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Gene distribution of *cagII* in *Helicobacter pylori*-infected patients of Zhejiang Province

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Abstract

AIM: To determine the prevalence of genotypes of *cagII* in *Helicobacter pylori* (*H pylori*)-infected patients in Zhejiang Province and investigate the relationship between these genotypes and the types of gastroduodenal diseases.

METHODS: One hundred and seventy one clinical isolates were collected from 70 chronic superficial gastritis, 31 chronic atrophic gastritis, 41 gastric ulcer, 21 duodenal ulcer, 3 gastric and duodenal ulcer, and 5 gastric adenocarcinoma patients. Polymerase chain reaction assays were performed for analysis of *cagT, ORF13* and *ORF10* genes in the *cagII* region.

RESULTS: Of 171 *H pylori* isolates from Zhejiang patients, 159(93.0%) were positive for all the three loci. One isolate (0.6%) was negative for all the three loci, and 11(6.4%) were partially deleted in *cagII*. The positive rates of *cagT, ORF13* and *ORF10* genes were 97.1%, 94.7% and 99.4%, respectively. In the strains isolated from the patients with diseases including chronic superficial gastritis, chronic atrophic gastritis, gastric ulcer and duodenal ulcer, the sitive rates of *cagT* were 95.7%, 100.0%, 95.1% and 100.0%, respectively. The positive rates of *ORF13* were 94.3%, 93.5%, 95.1% and 100.0%, respectively. The sitive rates of *ORF10* were 98.6%, 100.0%, 100.0% and 100.0%, respectively. The three genes were all positive in the three *H pylori* strains isolated from the patients with both gastric and duodenal ulcer. In the five strains isolated from the patients with gastric adenocarcinoma, only one isolate was negative for *ORF13*. There were no significant differences of the *cagT*, *ORF13* and *ORF10* genes among the different gastroduodenal diseases including chronic superficial gastritis, chronic atrophic gastritis, gastric ulcer, duodenal ulcer, both gastric and duodenal ulcer and gastric adenocarcinoma (χ^2=3.098, P>0.05 for *cagT*; χ^2=3.935, P>0.05 for *ORF13* and χ^2=6.328, P>0.05 for *ORF10*).

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.

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INTRODUCTION

Although more than 50% of the world population are infected with *Helicobacter pylori* (*H pylori*), most of the carriers are asymptomatic[1,2]. 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 the gastroduodenal diseases. The genes of *cagT*, *ORF13* and *ORF10* that have representative spacing sequences 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 ℃ for 5 d under 100% humidity and microaerophilic conditions (50 mL/L O_2, 100 mL/L CO_2, and 850 mL/L N_2). *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.

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 mmol/L $MgCl_2$, 2.5 μL of 2 mmol/L dNTPs, 0.2 μL of *Taq* DNA polymerase, 0.5 μL of 20 μmol/L primer sets (Table 1), 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 ℃ for 3 min; 30 cycles of at 94 ℃ for 30 s, at 56 ℃ for 30 s and at 72 ℃ for 45 s; and a final extension at 72 ℃ for 7 min. PCR was performed in a thermal cycle (GeneAmp PCR system 9 600; 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' TCTAAAAAGATTACGCTCATAGGCG 3'	490
	-	5' CTTTGGCTTGCATGTTCAAGTTGCC 3'	
ORF13	+	5' CGTTCATGTTCCATACATCTTTGGC 3'	617
	-	5' GATTTATAGCGATCTAAGAAACCGC 3'	
ORF10	+	5' AATAGTGCTTTCTTTAGGATTAGCG3'	658
	-	5' CCGATTTAATCCTTTCGCTTATGTG 3'	

Statistical analysis

Statistical analysis was performed using the χ^2 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 1.7% agarose gels, and stained with ethidium bromide (Figure 1).

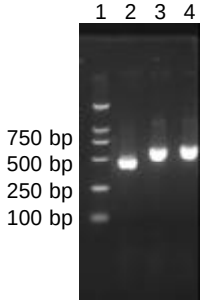

Figure 1 Electrophoresis of *cagT*, *ORF13* and *ORF10* after PCR. Lane 1:100 bp DNA ladder; Lane 2: *cagT* (490 bp); Lane 3: *ORF13*(617 bp); Lane 4: *ORF10*(658 bp).

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 (χ^2=3.098, $P>0.05$ for *cagT*; χ^2=3.935, $P>0.05$ for *ORF13* and χ^2=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[8,9]. It is also closely related with gastric adenocarcinoma, gastric mucosa-associated lymphoid tissue lymphoma and primary gastric non-Hodgkin's lymphoma[10]. The *cag PAI* is an approximately 40-kb cluster of genes on the *H. pylori* chromosome[3,11] 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 flexnerii* 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[12], 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 of the Ti plasmid DNA from the bacterium to the nucleus of the plant cell[1, 13]. The products of the *virB7, virB10* and *virD4* genes are considered to be important components in type IV secretion system[14]. 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[2,15,16]. Deletion of the *cag*II 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[13,17,18]. 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[19,20]. These

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

Group	*n*	cagT *n*	%	*ORF*13 *n*	%	*ORF*10 *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[21].

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[16]. In Taiwanese, all strains were positive for *cagT* and *ORF13* genes[22]. 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[21]. In Europe the prevalence of *cagT*, *ORF13* and *ORF10* in clinical isolates was 79.5%, also lower than the one of our report[23]. 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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