

***Helicobacter pylori* vacA genotypes and cagA status and their relationship to associated diseases**

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INTRODUCTION

Helicobacter pylori (*H. pylori*) is a major causative bacterium of chronic gastritis, peptic ulcer and mucosa-associated lymphoid tissue lymphoma in humans, and associated with an increased risk of gastric cancer^[1-8]. An important virulent factor of *H. pylori* is the vacuolating cytotoxin (VacA) encoded by vacA that induces cytoplasmic vacuolation in target cells both *in vitro* and *in vivo*^[9-11]. VacA is produced as a 140 kDa precursor which contains an N-terminal signal peptide and an approximately 33 kDa C-terminal outer membrane exporter. The precursor is cleaved at both N-terminal and C-terminal and secreted into the extracellular milieu as a 95 kDa mature protein. The mature protein further undergoes specific cleavage to yield 37 kDa and 58 kDa subunits^[12-14]. Although vacA is present in all *H. pylori* strains, only about 50% to 60% of strains can induce vacuolation of epithelial cells as assessed by the HeLa cell assay. vacA shows considerable genetic variation in *H. pylori* isolated from all over the world and contains at least two variable regions. The s region exists as s1 or s2 allelic types. Among type s1 strains, subtypes s1a and s1b have been identified. The m region occurs as m1 or m2 allelic types. Specific vacA genotype of *H. pylori* strains are associated with the production of the cytotoxin *in vitro*, epithelial damage *in vivo*, and clinical consequences^[15-27]. The other virulent factor is the cytotoxin-associated protein (CagA) encoded by the cytotoxin-associated gene (cagA). The cagA gene is present in about 60% to 70% of strains and all of these

strains express the cagA. The presence of cagA is also associated with the production of the cytotoxin *in vitro*, and clinical outcome^[24-30]. The aim of this study was (i) to identify vacA genotypes and cagA status of *H. pylori* isolated from Chinese patients; (ii) to evaluate the relationship between vacA genotypes, cagA status and related gastroenterological disorders.

MATERIALS AND METHODS

Patients

Seventy-four clinical isolates of *H. pylori* were obtained from patients which underwent gastroduodenoscopy in Changhai Hospital, Shanghai, China. *H. pylori* strain CCUG17874 and G50 obtained from IRIS, Italy were also used in this study. No patient had received nonsteroidal anti-inflammatory drugs or antacids. Of these, 31 patients (mean age 47 years) had peptic ulcer including 21 duodenal ulcers, 5 gastric ulcers and 5 complete ulcers; 39 patients (mean age 41 years) had gastritis including 18 suppur gastritis, 6 atrophic gastritis and 15 erosive gastritis; and 4 patients (mean age 56 years) had gastric adenocarcinoma.

Isolation and culture of H. pylori

Two gastric biopsies were obtained respectively from gastric antrum and corpus of each patient by using endoscopy. Each specimen was placed in a transport medium and sent to the laboratory within 3 h. The specimens were then incubated onto Campylobacter selective agar (MERCK) containing 10% sheep blood for 3 d at 37°C under microaerobic conditions (10% CO₂, 5% O₂, 85% N₂). All *H. pylori* strains were positive for urease, oxidase, catalase and were identified by Gram and Giemsa staining under light microscopy. Colonies directly harvested from the plates were used for RNA extraction.

Preparation of total RNA and RT-PCR amplification

The extraction of total RNA was performed using Promega's SV total RNA isolation system according to manufacturer's instructions. The DNA sequences of the primer oligonucleotides used for RT-PCR and the size of the corresponding PCR products are listed in Table 1. RT-PCR reactions were performed as follows: denaturation at 95°C for 2 min, followed by 30 cycles consisting of 95°C for 40 s, 55°C for 1 min, and 67°C for 2 min, and final extension at 67°C for 7 min. The RT-PCR products were electrophoretically separated on a 1.5% agarose gel and stained with ethidium bromide.

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Statistical methods

Analysis of data was performed using the χ^2 test. Probability levels (P) of <0.05 were considered statistically significant.

Table 1 Oligonucleotide primers used in this work

Region amplified	Primer sequence	Size of PCR product
cagA	5'ATAATGCTAAATTAGACAACCTTGAGCG 3' 5'TTAGAATAATCAACAAACATCAGCCA 3'	297 bp
vacA s1a	5'ATGGAAATACAACAAACACAC 3' 5'GTCAGCATCACACCGCAAC 3'	190 bp
s1b	5'ATGGAAATACAACAAACACAC 3' 5'AGCGCCATACCGCAAGAG 3'	187 bp
s2	5'ATGGAAATACAACAAACACAC 3' 5'CTGCTTGAATGCGCCAAAC 3'	286 bp
m1	5'GGTCAAAATGCGGTCATGG 3' 5'CCATTGGTACCTGTAGAAAC 3'	290 bp
m2	5'GGAGCCCCAGGAAACATTG 3' 5'CATAACTAGCGCCTTGAC 3'	352 bp

RESULTS

vacA genotypes of *H. pylori* strains

Typing of the *vacA* gene of 74 clinical isolates and strain CCUG17874 and G50 was performed based on signal and middle sequences by RT-PCR as described in materials and methods. All of the 74 clinical isolates and strain CCUG17874 were s1a type whose fragment was 259 bp products as predicted by RT-PCR-amplification. Strain G50 was s1b type whose fragment was 286 bp products predicted by RT-PCR-amplification. No clinical isolates was of type s1b. Neither strain CCUG17874 and G50 nor clinical isolates was of type s2. Seven isolates (9.5%) and strain CCUG17874 were of m1 type whose fragment was 290 bp products. Sixty-seven isolates (90.5%) and strain G50 were of m2 type whose fragment was 352 bp products. Overall, 7 strains (9.5%) were type s1a/m1, 67 isolates (90.5%) were type s1a/m2. An s1a/m2 type strain was predominantly found in clinical isolates from Chinese patients living in Shanghai.

cagA positivity of *H. pylori* strains

The *cagA* gene was determined in 74 isolates by RT-PCR. Sixty-nine Chinese *H. pylori* strains (93.2%) were *cagA* positive. When the *vacA* genotype was compared with *cagA* status, all (100%) of 7 s1a/m1 type strains and 62 (92.5%) of 67 s1a/m2 type strains were *cagA* positive.

Relationship between *vacA* genotype, *cagA* status and related gastroenterological disorders

Infection with type s1a/m1 strain was found in 4 (12.9%) of 31 patients with peptic ulcers compared with 3 (7.7%) of 39 patients with gastritis. Infection with s1a/m2 strain was found in 27 (87.1%) of 31 patients with peptic ulcer compared with 36 (92.3%) of 39 patients with gastritis. There was no statistical significance between *vacA* genotype and the clinical outcome. ($P > 0.05$). *cagA* positivity was found in 29 (93.5%) of 31 patients with peptic ulcer compared with 37 (94.9%) of 39 patients

with gastritis. There was also no relationship between *cagA* status and related gastroenterological diseases ($P > 0.05$).

DISCUSSION

Based upon the methodology for typing *vacA* gene developed by Atherton *et al*^[19], we analysed and typed *vacA* genotype of clinical isolates from Chinese patients living in Shanghai by using RT-PCR. The product amplified by RT-PCR was obtained in all clinical isolates, CCUG17874 and G50 strains. The results suggested that *vacA* transcription occurred in each of *H. pylori* strains tested and was in agreement with previous reports^[19]. According to typing of *vacA* alleles, these Chinese patients infected prevalently with *H. pylori* contained the type s1a/m2 of *vacA* alleles. The finding reported here was different from that in United States, Germany, and Japan^[15-26]. The reason why the s1a/m2 strains have accumulated in Shanghai, China is unclear. It is known that genomic rearrangement may be associated with the uptake of DNA by natural transformation. Chinese have the same habits and customs, and little intermarriages with other nations. The high incidence of the s1a/m2 allele in Chinese population may indicate that it was first acquired in this population and the chance for genomic diversity may be little. Alternatively, the s1a/m2 allele may have a selective advantage in the Chinese population due to human polymorphism and ethnic background.

Recently, van Doorn *et al* found a novel subtype of *vacA* signal region from s1a subtype, designated as s1c. Type s1c allele was obtained exclusively in isolates from East Asia^[32]. We have done *vacA* gene sequencing from 5 clinical isolates (in press). The s region was considered as type s1a. Compared with s1c sequence, two of the five strains were of type s1c. These results indicated that s1c subtype could be identified as subtype s1a. Whether s1c strains are phenotypically different from s1a strains remains to be determined.

The findings here showed that all strains but five possessed the *cagA* gene and suggested that *cagA* positive strains were predominantly found in Shanghai also. The relationship between type s1a *vacA* allele and *cagA* positive has been described^[31]. All (100%) of Chinese (Shanghai) *H. pylori* strains were of type s1a *vacA* allele and 93.2% of strains were *cagA* positive and were highly prevalent in China.

Subtype analysis of *vacA* alleles and *cagA* positivity demonstrated that the *vacA* subtype and *cagA* status was not independently associated with the clinical outcome of *H. pylori* infection. The factors affecting the clinical consequences need further studies.

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