Gene distribution of cagI in Helicobacter pylori-infected patients of Zhejiang Province

Hai-Yan Liu, Ping-Chu Fang, Yun-Shui Jiang, Ran Tao, Jin Chen

INTRODUCTION

Although more than 50% of the world population are infected with Helicobacter pylori (H. pylori), most of the carriers are asymptomatic. Only a minority of infected persons may develop serious gastroduodenal diseases. Though the pathogenesis of H. pylori infection is not well understood, there are several putative virulence factors that may contribute to mucosal damage by H. pylori infection such as the cytotoxin associated gene (cag) pathogenicity island (PAI) [3]. The cag PAI was reported to be a major virulence factor of H. pylori [4,5]. The cagII is located on the left of cag PAI. There is growing evidence that genetic differences among strains determine the clinical outcome of infection [6-7]. Some of the genes in cagII are believed to encode proteins that have similarities to recognized virulence factors in other bacteria. However in mainland China the distribution of these genes in cagII of H. pylori and their relationship with gastroduodenal diseases remain unclear. In this work, we attempted to determine the structure of cagII of H. pylori isolated from Zhejiang Province and the relationship between the genes in cagII and the types of gastroduodenal diseases. The genes of cagT, ORF13 and ORF10 that have representative sequencing along the cagII were selected and amplified by polymerase chain reaction (PCR) to evaluate the cagII distribution in 171 isolates from H. pylori-infected patients with different gastroduodenal diseases in Zhejiang Province.

MATERIALS AND METHODS

H. pylori isolates

A total of 171 H. pylori isolates were obtained from H. pylori-infected adults who had undergone upper gastrointestinal endoscopy at the Second Affiliated Hospital of Zhejiang University and the Hospital of Daishan County in Zhejiang Province. The patients consisted of 115 men and 56 women with a mean age of 42.9 years (ranging from 16 to 71 years). The patients were classified into 6 groups of chronic superficial gastritis (n=70), chronic atrophic gastritis (n=31), gastric ulcer (n=41), duodenal ulcer (n=21), both gastric and duodenal ulcer (n=3) and gastric adenocarcinoma (n=5). The classification of patients was based on the results of endoscopic and histological examinations.

Culture of H. pylori

Biopsy specimens were cultured on ECY-selective agar plates at 37°C for 5 days under 100% humidity and microaerophilic conditions (50 mL/L O₂, 100 mL/L CO₂, and 850 mL/L N₂). H. pylori was identified by the following criteria: characteristic of colony, rapid urease test, catalase test and morphology on Gram staining.

Genomic DNA extraction

H. pylori genomic DNA was extracted by phenol/chloroform method.

CONCLUSION: The cagII is not a uniform and conserved entity. Although the genes in cagII are highly associated with the gastroduodenal diseases, the clinical outcome of H. pylori infection is not reliably predicted by the three genes in cagII in patients from Zhejiang Province.
Detection of cagT, ORF13 and ORF10 with PCR

For the detection of cagT, ORF13 and ORF10 genes, PCR was performed in a volume of 25 μL containing 2.5 μL of 10× buffer, 2 μL of 25 mM M Month Cl, 2 μL of 2 mM dNTPs, 0.2 μL of Tau DNA polymerase, 0.5 μL of 0.2 μL inhibitor, 1 μL of genomic DNA, 15.8 μL of water. The primers for cagT, ORF13 and ORF10 were synthesized as described in Table 1. The PCR amplification of cagT, ORF13 and ORF10 genes was as follows: initial denaturation at 95 °C for 3 min; 30 cycles of 94 °C for 30 s, at 56 °C for 30 s and at 72 °C for 45 s; and a final extension at 72 °C for 7 min. PCR was performed in a thermal cycle (GeneAmp PCR system 9600; Perkin-Elmer, Norwalk, Conn, USA). After amplification, 5 μL of PCR products was electrophoresed on 17 g/L agarose gel and examined under UV illumination.

### Table 1 PCR primers for amplification of cagT, ORF13 and ORF10

| Gene | Strand | Primer sequence | Length (bp) |
|------|--------|----------------|-------------|
| cagT | +      | 5'-TCTAAAAAGATTAAGGCTCATAGGCG-3' | 490         |
|      | -      | -              |             |
| ORF13| +      | 5'-CGTCTAGTGTCATCACTTTGGC-3' | 617         |
|      | -      | -              |             |
| ORF10| +      | 5'-AATAGGTGCTCTTCTTAGGATTAGCG-3' | 658         |
|      | -      | -              |             |

Statistical analysis

Statistical analysis was performed using the χ² test. Values of P<0.05 were considered to be statistically significant.

RESULTS

Amplification of cagT, ORF13 and ORF10 genes

After PCR amplification of the cagT, ORF13 and ORF10 genes, the products were electrophoresed on 17% agarose gel, and stained with ethidium bromide (Figure 1).

![Figure 1](Image)

**Figure 1** Electrophoresis of cagT, ORF13 and ORF10 after PCR. Lane 1:100 bp DNA ladder; Lane 2: cagT (490 bp); Lane 3: ORF13 (617 bp); Lane 4: ORF10 (658 bp).

Distribution of selected genes within cagII in *H pylori* isolates from patients with gastroduodenal diseases

Of 171 *H pylori* isolates from Zhejiang Province, 159(93.0%) were positive for all the three loci. One isolate (0.6%) from a patient with chronic superficial gastritis was negative for all the three loci, and 11(6.4%) were partially deleted in cagII. Among the latter 11 isolates, 6 were from chronic superficial gastritis, 2 from chronic atrophic gastritis, 3 from gastric ulcer and 1 from gastric adenocarcinoma. The positivity rates of cagT, ORF13 and ORF10 gene expression and their relationship with gastroduodenal diseases are listed in Table 2. There were no significant differences among the three selected genes in different gastroduodenal diseases (χ²=3.098, P>0.05 for cagT; χ²=3.935, P>0.05 for ORF13 and χ²=6.328, P>0.05 for ORF10).

DISCUSSION

*H pylori* is a Gram-negative, spiral-shaped, microaerophilic bacterium that infects human gastric mucosa and is recognized as a major cause of chronic active gastritis and most peptic ulcer diseases. It is also closely related with gastric adenocarcinoma, gastric mucosa-associated lymphoid tissue lymphoma and primary gastric non-Hodgkin’s lymphoma. The cag PAI is an approximately 40-kb cluster of genes on the *H. pylori* chromosome and divided into two regions, cagI and cagII. There are 14 open reading frames in cagII. Some of the genes within cagII are believed to encode proteins, which have homologue of recognized virulence factors in other bacteria by amino acid database search and analysis. The protein encoded by cagT gene is similar to *Shigella flexneri* 42-kDa surface antigen IPAC. It was reported that IPAC of *Shigella* was essential for initial bacterial entry into epithelial cells by interacting with beta-catenin and destabilizing the cadherin-mediated cell adhesion complex, thus the epithelial cell-cell tight adhesion was disrupted. These events might facilitate the further basolateral invasion of bacteria through the disrupted space and/or modulate the cell-to-cell spread of *Shigella*. We propose that cagT may play a similar role in the pathogenesis of *H pylori*. Moreover the proteins encoded by cagT, ORF13 and ORF10 are similar to virB7, virB10 and virD4 of *Agrobacterium tumefaciens* that are needed for the transferring the Ti plasmid DNA from the bacterium to the nucleus of the plant cell. The products of the virB7, virB10 and virD4 genes are considered to be important components in type IV secretion system. Several lines of evidence suggest that the type IV secretion system encoded by the *cag PAI of H pylori* is recognized as a major virulence determinant, governing the translocation of the CagA protein to eukaryotic cells and inducing strongly the expression and secretion of IL-8 in gastric epithelial cells. Deletion of the cagII segment from strain 26695 reduced IL-8 synthesis to about 10-20% of the wild-type control. Inactivation of *ORF13 or cagT* also caused similar reduction in IL-8 synthesis after infection. In addition, the products of cagT, ORF13 and ORF10 were absolutely essential for the translocation of CagA and tyrosine phosphorylation. IL-8, a potent neutrophil and T-cell chemoattractant and activator, is believed to play a key role in the pathogenesis of *H pylori*-induced tissue damage. These

### Table 2 Relationship between cagT, ORF13, ORF10 gene expression and clinical diagnosis in patients of Zhejiang Province

| Group                  | n  | cagT n | %  | ORF13 n | %  | ORF10 n | %  |
|------------------------|----|--------|----|---------|----|---------|----|
| Chronic superficial gastritis | 70 | 67     | 95.7 | 66      | 94.3 | 69      | 98.6 |
| Chronic atrophic gastritis | 31 | 31     | 100.0 | 29      | 93.5 | 31      | 100.0 |
| Gastric ulcer          | 41 | 39     | 95.1 | 39      | 95.1 | 41      | 100.0 |
| Duodenal ulcer         | 21 | 21     | 100.0 | 21      | 100.0 | 21      | 100.0 |
| Both gastric and duodenal ulcer | 3  | 3      | -   | 3       | -   | 3       | -   |
| Gastric adenocarcinoma | 5  | 5      | -   | 4       | -   | 5       | -   |
| Total                  | 171| 166    | 97.1| 162     | 94.7 | 170     | 99.4 |
results indicate that the genes in cagII participate in the translocation of CagA and induction of IL-8 synthesis and then a resultant severe inflammatory response. The presence of cagII is highly associated with the gastroduodenal diseases.22

In the present study, we have shown that the overall prevalence of the cagT, ORF13 and ORF10 is 97.1%, 94.7% and 99.4%, respectively. Although the genes in cagII are highly associated with the gastroduodenal diseases, the clinical outcome of H pylori infection is not reliably predicted by the genes of cagT, ORF13 and ORF10 in the cag II in Zhejiang Province. These results are in agreement with those of studies in Japanese and Taiwanese. The distribution of presence of cagT, ORF13 and ORF10 in Japan has been shown to be about 94%, 98.4% and 98.4%, respectively.23 In Taiwan, all strains were positive for cagT and ORF13 genes.24 However, in South Africa the overall positivity rate of cagT in clinical isolates was 81.7%, lower than our report. And the prevalence of cagT in patients with peptic ulceration and gastric adenocarcinoma was significantly higher than that in gastritis.25 In Europe the prevalence of cagT, ORF13 and ORF10 in clinical isolates was 79.5%, also lower than the one of our report.26 These results indicate that H pylori isolated from Asia is different from the ones isolated from South Africa and Europe. In the present study, of 171 H pylori isolates from Zhejiang patients, 159(93.0%) were positive for all the three loci. One isolate (0.6%) from a patient with chronic superficial gastritis was negative for all the three loci, and 11(6.4%) were partially deleted in cagII. It appears that the cagII is not a uniform, conserved entity.

In conclusion, we speculate that the distribution of cagT, ORF13 and ORF10 in Zhejiang Province is in accordance with those in other Asian countries. The clinical outcome of H pylori infection can not be reliably predicted by the genes of cagT, ORF13 and ORF10 in cag II. Many factors such as the genetic factors of both H pylori and the host cell and the circumstance may contribute to the clinical outcome of H pylori infection. Nevertheless, Further work is required to illustrate pathogenesis of cagII in H pylori associated gastroduodenal diseases.

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