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Original Article

Association of different genotypes of *helicobacter pylori* with CDX2 expression in intestinal metaplasia and gastric cancer

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Abstract: Objective: The goal of this study was to clarify the effect of *Helicobacter pylori* (*H. pylori*) genotypes on CDX2 expression by detecting expression of CDX2 in *H. pylori*-positive gastric cancer (GC) and gastric intestinal metaplasia (GIM). Methods: CDX2 expression was evaluated by immunohistochemistry in 293 *H. pylori*-positive gastric tissues, including 38 cases of superficial gastritis (GS), 82 cases of GIM, and 173 cases of GC. The samples were subjected to PCR for detection and identification of *cagA* and *vacA* genes. Results: The frequency of the *vacA* genotypes *vacA* s1 (87.8%), *vacA* m2 (42.7%), *vacA* s1m2 (41.5%), *cagA*+ (75.6%), *cagA*+ *vacA* s1 (63.4%), *cagA*+ *vacA* m2 (34.1%), and *cagA*+ *vacA* s1m2 (32.9%) were higher than others in GIM. The frequency of the *vacA* genotypes *vacA* s1 (63.0%), *vacA* m1 (37.0%), *vacA* s1m1 (25.4%), *cagA*+ (58.4%), *cagA*+ *vacA* s1 (39.9%), *cagA*+ *vacA* m1 (28.3%), and *cagA*+ *vacA* s1m1 (21.4%) were higher than others in GC. CDX2 expression was decreased in genotypes *vacA* s1, *vacA* m1, *vacA* s1m1, *cagA*+, *cagA*+ *vacA* s1, *cagA*+ *vacA* m1, and *cagA*+ *vacA* s1m1 in the GC group ($P < 0.05$). CDX2 expression was higher in the age (> 60, $P = 0.003$), well differentiated ($P < 0.001$), and intestinal type GC ($P < 0.001$) groups than the other groups. The predominant genotypes were positive in poorly differentiated GC ($P < 0.05$). Conclusions: The predominant genotype was *cagA*+ *vacA* s1m2 in GIM, which was not associated with CDX2 expression; however, the predominant genotype was *cagA*+ *vacA* s1m1 in GC, which was negatively associated with CDX2 expression and differentiation.

Keywords: Intestinal metaplasia, gastric cancer, *Helicobacter pylori*, *vacA*, *cagA*

Introduction

Caudal type homeobox 2 (CDX2) is a transcription factor that is involved in intestinal differentiation in normal and aberrant locations [1-3]. Ectopic expression of CDX2 has been frequently observed in gastric cancer (GC) and gastric intestinal metaplasia (GIM) [4, 5]. Many studies, including our previous results, have reported that CDX2 may have a suppressive role in the progression and carcinogenesis of GC [6]. At the same time, some reports demonstrated that negative CDX2 expression was associated with poor survival in GC patients. During the GIM stage, low expression of CDX2 had a close relationship with GC in our previous study [7, 8]. However, the mechanism for this is still unclear. It was hypothesized that the different genotypes of *Helicobacter pylori* (*H. pylori*) may have an important role in the progression of GIM to GC.

H. pylori is a gram-negative bacterium that colonizes the gastric mucosa in 50% of humans [9]. Colonization of these organisms consistently induces gastric mucosal inflammation and is associated with an increased risk of GIM and GC. The main virulence factors, including cytotoxin-associated gene A (*cagA*) and vacuolated cytotoxic (*vacA*), have a close relationship with the progression of GIM and GC [10, 11]. The *cagA* gene is not present in all *H. pylori* strains but it is a major virulence factor for gastric disease. The *vacA* cytotoxin is present in all *H. pylori* strains and comprises two variable parts: the s-region (signal), with s1 or s2 variable alleles, and the m-region (middle), with m1 or m2 variable alleles. There are four chimeric proteins: s1m1, s1m2, s2m1, and s2m2. Different combinations of alleles determine the pathogenicity of isolates by means of cytotoxin production. Subsomwong et al. [12] and Vilar et al. [13] showed that *cagA*-positive expression can be detected in GIM, atypical hyperplasia, and GC.

Table 1. PCR primers in this study

Application	Primer	Size
cagA	cagA F: 5' CGATAGGGATAACAGGCAAGCTT 3' cagA R: 5' CTGAAAGCTCTTTGTGGAAGATTC 3'	181 bp
vacA s1	s1F1: 5' GTGGAGCAAGCACAGCTAAGGTTTTA 3' s1R1: 5' CAAAATCGCTACAACATTTTATGGGT 3'	141 bp
vacA s2	s2F2: 5' CTGGTCTAAAGTCGCACCCTTTGTGC 3' s2R2: 5' CAATGGCTGGAATGATCACGGTTGTA 3'	153 bp
vacA m1	m1F1: 5' CAACAATCAAGGCACTATCAACTA 3' m1R1: 5' CCGCATGCTTTTAATGTCATCAG 3' m1F2: 5' TGGTCCGAGGCGGGAAAGT 3' m1R2: 5' GACAAAAAGATTCATCGTGCCTT 3'	107 bp
vacA m2	m2F1: 5' TTTGGAGCTCCAGGAAACATTG 3' m2R1: 5' CTACACGCCCATCTTGGACAA 3' m2F2: 5' ACCCTAAATAGCAACGCAAGC 3' m2R2: 5' GACAAAAAGATTCATCGTGCCTT 3'	102 bp

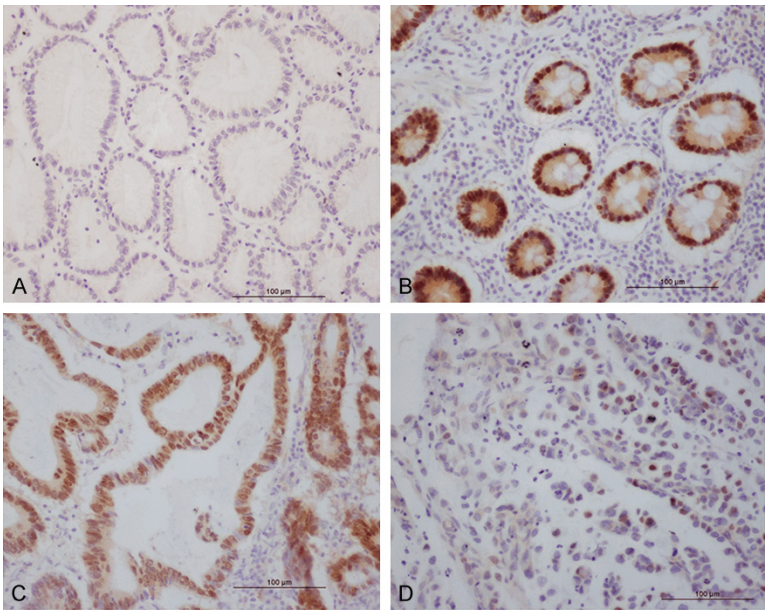

Figure 1. CDX2 expression in GIM and GC. A. Negative expression in no-atrophy gastric. B. Strongly positive expression (+++) in GIM. C. Strongly positive expression in well differentiated GC. D. Weakly positive expression (+) in poorly differentiated GC. Magnification, 200 ×.

The *vacA* genotype, especially *vacA* s1m1, is a marker of the pathogenicity of *H. pylori* strains, and causes severe epithelial cell damage, peptic ulcers, GIM [14], and GC [15]. Therefore, further studies are required to elucidate the genotypes of *H. pylori* and their relationship with CDX2 expression in GIM and GC.

Materials and methods

Tissue specimens

The study population consisted of 293 *H. pylori*-positive paraffin-embedded gastric tissue specimens, including 120 non-gastric cancer tissues obtained by endoscopy and 173 gastric cancer tissues excised by surgery. All the tissues were obtained from Shenyang Medical College between 2014 and 2016 from 198 men and 95 women aged 25-88 years, with a median age of 60 years. GC patients had not been treated with radiotherapy or chemotherapy before surgery. There was no statistically significant difference in age and sex between the gastric cancer and control groups ($P > 0.05$). This study was approved by the Ethics Committee of the Shenyang Medical College and informed consent was obtained from each patient involved in the study.

Histopathology

All gastric biopsy specimens and surgical specimens were immersed in 10% formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin (H.E.) and all specimens were accepted for histological assessment by two experienced pathologists who were blinded to the clinical information of each patient. The histopathological parameters were classified according to the criteria described in the updated Sydney's classification system [16]. Gastric carcinoma cases were categorized according to Lauren classification.

DNA isolation and polymerase chain reaction

Genomic DNA was extracted from samples using a standard kit-based method (TIANamP FFPE DNA Kit, TianGen, and No. DP331-02, Beijing, China). DNA concentration was adjusted to 50 μM by TE buffer, and all DNA preparations were stored at -20°C until use.

PCR was performed to detect the genotypes of *vacA* and *cagA*. Genotype-specific PCR primers

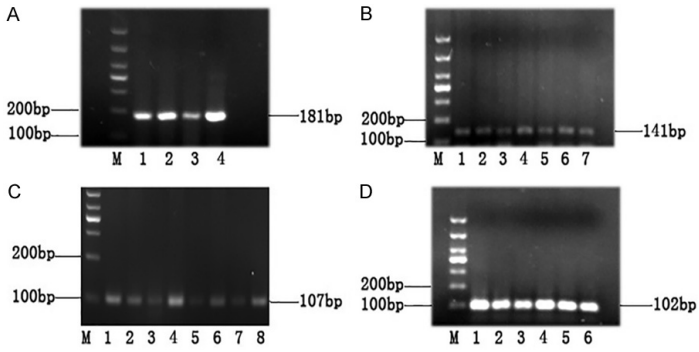

Figure 2. Detecting the genotypes of *vacA, cagA* by PCR. A. *CagA* positive product 181 bp. B. *VacAs1* positive product 141 bp. C. *VacAm1* positive product 107 bp. D. *VacAm2* positive product 102 bp (M: DNA marker LD 1000, 1-8 positive lane).

Table 2. Distribution of *H. pylori* virulence factors in GIM and GC

Virulence factor	GIM n (%)	GC n (%)
vacA s1	72 (87.8)[a,b]	109 (63.0)[a]
vacA s2	0 (0)	0 (0)
vacA s-	10 (12.2)	64 (37.0)
vacA m1	11 (13.4)	64 (37.0)[d,e]
vacA m2	35 (42.7)[c,f]	29 (16.8)
vacA m1m2	25 (30.5)	39 (22.5)
vacA m-	11 (13.4)	41 (23.7)
vacA s1m1	8 (9.8)	44 (25.4)[h]
vacA s1m2	34 (41.5)[g]	11 (6.4)
vacA s1m1m2	19 (23.2)	28 (16.3)
vacA s1m-	11 (13.4)	26 (15.0)
vacA s-m1	3 (3.7)	21 (12.1)
vacA s-m2	6 (7.3)	19 (11.0)
vacA s-m1m2	1 (1.1)	12 (6.9)
vacA s-m1-m2-	0 (0.0)	12 (6.9)
cagA+	62 (75.6)[i]	101 (58.4)
cagA+ vacA s1	52 (63.4)	69 (39.9)
cagA+ vacA m1	7 (8.5)	49 (28.3)
cagA+ vacA m2	28 (34.1)	10 (5.8)
cagA+ vacA s1m1	4 (4.9)	37 (21.4)
cagA+ vacA s1m2	27 (32.9)	4 (2.3)
cagA+ vacA s1m1m2	15 (18.3)	18 (10.4)

a *vacA s1* vs. *vacA s2, vacA s-* in different group, $P < 0.001$; b *vacA s1* in GIM-1 vs. *vacA s1* GC, $P < 0.001$; c In GIM, *vacA* m2 vs. *vacA* m1, $P < 0.001$; *vacA* m2 vs. *vacA m-*, $P < 0.001$; d In GC, *vacA* m1 vs. *vacA* m2, $P < 0.001$; *vacA* m1 vs. *vacA* m-, $P = 0.007$; *vacA* m1 vs. *vacA* m1m2, $P = 0.003$; e *vacA* m1 GC vs. *vacA* m1 in GIM, $P < 0.001$; f *vacA m2* in GIM vs. *vacA m2* in GC, $P < 0.001$; g *vacA s1m2* vs. others in GIM-1, $P < 0.05$; h *vacA s1m1* vs. others in GC, $P < 0.05$; *cagA+* in GIM-1 vs. *cagA+* in GC, $P = 0.007$.

for *cagA, vacA s1*, *vacAs 2*, *vacA m1*, and *vacA m2* were deduced from the sequence alignments (**Table 1**). PCR was performed in a volume of 25 μl containing 12.5 μl Taq Master Mix (including Dye Plus, 10 mM Tris-HCl, 50 mM KCl, 2.5 mM $MgCl_2$, 200 μM dNTPs, and 1.5 U Taq), and 50 pmol of both forward and reverse primers. PCR was performed in an automated thermocycler (AG 22331; Eppendorf, Hamburg, Germany) for *vacA s1/s2* and *cagA*, with a 5-min pre-denaturation at 95°C, followed by 35 cycles of 1 minute at 95°C, 1 minute at 54°C, and 1 minute at 72°C. Final extension was performed for 1 minute at 72°C and PCR products were stored at 4°C. The other genes were amplified by nested PCR. The conditions for the first PCR were as follows: initial denaturation at 95°C for 5 minutes; 35 cycles of 1 minute at 95°C, 1 minute at 54°C, and 1 minute at 72°C; and finally, 72°C for 1 minute. A total of 2 μl of product from the first PCR was used as the template for the second. The conditions for the second round of PCR were as follows: initial denaturation at 95°C for 5 minutes; 30 cycles of 1 minute at 95°C, 1 minute at 54°C, and 1 minute at 72°C; and finally, 72°C for 1 minute. The PCR products were resolved by electrophoresis on 2% agarose gels (No. 111860; Biowest, Spain), which were then stained with ethidium bromide for 10 minutes and photographed under UV light.

Immunohistochemistry (IHC)

Formalin-fixed paraffin-embedded (FFPE) specimens were cut into 4 μm-thick sections and subjected to IHC using monoclonal antibody against CDX2 (1:100, No. MA516347; Thermo Scientific, USA) according to the kit's instructions (No. KIT-9901; Elivision Plus, Fujian, China). For the negative control, sections were incubated with normal mouse IgG1 and no immunoreactivity was observed.

Positivity was judged by the intensity of brown coloration (score 1, light brown; score 2, brown; score 3, deep brown) and the number of cells with brown coloration (score 1, stained cells < 30%; score 2, stained cells 30%-70%; score 3,

Table 3. Relationship between predominant *H. pylori* virulence factors and CDX2 expression in GIM and GC

Virulence factor		GIM				GC			
		CDX2 (+)/total N (%)	*P*-value	OR	95% CI	CDX2 (+)/total N (%)	*P*-value	OR	95% CI
vacA s1	Positive	56/72 (77.8)	0.679	0.389	(0.046-3.303)	54/109 (49.5)	0.024	0.479	0.252-0.912
	Negative	9/10 (90.0)				43/64 (67.2)			
vacA m1	Positive	9/11 (81.8)	1.000	1.205	(0.235-6.181)	27/64 (42.2)	0.005	0.407	0.216-0.765
	Negative	56/71 (78.9)				70/109 (64.2)			
vacA m2	Positive	28/35 (80.0)	0.888	0.925	(0.313-2.733)	18/29 (62.1)	0.476	1.346	0.594-3.593
	Negative	37/47 (78.7)				79/144 (54.9)			
vacA s1m1	Positive	6/8 (75.0)	0.754	0.763	(0.140-4.165)	15/44 (34.1)	0.001	0.296	0.144-0.609
	Negative	59/74 (79.7)				82/129 (63.6)			
vacA s1m2	Positive	27/34 (79.4)	0.978	1.015	(0.343-3.003)	6/11 (54.5)	0.916	0.936	0.275-3.193
	Negative	38/48 (79.2)				91/162 (56.2)			
cagA+	Positive	50/62 (80.6)	0.588	1.389	(0.422-4.575)	48/101 (47.5)	0.007	0.425	0.226-0.799
	Negative	15/20 (75.0)				49/72 (68.1)			
cagA+ vacA s1	Positive	41/52 (78.8)	0.901	0.932	(0.306-2.842)	29/69 (42.0)	0.002	0.384	0.205-0.718
	Negative	24/30 (80.0)				68/104 (65.4)			
cagA+ vacA m1	Positive	6/7 (85.7)	0.660	1.627	(0.182-14.508)	19/49 (38.8)	0.004	0.374	0.189-0.738
	Negative	59/75 (78.7)				78/124 (62.9)			
cagA+ vacA m2	Positive	24/28 (85.7)	0.300	1.902	(0.557-6.500)	5/10 (50.0)	0.750	0.772	0.215-2.769
	Negative	41/54 (75.9)				92/163 (56.4)			
cagA+ vacA s1m1	Positive	3/4 (75.0)	0.829	0.774	(0.075-7.949)	12/37 (32.4)	0.001	0.288	0.133-0.623
	Negative	62/78 (79.5)				85/136 (62.5)			
cagA+ vacA s1m2	Positive	23/27 (85.2)	0.354	1.780	(0.520-6.093)	3/4 (75.0)	0.632	2.394	0.244-23.482
	Negative	42/55 (76.4)				94/169 (55.6)			

stained cells > 70%). According to the sum of the two indexes, several comprehensive scores were made. A comprehensive score 0 was defined as negative expression, comprehensive scores of 2-3 were defined as weakly positive (+), comprehensive scores of 4 were defined as mildly positive expression (++), and comprehensive scores above 5 were defined as strongly positive expression (+++) [17].

Statistical analysis

Baseline descriptive data were analyzed and reported as a percentage and frequency for categorical variables. Chi-square and Fisher's exact tests were used to compare the positive rate between the different groups. Odds ratios (ORs) and 95% confidence intervals (CIs) for the association between CDX2-positive expression and the risk of GC were obtained using an unconditional logistic model adjusting for sex and age at diagnosis. Trend statistics for risk of GC in association with CDX2-positive expression were obtained using multivariate models. All tests were two sides, and all statistical analyses were performed using Statistical Analysis System software (SPSS 17.0).

Results

Infection of H. pylori and GIM evaluation

By *H. pylori*-specific PCR (IgM) and methylene borate staining, 173 cases of gastric cancer (GC) and 120 non-gastric cancer endoscopy specimens were determined to be *H. pylori*-positive. By H&E staining, 82 cases of gastric endoscopy specimens were diagnosed with GIM, and the other 38 cases tissues were non-GIM as the control group.

Expression of CDX2 in different groups

All the cases exhibited negative expression of CDX2 (**Figure 1**). The positivity rate was higher in GIM (65/82, 79.3%) and GC (97/173, 56.1%) groups compared with the control group ($P < 0.05$). The percentage of CDX2 positivity in GIM was higher than in the GC group ($P < 0.001$).

Distribution of H. pylori virulence factors in GIM and GC

VacA and *cagA* status was analyzed for all 293 *H. pylori*-infected GIM and GC patients (**Figure 2** and **Table 2**). The virulence factor of *vacA* s1

Table 4. Correlation between clinicopathological characteristics and CDX2 expression in GC

Characteristics	N (%)	CDX2 negative N (%)	CDX2 positive N (%)	*P*-value
Gender				
Male	137 (79.2)	58 (42.3)	79 (57.7)	0.410
Female	36 (20.8)	18 (50.0)	18 (50.0)	
Age				
≤ 60	78 (45.1)	44 (56.4)	34 (43.6)	0.003
> 60	95 (54.9)	32 (33.7)	63 (66.3)	
Diameter				
< 5 cm	86 (49.7)	34 (39.5)	52 (60.5)	0.247
≥ 5 cm	87 (50.3)	42 (48.3)	45 (51.7)	
Differentiation				
Well	25 (14.5)	2 (8.0)	23 (92.0)	< 0.001
Moderate	67 (38.7)	28 (41.8)	39 (58.2)	
Poor	81 (46.8)	46 (56.8)	35 (43.2)	
Gross				
Early gastric	3 (1.7)	1 (33.3)	2 (66.7)	0.056
B1	3 (1.7)	0 (0.0)	3 (100.0)	
B2	4 (2.3)	2 (50.0)	2 (50.0)	
B3	140 (80.9)	56 (40.0)	84 (60.0)	
B4	23 (13.4)	17 (73.9)	6 (26.1)	
Depth of invasion				
SM	1 (0.5)	1 (100.0)	0 (0.0)	0.455
MP	20 (11.6)	8 (40.0)	12 (60.0)	
SS	27 (15.6)	9 (33.3)	18 (66.7)	
SE	117 (67.6)	55 (47.0)	62 (53.0)	
SI	8 (4.6)	3 (37.5)	5 (62.5)	
N stage				
No	45 (26.0)	23 (51.1)	22 (48.9)	0.259
Yes	128 (74.0)	53 (41.4)	75 (58.6)	
Lauren				
Intestinal type	93 (53.8)	29 (31.2)	64 (68.8)	< 0.001
Diffuse type	80 (46.2)	47 (58.7)	33 (41.3)	

M, musical; SM, submucosal; MP, muscularis; SS, subserosal; SE, serosal exposure; SI, serosal invasion.

was obtained in 109 (63%) of *H. pylori*-infected GC, which was lower than in GIM (72/82, 87.8%, $P < 0.001$). The distribution of *vacA* m2 was higher in GIM (35/82, 42.7%) than GC ($P < 0.05$). At the same time, *vacA* m1 in GC was higher than GIM compared with the other virulence factors (*vacA* m2, *vacA* m1m2, and *vacA* m-, all $P < 0.05$).

Combining *vacA* s and *vacA* m to analyze the distribution of *H. pylori* virulence, *vacA* s1m2 was the main genotype in GIM ($P < 0.05$), and the ratio was much higher than in the GC group ($P < 0.05$); however only *vacA* s1m1 was higher than the other genotypes in GC ($P < 0.05$).

The influence of *cagA* factor was higher in GIM than in the GC group ($P < 0.05$). Combining *cagA* and *vacA*, the *cagA*+ *vacA* s1, *cagA*+ *vacA* m2, and *cagA*+ *vacA* s1m2 genotypes were the most frequent genotype in GIM ($P < 0.05$). However, *cagA*+ *vacA* s1, *cagA*+ *vacA* m1, and *cagA*+ *vacA* s1m1 were the predominant genotypes in GC ($P < 0.05$).

Relationship between the predominant H. pylori virulence factors and CDX2 expression in GIM and GC.

In the GIM group, there was no association between the expression of CDX2 and predominant *H. pylori* virulence factors (**Table 3**). While in GC, the expression of CDX2 was lower in the *vacA* s1-, *vacA* m1-, *vacA* s1m1-, *cagA*+, *cagA*+ *vacA* s1-, and *cagA*+ *vacA* s1m1-positive groups than the negative group ($P < 0.05$).

CDX2 expression is associated with clinicopathological factors in GC

Characteristics of patients subjected to CDX2 expression analysis are summarized in **Tables 4** and **5**. The percentage of positive CDX2 expression was significantly higher in the > 60 years age group (median age) compared with the ≤ 60 years age group ($P = 0.003$) and was mainly in the (+) group (47.6% vs. 26.5%, $P = 0.043$). Furthermore, it was significantly higher in the well-differentiated histological type compared with the moderate and poor histological types ($P < 0.001$ and $P < 0.001$, respectively), especially the (+++) group (73.9% vs. 5.7%, $P < 0.001$), and also the intestinal type compared with the diffuse type ($P < 0.001$), especially the (+++) group (40.7% vs. 9.1%, $P = 0.001$).

Univariate regression analysis demonstrated that positive expression of CDX2 was significantly associated with differentiation (OR =

Table 5. Association of CDX2 expression intensity with patients' characteristics in GC

Characteristics	Expression intensity of CDX2		
	+	++	+++
	N (%)	N (%)	N (%)
Age			
≤ 60	9 (26.5)	14 (41.2)	11 (32.3)
> 60	30 (47.6)[a]	15 (23.8)	18 (28.6)
Differentiation			
Well	4 (17.4)	2 (8.7)	17 (73.9)[b,c]
Moderate	18 (46.2)	11 (28.2)	10 (25.6)
Poor	17 (48.6)	16 (45.7)	2 (5.7)
Lauren			
Intestinal	23 (35.9)	15 (23.4)	26 (40.7)[d]
Diffuse	16 (48.5)	14 (42.4)	3 (9.1)

[a] > 60 (+) vs. ≤ 60 (+), $P = 0.043$; [b]Well differentiated histological type (+++) vs. moderately differentiated histological type (+++), $P < 0.001$; [c]Well differentiated histological type (+++) vs. poor differentiated histological type (+++), $P < 0.001$; [d]Intestinal type (+++) group vs. diffused type (+++), $P = 0.001$.

15.114, 95% CI: 3.338-68.432) and intestinal type (OR = 3.143, 95% CI: 1.682-5.872). In contrast, negative expression of CDX2 was significantly associated with different virulence factors of *H. pylori*, especially multiple infection with *vacA* s1m1 (OR = 3.373, 95% CI: 1.643-6.924) and *cagA*+ *vacA* s1m1 (OR = 3.472, 95% CI: 1.606-7.506).

Association of predominant H. pylori virulence factors with clinicopathological features

In a comparison of the virulence factors of H. pylori and clinicopathological characteristics, only differentiation- and sex-related virulence factors were identified. Among them, vacA m1, vacA s1m1, cagA+, cagA+ vac Am1, and cagA+ vacA s1m1 genotypes had a close relationship with differentiation (**Table 6**), especially moderate and poor differentiation. Only vacA m1 had a close relationship with males (56/64, 87.5%), which was higher than females (8/64, 12.5%), P = 0.039, and there were no statistically significant associations in other groups.

Discussion

These results show that CDX2 is expressed in GIM and GC. The positivity rate of GIM was significantly higher than in the GC group, consistent with previous data. During progression of GIM to GC, *H. pylori* is an important risk factor. However, there are discrepancies between CDX2 expression and the status of *H. pylori* [18-20]. The main reason is that the strain variation of *H. pylori* was not taken into account in previous studies, especially with respect to *vacA* and *cagA* virulence factor-encoding genes, which have been proposed as a means of identifying strains with the highest degrees of pathogenicity, and consequently, individuals with the highest risk of disease.

The distribution of *H. pylori* virulence factors was analyzed in GIM and GC. The results show that the distribution of *vacA* s1 and *vacA* m2 was higher in GIM. Combing the m-region and s-region, the genotype of *vacA* s1m2 was the predominant factor in GIM patients. However, the predominant virulence factor of *H. pylori* was *vacA* s1m1 in GC. Additionally, *cagA*+ was another virulence factor that was noted at high infection levels in GIM and GC. The distribution of genotypes in *H. pylori* suggested that *cagA*+ *vacA* s1m2 was the predominant virulence factor in GIM, while *cagA*+ *vacA* s1m1 was the main genotype of GC. In other words, the predominant virulence factor of *H. pylori* was different in GIM and GC. *vacA* is present in each *H. pylori* strain, and *vacA* s1 strains secrete larger amounts of cytotoxin than *vacA* s2 strains *in vitro*, the latter supposedly being less virulent. *H. pylori vacA* s1 and *vacA* m1 strains are associated with enhanced gastric mucosal inflammation and increased risk of atrophy, intestinal metaplasia, and carcinoma in comparison with *vacA* s2 and m2 strains [21-23]. Although the *cagA* gene is not present in all *H. pylori* isolates, many data support the idea that infection with a *cagA*-positive isolate increases the risk of GC [24]. Recently, a meta-analysis reported that infection by *H. pylori* strains with *vacA* s1m1 and *cagA* genes could significantly increase the risk of GC [25]. Different predominant virulence factors were considered that could promote variable cell differentiation.

To confirm these observations, the relationship between the expression of CDX2 and the genotypes of *H. pylori* in GIM and GC was analyzed. It was interesting to note that there was no relationship between the predominant virulence factor of *H. pylori* and expression of CDX2 in GIM. However, in GC, the *vacA* s1, *vacA* s1m1, *cagA*+, *cagA*+ *vacA* s1, *cagA*+ *vacA* m1, and

Table 6. Relationship between predominant *H. pylori* virulence factors and degree of differentiation in GC

Virulence factor		N (%)	Differentiation			P	OR	95% CI
			Well	Moderate	Poor			
vacA m1	Negative	109 (63.0)	21 (19.3)	34 (31.2)	54 (49.5)	0.009	2.625	0.819-8.414
	Positive	64 (37.0)	4 (6.2)	33 (51.6)	27 (42.2)			
vacA s1	Negative	64 (37.0)	9 (14.1)	27 (42.2)	28 (43.8)	0.768	1.065	0.417-2.716
	Positive	109 (63.0)	16 (14.7)	40 (36.7)	53 (48.6)			
vacA s1m1	Negative	129 (74.6)	23 (17.8)	44 (34.1)	62 (48.1)	0.031	3.524	0.760-16.334
	Positive	44 (25.4)	2 (4.5)	23 (52.3)	19 (43.2)			
cagA+	Negative	72 (41.6)	16 (22.2)	23 (31.9)	33 (45.9)	0.036	2.586	1.021-6.548
	Positive	101 (58.4)	9 (8.9)	44 (43.6)	48 (47.5)			
cagA+ vacA m1	Negative	124 (71.7)	23 (18.5)	41 (33.1)	60 (48.4)	0.011	4.025	0.873-18.551
	Positive	49 (28.3)	2 (4.1)	26 (53.1)	21 (42.8)			
cagA+ vacA s1	Negative	104 (60.1)	19 (18.3)	36 (34.6)	49 (47.1)	0.151	2.068	0.746-5.736
	Positive	69 (39.9)	6 (8.7)	31 (44.9)	32 (46.4)			
cagA+ vacA s1m1	Negative	136 (78.6)	24 (17.7)	46 (33.8)	66 (48.5)	0.012	5.455	0.683-43.550
	Positive	37 (21.4)	1 (2.7)	21 (56.8)	15 (40.5)			

cagA+ vacA s1m1 groups had a negative relationship with CDX2 expression. Above all, the predominant virulence factor of *H. pylori* could not change the expression of CDX2 during GIM. However, it could reduce the expression of CDX2 in GC, and furthermore, the tissues would dedifferentiate. Some reports showed *H. pylori*, especially *cagA+*, could upregulate CDX2 mRNA levels and protein expression in AGS, NUGC-4, and KATOIII gastric cancer cell lines. However, the results of CDX2 expression were not consistent in gastric tissue. *H. pylori* infection has relationship with negative expression of CDX2 in gastric cardia adenocarcinoma [26]. Nonetheless, among these reports, the genotypes of *cagA+* and *vacA* were neglected. Thus, these genotypes should be considered when detecting the effect of *H. pylori* on the expression of CDX2. Until now, the mechanism has been unknown. Malik et al. reported that increased expression and activity of the BMP pathway accompanied by CDX2 upregulation and SOX2 downregulation were observed in AGS cells cocultured with *H. pylori* or BMP2 [27]. Ren et al. reported that the signaling pathways activated by IL-6 had a crucial role in the regulation of CDX2 and was a critical factor in the process of gastric carcinogenesis [28]. Of course, further *in vitro* and animal studies are needed to confirm these results

Based on these results, continuous analysis of the relationship between the genotypes of *H. pylori* and the clinical characteristics of CDX2 in the GC group is required. Univariate analysis showed that positive expression of CDX2 had a close relationship with age, Lauren type, and especially the degree of differentiation (OR (95% CI) 15.114 (3.338-68.432)). These results suggest that GIM occurs in older people of over 60 years. CDX2 was also a good marker of GC differentiation. CDX2 is mainly expressed in well and moderately differentiated GC and intestinal type GC. Poorly and moderately differentiated GC was associated with the genotypes of *vacA* m1, *vacA* s1m1, *cagA*, *cagA+ vacA* m1, and *cagA+ vacA* s1m1.

Therefore, loss of CDX2 is associated with these genotypes of *H. pylori*, resulting in poor outcome. Other studies also reported that CDX2-positive gastric carcinomas were more likely to be resectable and patients with CDX2-positive tumors have significantly better survival.

Conclusion

The predominant genotype was *cagA+ vacA* s1m2 in GIM, which was not associated with CDX2 expression. However, the predominant genotype in GC (*cagA+ vacA* s1m1) was negatively associated with CDX2 expression and differentiation.

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Disclosure of conflict of interest

None.

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