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Helicobacter pylori vacA affects the expression of COX-2 in the duodenal mucosa of patients with duodenitis

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Summary. Duodenitis refers to inflammation that occurs in the duodenum. *Helicobacter pylori* (Hp) is a known risk factor for duodenitis. This paper attempted to analyze the correlation between Hp virulence genotypes and the initiation and development of duodenal bulbar inflammation (DBI) to lay the foundation for the management of duodenitis induced by Hp infection. Total RNA was extracted from duodenal samples of 156 Hp-positive patients [70 with DBI and 86 with duodenal bulbar ulcer (DBU)] and 80 Hp-free DBI patients, followed by RT-qPCR detection of COX-2 mRNA expression and the presence of virulence factors. The cagA positive (62.2%), vacAs1 (21.79%), vacAm2 (23.72%), vacAs1m2 (19.87%) and iceA1 (55.80%) genotypes were dominant in 156 Hp-positive samples. Statistical difference was observed in vacAs and vacA mixtures between DBI and DBU patients. Gastric metaplasia had an association with vacA allelotypes, and its occurrence had strong correlations with vacAs1 and vacAs1m2 genotypes. The vacAs1 and vacAs1m2 genotypes were correlated with gastric metaplasia occurrence (all $p<0.05$). There were significant correlations between vacAs and vacA mixtures with cagA genotypes, and between iceA genotypes with vacA mixtures (all $p<0.05$). COX-2 was strongly expressed in Hp-infected duodenal mucosa and showed correlations with vacA genotype. COX-2 was differentially expressed in vacAs1- and vacAs2-positive patients. COX-2 was more highly upregulated in vacAs1m1- and vacAs1m2-positive patients than vacAs2m2-positive patients. Overall, Hp virulence genotype vacA was correlated with DBI and DBU initiation and development.

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Introduction

Duodenitis refers to inflammation in the duodenum and is classified as a digestive tract disease with high incidences in clinics (Terra et al., 2021). In 1977, Thomson et al. (1977) pointed out that duodenitis belongs to a unique entity possibly representing the precursor lesion of duodenal ulcer or a stage of duodenal ulcer recovery. With the increased knowledge due to the gastroscope introduction, clinicians have identified the duodenal bulb as the area most susceptible to inflammation according to the research data and endoscopy statistics (Paoluzi et al., 1982). Duodenitis is considered a threat to multiple strictures of the duodenum (Somani et al., 2017). Thereby, active diagnosis and treatment of duodenitis are of utmost importance for human health.

Duodenitis can be attributed to pathogen infection, abdominal disease, drug use, and dietary and living habits (Owen and Owen, 2018). *Helicobacter pylori* (Hp) is a spiral or curve-shaped and microaerophilic Gram-negative bacterium with flagella, which was successfully isolated from gastric mucosa biopsy tissues of patients with chronic active gastritis and cultured for the first time by Marshall and Warren in 1983 (Marshall and Warren, 1984). As one of the most common infections, Hp infection is closely tied up with the initiation of chronic gastritis, peptic ulcer, mucosa-associated lymphoid tissue lymphoma, and gastric cancer (Kong et al., 2020; Teng et al., 2020; Ford et al., 2022). To date, the identified Hp virulence genes include cytotoxin-associated gene A (cagA), vacuolating toxin gene A (vacA), and induced by contact with epithelium A (iceA), among which cagA is the major virulence genotype of cell canceration and accounts for 60% of Hp infection (El Khadir et al., 2020). Different from cagA, vacA is a type of secreted protein encoded by the vacA

gene and released by the type II secretory system and maintains Hp colonization in gastric mucosa, and diverse genotypes including s1m1, s1m2, s2m2, and s2m1 can be formed via a combination of different alleles (Erzin et al., 2006). And s1m2 is the closest genotype in association with gastric cancer (Homan et al., 2009). The last Hp virulence gene iceA consists of two alleles, iceA1, and iceA2, and researchers have found an association between the former and a higher risk of gastrointestinal disorders (Farsimadan et al., 2021). There is evidence for increased levels of IL-8, IL-1β, and cyclooxygenase 2 (COX-2) in gastric mucosa of patients with chronic gastritis and their correlation with cagA and vacAs1m1 infection (Bartchewsky et al., 2009). Han et al. (2016) revealed that COX-2 expression is considerably augmented in Hp-positive gastritis tissues relative to that in Hp-negative gastritis tissues. Despite the knowledge that there exists a vicious circle among Hp infection, gastric metaplasia, and duodenal mucosal inflammation (Ciancio et al., 2002, Ford and Talley, 2009), the specific mechanism remains unknown. The research regarding the association between Hp virulence genotypes and the occurrence and development of duodenal bulbar inflammation (DBI) is scarce and thus requires further explanation. This study attempted to analyze the correlation between Hp virulence genotypes and DBI initiation and development by collecting duodenal bulbar mucosal tissues of Hp-positive patients and patients without Hp infection and detecting the expression of Hp virulence genes in different groups, with the expectation to offer new rationale for the management of Hp-infected duodenitis.

Materials and methods

Study subjects

This study enrolled 156 Hp-positive patients diagnosed and treated in Shanghai Fifth People's Hospital, Fudan University from January 2021 to February 2022, among whom 70 cases were diagnosed with DBI and 86 cases with duodenal bulbar ulcer (DBU). At the same time, 80 DBI patients free from Hp infection were registered as controls.

Inclusion criteria (Tytgat, 1991): 1) patients with symptoms of abdominal pain and distension, acid reflux, and loss of appetite; 2) patients with bulbar mucosal hyperemia, edema, erosion, exudation with inflammatory secretion, hemorrhage, vessel exposure, mucosal roughness, thick and granular mucosal folds with polypoid change according to endoscopy; 3) patients with astrophy and degeneration of villous epithelium, a large number of inflammatory cells infiltrated in the lamina propria, and gastric metaplasia according to mucosal biopsy; 4) patients with Hp-positive result confirmed by at least one among the ^{14}C breath test, rapid urease test, and pathological Giemsa staining.

Exclusion criteria: 1) patients with a history of drug use such as oral proton pump inhibitor, bismuth, and antibiotics that affect Hp detection in recent 3 months; 2) patients with a history of upper gastrointestinal operation such as subtotal gastrectomy and esophageal surgery; 3) patients complicated with other diseases such as peptic ulcer, acute cholecystitis, and reflux esophagitis; 4) patients featured by esophageal stenosis and gastric outlet stenosis that affect endoscopy and observation; 5) patients aged more than 80 years or less than 14 years; 6) patients complicated with organ dysfunction such as renal and liver dysfunction; 7) patients complicated with tumor; 8) patients complicated with psychiatric disorders and incapable of coordination.

Endoscopy and sample collection

Routine examination was conducted using OLYMPUS-GIF-H260 PENTAX-EG-2990i gastroscope. Two to three pieces of bulbar tissues were subjected to biopsy. Rapid urease test was carried out on the spot. One of the specimens was sent to the pathology department for pathological diagnosis, and the rest was collected in the cryopreservation tubes containing brain heart infusion and preserved at -80°C.

Histopathology

Firstly, the biopsy specimens were fixed with 10% formaldehyde, embedded in paraffin, and serially sliced at 4 μm, followed by routine hematoxylin & eosin staining. Secondly, Hp infection was detected by modified Giemsa staining. Finally, gastric metaplasia was monitored by Alcian blue/Periodic acid-Schiff (AB/PAS) staining of the duodenal mucosa. Endoscopy was operated by researchers with senior professional titles, over 10 years of work experience, and more than 4000 gastroscopy operations. After routine fixation and staining, histopathological results were read separately by two pathologists with over 10 years of work experience.

Bacterial culture

The powders of brain heart infusion solid medium were dissolved in sterile purified water and sterilized for 15 min in an autoclave under high pressure and high temperature. Following 2h water bath at 60°C, the sterilized medium was added with 7% defibrillated sheep blood and Hp selective antibiotic additive and placed in a sterile culture dish (15-20 mL per dish) and stored at 4°C after cooling. The mucosal biopsy specimens were melted on ice and evenly applied to the dish using a culture ring. The culture plate was placed in anaerobic jars with the addition of a microaerobic bag inside of a constant temperature (37°C) incubator for 5-7 days. After that, the culture dish was taken out to observe the colony morphology under the light. Some colonies on the surface layer of the medium were scraped off using an inoculation loop and put in a urease reagent for 1 min to turn the reagent red. A few colonies

were spread in normal saline and applied to the slide to observe bacterial morphology under a microscope and confirm the bacterium as Hp. The bacteria were harvested and conserved in cryopreservation tubes at -80°C after passage.

PCR amplification

The biospin tissue genomic DNA extraction kit (BioFlux, Tokyo, Japan) was utilized to extract DNA. Polymerase chain reaction (PCR) was conducted by performing 35 cycles of 30s denaturation at 94°C, 30s annealing at 56°C, 30s extension at 72°C, and one final extension for 5 min at 72°C to detect vacA, cagA, iceA1, and iceA2. The sequences of primers used are presented in Table 1.

RT-qPCR

Total RNA was extracted from the duodenal mucosa utilizing the TRIzol kit (Thermo Fisher Scientific, Rockford, IL, USA) as per the manufacturer's protocols, and reversely transcribed into cDNA as instructed by the reverse transcriptase assay kit (Takara, Dalian, China). Reverse transcription-quantitative PCR (RT-qPCR) was carried out employing SYBR Green real-time PCR Master Mix (Takara) and miScript SYBR Green PCR kit (Qiagen, Hilden, Germany) on ABI 7500 rapid real-time PCR system (ABI, Foster City, CA, USA) under the following conditions: 94°C for 30s and 40 cycles of 60°C for 30s and 72°C for 30s. The relative expression of COX-2 after normalization with the internal reference GAPDH was calculated using the $2^{-\Delta\Delta Ct}$ method. Primer sequences are listed in Table 2.

Statistical analysis

Statistical data analysis and plotting were implemented by adopting SPSS 21.0 statistical software (IBM Corp., Armonk, NY, USA) and GraphPad Prism 8.01 software (GraphPad Software Inc., San Diego, CA, USA). The data were depicted in the form of mean ± standard deviation. Pairwise comparison was processed utilizing the t test, and multi-group comparison was processed utilizing one-way ANOVA with Tukey's multiple comparisons test as post hoc test. Categorical data (between virulence factors) were described as frequencies and compared through Chi-square or Fisher exact test. Statistical significance was defined as $p<0.05$.

Results

Clinical baseline characteristics of the population enrolled

The demographic characteristics and clinical complications of Hp-positive and Hp-negative patients are presented in Table 3. There were 70 cases of Hp-positive DBU cases with a male-to-female ratio of 39:31 and an average age of 49.3±9.5, whereas the Hp-positive DBU group consisted of 86 patients with a male-to-female ratio of 49:37 and an average age at 52.6±10.3.

Table 1. Primer sequences in PCR amplification.

Gene	Primer	Primersequence(5′–3′)	PCR product(bp)	References
cagA	Forward	GATAACAGGCAAGCTTTTGAGG	349	Van Doom et al., 1998
	Reverse	CTGCAAAAGATTGTTTGGCAGA		
vacAs1/s2	Forward	ATGGAAATACAACAAACACAC	259 (s1)	Carlosama-Rosero et al., 2019
	Reverse	CTGCTTGAATGCGCCAAAC	286 (s2)	
vacAm1/m2	Forward	CAATCTGTCCAATCAAGCGAG	570 (m1)	Carlosama-Rosero et al., 2019
	Reverse	GCGTCTAAATAATTCCAAGG	645 (m2)	
iceA1	Forward	GTGTTTTTAACCAAAGTATC	247	van Doom et al., 1998
	Reverse	CTATAGCCASTYTCTTTGCA		
iceA2	Forward	GTTGGGTATATCACAATTTAT	229 or 334	van Doom et al., 1998
	Reverse	TTRCCCTATTTTCTAGTAGGT		

PCR, polymerase chain reaction; cagA, cytotoxin-associated gene A; vacA, vacuolating toxin gene A; iceA, induced by contact with epithelium A.

Table 2. Primer sequences in RT-qPCR.

Gene	Forward 5'-3'	Reverse 5'-3'
COX-2	TGGCTACAAAAGCTGGGAAG	GGGGATCAGGGATGAACTTT
GAPDH	GAGTCAACGGATTTGGTCGT	ATCCACAGTCTTCTGGGTG

RT-qPCR, reverse transcription-quantitative polymerase chain reaction; COX-2, cyclooxygenase 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Additionally, no remarkable difference was observed in gender, age, smoking, drinking, abdominal pain, and anorexia among groups.

Distribution of cagA, vacA, and iceA allelotypes

To figure out the virulence genotypes that trigger DBI and DBU, we analyzed the distribution of Hp cagA, vacA, and iceA allelotypes. As shown in Table 4, cagA positive (62.20%), vacAs1 (21.79%), vacAm2 (23.72%), vacAs1m2 (19.87%) and iceA1 (55.80%) genotypes were dominant in Hp-positive samples. The difference in vacAs and vacA mixtures between DBI and DBU patients meant statistical significance ($p<0.05$). However, there was no statistically significant correlation observed in cagA, vacAm and iceA between DBI and DBU patients ($p>0.05$).

Table 3. Clinical baseline characteristics of the population enrolled.

Parameters	Control	DBI	DBU
Number of cases	80	70	86
Gender (male/female)	36/44	39/31	49/37
Age (years)	51.5±12.4	49.3±9.5	52.6±10.3
Cases of smoking (%)	35 (43.8)	27 (38.6)	32 (37.2)
Cases of drinking (%)	27 (33.8)	28 (40.0)	24 (28.0)
Cases of abdominal pain (%)	42 (52.5)	36 (51.4)	49 (57.0)
Cases of anorexia (%)	8 (10.0)	6 (8.6)	9 (10.5)

DBI, duodenal bulbar inflammation; DBU, duodenal bulbar ulcer.

Correlation of vacA allelotypes with gastric metaplasia

Hp is most commonly found in the gastric metaplasia area (Wyatt et al., 1987). Since we have discovered the different distribution of vacA allelotypes in DBI and DBU patients, we subsequently analyzed the correlation between the occurrence of gastric metaplasia and vacA allelotypes in Hp-positive DBI and Hp-positive DBU patients. As presented in Table 5, there were 17 cases of gastric metaplasia (24.30%) in the biopsy specimens of 70 cases of Hp-positive DBI and 48 cases of gastric metaplasia (55.80%) in 86 cases of Hp-positive DBU. Moreover, there was a correlation of vacAs1 and vacAs1m2 genotypes with gastric metaplasia initiation (all $p<0.05$).

Correlations of vacA allelotypes with cagA, iceA1 and iceA2 genotypes

The correlations of vacA allelotypes and cagA,

Table 4. Distribution of cagA, vacA, and iceA allelotypes.

Genotypes	DBI (n=70)	DBU (n=86)	Total (n=156)	*p* value
cagA Positive	38 (54.30%)	59 (68.60%)	97 (62.20%)	*p*=0.0709
cagA Negative	32 (45.70%)	27 (31.40%)	59 (37.80%)	
vacAs1	10 (14.29%)	24 (27.91%)	34 (21.79%)	*p*=0.0491
vacAs2	9 (12.86%)	5 (5.81%)	14 (8.97%)	
vacAm1	8 (11.43%)	10 (11.63%)	18 (11.54%)	*p*=0.7753
vacAm2	19 (27.14%)	18 (20.93%)	37 (23.72%)	
vacA s1m1	13 (18.57%)	4 (4.65%)	17 (10.90%)	*p*=0.0027
vacA s1m2	8 (11.43%)	23 (26.74%)	31 (19.87%)	
vacA s2m2	3 (4.29%)	2 (2.33%)	5 (3.21%)	
iceA1	39 (55.70%)	48 (55.80%)	87 (55.80%)	*p*>0.9999
iceA2	31 (44.30%)	38 (44.20%)	69 (44.20%)	

DBI, duodenal bulbar inflammation; DBU, duodenal bulbar ulcer; cagA, cytotoxin-associated gene A; vacA, vacuolating toxin gene A; iceA, induced by contact with epithelium A.

Table 5. Correlation between vacA and gastric metaplasia.

Genotype	DBI (n=70)		*p* value	DBU (n=86)		*p* value
	No gastric metaplasia	Gastric metaplasia		No gastric metaplasia	Gastric metaplasia	
vacAs1	8	7	0.0226	5	15	0.0486
vacAs2	10	3	0.9103	8	4	0.0909
vacAm1	8	0	0.0887	7	4	0.1642
vacAm2	9	0	0.0687	6	4	0.2841
vacAs1m1	8	1	0.3235	6	3	0.1512
vacAs1m2	6	6	0.0225	3	13	0.0232
vacAs2m2	4	0	0.2434	3	5	0.6893

DBI, duodenal bulbar inflammation; DBU, duodenal bulbar ulcer; vacA, vacuolating toxin gene A.

iceA1 and iceA2 genotypes were further analyzed. As described in Table 6, in 97 cagA-positive Hp samples, 27 samples showed correlation with vacAs1, 21 samples with vacAm2, and 25 samples with vacAs1m2; in 87 iceA1-positive Hp samples, 21 samples showed association with vacAs1, 26 samples with vacAm2, and 9 samples with vacAs1m2; in 69 iceA2-positive Hp samples, 13 samples were correlated with vacAs1, 11 samples with vacAm2, and 22 samples with vacAs1m2. Moreover, vacAs and vacA genotype mixtures were significantly correlated with cagA genotype ($p<0.05$), and iceA1 genotype was considerably correlated with vacA genotype mixtures ($p<0.05$).

COX-2 was highly expressed in Hp-infected duodenal mucosa

COX-2 is known to be upregulated after Hp infection (Han et al., 2016). We herein verified the expression pattern of COX-2 in Hp-infected duodenal mucosa through RT-qPCR, which manifested the presence of COX-2 expression in biopsy specimens of both Hp-positive and Hp-negative patients, and a higher expression of COX-2 in Hp-positive patients than that in Hp-negative patients (Fig. 1, $p<0.01$).

Effect of allelotypes on COX-2 expression in Hp-infected duodenal mucosa

We subsequently measured COX-2 expression in patients infected by cagA, vacA and iceA allelotypes using RT-qPCR to further understand the action of allelotype on COX-2 expression in Hp-infected duodenal mucosa, and noticed the correlation between mucosa COX-2 expression and vacA genotypes in Hp-infected patients, evidenced by higher COX-2 expression in vacAs1-positive patients than that in vacAs2-positive patients (Fig. 2A, $p<0.01$). COX-2 expression showed no distinct difference between vacAm1-positive patients and vacAm2-positive patients (Fig. 2B, $p>0.05$). COX-2 expression was prominently increased in mucosa of vacAs1m1- and bacAs1m2-positive patients in comparison to that of vacAs2m2-positive patients (Fig. 2C, $p<0.01$). However, there was no obvious difference in COX-2 expression between cagA-positive and cagA-negative patients (Fig. 2D, $p>0.05$) and between iceA1-positive and iceA2-positive patients (Fig. 2E, $p>0.05$). Based on these results, we concluded that COX-2 expression in Hp-infected duodenal mucosa was correlated with vacA allelotypes.

Discussion

Peptic duodenitis could be traced back to excessive gastric acid production owing to gastric Hp infection (Owen and Owen, 2018). Although peptic duodenitis is not fatal, it happens periodically and repeatedly and impairs the quality of life in a significant way (Graham, 2014). The virulence factors produced by Hp isolates are implicated in the progression of diseases associated with

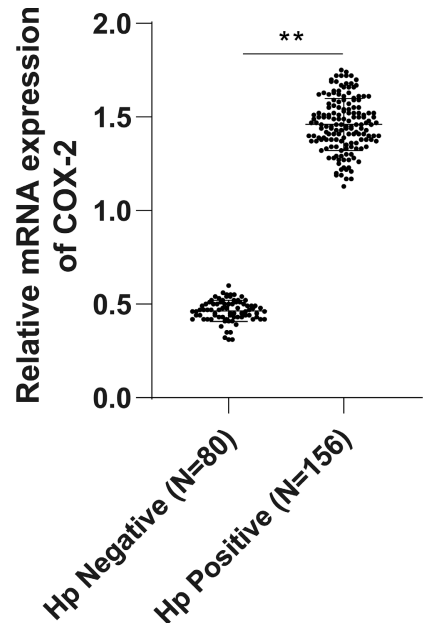

Fig. 1. COX-2 was highly expressed in Hp-infected duodenal mucosa. COX-2 expression in Hp-positive and HP-negative patients was measured by RT-qPCR. The data were depicted in the form of mean ± standard deviation. Pairwise comparison was processed utilizing the independent sample t test. **$p<0.01$.

Table 6. Correlations of vacA allelotypes with cagA, iceA1, and iceA2 genotypes.

vacA genotype	cagA Positive	cagA Negative	*p* value	iceA1 Positive	iceA2 Positive	*p* value
vacAs1	27	7	0.0192	21	13	0.5282
vacAs2	6	8		7	7	
vacAm1	8	10	0.5656	10	8	0.3677
vacAm2	21	16		26	11	
vacAs1m1	8	9	0.0274	11	6	0.0427
vacAs1m2	25	6		9	22	
vacAs2m2	2	3		3	2	

cagA, cytotoxin-associated gene A; vacA, vacuolating toxin gene A; iceA, induced by contact with epithelium A.

Hp infection (Roszczenko-Jasinska et al., 2020). This study therefore discussed the correlation between Hp virulence genotypes cagA, vacA, and iceA and the onset and progression of DBI and DBU.

A former study has found 80% cagA-positive cases in duodenal ulcers (Akeel et al., 2019). One of the allelic variants of iceA, iceA1 shows a connection with peptic ulcer disease (Miehlke et al., 2001). Moreover, vacA genotype is well associated with the risk of duodenal ulcer progression (Zhang et al., 2014). Therefore, we enrolled 70 Hp-positive DBI patients and 86 Hp-positive DBU patients with 80 Hp-negative patients as controls and analyzed the distribution of iceA, vacA, and cagA allelotypes in the clinical samples. Intriguingly, cagA positive, vacAs1, vacAm2, vacAs1m2 and iceA1 dominated the Hp-positive samples. The vacA virulence gene plays a critical role in the progression of gastroduodenal diseases (Junaid et al., 2016). It's widely known that some Hp strains produce an extracellular cytotoxin, which leads to vacuolation in various mammalian cells (Phadnis et al., 1994). Among the bacterial virulence factors, vacA acts as an important determinant of pathogenicity, and vacA allelotypes play different roles in Hp pathogenicity, with the most important biological effects of vacA activity on host cells being the formation of membrane pores and the induction of vacuole formation (Junaid et al., 2016). Early on, researchers reported that the strains containing the s2 sequence of vacA are unable to produce detectable vacuolar cytotoxic activity *in vitro*, which can only be

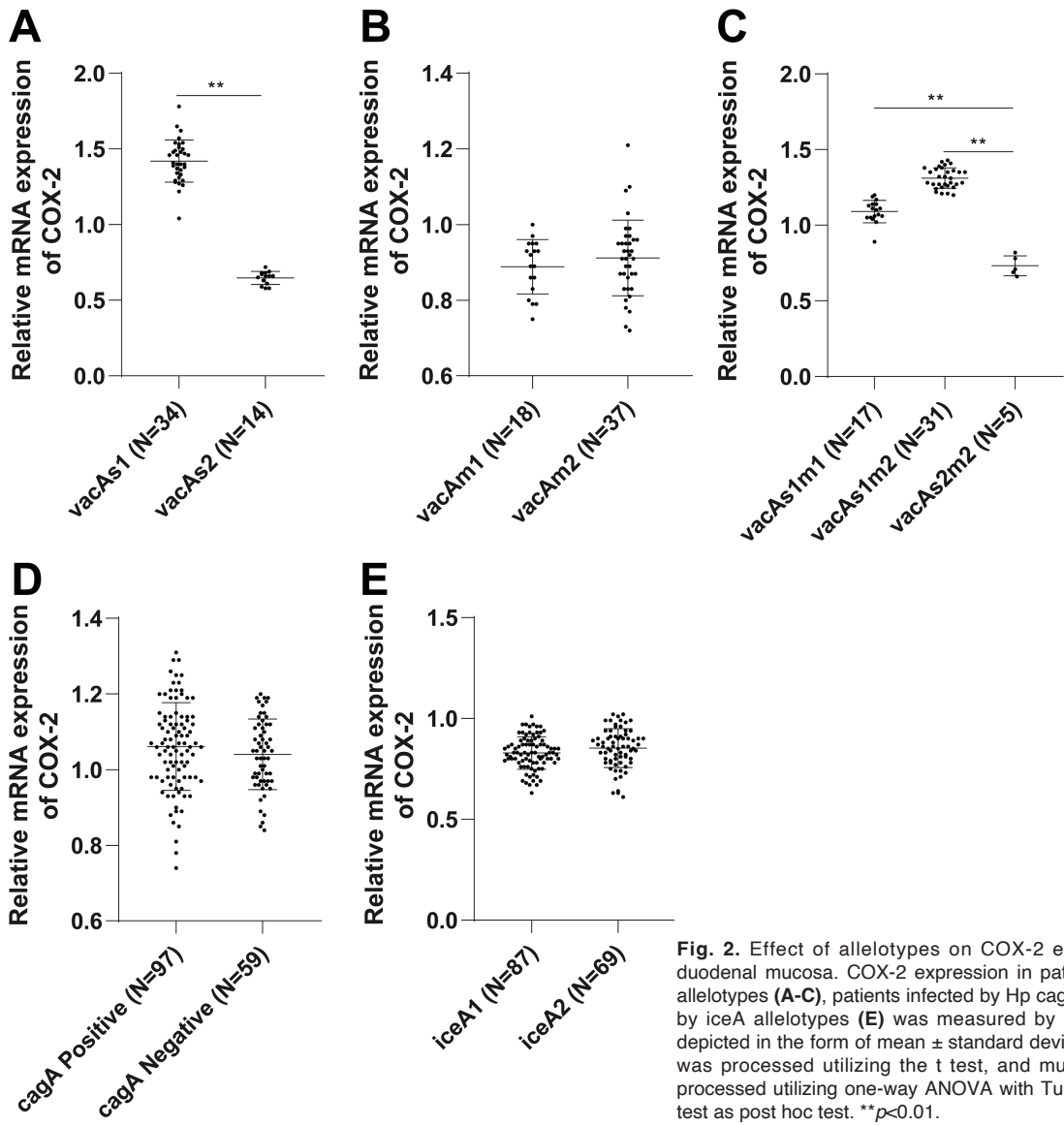

Fig. 2. Effect of allelotypes on COX-2 expression in Hp-infected duodenal mucosa. COX-2 expression in patients infected by Hp vacA allelotypes **(A-C)**, patients infected by Hp cagA **(D)** and patients infected by iceA allelotypes **(E)** was measured by RT-qPCR. The data were depicted in the form of mean ± standard deviation. Pairwise comparison was processed utilizing the t test, and multi-group comparison was processed utilizing one-way ANOVA with Tukey's multiple comparisons test as post hoc test. $^{**}p<0.01$.

produced by the vacAs1 strain (Atherton et al., 1995). Hp strains possessing vacAs1 genotype are associated with more severe gastritis (Molaei et al., 2010). Previous evidence shows that vacAm2 genotype is correlated with peptic ulcer and exists universally in people with high incidence of peptic ulcer and gastric cancer (Pagliaccia et al., 1998). The vacAs1m2 genotype has been found to be the major subtype in chronic gastritis and duodenal ulcer (Caner et al., 2007). There is a close association between intestinal metaplasia and vacAs1/m2 genotype (Ghalehnoei et al., 2016). Our results further revealed the dominance of vacAs1, vacAm2 and vacAs1m2 in Hp-positive samples and the correlation of vacAs1 and vacAs2m2 with gastric metaplasia. Additionally, vacAs exhibited statistical significance in DBI and DBU patients. Furthermore, we noticed significant correlations of vacAs and vacA genotype mixtures with cagA genotypes and the remarkable association between iceA1 genotypes and vacA genotype mixtures.

Emerging evidence suggests that COX-2 expression is elevated in Hp-evoked inflammation (He et al., 2021). We hence assessed the expression of COX-2 in Hp-infected duodenal mucosa and the result presented that the expression of COX-2 could be detected in both Hp-positive and Hp-negative samples, and the expression was significantly increased in Hp-positive ones relative to Hp-negative ones. In addition to the association between Hp and COX-2 expression, we subsequently analyzed the relationship between COX-2 expression and vacA, cagA, and iceA allelotypes. In previous research, vacA has been perceived as an inducer of COX-2 expression (Hisatsune et al., 2007). Our results elucidated that vacA genotype was correlated with mucosal COX-2 expression, evidenced by increased COX-2 expression in vacAs1-positive patients relative to that in vacAs2-positive patients, and decreased COX-2 expression in vacAs1m2-positive patients compared with that in vacAs1m1-positive patients. No correlation of iceA and cagA 3' repeat region with COX-2 expression was identified in the research conducted by Chang et al. (2004), which was consistent with our findings. Collectively, COX-2 expression in Hp-infected duodenal mucosa was associated with vacA allelotypes.

In summary, this study evinced that the Hp virulence genotype vacA affected the expression of COX-2 in Hp-infected duodenal mucosa, and was associated with the initiation and development of DBU and DBI, thus laying the foundation for the management of Hp-infected duodenitis. Nonetheless, this study was imperfect due to the insufficient number of cases and events discussed, unspecified relationship between Hp virulence genotypes and DBI, and undetermined changes of Hp virulence genotypes during treatment. Therefore, future studies shall be carried out in multiple centers using a larger sample size to further clarify the relationship between Hp virulence genotypes and DBI and the alteration of Hp virulence genotypes in the process of treatment.

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Ethics approval and consent to participate. This study had formal permission from the Academic Ethics Committee of Shanghai Fifth People's Hospital, Fudan University(Approval number: 2021008). Each participant was informed of the purpose of this study and signed the informed consent. All procedures were strictly implemented according to the Declaration of Helsinki.

Consent for publication. Not applicable.

Availability of data and materials. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests. All authors declare that there is no conflict of interests in this study.

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Authors' contributions. FYC is guarantor of integrity of the entire study; QCP is responsible for study concepts; YC is responsible for study design, definition of intellectual content, experimental studies, data analysis, statistical analysis, manuscript preparation, manuscript editing, manuscript review; NC is responsible for literature research; XPL is responsible for clinical studies; JF is responsible for data acquisition; All authors read and approved the final manuscript.

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