Combined Use of *Helicobacter pylori* Genotyping and CDX2 Expression as a Predictor of Malignant Potential in Gastric Intestinal Metaplasia

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Abstract. ***Objective***. Gastrointestinal metaplasia (GIM) has a close relationship with gastric cancer (GC), but it is unclear how to judge which GIM could develop into GC. This study aimed to assess the role of CDX2 and its association with *Helicobacter pylori* (*H.pylori*) genotypes in GIM. ***Methods***. *CagA* and *vacA* genes were identified via PCR in 466 *H. pylori*-positive gastric tissues, including gastritis (n=104), GIM diagnosed endoscopically (GIM-1; n=82), gastric cancer (GC; n=173), and paired adjacent GIM tumors resected surgically (GIM-2; n=107). GIM was subclassified per the HID- AB pH2.5-PAS as follows: type I (n=23), type II (n=43), and type III (n=16) in GIM-1; type I (n=8), type II (n=40), and type III (n=59) in GIM-2. CDX2 expression was evaluated immunohistochemically. ***Results***. In GIM-1, the infection rate of *vacA*m2 (55.8%) and *vacA*s1m2 (53.5%) was higher in subtype II than in others (P<0.05), while that of *vacA*m1 (49.2%) and *vacAs*1m1 (33.9%) was higher in subtype III than in others. The *cagA*$^+$ rate was higher in subtypes I (75.0%) and III (64.4%) than in subtype II (40.0%; P<0.05) respectively. *CDX2* was upregulated in subtype I than in subtypes II and III in GIM-1 and GIM-2. In GIM-2 and GC, *CDX2* was downregulated in *vacA*m1, *vacA*s1m1, and *cagA*$^+$ (P<0.05). The predominant genotype was *vacA*s1m2 in subtype II of GIM-1, CDX2 expression remaining unaltered; however, the predominant genotype was *cagA*$^+$ *vacA*s1m1 in subtypes II and III of GIM-2, negatively correlated with *CDX2* expression. ***Conclusion***. These GIM subtypes (*cagA*$^+$ *vacA*s1m1 *H. pylori*-positive GIM with negative *CDX2* expression) resemble GC and should be evaluated similar to cancerous GIM.

Key words: Gastric, intestinal metaplasia, *Helicobacter pylori*, vacuolated cytotoxic, cytotoxin-associated gene A.

Introduction

The mortality rate of gastric cancer (GC) has decreased owing to curative treatment via endoscopic submucosal dissection (ESD) in the early stages of GC in some countries [1]. However, gastric cancer is still a leading global health concern owing to its high mortality, especially in China. Furthermore, a standard method for the early detection of gastric cancer in low-prevalence regions (5-7 per 100 000 individuals) has not been established [2,3]. Many studies suggest selecting a high-risk patient for surveillance endoscopy in daily clinical practice [4-8]. Patients diagnosed with gastric intestinal metaplasia (GIM) belong to the high-risk category because GIM is a premalignant stage in Correa's cascade and recognized as the committed step in gastric carcinogenesis [9]. In the clinical setting, not all GIM subtypes develop into GC. Therefore, developing methods to assess malignant potential in GIM is challenging due to its potential progression to GC.

Until now, conventional histochemical (HID-ABpH2.5-PAS) staining is the primary diagnostic method for GIM subtypes. Based on morphological characteristics, GIM is divided into complete and incomplete subtypes, or subtypes I, II, and III. Subtype III and incomplete GIM are markedly associated with GC [9-12]. A 5-year follow-up study

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0091-7370/24/0100-009.

reported that patients with incomplete GIM (characterized by sulfomucin expression, including II and III) were at the most considerable risk of developing dysplasia or early gastric cancer [11]. Despite its lasting clinical success, GIM subtyping has numerous issues in the clinical setting, such as accuracy and objectivity in diagnosing GIM subtypes. New biomarkers are required to determine the exact subtypes of GIM.

H.pylori is a significant marker associated with GC and GIM. Infections of these bacteria consistently induce gastric mucosal inflammation and are associated with an increased risk of GIM and gastric adenocarcinoma. Thus far, numerous studies have reported different levels of virulence of *H.pylori*, thereby yielding inconsistent results. The primary virulence factors include cytotoxin-associated gene A (*CagA*) and vacuolated cytotoxic (*VacA*) and are closely associated with the progression of GIM and GC [13-15]. CagA is not present in all *H.pylori* strains; however, it is a major virulence factor for gastric diseases. VacA cytotoxin is present in all *H. pylori* strains and comprises two variable parts in the *vacA* gene: the s-region (signal), with s1 or s2 variable alleles and the m-region (middle), with m1 or m2 variable alleles. These consist of four chimeric forms: s1/m1, s1/m2, s2/m1, and s2/m2. Different combinations of alleles help determine the pathogenicity of isolates based on cytotoxin production. We hypothesize that the different genotypes of *H.pylori* play an essential role in the progression from GIM to GC.

Caudal type homeobox 2 (CDX2) is a transcription factor, involved in intestinal differentiation in normal and in unusual locations. Numerous studies, including our previous study, have reported that CDX2 is a suitable biomarker for determine GIM subtypes. Simultaneously, some studies have reported that positive CDX2 expression is associated with well differentiation GC and enhanced survival of GC patients. CDX2 is considered a suppressor of carcinogenesis [16,17].

Therefore, the present study aimed to elucidate the genotypes of *H.pylori* and the association between CDX2 expression in GIM and subtypes of GIM and to distinguish malignant GIM based on the two biomarkers.

Materials and Methods

Samples. Four hundred sixty-six formalin-fixed, *H. pylori*-positive paraffin-embedded samples were collected from the Affiliated Hospital of Shenyang Medical College between 2014 and 2016 from 293 individuals (198 men, 95 women; median age, 59 [range, 34-87] years), including 120 endoscopic biopsy samples, 173 gastric carcinoma and 173 paired adjacent non-tumor tissue specimens. The rapid urease test (RUT) was administered to confirm the presence of *H. pylori* infection. Patients with positive results from both the histological examination and RUT test were included. The patients had not received non-steroidal anti-inflammatory drugs or any anti-Helicobacter therapy at least for 3 months prior to endoscopy. GC patients had not been treated with radiotherapy or chemotherapy before surgery. There were no significant differences in age and sex between the gastric cancer group and the endoscopy group ($P>0.05$). This study was approved by the Ethics Committee of the Shenyang Medical College, and informed consent was obtained in writing from each patient involved in the study.

Histology and HID-ABpH2.5-PAS stain for GIM classification. Serial sections were cut, stained with hematoxylin and eosin (H.E); high-iron diamine and Alcian blue at pH 2.5, periodic acid-Schiff stain (HID-ABpH2.5-PAS), and modified methylene borate stain. All specimens were deemed acceptable for histological assessment by two experienced pathologists, blinded to the clinical information of each patient. The histopathological parameters were classified in accordance with the criteria described in the updated Sydney's classification system [18].

HID-ABpH2.5-PAS staining was performed in accordance with the protocols established in our laboratory previously [19]. In brief, deparaffinized sections were treated with HID for 24 h at room temperature followed by incubation with AB solution for 20 min in a humified chamber. After washing with distilled water, sections were treated with PAS at pH 2.5 for 1-2 min (NO. DG0039, DG0007, DG0004; Leagene Biology Technology Company, Beijing, China) and then washed as above. The slides were sealed with neutral gum.

Upon histology and HID-ABpH2.5-PAS staining, GIM was divided into I, II, and III subtypes. Type I (complete): straight crypts and regular architecture. The epithelium consists of mature absorptive and goblet cells, the latter secreting sialomucins (blue); Type II (incomplete): The crypts are tortuous and there is mild architectural distortion. GIM cells with few or absent absorptive cells; presence of columnar "intermediate" cells in

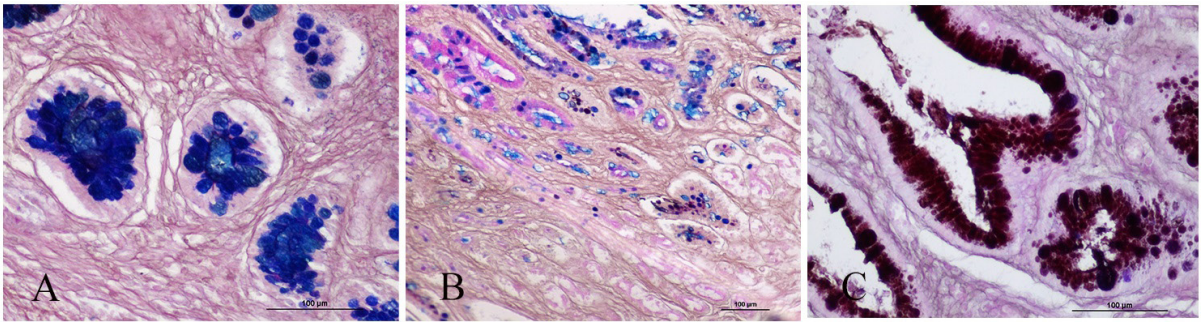

Figure 1. *The subtypes of GIM by HID-ABpH2.5-PAS staining.* ***(A)*** *subtype I,straight crypts and regular architecture, goblet cells secreting sialomucins (blue) (x200),* ***(B)*** *subtype II, The glands are tortuous and there is mild architectural distortion,secreting neutral (red),acid sialomucins (blue) and sulphomucins (black)(x200), and* ***(C)*** *cells secrete predominantly sulphomucins(black) (x200).*

various stages of differentiation, secreting neutral (red) and acid sialomucins (blue); goblet cells secreting sialomucins and/or occasionally sulphomucins (black); Type III (incomplete): a variable degree of disorganized distortion of glandular architecture is more pronounced cell atypia and loss of differentiation is more marked than in type II; "intermediate" cells secrete predominantly sulphomucins **(Figure 1)**.

DNA Isolation and polymerase chain reaction. Genomic DNA was extracted from all samples, using a standard-kit-based method (TIANamP FFPE DNA kit, TianGen, and No. DP331-02, Beijing, China). Detection the genotype of *vacA*, *cagA* were performed in accordance with the protocols established in our laboratory previously (14).The DNA concentration was adjusted to 50 μM with TE buffer, and all DNA samples were stored -20°C until use.

Genotype-specific PCR primers for *cagA, vacA*s1*, vacA*s2*, vacA*m1, and *vacA*m2 were obtained from the sequences and conditions presented in **Table 1**.

Immunohistochemistry (IHC). Sections 4 μm thick were cut, deparaffinized in xylene, and then dehydrated in descending dilutions of ethanol. For the antigen retrieval regimen, all slides were at 120°C microwaved in 10 mmol/L sodium citrate buffer (pH 6.0) at 10 min intervals for a total of 20 min. Thereafter, the specimens were cooled for 60 min at about 25°C. Then all the sections were treated with 3% hydrogen peroxide in methanol for 15 min to block endogenous peroxidase at room temperature. After washing in PBS, the sections were incubated with monoclonal mouse anti-human antibodies CDX2 antibody (1:100, No. MA516347, Thermo Scientific, MA, USA) in a moist chamber overnight at 4°C.The sections were washed with PBS and incubated with polymerase auxiliaries for 20 min. After washing in PBS, the sections were incubated with biotinylated secondary antibody for 30 min at room temperature and finally DAB was visualized. Tissues were counterstained with hematoxylin. A negative control was designed by using PBS instead of primary antibody.

Statistical analysis. The observed frequencies of *cagA, vacA* subtypes and genotypes in different diseases were compared using chi-squareor Fisher's exact tests. Using the same methods to assess the CDX2 expression among the different groups and subtypes of GIM, and the CDX2 expression in different *H. pylori* genotypes. Statistical significance was set at $P<0.05$. Statistical analyses were performed using SPSS program (version 19.0; SPSS Inc., CA, USA).

Results

Detection of GIM in different groups and subtype distribution of GIM. The detection rate of IM lesions in gastric endoscopy specimen (GIM-1) was 82/120 (68.3%), and that in the resected tissues adjacent to GC (GIM-2) was 107/173 (61.8%), other cases all were diagnosed chronic gastritis (GS).

Upon histology and HID-ABpH2.5-PAS staining, GIM-1 was divided into 23 cases type I; type II, 43; type III, 16 and 8 cases were of type I; type II, 40; type III, 59 in GIM-2.The ratio of subtype III was significantly lower in GIM-1 (16/82, 19.6%) than in GIM-2 (59/107, 55.1%; $P=0.016$).

Distribution of H. pylori virulence factors in GIM and GC. *VacA* and *cagA* status was analyzed for all 362 *H. pylori*-infected GIM and GC patients **(Figure 2A)**. The virulence factor *vacA* s1 was

Table 1. *Primer used for PCR application in this study.*

Application	primer	PCR conditions	size
cagA	F: 5′CGA TAG GGA TAA CAG GCA AGC TT 3′ R: 5′CTG AAA GCT CTT TGT GGA AGA TTC 3′.	95°C, 5 min; 54°C, 1 min; 72°C, 1 min (35 cycles)	181bp
*vacA*s1	F1: 5′GTG GAG CAA GCA CAG CTA AGG TTT TA 3′ R1: 5′CAA AAT CGC TAC AAC ATT TTA TGG GT 3′	95°C, 5 min; 54°C, 1 min; 72°C, 1 min (40 cycles)	141bp
vacAs2	F2:5′CTG GTC TAA AGT CGC ACC CTT TGT GC 3′ R2:5′CAA TGG CTG GAA TGA TCA CGG TTG TA 3′	95°C, 5 min; 54°C, 1 min; 72°C, 1 min (35 cycles)	153bp
*vacA*m1	F1: 5′CAA CAA TCA AGG CAC TAT CAA CTA 3′ R1: 5′CCG CAT GCT TTTA ATG TCA TCA G 3′	95°C, 5 min; 54°C, 1 min; 72°C, 1 min (35 cycles)	107bp
*vacA*m2	F2: 5′TGG TCC GAG GCG GGA AAG T 3′ R2: 5′GAC AAA AAG ATT CAT CGT GCC TT 3′ F1:5′TTT GGA GCT CCA GGA AAC ATT G 3′ R1:5′CTA CAC GCC CAT CTT GGA CAA 3′	95°C, 5 min; 54°C, 1 min; 72°C, 1 min (30 cycles) 95°C, 5 min; 54°C, 1 min; 72°C, 1 min (35 cycles)	102bp
	F2:5′ACC CTA AAT AGC AAC GCA AGC 3′ R2:5′GAC AAA AAG ATT CAT CGT GCC TT 3′	95°C, 5 min; 54°C, 1 min; 72°C, 1 min (30 cycles)	

detected in 109 (63%) *H. pylori*-positive gastric cancer patients, which resembled 69 (64.5%) patients in the GIM-2 group. The distribution of *vacA* s1 (72/82, 87.8%) was more prevalent in GIM-1 than in GIM-2 and GC (P<0.001, P<0.001, respectively).

The distribution of *vacA* m1 was significantly higher in GIM-2 (40/107, 37.4%) and GC (64/173, 37.0%) than in GIM-1 (P<0.05), while that of *vacA*m2 was higher in GIM-1 than in GIM-2 and GC (P<0.05). Simultaneously, *vacA*m1 was significantly higher in GIM-2 and GC than other virulence factors (*vacA*m2, *vacA*m1m2, *vacA*m-(P<0.05).

On combining *vacA* s and *vacA* m to analyze the distribution of *H. pylori* virulence, *vacA* s1m2 was the primary genotype in GIM-1 (P<0.05), and the ratio was significantly higher than that in the GIM-2 and GC group (P<0.05); however, in the GIM-2 group, *vacA*s1m1, and *vacA*s1m1m2 was significantly more prominent than other genotypes (P<0.05). Only *vacA*s1m1 was significantly more prominent than other genotypes in the GC group (P<0.05).

The influence of *cagA* factor was significantly more prominent in GIM-1 than that in GIM-2 and GC (P<0.05).

Distribution of H. pylori virulence factors in subtypes GIM. In GIM-1, the infection rates of *vacA*m2 and *vacA*s1m2 was significantly higher in subtype II (P<0.05). However, in GIM-2, the infection rate of vacAm1 and *vacA*s1m1 was higher than those of others in subtype III. *CagA*$^+$ was significantly higher in subtypes I and III than in subtype II (P<0.05) **(Figure 2B)**.

CDX2 protein expression in different groups and different subtypes of GIM. All GS cases displayed CDX2 expression. The positivity rate was significantly higher in the GIM-1 (65/82, 79.3%), GIM-2 (67/107, 62.6%), and GC (97/173, 56.1%) group compared to the GS group (P<0.05). The positive rate of CDX2 was significantly higher in GIM-1 than in GIM-2 (P=0.016) and the GC group (P<0.05).

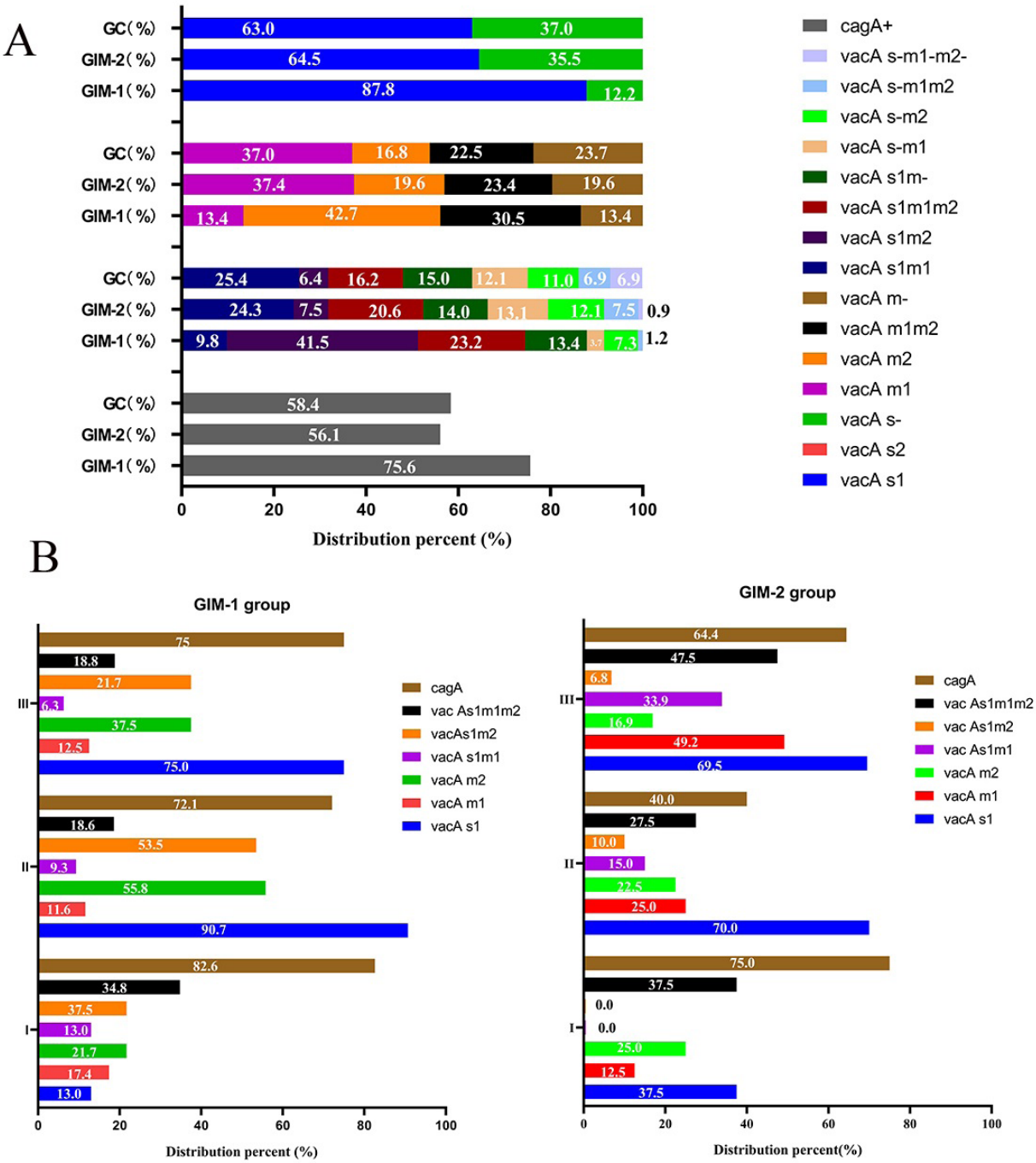

Figure 2. *The distribution of H.pylori genotype.* ***(A)*** *The positive rate of different H.pylori genotypes in GC, GIM-1 and GIM-1.* ***(B)*** *The distribution of H.pylori-predominant virulence factors in subtypes of GIM-1(left) and GIM-2 (right).*

The positive expression rate of CDX2 was significantly higher in subtypes I (21/23, 91.3%) and II (37/43, 86.0%) than in subtype III (7/16, 43.8%) in the GIM-1 group (P<0.001 and P<0.01, respectively).

In GIM-2, the positive rate for CDX2 expression was slightly but not significantly higher in subtype I (7/8, 97.5%) than in subtypes II (29/40, 72.5%) and III (31/59, 52.5%; P>0.05) **(Figure 3)**.

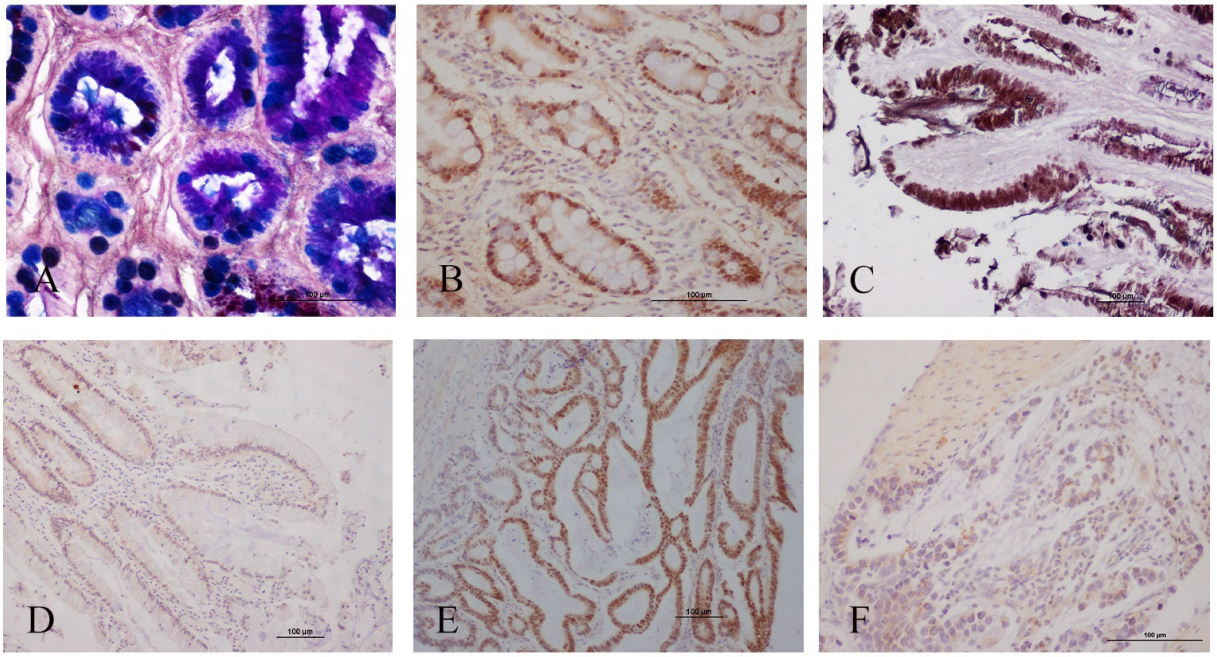

Figure 3. *CDX2 expression in GIM and GC by immunohistochemistry. The positive site is in the nucleus(brown color).* ***(A)*** *Subtype I by HID-ABpH2.5-PAS staining (x200),* ***(B)*** *CDX2 positive expression in subtype I GIM (x200),* ***(C)*** *Subtype III by HID-ABpH2.5-PAS staining (x200),* ***(D)*** *CDX2 negative expression in subtype III GIM (x200),* ***(E)*** *CDX2 positive expression in well differentiated GC (x100), and* ***(F)*** *CDX2 negative expression in poorly differentiated GC (x100).*

Association between H. pylori predominant virulence factors and CDX2 expression. In GIM-1, CDX2 expression was not associated with *H. pylori*-predominant virulence factors **(Table 2)**, while in GIM-2 and GC, CDX2 was significantly down-regulated in the *vacA*m1, *vacA*s1m1, and *cagA*+ genotype ($P<0.05$).

Discussion

In the present study, GIM-adjacent GC mostly resembled GC; accordingly, we obtained 107 GIM-adjacent GC (GIM-2) specimens and compared them with GIM diagnosed via routine endoscopy (GIM-1). The ratio of subtype III was significantly higher in GIM-2 than in GIM-1. These results suggest that most GIM-adjacent GC tissues were incomplete GIM (partly secretory acidic sialomucins). Nevertheless, it remains unclear as to whether this type of GIM develops into a malignant state.

Strain variations in *H. pylori*, especially those related to *vacA* and *cagA* virulence factor-encoding genes, have been proposed as a means to identify strains with the highest degree of pathogenicity and consequently, individuals with the highest risk of diseases [20].We analyzed continuously the distribution of *H. pylori* virulence factor in GIM-1, GIM-2, and GC. The results also showed that distribution of *vacA*s1 and *vacA*m2 were higher in GIM-1. On Combining the m-region and s-region, the genotype of *vacA*s1m2 was the predominant factor in GIM-1 patients. However, the predominant virulence genotype of *H. pylori* was the *vacA*s1m1 in GC, along with genotype *vacA*s1m1m2 in the patients of GIM-2. Simultaneously, *cagA*$^+$ was another virulence factor, which was highly expressed during *H. pylori* infection in GIM-1, GIM-2, and GC. The distribution of genotypes in *H. pylori* suggested that the *cagA*$^+$ *vacA*s1m2 genotype was the predominant virulence genotype in GIM-1; however, *cagA*$^+$ *vacA*s1m1 and *cagA*$^+$ *vacA*s1m1m2 were the predominant virulence in GIM-2, only the *cagA*$^+$ *vacA*s1m1 was the primary genotype of GC.

Furthermore, the predominant virulence factors of *H. pylori* in GIM-2 resemble those in GC. *VacA* was present in each *H. pylori* strain. However, the level of cytotoxin production *in vitro* and the risk of disease are related to the strain's particular *vacA* genotype. *VacA*s1 strains secrete larger amounts of

Table 2. *Association between H. pylori-predominant virulence factors and CDX2 expression.*

Virulence factor		GIM-1 CDX2(+)/total N (%)	*P*-value	OR	95%CI	GIM-2 CDX2(+)/total N (%)	*P*-value	OR	95%CI	GC CDX2(+)/total N (%)	*P*-value	OR	95%CI
vacA s1	positive	56/72(77.8)	0.679	0.389	(0.046-3.303)	37/72(51.4)	0.001	0.176	(0.061-0.505)	54/109(49.5)	0.024	0.479	(0.252-0.912)
	negative	9/10(90.0)				30/35(85.7)				43/64(67.2)			
vacA m1	positive	9/11(81.8)	1.000	1.205	(0.235-6.181)	22/40(55.0)	0.208	0.598	(0.267-1.366)	27/64(42.2)	0.005	0.407	(0.216-0.765)
	negative	56/71(78.9)				45/67(67.2)				70/109(64.2)			
vacA m2	positive	28/35(80.0)	0.888	0.925	(0.313-2.733)	15/21(71.4)	0.352	1.635	(0.577-4.628)	18/29(62.1)	0.476	1.346	(0.594-3.593)
	negative	37/47(78.7)				52/86(60.5)				79/144(54.9)			
vacA s1m1	positive	6/8(75.0)	0.754	0.763	(0.140-4.165)	9/26(34.6)	0.001	0.210	(0.082-0.538)	15/44(34.1)	0.001	0.296	(0.144-0.609)
	negative	59/74(79.7)				58/81(71.6)				82/129(63.6)			
vacA s1m2	positive	27/34(79.4)	0.143	1.015	(0.343-3.003)	4/8 (50.0)	0.443	0.571	(0.135-2.424)	6/11(54.5)	0.916	0.936	(0.275-3.193)
	negative	38/48(79.2)				63/99(63.6)				91/162(56.2)			
vacA s1m1m2	positive	14/19(73.7)	0.493	0.659	(0.199-2.186)	10/22(45.5)	0.062	0.409	(0.158-1.062)	15/28(53.6)	0.771	0.886	(0.394-1.997)
	negative	51/63(81.0)				57/85(67.1)				82/145(56.6)			
cagA$^+$	positive	50/62(80.6)	0.588	1.389	(0.422-4.575)	30/60(50.0)	0.002	0.270	(0.114-0.640)	48/101(47.5)	0.007	0.425	(0.226-0.799)
	negative	15/20(75.0)				37/47(78.7)				49/72 (68.1)			

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 with an 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, several data support the premise that an infection with a *cagA*-positive isolate increases the risk of GC [24]. A recent meta-analysis reported that infection with *H. pylori* strains with positive *vacA*s1m1 and the *cagA* genes could significantly increase the risk of GC [25]. We considered that *cagA*$^+$ *vacA*s1m1 positive-GIM could quickly develop into a malignant state.

Further, the distribution of genotypes of *H. pylori* in different subtypes of GIM has shown that the predominant virulence genotypes of *H. pylori* were *vacA*m2 and *vacA*s1m2 in subtype II (incomplete GIM) of GIM-1. However, in GIM-2, *vacA*m1 and *vacA*s1m1 were closely associated with subtypes II and III (incomplete GIM), which resembled those of GC. Therefore, we considered that if GIM tissues were infected by *vacA*s1m1 *H. pylori*, they might closely resemble GC tissues.

Interestingly, the positivity rate of *cagA*$^+$ was high in all subtypes of GIM-1 and GIM-2, with a significant difference only between subtypes I and III of GIM-2; the underlying reason is unclear. Numerous studies have reported that toxin of *cagA*$^+$ is determined based on variation in the number and sequences of Glu-Pro-Tyr-Ala (EPIYA) tyrosine phosphorylation motifs in C-terminal regions, including EPIYA-A, EPIYA-B, EPIYA-C, and EPIYA-D. Varizi et al. [26] reported that the ABCCC type could induce GIM; however, they did not report the subtypes of GIM. Further studies are warranted to explain this phenomenon.

Furthermore, we detected the CDX2 distribution in different subtypes of GIM. CDX2 is an essential biomarker for mature GIM. The results indicate that CDX2 was expressed in the GIM-1, GIM-2, and GC groups. The positivity rate was significantly higher in GIM-1 than in GIM-2 and GC group, with no significant difference between the GIM-2 and GC groups. The GIM-2 group resembled the GC group. Furthermore, CDX2 was prominently expressed primarily in subtype I GIM, especially in GIM-1. Despite the lack of significant differences

among the subtypes of GIM-2, the tendency of CDX2 expression in subtypes of GIM was consistent with that in GIM-1. CDX2 tended to be gradually downregulated. These results show that CDX2 can be assessed to determine the differentiation state of GIM, and GIM with negative CDX2 expression may be malignant. Further, we considered that the genotypes of *H. pylori* played a vital role in the differential regulation of CDX2 expression in the subtypes of GIM-1 and GIM-2.

Then we analyzed the association between CDX2 expression and the genotypes of *H. pylori* in the subtype of GIM. Interestingly, CDX2 expression was not associated with the GIM-1 genotypes. However, *vacA*s1, *vacA*s1m1, and *cagA*$^+$ groups displayed a robust negative association with CDX2 expression in the GIM-2 genotype, similar to that observed in the GC group.

Together, the present results suggest that *vacA*s1m2, *vacA*s1m1 and *cagA*$^+$ were the predominant virulence genotypes of *H. pylori* in subtypes II and III GIM (incomplete one); however, only *vacA*s1m1 and *cagA*$^+$ genotypes of *H. pylori* were negatively associated with CDX2 expression, thereby mostly resembling GC. Further cellular and animal studies are warranted to confirm the present results.

Acknowledgements

This work was supported by the Natural Science Foundation of Liaoning (No.2019-ZD-0319) and the Education Department of Liaoning Province (SYYX202007).

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