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Inverse relationship between *cagG*-positive *Helicobacter pylori* status and risk of gastric ulcer

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Helicobacter pylori is a spiral-shaped gram-negative bacterium that infects the human gastric mucosa and is associated with the development of gastrointestinal diseases such as chronic atrophic gastritis, peptic ulceration, and gastric adenocarcinoma. About 10–15% of *H. pylori* infections have been reported to lead to peptic ulceration, and *H. pylori* is responsible for 70–85% of the gastric ulcers and 90–95% of the duodenal ulcers [1]. The *cag* pathogenicity island (*cag*PAI) of *H. pylori* contains some 32 different genes, encoding a type IV secretion system (T4SS). The presence of *cag*PAI is associated with the development of peptic ulcerations [2,3]. The *cag*PAI locus has a length of about 40 kb and includes two sections: the cagI region containing 16 genes and the cagII region containing 14 genes. The two *cagA* and *cagE* genes from the cagI region, and the *cagT* and *cagM* genes, as markers of the cagII region, play an important role in gastroduodenal diseases, such as peptic ulceration and gastric cancer [4–8]. Moreover, *cagA*-positive *H. pylori* status is associated with an increased risk of developing gastric and duodenal ulceration [9,10]. CagL and CagH are two other proteins from *cag*PAI that are essential for the transfer and injection of CagA into host epithelial cells as well as the formation of T4SS pili [2]. In our hands, the *cagL*, but not the *cag*H gene is strongly associated with the risk of peptic ulceration [2], and elsewhere this genotype is associated with the risk of duodenal ulcer [11]. The *cagG* and *orf17* genes are two other genes from *cag*PAI. The *cagG* gene is located upstream of the *cagA* gene and encodes the CagG protein, which plays a role in colonization and inflammation. Although *orf17* has no significant relationship with gastric cancer, it is related to the risk of peptic ulceration [2]. Accordingly, pyloric ulcers-related risk factors should be considered separately for duodenal and gastric ulceration. Therefore, we hypothesised an associations between *H. pylori cag* PAI genotypes (i.e. *cagL*, *orf17*, *cagG*, and *cagH*) with the risk of either gastric or duodenal ulceration.

Two hundred *H. pylori* isolates were obtained from gastric biopsy cultures of 145 (72.5%) patients with endoscopically defined non-atrophic gastritis, 27 (13.5%) with gastric ulceration and 28 (14.0%) with duodenal ulceration. Informed written consent was obtained from each participant, and the study was approved by the local research ethics committee. Biopsies were cultured and identified on selective Brucella agar plates supplemented with antibiotics, under microaerobic conditions at 37 °C for a maximum of 5–7 days. Colonies were identified as *H. pylori* according to standard criteria including negative Gram staining, typical cell morphology, and positive reactions to catalase, oxidase, and urease, as well as PCR amplification of *H. pylori, 16S rDNA*. Single colonies were isolated to ensure that each strain consisted of only a single genotype. DNA was extracted from *H. pylori* isolates with the Genomic DNA Purification kit (Fermentas, UK). The presence of the *cag*PAI genes was determined by PCR amplification and sequencing. The specific primers of each gene and their optimized annealing temperature, which were used for PCR amplification and sequencing, are listed in Table 1. The total volume of the reaction was 30 µL and included PCR buffer 3 µL, $MgCl_2$ 1 µL, dNTP mix 0.5 µL, primers (reverse and forward mixture) 1 µL, template DNA (5 µL) 25 ng, *Taq* DNA polymerase 0.2 µL, and distilled water 19.3 µL. The gene amplification conditions were previously described [2].

Pearson's Chi-square and Fisher's exact test were used to determine differences in the frequency of each gene. The effect of each pathogenic gene on the risk of gastric or duodenal ulceration was assessed using simple logistic regression analysis by the *Enter* method. Multiple logistic regression was used to analyse the relationship between each gene and gastrointestinal diseases under the control of host factors such as age and sex. A P value of <0.05 indicated statistically significant level. Odds ratios (ORs) and the corresponding 95% confidence intervals (CIs) were also computed. Data were collected and analysed using SPSS version 23.

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Table 1. Primer sequence and conditions of PCR.

Genes	Primers	Sequences (From 5′ to 3′)	Size of PCR products (bp)	Optimized annealing temperature (°C)
16S rDNA	HP1	GCAATCAGCGTCAGTAATGTTC	519	55
	HP2	GCTAAGAGATCAGCCTATGTCC		
cagH	Forward	ATGGCAGGTACACAAGCTAT	1113	52
	Reverse	TCACTTCACGATTATTTTAG		
cagL	Forward	AAAACACTCGTGAAAAATACCATATC	263	54
	Reverse	TCGCTTCAAAATTGGCTTTC		
cagG	Forward	TTATAAAATTAAATTACTATTTGC	398	50
	Reverse	GTGGTAAAAACGATGAATCTG		
orf17	Forward	CTTGATTGATGAAAATTTGGTTG	546	50
	Reverse	TTAGTGATATATTCATAATTTTCC		

Table 2. Frequency of the *cag* pathogenicity island genes in patients with gastrointestinal disorders.

Clinical status	Genotype frequency (n, %)			
	cagH$^+$	*cagL*$^+$	*cagG*$^+$	*orf17*$^+$
Non-atrophic gastritis (n = 145)	86 (59.3)	120 (82.2)	110 (75.9)	88 (60.7)
Any ulceration (n = 55)	41 (74.5)	54 (98.2)	38 (69.1)	44 (80.0)
P value	0.046	0.004	0.330	0.01
Gastric ulceration (n = 27)	21 (77.8)	27 (100.0)	14 (51.8)	21 (77.8)
Duodenal ulceration (n = 28)	20 (71.4)	27 (96.4)	24 (85.7)	23 (82.1)
P value	0.589	1.00	0.007	0.686

The total of *cagH, cagL, cagG*, and *orf17* in the three groups of patients are shown in Table 2. The frequency of *cagH, cagL* and *orf17* was higher in the combined group of patients with any ulceration compared to patients with non-atrophic gastritis. The frequency of *cagG* was higher in patients with duodenal ulceration compared with those with gastric ulceration. Simple logistic regression analysis demonstrated that the *cagG* gene was inversely associated with gastric ulceration. *orf17* was significantly associated with an increased risk of duodenal ulceration. The OR (95% CI) for *cagG* and *orf17* was 0.34 (0.15–0.80) and 2.98 (1.07–8.29), respectively (P = 0.013 for *cagG*, P = 0.036 for *orf17*). No significant association was found between the *cagH* and *cagL* genes and the risk of gastric or duodenal ulceration. Multiple logistic analysis showed that the *cagG* genotype in the presence of sex was inversely related to the risk of gastric ulceration (OR (95% CI) 0.44 (0.17–0.98), P = 0.044). When duodenal ulceration was considered a dependent variable in multiple logistic regression analysis, no genotypes remained in the final model after controlling for age and sex.

H pylori cag pathogenicity island genes are strongly implicated in gastrointestinal disease [4]. In the present study, only 4% of the strains did not have *cag*PAI: the highest frequency was the *cagL* (87.0%), similar to studies from India, Malaysia, Singapore, and Taiwan, where >85% of the strains carried *cagL* [2]. Our simple logistic regression analysis did not show any relation between *cagL* or cagH and either form of ulceration. A study of 100 patients with gastrointestinal disorders (47 with peptic ulceration, 23 with gastric cancer, and 30 with gastritis) showed that the D58 polymorphism in *cagL* increased the risk of peptic ulceration 6.5 fold (95% CI 1.2–35.7) [12]. Ninety-seven % of strains from Japan and 91.7% of strains from China were positive for *cagG*, although no significant links with gastric or duodenal ulceration were found [2]. However, in the present study, *cagG* in the simple logistic regression analysis revealed an inverse relationship with gastric ulceration, but not duodenal ulceration. A small study of 38 patients with duodenal ulceration showed that the frequency of the *cagH* (65.2%) was higher than that in a control group (28.6%) [11], whereas we failed to find a significant relationship between *cagH* and the risk of gastric or duodenal ulceration. We have previously shown that *orf17* is not linked with gastric cancer, but with peptic ulceration [2], and now show that *orf17* is linked to duodenal ulceration, but not gastric ulceration. Moreover, *cagG* was inversely associated with the risk of gastric ulceration in multiple logistic regression analysis after controlling for sex. Our study in limited by the small number of patients with ulcers. Nevertheless, it represents an advance in biomedical science as it suggests that infection with *cagG*$^+$ strains could be benefit biomarker for reduced risk of gastric ulceration, whereas strains carrying the *orf17* might increase the risk of duodenal ulceration.

Disclosure statement

No potential conflict of interest was reported by the authors.

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