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• *H pylori* •

Distribution of *cagG* gene in *Helicobacter pylori* isolates from Chinese patients with different gastroduodenal diseases and its clinical and pathological significance

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Abstract

AIM: To determine the distribution of *cagG* gene of *Helicobacter pylori* (*H pylori*) isolates cultured from patients with various digestive diseases and its relationship with gastroduodenal diseases.

METHODS: *cagG* was amplified by polymerase chain reaction in 145 *H pylori* isolates cultured from patients with chronic gastritis (*n*=72), duodenal ulcer (*n*=48), gastric ulcer (*n*=17), or gastric and duodenal ulcer (*n*=8), and the relationship between *cagG* status and the grade of gastric mucosal inflammation was determined.

RESULTS: *cagG* was present in 91.7 % of the 145 *H pylori* isolates, with the rates were 90.3 %, 93.8 %, 88.2 % and 100.0 %, respectively, in those from patients with chronic gastritis, duodenal ulcer, gastric ulcer, and gastric and duodenal ulcer. There was no significant difference among the four groups ($P>0.05$). The average grade of gastric mucosal inflammation in the antrum and corpus was 1.819 ± 0.325 and 1.768 ± 0.312, respectively in *cagG* positive patients, whereas the average inflammation grade was 1.649 ± 0.297, 1.598 ± 0.278 respectively in *cagG* negative cases ($P>0.05$).

CONCLUSION: *cagG* gene of *H pylori* was quite conservative, and most *H pylori* strains in Chinese patients were *cagG* positive. *cagG* status was not related to clinical outcome or the degree of gastric mucosal inflammation. Therefore, *cagG* can not be used as a single marker for discrimination of *H pylori* strains with respect to a specific digestive disease.

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INTRODUCTION

Helicobacter pylori is a well-recognized pathogen that chronically infects more than 50 % of the world population. *H pylori* is associated with the development of acute or chronic gastritis, peptic ulcer diseases, gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue (MALT) lymphoma. Most infected subjects will remain asymptomatic throughout life with only about 20 % developing peptic ulcer diseases or gastric carcinoma[1-5]. What determines the outcome of an infection remains unclear. The reasons for these different outcomes of *H pylori* infection may be related to both bacterial factors and host responses. The major *H pylori* disease-associated genetic factor is the whole *cag* pathogenicity island (PAI), which contains 25 open reading frames and at least 30 genes. The *cag* PAI is associated with increased interleukin (IL)-8 production by gastric epithelial cells[6,7].

The cytotoxin-associated gene A (*cagA*) is located in the most downstream part of the *cag* PAI. The presence of this gene or its encoded protein, CagA, has been regarded as a marker for the *cag* PAI. Many clinical studies have demonstrated that *cagA* gene or CagA protein is associated with a more severe clinical outcome. CagA was reported to increase the risk of development of duodenal ulcer, atrophic gastritis and gastric adenocarcinoma. In contrast to the *cagA*-negative patients, gastric mucosal inflammation of *cagA*-positive patients was more severe[8-10]. *cagG* is located within the *cag* PAI upstream of *cagA*. The distribution of this gene in *H pylori* strains isolated from Chinese digestive patients and its relation with the gastroduodenal diseases remain unclear. In the present study, a set of specific primers were designed to detect the *cagG* gene in 145 clinical *H pylori* strains, and the relationship between *cagG* and different digestive diseases was determined.

MATERIALS AND METHODS

H pylori isolates

H pylori isolates obtained from 145 patients (80 males, 65 females, aged 18-69 years, mean age 42.5 years old) who underwent upper endoscopy in our department were included in this study. These patients were diagnosed endoscopically as chronic gastritis (*n*=72), duodenal ulcer (*n*=48), gastric ulcer (*n*=17), or gastric and duodenal ulcer (*n*=8). Informed consents were obtained from all patients. The standard strains CCUG17874 (NCTC11638) and Tx30a were kindly provided by the Italian IRIS Research Center.

H pylori culture

Two antral biopsy specimens taken during endoscopy were immediately cultured on the *H pylori* selective agar plates with 10 % defibrillated sheep blood and antibiotics (Merck Company, Germany) at 37 ℃ under microaerophilic conditions with 5 % O_2 10 % CO_2 and 85 % N_2 for 3-6 days. The colonies were identified as *H pylori* if Gram stain morphology and biochemical tests were positive for urease, oxidase and catalase. All stock cultures were preserved at -80 ℃ in Brucella broth with 20 % glycerol, and subcultured for genomic DNA extraction. The passage number of *H pylori* used in this study averaged six.

Histopathologic examination
Two biopsy specimens each taken from the gastric corpus and antrum endoscopically were used for histopathologic examination to grade the severity of gastritis after they were embedded in paraffin and stained with hematoxylin and eosin. The severity of gastritis (i.e. mononuclear cell and polymorphonuclear leukocyte infiltration) was evaluated, and graded on a scale of 0-3 (i.e. 0=no, 1=mild, 2=moderate, and 3=marked) according to the updated Sydney system[11].

Genomic DNA extraction
Subcultured *H pylori* cells were collected from the agar plates, then genomic DNA was extracted and purified from each *H pylori* isolate using cetyltrimethyl ammonium bromide (CTAB), phenol-chloroform-isoamyl alcohol, and ethanol precipitation.

Detection of cagG with polymerase chain reaction (PCR)
The primers to amplify *cagG* gene and give a 497 base pair (bp) product were designed based on the published gene sequence[12]. *cagG*F: 5’ -GCCATGTTAACACCCCCTAG-3’ , and *cagG*R:5’ -TTAATGCGCTAGAATAGTGC-3’ . PCR was performed in an Eppendorf thermal cycler using a PCR kit (Takara, Dalian, China) according to the manufacturer’ s instructions. Briefly, the reaction was performed in a total volume of 50 μl containing 20 ng genomic DNA as a template and 200 μM each deoxynucleotide, 1.5U Taq polymerase, 0.4 μM each primer and PCR buffer. The PCR amplification program comprised at 95 ℃ for 5 min, then 35 cycles at 95 ℃ for 1 min, at 52 ℃ for 1 min and at 72 ℃ for 1 min, followed by at 72 ℃ for 7 min, then cooled at 4 ℃. The PCR products were analyzed on 1.5 % agarose gels with ethidium bromide. CCUG17874 was taken as a positive control, Tx30a as a negative control, and deionized water as a blank control.

Statistical analysis
The data were expressed as the mean ±SD. The *t* test and χ^2 test were used for statistical analysis. A *P* value <0.05 was considered to be statistically significant.

RESULTS

Amplification of cagG gene
After PCR amplification of the *cagG* gene, the products were electrophoresed on 1.5 % agarose gels, and stained with ethidium bromide. Under ultraviolet light, *cagG* appeared as a specific band with 497 bp (Figure 1).

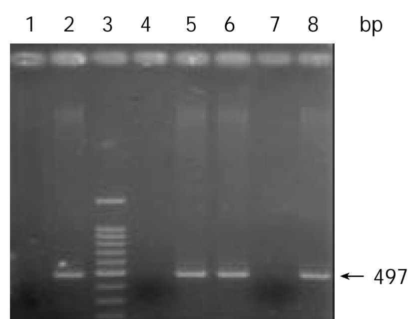

Figure 1 Electrophoresis of PCR products. Lane 3: 100 bp DNA Marker; Lanes 1, 2 and 4: controls: Tx30a, CCUG17874, and deionized water; Lanes 5, 6, 7 and 8: clinical *H pylori* isolates.

CagG status in H pylori isolates from patients with various diseases
H pylori cagG gene was detected in 91.7 % of the 145 isolates, the rate was 90.3 %, 93.8 %, 88.2 % and 100.0 % in patients with chronic gastritis, duodenal ulcer, gastric ulcer and gastric/duodenal ulcer, respectively (Table 1). There was no significant difference among the groups (*P*>0.05).

Table 1 *cagG* in *H pylori* isolates from patients with different gastroduodenal diseases

Group	Number	*cagG* positive	*cagG* negative	*cagG* positive rate (%)[a]
Chronic gastritis	72	65	7	90.3
Duodenal ulcer	48	45	3	93.8
Gastric ulcer	17	15	2	88.2
Gastric duodenal ulcer	8	8	0	100.0
Total	145	133	12	91.7

[a]*P*>0.05 between any two groups.

CagG status and gastric mucosal inflammation
The average grade of gastric mucosal inflammation in the antrum and corpus was 1.819±0.325 and 1.768±0.312 in *cagG* positive patients, respectively, whereas the average grade was 1.649±0.297, 1.598±0.278 in *cagG* negative group, respectively (*P*>0.05, both in the antrum and body).

DISCUSSION
H pylori infects human gastric mucosa which evokes a mucosal inflammatory response by neutrophil recruitment from the microcirculation. Persistent inflammation may lead to the development of digestive diseases such as chronic gastritis, peptic ulcer disease and gastric cancer. Although pathogenicity of *H pylori* infection is not well understood, there were several putative virulence factors that might contribute to mucosal damage by *H pylori* infection[13,14].

The *cag* PAI is an approximately 40-kb cluster of genes in *H pylori* chromosome, and a quite conservative entity. Many of *H pylori* strains had an intact *cag* PAI divided into two regions: *cag*I in downstream and *cag*II in upstream, and some with an insert sequence IS605 or IS606[15,16]. There were at least 14 and 16 open reading frames in *cag*I and *cag*II, several of which were virulence factors[17]. Several studies suggested that *cagA* gene could be used as a marker for *cag* PAI[16]. *cagG* is another gene in *cag*I region which is located upstream of *cagA* gene. The function of this gene is not well known. Recently, Hsu *et al*[17] reported that an intact *cag* PAI was identified in 95 % and 100 % of the strains that possessed *cagA* and *cagG* respectively, whereas the *cagA* and *cagG* genes were found in 100 % and 95 % of the strains containing a partial or complete set of *cag* PAI, indicating that *cagA* gene is not associated with a complete *cag* PAI in 5 % of the strains, and cannot be considered as an absolute marker for the presence of a complete set of *cag* PAI, but *cagG* gene may be a better indicator for the presence of an intact *cag* PAI.

Extensive studies of *cagA* gene indicated that CagA protein encoded by *cagA* gene was associated with severe clinical outcomes, such as peptic ulcer disease and gastric cancer. Therefore, it was considered as a main virulence factor of *H pylori*[16]. Some reports suggested that the presence of *cagE* gene within *cag*I might be related to more severe clinical outcomes. For example, Day *et al*[18] revealed that *H pylori* isolates containing *cagE* were associated with duodenal ulcer in Canadian children. Fallone *et al*[19] reported that *cagE*-positive isolates were more prevalent in Canadian adult patients with peptic ulcer or gastric cancer than in those with gastritis only. In the present study, we designed a set of primers to amplify *cagG* gene of 145 clinical *H pylori* isolates, and

determined the correlation of *cagG* status with endoscopic presentation, and histological findings. The results showed that *cagG* was present in 91.7 % *H pylori* isolates examined. 100 % of *H pylori* isolates from patients with gastric and duodenal ulcer were *cagG* positive, which was higher, but not statistically significant than that in other groups ($P>0.05$). Lack of difference in *cagG* positive rate might be due to patient selection or the relatively small number of patients with gastric and duodenal ulcer. Nevertheless, our study suggested that positive rate of *cagG* in *H pylori* was high and *cagG* was quite conservative in Chinese population, and that there was no difference in the frequencies of *cagG*-positive isolates among patients with gastritis, duodenal ulcer, gastric ulcer or gastric duodenal ulcer. Our results are supported by the study by Jenks *et al*[20] who demonstrated that no specific genes within the *cag* PAI could reliably predict the clinical outcome of *H pylori* infection in French patients, and also by Hsu *et al*[17] who concluded that any of the *cag* PAI genes such as *cagE* could not predict the clinical presentation in Korean patients.

Hsu *et al* reported that of the 120 clinical isolates from Korean patients with various gastrointestinal diseases, 86.7 % (104/120) were *cagG* positive[17]. Mizushima *et al*[21] used PCR and Southern blot to investigate the prevalence of *cagG* gene in 236 clinical *H pylori* isolates from Japanese patients, and found that *cagG* was present in 97 % of the isolates. These results were similar with ours. The same Japanese research group[21] further used flow cytometry to assay the ability of *H pylori* with or without *cagG* to adhere to KATOIII and ELISA to detect the IL-8 secreted from gastric epithelial cells induced by *H pylori.* They observed that in comparison with the *cagG*-positive strains, all *cagG*-deleted strains decreased adherence to KATOIII cells, and abolished IL-8 induction despite the presence of *cagE*, which was reported to be essential for IL-8 induction. *H pylori* genome is known to be diversified and may differ between geographic regions. However, there has no reports so far about *cagG* gene distribution in the Western countries.

Infection with *cagA* positive *H pylori* induces stronger gastric chemokine mRNA expression such as IL-8 in the antral mucosa, which may be relevant to the increased mucosal damage associated with *cagA* positive *H pylori* infection. The levels of the chemokines were correlated with cellular infiltration in the antrum and inflammation of the gastric mucosa[22,23]. We compared the severity of gastric mucosal inflammation in the antrum and corpus in *cagG*-positive and *cagG*-negative patients, and observed that, the average grade of inflammation was only slightly higher in *cagG*-positive group than that in *cagG* negative group both in the antrum and in the corpus ($P>0.05$). Therefore, the *cagG* status has no relation to the severity of gastritis.

In conclusion, *cagG* gene was quite conservative in clinical *H pylori* isolates from Chinese patients with different gastroduodenal diseases, since most *H pylori* isolates were *cagG* positive. There was no difference in the frequency of *cagG* -positive isolates among patients with different diseases. The *cagG* status was not related to gastric mucosal inflammation grade. Therefore, *cagG* cannot reliably predict the clinical and histological outcomes.

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