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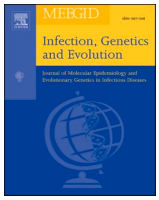

Genetic diversity of the *oipA* gene among *Helicobacter pylori* isolates and clinical outcome in Vietnam

Thi Hong Nhung Thai [a,d], Hong Phong Nguyen [b], Thi Hai Yen Nguyen [c], Thi Be Hai Nguyen [c], Thai Hoa Nguyen [d], Thi Mai Ngan Nguyen [e], Thi Minh Thi Ha [e,f,*]

[a] Department of Internal Medicine, University of Medicine and Pharmacy, Hue University, Hue, Viet Nam
[b] Department of Pathology and Forensic Medicine, Faculty of Medicine, Can Tho University of Medicine and Pharmacy, Can Tho, Viet Nam
[c] Department of Microbiology, Faculty of Medicine, Can Tho University of Medicine and Pharmacy, Can Tho, Viet Nam
[d] Department of Internal Medicine, Faculty of Medicine, Can Tho University of Medicine and Pharmacy, Can Tho, Viet Nam
[e] Department of Medical Genetics, University of Medicine and Pharmacy, Hue University, Hue, Viet Nam
[f] Institute of Biomedicine, University of Medicine and Pharmacy, Hue University, Hue, Viet Nam

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ABSTRACT

Outer inflammatory protein A (OipA), which is encoded by the *oipA* gene, can induce interleukin-8 secretion in gastric epithelial cells. The functional status of the *oipA* gene is regulated by the slipped-strand mispairing mechanism based on the CT dinucleotide repeat number in the 5′ region. This study aimed to investigate the *oipA* functional status ("on/off") of *Helicobacter pylori (H. pylori)* and its association with gastroduodenal diseases in southwestern Vietnam. The cross-sectional study was conducted on 173*H. pylori* isolates from 173 patients with gastroduodenal diseases. Sanger sequencing was used to determine the functional status of *oipA*. Multivariable logistic regression analysis was performed to identify the association between *oipA* status and gastroduodenal diseases. The *oipA* "on" status accounted for 96% of *H. pylori* isolates. Twenty-five CT repeat patterns of the *oipA* 5′ signal region were observed, five of which were novel CT repeat patterns. The *oipA* "on" status was found in 100%, 97.8%, and 86.8% of *H. pylori* isolates from patients with peptic ulcer, precancerous lesions, and chronic gastritis, respectively ($p < 0.01$). The *oipA* "on" status was related to gastric precancerous lesions versus chronic gastritis (adjusted OR = 7.39, 95% CI: 1.35–40.59, $p = 0.021$) and peptic ulcers versus chronic gastritis (adjusted OR = 12.79, 95% CI: 1.19–1760.32, $p = 0.033$). Our data show a high prevalence of the *oipA* "on" status, which was associated with precancerous gastric lesions and peptic ulcers. Moreover, genetic diversity in the number and pattern of CT dinucleotide repeat of *oipA* among Vietnamese *H. pylori* strains was identified.

1. Introduction

Helicobacter pylori (H. pylori) is a gram-negative bacterium discovered in 1983 by Marshall and Warren (Marshall and Warren, 1984). The prevalence of *H. pylori* infection worldwide is over 50% (Hooi et al., 2017). Despite this high rate of *H. pylori* infection, most individuals with *H. pylori* infection have no apparent symptoms and only show gastritis via endoscopy (Xu et al., 2020). However, chronic gastritis may develop into severe gastroduodenal diseases, including peptic ulcers, gastric mucosa-associated lymphoid tissue lymphoma, and gastric cancer (Suerbaum and Michetti, 2002). The stepwise cascade of *H. pylori*-induced gastric carcinogenesis starts with chronic gastritis, followed by atrophy, intestinal metaplasia, dysplasia, and finally, gastric cancer (Correa and Blanca Piazuelo, 2012). Currently, three main factors are thought to take part in the pathogenesis of *H. pylori*-associated gastroduodenal diseases, namely, *H. pylori* virulence factors, host factors, and environmental changes (Kao et al., 2016). The differences in bacterial virulence genotypes of *H. pylori* are considered to be important clues in determining which gastroduodenal disease may develop (Yamaoka, 2010).

The adherence of *H. pylori* to gastric epithelial cells is crucial to establish persistent colonization in the human stomach (Kao et al., 2016). The outer membrane proteins (OMPs) are mainly responsible for bacterial adherence (Yamaoka et al., 2006). Approximately 4% of the *H. pylori* genome are genes that encode OMPs (Alm et al., 2000), with at least 32 OMPs found (Tomb et al., 1997). Among these OMPs, OipA

* Corresponding author at: 6, Ngo Quyen street, Hue city 49100, Viet Nam.
E-mail addresses: htmthi@hueuni.edu.vn, htmthi@huemed-univ.edu.vn (T.M.T. Ha).

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(outer inflammatory protein A), which was discovered first in 2000 and belongs to the Hop family (*Helicobacter* OMPs), play a role in bacterial colonization and induce interleukin-8 (IL-8) secretion from gastric epithelial cells (Yamaoka et al., 2000a). The expression of OipA induces apoptosis and changes in the cytoskeleton of host gastric epithelial cells, promoting severe gastroduodenal diseases (Hedayati and Salavati, 2021). OipA is encoded by the *oipA* gene; however, not all *oipA* genes are expressed in *H. pylori.* The functional status of the *oipA* gene is regulated through a slipped-strand mispairing mechanism based on the number of CT dinucleotide repeats in the 5′ region (Yamaoka et al., 2000a, 2000b, 2002). This gene is "on" when it is in the reading frame but "off" when out of the frame. Some previous studies showed that the characteristics of *oipA* functional status differ significantly between geographical regions (Ando et al., 2002), (Kim et al., 2021). Although some recent studies found that *oipA* "on" status is associated with an increased risk of peptic ulcers and gastric cancer (Braga et al., 2019; Liu et al., 2013), other studies did not agree (Chiarini et al., 2009), (Farzi et al., 2018).

Some Southeast Asian countries, including Vietnam, have reported high *H. pylori* infection rates (Quach et al., 2018). Most studies on the *oipA* gene in this region have focused on the presence or absence of the gene, and only a small number have mentioned its functional status. The association between *oipA* functional status and gastroduodenal diseases has also been controversial. Therefore, our work aimed to study the functional status of the *oipA* gene and its association with gastroduodenal diseases in *H. pylori* strains from patients in Vietnam.

2. Material and methods

2.1. Ethics statement

The Ethics Committee of the University of Medicine and Pharmacy, Hue University, Vietnam, approved this study under the number H2021/389. Each patient signed a written informed consent form.

2.2. Study design and participants

The study was designed as a cross-sectional investigation and carried out at the Can Tho University of Medicine and Pharmacy Hospital from May 2021 to October 2022. This hospital is the University referral hospital that serves most of the population of the Mekong Delta area of Vietnam and provides technical support for and receives patients transferred from the provincial or district hospitals in this area. Patients with dyspepsia who underwent upper gastrointestinal endoscopy at the Centre of Endoscopy - Interventional Endoscopy, Can Tho University of Medicine and Pharmacy Hospital, during the study period, were assessed for eligibility. The inclusion criteria included patients with dyspepsia who had positive results of both the rapid urease test and culture for *H. pylori* infection and the result reports of endoscopic findings or histopathological abnormalities for gastroduodenal diseases. Patients were excluded if they had a history of gastric surgery, diseases of bleeding and coagulation, consumption of antibiotics or bismuth within 4 weeks or proton pump inhibitors within 2 weeks preceding their endoscopy or they declined to participate. Moreover, we excluded patients who did not have the sequencing results of the *H. pylori oipA* gene.

Upper gastrointestinal endoscopy was performed on each patient to record endoscopic findings, including gastritis, gastric ulcer, duodenal ulcer, or visible neoplastic suspicious lesions, as well as take gastric biopsies. During the gastroscopy, all patients had two biopsy specimens taken from the antrum and corpus for a rapid urease test and culture to determine their *H. pylori* infection status. If both assays were positive, then the patients were diagnosed with an *H. pylori* infection. If patients had visible neoplastic suspicious lesions, additional biopsies were also done in suspicious regions for histopathology. Patients with endoscopic gastritis had two additional biopsy samples taken from the antrum and corpus for histopathology. For histopathological examinations, biopsy specimens were stained with hematoxylin-eosin and evaluated by a pathologist blinded to the molecular characteristics of the studied *H. pylori* isolates. Chronic gastritis, atrophy, or intestinal metaplasia was scored as either absent or present using the updated Sydney System (Dixon et al., 1996). The presence of dysplasia was identified using the 2019 WHO Classification of Tumors (Kushima et al., 2019).

Patients in this study were divided into three groups based on the endoscopic and histopathological findings, including chronic gastritis, peptic ulcers, and precancerous gastric lesions. Chronic gastritis was defined as histological chronic gastritis only, without gastric precancerous lesions or peptic ulcers. Peptic ulcers, including gastric ulcer and/or duodenal ulcer, were diagnosed endoscopically. Gastric precancerous lesions (atrophy, intestinal metaplasia, or dysplasia) were determined via histopathology without peptic ulcers.

2.3. H. pylori culture and genomic DNA extraction

Two biopsy specimens from the antrum and corpus in each patient were transferred into a 0.5 mL transportation medium (20% glycerol, 0.9% NaCl in Milli-Q water) and brought to the laboratory within 4 h. These biopsy specimens were ground in a culture medium (100 μL of brain heart infusion solution supplemented with 10% fetal bovine serum). Then the homogenate was spread on plates of agar containing lysed horse blood and antibiotic mixture, including trimethoprim, vancomycin, amphotericin B, and polymyxin B (Nam Khoa Biotek Co., Ltd., Ho Chi Minh City, Vietnam). These plates were then incubated at 37 °C in a microaerobic environment of 85% N_2, 10% CO_2, and 5% O_2 for 3–10 days. *H. pylori* colonies were confirmed based on the morphology of colonies, gram-negative curved rod-like or seabird-like or spiral bacterium, and the positivity for oxidase, catalase, and urease. *H. pylori* colonies were stored at −80 °C in brain heart infusion media supplemented with bovine serum albumin and glycerol. Genomic DNA was extracted as previously described (Peek et al., 1995).

2.4. Genotyping and sequencing of oipA gene

The 5′ signal region of the *oipA* gene, including the CT dinucleotide repeats, was amplified by polymerase chain reaction (PCR) assay using a primer pair 5′-CAAGCGCTTAACAGATAGGC-3′ (forward) and 5′-GCTTCACGAGAAAACGCCTT-3′ (reverse) described by Yamaoka (Yamaoka et al., 2000a). Briefly, the PCR was performed in a total volume of 50 μL containing 25 μL of OneTaq 2× Master Mix (New England BioLabs, UK), 3 μL of each forward and reverse primer (10 pmol/μL), 150 ng DNA template, and nuclease-free water. The temperature conditions were as follows: an initial denaturation at 95 °C for 5 min followed by 35 cycles of 95 °C for 30 s, 60 °C for 50 s, 68 °C for 1 min, and a final cycle at 68 °C for 10 min. All PCR reactions were conducted with sterile water as a negative control and the DNA of the *oipA*-positive *H. pylori* strain identified previously as a positive control. PCR products were examined by electrophoresis at 80 V for 45 min in 1% agarose added RedView as DNA staining agent. The PCR yielded 400–450 bp fragments. PCR assay was performed in an Applied Biosystems 2720 Thermocycler at the Department of Medical Genetics, Hue University of Medicine and Pharmacy, Hue, Vietnam. A total of 40 μL of each PCR product was subjected to direct sequencing at the 1st BASE Laboratory (Seri Kembangan, Malaysia) using a BigDye Terminator v.3 Kit (Applied Biosystems, Foster City, CA, USA) on an Applied Biosystems 3730 Genetic Analyzer (Thermo Fisher Scientific).

The *oipA* genes of *H. pylori* strains were determined to be in or out of the reading frame on the nucleotide sequences. The *oipA* gene had an "on" status when it was in the reading frame and an "off" status when it was out of the frame. A CT repeat pattern was defined as non-continuous when the CT dinucleotide was separated by the dinucleotide "TT," "GT," or "GC" and not as a continuous one. The starting position of the CT repeat was at position +23 when following the "CTAA" nucleotide sequence or at position +19 when the alanine-alanine (AA) at position

+21, 22 changed to CT (Yamaoka et al., 2002).

2.5. Data analysis

The data were processed using the R Statistical Environment (v4.2.2; R Core Team, 2022). Wilson's score technique was used to estimate the 95% confidence interval (CI) for the prevalence of the *oipA* gene's functional status (Newcombe, 1998). The distribution of a categorical variable in a group was compared with the distribution in another group using the Chi-squared test or Fisher's exact test if the expected values were too low.

Some studies suggested that increasing age and male gender are related to precancerous gastric lesions or peptic ulcers (Nishizawa et al., 2017), (den Hoed et al., 2011), (Wang et al., 1996). Thus, we performed a multivariable logistic regression analysis to identify whether *oipA* "on" status was associated with gastroduodenal diseases after adjusting other confounders such as gender and age. Firth logistic regression was used for rare events (Heinze, 2006; Heinze and Schemper, 2002). A p-value <0.05 was considered statistically significant. Odds ratio (OR) and 95% CI were used to estimate the risk.

3. Results

3.1. General characteristics of the study participants

From May 2021 to October 2022, among 1087 patients being assessed for eligibility, we excluded 870 patients, leaving 217 patients who had *H. pylori* isolates genotyped and sequenced for the *oipA* gene. Moreover, we excluded 44 patients who did not have the sequencing results of the *H. pylori oipA* signal region. Finally, 173 patients were included in this present study to analyze the *H. pylori oipA* gene (Fig. 1).

The study included 173 patients (85 males and 88 females), whose mean age was 42.6, ranging from 16 to 85 years old. None of the patients were diagnosed with gastric carcinoma; and we found 93 patients with precancerous gastric lesions, 42 with peptic ulcers, and 38 with chronic gastritis (Table 1).

3.2. The oipA status

DNA sequencing was performed on 173*H. pylori* isolates. Based on the analysis of CT repeat patterns in the 5′ signal region, the prevalence of the *oipA* "on" status was 96% (166/173) (95% CI: 91.5–98.2) while only 4% were identified as "off."

From analyzing 173 signal sequences of the *oipA* gene, we observed 25 different CT repeat patterns (19 "on" and 6 "off"), ranging from 4 to 10 CT repeats. Among the 19 "on" CT repeat patterns, the "2 + 1 + 1 + 1" and "3 + 1" were predominant, accounting for 31.8% and 26%, respectively (Table 2). Five novel CT repeat patterns of the *oipA* "on" were recorded (Fig. 2).

Majority of the CT repeat patterns of the 173 *oipA* signal sequences began at position +19 (52%) and were non-continuous (88.4%). There was no significant difference in the starting position of the CT repeat of *oipA* "on" status (53% at +19 vs. 47% at +23, $p = 0.262$). The rate of *oipA* "on" with non-continuous CT repeat patterns was 91%,

Table 1
Demographic characteristics of 173 patients with gastroduodenal diseases.

Characteristics	Results
Mean age (years) ± SD	42.6 ± 15.1
Gender, n (%)	
Male	85 (49.1)
Female	88 (50.9)
Gastroduodenal diseases, n (%)	
Chronic gastritis	38 (22.0)
Peptic ulcers	42 (24.3)
Precancerous gastric lesions	93 (53.7)

Notes: SD: standard deviation.

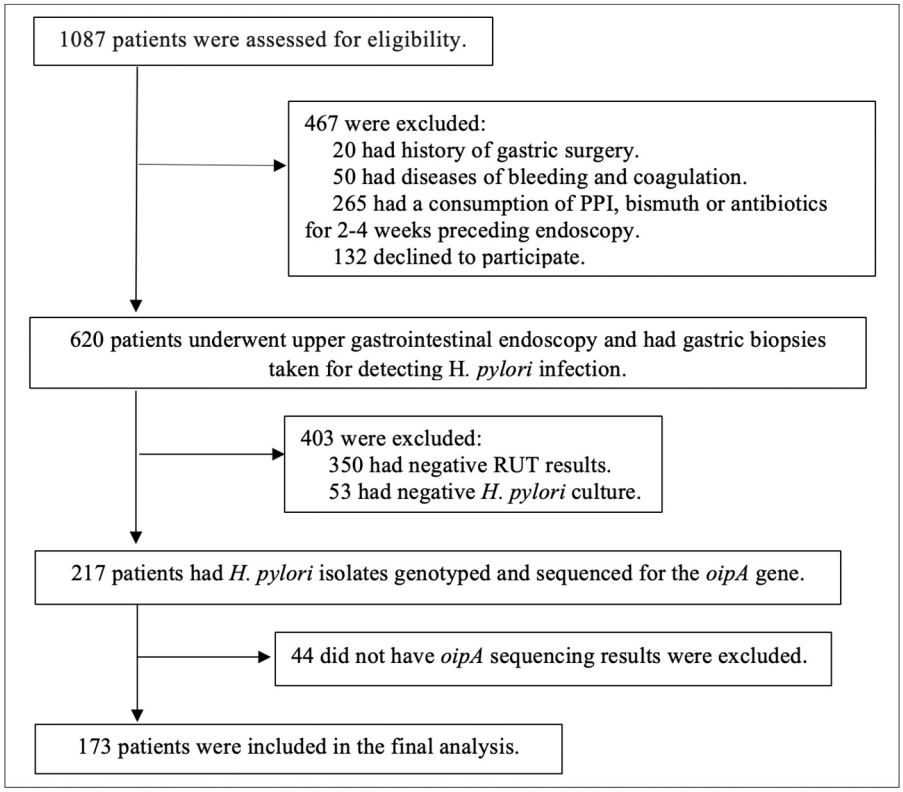

Fig. 1. Flowchart of recruitment.

Table 2
Prevalence of the CT repeat patterns in the 5′ signal region of the *oipA* gene among 173 Vietnamese *H. pylori* strains.

Status	Sequences of the *oipA* signal coding region (5′-3′)	CT repeats pattern	No. of isolates (%)
ON	ATGAAAAAAACCCTTTTA**CTCT**TT**CT**GT**CT**TT**CT**CGTTTTGGCTC… M K K T L L L F L S F S F W L …	2 + 1 + 1 + 1	55 (31.8)
	ATGAAAAAAACCCTTTTACTAA**CTCTCT**TTTT**CT**CGTTTTGGCTC… M K K T L L L T L F F S F W L…	3 + 1	45 (26)
	ATGAAAAAAACCCTTTTA**CTCT**TT**CTCTCT**TT**CT**TGTTTTGGCTC… M K K T L L L F L S F L F W L …	2 + 3 + 1	23 (13.3)
	ATGAAAAAAGCTCTCTTACTAA**CTCTCTCTCTCT**CGTTTTGGCTC… M K K A L L L T L S L S F W L …	6a	7 (4.0)
	ATGAAAAAAACCCTCTTACTAA**CTCTCTCTCTCT**TTTTTTGGCTC… M K K A L L L T L S L F F W L …	6b	3 (1.7)
	ATGAAAAAAGCTCTCTTACTAA**CTCTCT**TT**CTCT**CGTTTTGGCTC… M K K A L L L T L F L S F W L …	3 + 2	7 (4.0)
	ATGAAAAAAGCTCTCTTACTAA**CTCTCT**TT**CTCTCTCT**TTGGCTC… M K K A L L L T L F L S L W L …	3 + 4	3 (1.7)
	ATGAAAAAAGCTCTCTTACTAA**CT**TT**CTCTCTCT**CGTTTTGGCTC… M K K A L L L T F S L S F W L …	1 + 4	3 (1.7)
	ATGAAAAAAACCCTTTTA**CTCT**TT**CT**GT**CTCTCT**CGTTTTGGCTC… M K K T L L L F L S L S F W L …	2 + 1 + 3	2 (1.2)
	ATGAAAAAAGCCCTCTTACTAA**CTCTCTCTCTCTCTCTCT**CGTTTTGGCTC… M K K A L L L T L S L S L S F W L …	9	2 (1.2)
	ATGAAAAAAGCTCTCTTACTAA**CTCT**TT**CTCTCT**CGTTTTGGCTC… M K K A L L L T L S L S F W L …	2 + 3	2 (1.2)
	ATGAAAAAAGCTCTCTTACTAA**CTCTCTCTCT**TTTTTTTTGGCTT… M K K A L L L T L S L F F W L …	5	2 (1.2)
	ATGAAAAAAGCTCTCTTACTAA**CTCTCTCT**TT**CT**TTTTTTGGCTC… M K K A L L L T L S F F F W L …	4 + 1	2 (1.2)
	ATGAAAAAAGCCCTCTTA**CTCTCTCTCT**TT**CTCT**CGTTTTGGCTC… M K K A L L L S L F L S F W L …	5 + 2	1 (0.6)
	Novel "on" status patterns		
	ATGAAAAAAACCCTTTTA**CTCT**TT**CT**GTTTTT**CT**CGTTTTGGCTC… M K K T L L L F L F L S F W L …	2 + 1 + 1	4 (2.3)
	ATGAAAAAAACCCTTTTA**CT**TTTT**CT**GT**CT**TT**CT**CGTTTTGGCTC… M K K T L L L F L S F S F W L…	1 + 1 + 1 + 1	2 (1.2)
	ATGAAAAAAACTCTTTTA**CTCT**TT**CT**GC**CT**TT**CT**CGTTTTGGCTC… M K K T L L L F L P F S F W L …	2 + 1 + 1 + 1	1 (0.6)
	ATGAAAAAAACCCTTTTACTAA**CTCTCT**TT**CT**TTCGTTTTGGCTC… M K K T L L L T L F L S F W L …	3 + 1	1 (0.6)
	ATGAAAAAAGCTCTCTTACTAA**CTCTCTCTCTCTCTCT**TTGGCTC… M K K A L L L T L S L S L W L …	8	1 (0.6)
	ATGAAAAAAGCCCTCTTACTAA**CTCTCTCTCTCTCTCTCT**CGTTTTGGC… M K K A L L L T L S L S L S R F G …	10	1 (0.6)
	ATGAAAAAAGCCCTCTTACTAA**CTCTCTCTCTCT**TTTTTTGGC… M K K T L L L T L S L S F F G …	7	2 (1.2)
	ATGAAAAAAGCCCTCTTACTAA**CTCTCTCTCTCTCTCT**CGTTTTGGCT… M K K A L L L T L S L S L V L A …	8	1 (0.6)
OFF	ATGAAAAAAACCCTTTTACTAA**CTCTCTCTCT**TTTTTTGGCT… M K K T L L L T L S L F L A …	5	1 (0.6)
	ATGAAAAAAGCCCTCTTA**CTCTCTCT**TT**CTCT**CGTTTTGGCT… M K K A L L L S L S L V L A …	4 + 2	1 (0.6)
	ATGAAAAAAGCCCTCTTA**CTCTCTCTCTCT**TT**CTCT**CGTTTTGGC… M K K A L L L S L S F S R F G …	6 + 2	1 (0.6)

Notes: Non-continuous CT repeats patterns separated by the dinucleotide "TT", "GT", or "GC", and thus the CT repeat number was counted partially and linked with "+" sign.

significantly different from the 71.4% rate of *oipA* "off" with continuous CT repeat patterns (Table 3).

3.3. Association between oipA status of H. pylori and gastroduodenal diseases

There was a statistically significant difference in the distribution of *oipA* status, gender, and age between the three groups of gastroduodenal diseases. The prevalence of *oipA*"on" status accounted for 100%, 97.8%, and 86.8% of *H. pylori* isolates from patients with peptic ulcers, precancerous gastric lesions, and chronic gastritis, respectively ($p < 0.01$). The mean age of patients with peptic ulcer diseases was higher than that of patients with chronic gastritis or precancerous gastric lesions ($p < 0.001$). The percentage of male gender in patients with peptic ulcers was higher than that in those with chronic gastritis or precancerous gastric lesions (Table 4).

After we adjusted for gender and age, the multivariable logistic regression analysis identified that the *oipA* "on" status was associated with a 12.79-fold increase in the odds of peptic ulcers (95% CI: 1.19–1760.32, $p = 0.033$) and a 7.39-fold increase in the odds of precancerous gastric lesions (95% CI: 1.35–40.59, $p = 0.021$) when compared to chronic gastritis (Table 5).

4. Discussion

The *oipA* gene is considered an important virulence factor of *H. pylori*. Its functional status is regulated through a slipped-strand mispairing mechanism based on the number of CT dinucleotide repeats in the 5′ region (Yamaoka et al., 2000a, 2000b, 2002). However, the number and patterns of CT repeat in the signal peptide coding region of *oipA* have been shown to differ between *H. pylori* strains from various geographic regions (Ando et al., 2002). The association between *oipA* functional status and gastroduodenal diseases has been controversial as well. In this study on 173 Vietnamese *H. pylori* strains from patients with

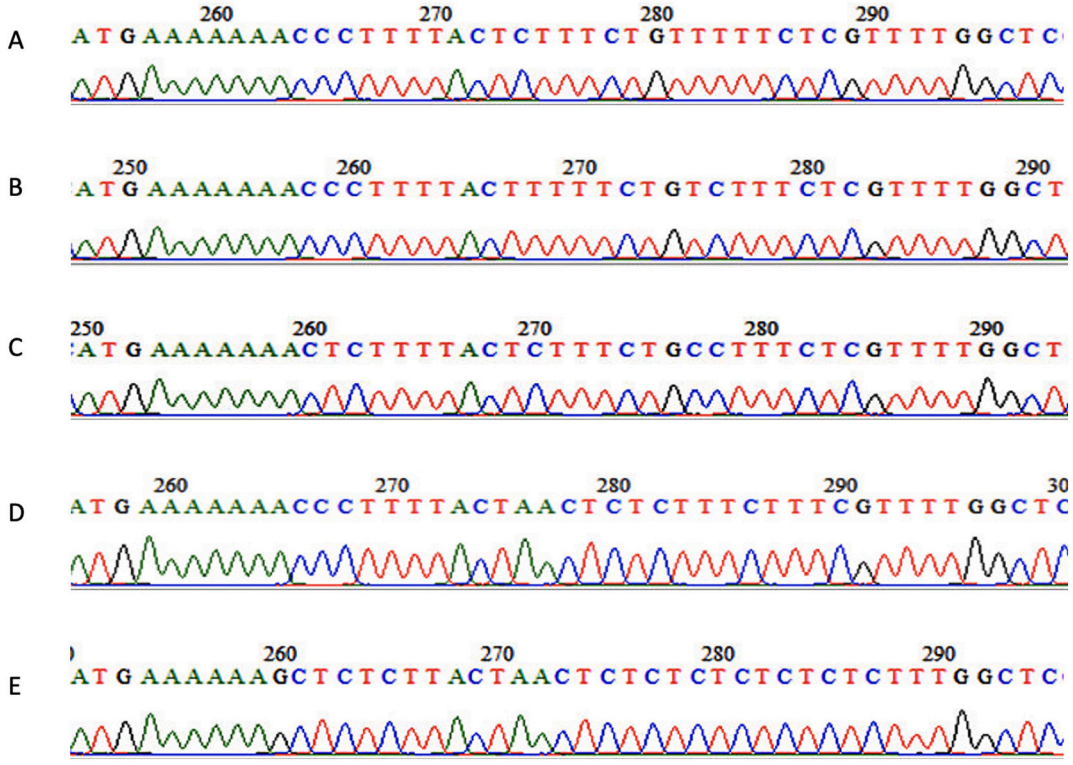

Fig. 2. Sequencing traces of novel CT repeat patterns in the 5′ signal region of the *oipA* gene.
A. "2 + 1 + 1" pattern, B. "1 + 1 + 1 + 1" pattern, C. "2 + 1 + 1 + 1" pattern, D. "3 + 1" pattern, E. "8" pattern.

Table 3
The distribution of the *oipA* status according to the starting position and the continuity of CT repeat pattern.

Characteristics	"on" status (%)	"off" status (%)	Total (%)	*P*-value
The starting position				
+19 position	88 (53.0)	2 (28.6)	90 (52.0)	0.262
+23 position	78 (47.0)	5 (71.4)	83 (48.0)	
The continuity				
Continuous pattern	15 (9.0)	5 (71.4)	20 (11.6)	< 0.001
Non-continuous pattern	151 (91.0)	2 (28.6)	153 (88.4)	
Total	166	7	173	

Table 4
Distribution of *oipA* status, gender, and age in groups of gastroduodenal diseases.

Factors	Gastroduodenal diseases, n (%)			P-value
	CG (n = 38)	PUD (n = 42)	PGL (n = 93)	
oipA status				
"on"	33 (86.8)	42 (100)	91 (97.8)	0.010
"off"	5 (13.2)	0 (0)	2 (2.2)	
Gender				
Male	15 (39.5)	29 (69.0)	41 (44.1)	0.011
Female	23 (60.5)	13 (31.0)	52 (55.9)	
Age				
Mean ± SD	40.4 ± 16.3	50.7 ± 14.2	39.8 ± 13.7	< 0.001

Notes: CG: chronic gastritis, PUD: peptic ulcer disease, PGL: precancerous gastric lesions.

different gastroduodenal diseases, we found that the *oipA* "on" status had a 96% prevalence (166/173). We recorded 25 CT repeat patterns, including 19 "on" and 6 "off," ranging from 4 to 10 CT repeats. The *oipA* "on" status was associated with peptic ulcers and precancerous gastric lesions when compared to chronic gastritis.

Table 5
Association between *H. pylori oipA* status and gastroduodenal diseases: the result of multivariable logistic analysis after age- and gender-adjusted.

Factors	PUD		PGL	
	aOR (95% CI)	*P*-value	aOR (95% CI)	*P*-value
oipA "on" vs. "off"	12.79 (1.2–1760.32)	0.033	7.39 (1.35–40.59)	0.021
Male vs. female	2.90 (1.09–7.92)	0.032	1.30 (0.59–2.90)	0.515
Age (+1 year)	1.03 (0.99–1.06)	0.082	0.99 (0.97–1.02)	0.739

Note: The reference was the chronic gastritis group. aOR: adjusted odds ratio, CI: confidence interval.

The prevalence of the *oipA* "on" status found in our study is compatible with that found in studies from East Asian countries, where >90% prevalence was reported. A study in China demonstrated that the frequency of the *oipA* "on" status was 100% (Zhao et al., 2020). Another study of 109*H. pylori* strains discovered that the *oipA* "on" status was 100% in strains from East Asian countries and India (Ando et al., 2002). A study on 233 Korean *H. pylori* isolates identified that the "on" status was prevalent in 98.7% of *oipA1* and 99.1% of *oipA2* (Kim et al., 2021). However, several studies on *H. pylori* strains from Western populations showed a lower frequency of the *oipA* "on" status compared to that in our study, such as Germany (59%) (Dossumbekova et al., 2006), Bulgaria (81%) (Markovska et al., 2011), Brazil (81.1%) (Braga et al., 2019), and Venezuela (83%) (Torres et al., 2014). The discrepancy could be due to the differences in virulence genotypes of *H. pylori* based on the geographic regions (Yamaoka et al., 2000b), (Yamaoka, 2010). Moreover, the high prevalence of *oipA* "on" among *H. pylori* strains in our study may contribute to the theory that *H. pylori* strains from East Asian countries are more virulent than those from Western ones.

We discovered 19 CT repeat patterns among 166*H. pylori* strains with the *oipA* "on" status. Some studies on East Asian *H. pylori* strains showed

the number of CT repeat patterns of *oipA* "on" was 7 (in 237 *oipA* "on" strains) (Xue et al., 2021), 12 (in 177 *oipA* "on" strains) (Zhao et al., 2020), or 13 (in 230 *oipA1* "on" strains) (Kim et al., 2021). Several studies on Western *H. pylori* strains showed the number of CT repeat patterns of *oipA* "on" was 5 (in 70 *oipA* "on" strains) (Markovska et al., 2011), 5 (in 88 *oipA* "on" strains) (Torres et al., 2014), or 3 (in 77 *oipA* "on" strains) (Braga et al., 2019). From those results, we realize that the number of *oipA* "on" CT repeat patterns in East Asian studies is higher than those in Western studies. Our data were even greater than those of some East Asian countries. Since *H. pylori* is a highly genetically diverse bacterium, this disparity may be due to the differences in the geographical genetic characteristics of *H. pylori*.

In this study, the two predominant CT repeat patterns of *oipA* "on" were "2 + 1 + 1 + 1" and "3 + 1". Our result is quite similar to other studies from East Asian countries (Ando et al., 2002; Kim et al., 2021; Xue et al., 2021; Zhao et al., 2020) but different from those from Western countries where the "6" CT repeat pattern is the most common (Braga et al., 2019; Dossumbekova et al., 2006; Markovska et al., 2011; Torres et al., 2014). Interestingly, we found "2 + 3 + 1" as the third prevalent motif; it was reported to appear in two *H. pylori* strains from Chinese patients and one strain from a Columbian patient (Yamaoka et al., 2002; Zhao et al., 2020). Moreover, some patterns that only appeared in strains from Western countries, such as "3 + 4," "5 + 2," "1 + 4," and "9" CT (Dossumbekova et al., 2006; Markovska et al., 2011; Yamaoka et al., 2002), were observed in our current study. Five new "on" CT repeat patterns that were not reported in previous reference studies were also detected. The CT repeat patterns of the *oipA* "on" status of *H. pylori* strains in our study are significantly different from those in Western countries and slightly distinct from those in other East Asian countries. Previous research found differences in CT repeat patterns in the signal peptide coding region of *oipA* between strains from different geographical regions (Ando et al., 2002; Kim et al., 2021; Yamaoka et al., 2002). Accordingly, the difference in CT repeat pattern between *H. pylori* strains in our study and those in Western countries is so apparent. Two possible explanations exist for why our *H. pylori* strains differ slightly from other East Asian strains. The first possible reason is that foreign tourists from abroad may import other regional *H. pylori* strains into Vietnam. The second reason may be the evolution of bacteria to adapt to environmental conditions. Interestingly, we likewise noted that most of the *oipA* "on" status of *H. pylori* have non-continuous CT repeat patterns. This is comparable to the finding of Yamaoka, who suggested that non-continuous CT repeat patterns may result from a purposeful change in status during the evolution of the bacteria to prevent the switch from being readily turned "off" (Yamaoka, 2012).

Our data identified that *oipA* status was associated with peptic ulcers or gastric precancerous lesions versus chronic gastritis in a multivariable logistic regression after adjusting for gender and age. Our results are in agreement with some studies. For instance, Liu et al. showed that *oipA* "on" is associated with an increased risk of peptic ulcers and gastric cancer versus gastritis (Liu et al., 2013). Braga et al. also mentioned the association between *oipA* "on" status and gastric cancer versus gastritis (Braga et al., 2019). Yamaoka et al. looked at *oipA* "on" status as an independent predictor of duodenal ulcer versus gastritis (Yamaoka et al., 2002). The association between *oipA* "on" status and peptic ulcers and gastric cancer may be explained by the ability of *OipA* to induce IL-8 production from gastric mucosa. IL-8 is a proinflammatory factor secreted by epithelial gastric cells that function as an activating and chemotactic peptide for neutrophils (Remick, 2005; Yamaoka et al., 2000a). The expression of IL-8 is significantly associated with tumorigenicity and the metastasis of tumors (Waugh and Wilson, 2008). Yamaoka et al. showed that the *oipA* "on" is associated with increased IL-8 secretion by a gastric cancer cell line (Yamaoka et al., 2000a) and with high levels of IL-8 in the antral mucosa (Yamaoka et al., 2002). Furthermore, Graham et al. demonstrated the role of *oipA* "on" in inducing increased IL-8 secretion in subjects carrying *H. pylori* strains that lack *cagA* (Graham et al., 2004). Yamaoka et al. further revealed that *OipA* is essential for activating the IL-8 promoter (Yamaoka et al., 2004). Gastric precancerous lesions, such as atrophy, intestinal metaplasia, or dysplasia, are involved in the carcinogenesis process and have been linked to an increased risk of gastric cancer (Correa and Blanca Piazuelo, 2012), (Banks et al., 2019). Therefore, the association of *oipA* "on" status with peptic ulcers and gastric precancerous lesions versus chronic gastritis may be explained by its role in inducing IL-8 production.

Our data from 173*H. pylori* strains from Vietnamese patients with gastroduodenal disease showed a high prevalence of *oipA* "on" status and genetic diversity in the number and patterns of CT repeats in the signal peptide coding region of the *oipA* gene. We also recognized an association between *oipA* "on" status and precancerous gastric lesions or peptic ulcers versus chronic gastritis. *H. pylori* infection is a global health problem. In Southeast Asia, the prevalence of *H. pylori* infection is high, but studies on the functional status of *H. pylori oipA* have yet to be conducted. As a result, our findings may support the knowledge of this virulence gene of *H. pylori* in this geographic region.

Our findings should be interpreted within the context of potential strengths and weaknesses. This study was well designed, with patients and bacterial strains collected through a standardized sampling scheme. The patients were evaluated for gastroduodenal lesions both endoscopically and histologically. *H. pylori* strains were cultured, genotyped, and sequenced for the *oipA* gene according to standardized procedures. Despite the fact that our study did not include *H. pylori*-positive patients with normal gastric mucosa or gastric cancer, we did subgroup patients with single chronic inflammation of the gastric mucosa or precancerous gastric lesions to evaluate the relationship more objectively between *oipA* status and gastroduodenal diseases. To the best of our knowledge, this is the first study on the *oipA* functional status of *H. pylori* isolates from Vietnam. Further large-scale studies on the functional status of the *oipA* gene in Vietnam are needed to confirm our findings.

5. Conclusions

In summary, our data identified the high prevalence of *oipA* "on" status among Vietnamese *H. pylori* strains. A genetic diversity was observed in the number and pattern of CT repeats in the signal peptide coding region of the *oipA gene*. The *oipA* "on" status was associated with precancerous gastric lesions and peptic ulcers when compared to chronic gastritis.

Author contributions

Thi Hong Nhung Thai performed gastroduodenal endoscopy, obtained gastric biopsy specimens, wrote original draft. Hong Phong Nguyen performed histopathological examination, Thi Hai Yen Nguyen and Thi Be Hai Nguyen conducted *H. pylori* culture, Thi Mai Ngan Nguyen carried out molecular biology techniques. Thai Hoa Nguyen: collected data and performed formal analyses. Thi Minh Thi Ha: designed this research, evaluated Sanger sequencing data, revised the draft, and supervised the study.

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Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

I have shared my data at the Attach file step

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2023.105438.

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