88	52.5 \pm 15.61	65	32	56	(57.7)

TABLE 2
Primers Used for Amplification of the *H. pylori* 16S rRNA, *cagA* and *vacA* Genes

Designation	Target sequence	Primer sequence	Annealing condition	Size (bp)	Ref	
16S-1	16S rRNA	5'-GCTAAGAGATCGGCCCTATGTCC-3'	55°C, 60 sec	522	17	
16S-2		5'-TGGCAATCAGCGTCAGGTAATG-3'				
cagA-1	<i>cagA</i>	5'-ACCCTAGTCGGTAATGGGTTA-3'	50°C, 60 sec	642–651 (type A) 756 (type B) 810 (type C) 756 (type D)	11	
cagA-2		5'-GTAATTGTCTAGTTTCGC-3'				
VA1-F	<i>vacA</i> s1	5'-ATGGAAATACAACAAACACAC-3'	55°C, 30 sec		259	13
VA1-R		5'-CTGCTTGAATGCGCCAAAC-3'				
VA4-F	<i>vacA</i> m2	5'-GGAGCCCCAGGAAACATTG-3'	59°C, 60 sec	352	13	
VA4-R		5'-CATAACTAGCGCCTTGCAC-3'				

(56/97). And 36.4% (4/11), 56.1% (37/66) and 75.0% (15/20) of our patients who suffered from gastritis, gastric ulcer and duodenal ulcer, respectively, were *H. pylori* positive (Table 1).

The *cagA* genotypes of our *H. pylori*-positive samples were investigated according to standard methods (11). Prevalence of *cagA*⁺ strains in our *H. pylori*-infected patients was 71.4% (40/56); the *cagA* genotype of *H. pylori* from all the *H. pylori*-infected patients was type A (Table 3). No other genotypes were detected in our patients. The distribution of *cagA*⁺ strain in patients with gastritis, gastric ulcer and duodenal ulcer was 50% (2/4), 73.0% (27/37) and 73.3% (11/15), respectively (Table 3). Our results showed that Taiwanese patients infected with *cagA*⁺ *H. pylori* strains were more susceptible to gastric ulcer and duodenal ulcer than were individuals with *cagA*⁻ *H. pylori* strains ($p < 0.05$, Table 3).

The *vacA* genotypes of 56 *H. pylori*-positive samples were investigated as described by Atherton et al. (13). The prevalence of s1 genotype in our patients was 98.2% (55/56; Table 4). And 75% (3/4), 100% (37/37) and 100% (15/15) of our patients who suffered from gastritis, gastric ulcer

TABLE 3
Prevalence of *cagA* Genotypes in *H. pylori*-Infected Patients

Diagnosis	n	<i>cagA</i> Type A genotype Positive n (%)	Negative n (%)	<i>p</i> *
Gastritis	4	2 (50.0)	2 (50.0)	
Gastric ulcer	37	27 (73.0)	10 (27.0)	<0.05
Duodenal ulcer	15	11 (73.3)	4 (26.7)	
Total	56	40 (71.4)	16 (29.6)	

* χ^2 test with Yates's correction, comparison of the percentage between patients infected with *cagA*⁺ and *cagA*⁻ *H. pylori* strains.

and duodenal ulcer, respectively, were infected with *H. pylori* strains of *vacA* s1 genotype (Table 4). Our results demonstrated that patients infected with *H. pylori* strain carrying *vacA* s1 genotype were more susceptible to gastritis, gastric ulcer and duodenal ulcer than were individuals with *H. pylori* strains without *vacA* s1 genotype ($p < 0.05$, Table 4).

The prevalence of *vacA* m2 genotypes of 56 *H. pylori*-positive samples was subsequently exam-

TABLE 4
Prevalence of *vacA* s1 and m2 Genotypes in *H. pylori*-Infected Patients

Diagnosis	n	<i>vacA</i> s1 positivity n (%)	<i>p</i> *	m2 positivity n (%)	<i>p</i> †
Gastritis	4	3 (75.0)*		1 (25.0)†	
Gastric ulcer	37	37 (100)*	<0.05	20 (54.1)†	<0.05
Duodenal ulcer	15	15 (100)*		9 (60.0)†	
Total	56	55 (98.2)		30 (53.6)	

* χ^2 test with Yates's correction, comparison of the percentage between patients infected with *vacA* s1 genotype-positive and -negative *H. pylori* strains.

† χ^2 test with Yates's correction, compared with the percentage between patients infected with *vacA* m2 genotype-positive and -negative *H. pylori* strains.

ined. The prevalence of m2 genotype in our patients was 53.6% (30/56; Table 4). And 25.0% (1/4), 54.1% (20/37) and 60.0% (9/15) of our patients who suffered from gastritis, gastric ulcer and duodenal ulcer, respectively, were infected with *H. pylori* strains of *vacA* m2 genotype. Similar to the results regarding *vacA* s1 genotype, patients infected with *H. pylori* strains carrying *vacA* m2 genotype were more susceptible to gastric ulcer and duodenal ulcer than were individuals with *H. pylori* strains without *vacA* m2 genotype ($p < 0.05$, Table 4).

We then statistically analyzed the putative correlation between the coexistence of *cagA* and *vacA* s1/m2 genotypes and various *H. pylori*-related gastroduodenal diseases by the Mann-Whitney test. No significant correlation between the coexistence of *cagA* and *vacA* genes with the outcome of *H. pylori*-infection was observed ($p = 0.162$, data not shown).

Discussion

Our study demonstrated that the frequency of *H. pylori* infection in a sample of Taiwanese patients with gastroduodenal diseases was 57.7%. The distribution of *cagA*⁺ *H. pylori* strains in our patients with gastritis, gastric ulcer and duodenal ulcer was 50%, 73.0% and 73.3%, respectively (Table 3). The genotype of all of the *cagA*⁺ *H. pylori* strains among our patients was type A. There was a discrepancy between our study and previous reports, in which the prevalence of *cagA*⁺ *H. pylori* strain was as high as 98–100% in Taiwan (21, 22). There are two possibilities to explain this discrepancy. First, we investigated *cagA* genotypes directly from gastric biopsy instead of using clinical isolates. It is possible that some *cagA*⁻ strains were missing due to negative culture results. And second, there might be a difference in the distribution of *H. pylori* strains among different geographical regions in Taiwan. Our results are comparable to the study from mainland China which found that only 68% of *H. pylori* isolates from patients with gastroduodenal diseases were *cagA*⁺ strain (23). The results of this report indicated that 55.6%, 54.2% and 63.3% of patients who suffered from gastritis, gastric ulcer and gastric cancer, respectively, were *H. pylori*-positive. In terms of the distribution of *cagA* genotypes, Maeda et al. (8) reported that the frequency of type A, type B, type C and type D genotype in Japanese *H. pylori* isolates is 93.5%, 0.12%, 4.5% and 0.64%, respectively. Taken together, these studies suggest that *cagA*⁺ *H. pylori* with type A genotype may be the most prevalent *H. pylori* strain in eastern Asia.

Regarding the *vacA* gene, 98.2% of our *H. pylori*-positive patients were infected with *vacA*⁺ *H.*

pylori strains. And 75%, 100% and 100% of our patients who suffered from gastritis, gastric ulcer and duodenal ulcer, respectively, were infected with *H. pylori vacA*⁺ strains carrying s1 genotype (Table 4). This indicated that individuals infected with *H. pylori* carrying *vacA* s1 genotype were especially susceptible to the development of ulcers. In addition, 25%, 54% and 60% of our patients who suffered from gastritis, gastric ulcer and duodenal ulcer, respectively, were infected with *H. pylori vacA*⁺ strain carrying m2 genotype. This observation indicates that the correlation between *vacA* m2 genotype and peptic ulcer is less significant than that between s1 genotype and peptic ulcer. Wang et al. (21) reported that the s1a genotype (100%) and m2 genotype (87%) of *H. pylori vacA*⁺ strain were most prevalent in Taiwanese patients with gastroduodenal diseases. Our result of 98.2% prevalence of *vacA* s1 genotype and 53.6% of m2 prevalence was both similar to and significantly different from theirs. This discrepancy reflected our previous hypothesis that the distribution of *H. pylori* strains might differ among the various geographical regions in Taiwan.

No significant difference was discovered between the coexistence of *cagA* and *vacA* genes with the *H. pylori*-infection manifestations (Mann-Whitney test, $p = 0.162$, data not shown). Tummuru et al. (24) reported that CagA protein-secreting *cagA*⁺ *H. pylori* can promote VacA production. However, the authors proposed that the *H. pylori* strain carrying a *cagA* mutant gene could still secrete VacA (25). Moreover, VacA activity can still be detected in some *cagA*⁺ strains that do not express CagA protein (26). Some studies show that *H. pylori* strains without the ability to produce CagA and VacA cannot induce gastric epithelial cells to secrete IL-8; however, contradictory results have been observed (27, 28). It seems that it is not absolutely necessary for CagA and VacA proteins to coexist for induction of inflammation in gastric epithelial cells.

Summary

Our study leads to the following conclusions: (a) The frequency of *H. pylori* infection in our patients with gastroduodenal diseases was 57.7%. (b) Our data supported previous reports that the pathogenic ability of *cagA*⁺ *H. pylori* strain was much greater than that of *cagA*⁻ strains. (c) Type A genotype of *H. pylori cagA* correlated with gastroduodenal diseases. (d) s1 and m2 genotypes of *H. pylori vacA* correlated with development of gastroduodenal diseases, compared to that of *H. pylori* without s1 and m2

genotypes. Above all, although the relationship between *H. pylori* infection and gastroduodenal diseases has been established, the pathogenic factors as well as the host immune responses that dictate the fate of the host still await further investigation, since only some of the infected individuals eventually develop gastroduodenal diseases while others do not.

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