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The association of polymorphisms in virulence factor of *Helicobacter pylori* and gastroduodenal diseases in South Korea

한국인에서 ***Helicobacter pylori*** 독성 인자의 유전적 다양성과 소화기 질병의 연관성 연구

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Abstract

Background: *Helicobacter pylori* (*H. pylori*) has been suggested to be associated with development of several gastroduodenal diseases. Clinical outcomes of *H. pylori* infection have been shown to be dependent on the variability of virulence factors in addition to host and environmental factors.

Aims: The aims of this study were to evaluate the prevalence of each virulence factor and the association between polymorphisms in virulence factors of *H. pylori* and clinical outcome of gastroduodenal diseases in South Korea

Methods: Four hundreds and thirteen *H. pylori* colonies were analyzed [78 colonies from 47 controls; 68 colonies from 37 benign gastric ulcer (BGU) patients; 104 colonies from 55 duodenal ulcer (DU) patients; 119 colonies from 75 stomach cancer (SC) patients; 44 colonies from 35 dysplasia patients]. PCR amplifications for *vacA*, *cagA*, *iceA*, *oipA* and *dupA* were performed using DNA extract from *H. pylori* isolates cultured from mucosal biopsy specimens.

Results: Most colonies were composed of *vacA* s1 (100.0%), i1 (98.3%) and m1 (92.7%), *cagA* (85.9%), *iceA*1 (95.6%), *oipA* (89.5%) and *dupA* (90.3%) genotype. *dupA* was expressed more frequent in BGU (98.4%), DU (99.0%) and SC (97.6%) than control (68.8%) (p<.001). Infection by *H. pylori* with *dupA* showed an increased risk of BGU (OR 4.43, 95% CI 4.17-284.33), DU (OR 36.99, 95% CI 4.56-300.10) and SC (OR 16.28, 95% CI 3.44-77.02).

Conclusion: *H. pylori* infection in South Korea appeared to be closely related to highly virulent strains [*vacA* s1/i1/m1, *cagA*(+), *iceA*1(+), *oipA*(+) and *dupA*(+)]. It was considered that *dupA* had intimate association with the development of peptic ulcer disease and stomach cancer.

Keywords: *Helicobacter pylori*; Virulence factors; Polymorphism; *dupA*; Gastroduodenal disease

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Introduction

Helicobacter pylori (*H. pylori*) is a Gram-negative, microaerophilic bacterium possessing a characteristic helix shape which can infect the gastric mucosa.[1] It has been reported that over half of the world's population is infected by *H. pylori*[2] and this infection persists, if no appropriate treatment is provided.[3] *H. pylori* infection is associated with several gastroduodenal diseases, such as gastritis, benign gastric ulcer (BGU), duodenal ulcer (DU) and stomach cancer.[4] It is considered that gastroduodenal diseases were developed through mucosal inflammation where virulence factors of *H. pylori* played an important role in its induction.[5] However, only about 10% of infected individuals develop severe diseases and *H. pylori* infection does not lead to identical diseases.[6] It has been suggested that these were associated with bacterial and host factors, especially, the diversity of bacterial virulent factors.[7]

Several virulence factors were identified and their mechanism and relationship to the gastroduodenal diseases were evaluated. However, effect of these virulence factors on gastroduodenal diseases was controversial based on geographic differences, especially between Western and Eastern areas. The cytotoxin-associated gene A (*cagA*) and vacuolating cytotoxin gene (*vacA*) were the most extensively studied *H. pylori* virulence factor. Several studies have led to an increasing knowledge of *cagA* as a bacteria-derived pathogen and carcinogen in particular in Western countries.[8] In Asia however, it is difficult to explain that the presence of *cagA* gene alone simply gives rise to diseases, as most strains of *H. pylori* have *cagA* gene.[9]

Recently, other novel virulence factors were also studied. The induction by contact with epithelium gene (*iceA*) has two main allelic variants, *iceA*1 and *iceA*2.[10] *iceA*1 demonstrated sequence homology with a gene from *Neisseria*

lactamica which encodes a CTAG-specific restriction endonuclease, but *iceA*2 had no such homology and its function remains unclear.[11] It was reported that *iceA*1 strains were significantly associated with peptic ulceration and increased mucosal concentrations of IL-8.[11]

The outer inflammatory protein gene (*oipA*), which encodes one of the outer membrane proteins is an inflammation-related gene located approximately 100 kb from the *cag* PAI on the *H. pylori* chromosome.[12] Recent studies revealed that *oipA* has a function of inducing inflammation and actin dynamics through the phosphorylation of multiple signaling pathways that usually interact with *cag* PAI-related pathways.[13, 14] It was reported that *oipA* functional status was related to clinical presentation, *H. pylori* density and gastric inflammation and *cag* PAI, or *vacA* status appear important as surrogate markers for a functional *oipA* gene.[15]

In 2005, novel virulence factor, duodenal ulcer promoting gene (*dupA*), which was located in the plasticity region of the *H. pylori* genome was identified.[16] It was suggested that *dupA* is associated with an increased IL-8 production by the gastric epithelial cells.[17] It was also reported that *dupA* is associated with an increasing risk of developing DU and protective effect on gastric atrophy, intestinal metaplasia and gastric cancer.[16] Recently, *dupA* is receiving attention for its association which the development of gastroduodenal diseases[18] and being an independent risk factor for eradication failure.[19] But, dupA was never studied in South Korea, before.

In South Korea, prevalence and clinical burden of *H. pylori* infection and the diseases associated with *H. pylori* infection, such as stomach cancer were considerable.[20, 21] However, only a few studies have reported about virulence factors of *H. pylori*. The aims of the present study were to evaluate the prevalence of each virulence factor and to evaluate the effect of polymorphisms in virulence

factor of *H. pylori* based on the clinical outcome of gastroduodenal diseases in South Korea.

Materials and Methods

Patients

The present study was conducted prospectively. Two hundred forty-nine patients (159 men and 90 women; mean age, 54.3 years) who registered at Seoul National University Bundang Hospital from July 2003 to February 2012 with a positive culture result (CLO testing, Delta-West, Bentley, Australia) and histology finding (modified Giemsa staining) for *H. pylori* infection were enrolled. We enrolled the subjects who wanted to undergo endoscopy because of their symptoms or family history of gastroduodenal diseases, such as stomach cancer, and had no histological diseases as control group. BGU, DU, stomach cancer and dysplasia were determined by histological diagnosis. The clinical statuses of subjects were as follows: control in 47, BGU in 37, DU in 55, stomach cancer in 75, and dysplasia in 35. Patients who had taken nonsteroidal anti-inflammatory drugs or acid-suppressing drugs within 4 weeks of endoscopy were excluded, as were those who had previously undergone *H. pylori* eradication therapy. The characteristics of the patients that we analyzed were described in Table 1. All patients provided informed consent, and the ethics committee of Seoul National University College of Medicine approved the study protocol.

Table 1 Characteristics of patients

	Control	BGU	DU	Stomach cancer	Dysplasia	Total
No. of patients	47	37	55	75	35	249
Gender (male : female)	33 :45	55 : 13	74 : 30	75 :44	31 : 13	268 : 145
Mean age (years± SD)	52.3 ± 9.9	55.1 ±11.9	47.9 ± 15.6	58.6 ± 12.7	60.5 ± 8.2	54.3 ± 13.2
No. of *H. pylori* isolates in the body (mean no. per host)	78 (1.66)	68 (1.84)	104 (1.89)	119 (1.59)	44 (1.26)	413 (1.66)

No., number; SD, standard deviation; BGU, benign gastric ulcer; DU, duodenal ulcer; *H. pylori*, *Helicobacter pylori*

Cell culture of *H. pylori* and DNA extraction from *H. pylori* isolates

A total of 413 colonies [control 78 colonies from 47 patients, BGU 68 colonies from 37 patients, DU 104 colonies from 55 patients, stomach cancer 119 colonies from 75 patients and dysplasia 44 colonies from 35 patients] were analyzed. These colonies were obtained from gastric antrum and body mucosa. The biopsy specimens from gastric mucosa were cultured at 37℃ on brain heart infusion (Difco Laboratories, Detroit, MI) plates containing 7% horse blood under microaerobic conditions (5% O2; 10% CO2; 85% N2) for three to five days. Organisms were identified as *H. pylori* by gram staining, colony morphology, and by positive oxidase, catalase, and urease reactions.

To extract genomic DNA from these 'single-colony isolates' (1-8 *H. pylori* isolates per host), we used phenol-chloroformisoamyl alcohol, and precipitated by adding isopropanol and cold ethanol. DNA pellets were washed in 70% ethanol and dissolved in Tris-EDTA buffer (pH 8.0) after centrifugation (14,000 rpm for 10 minutes). Supernatants were stored at -20℃ until required for polymerase chain reaction (PCR) amplification. Great care was taken at all times to prevent cross-contamination.

PCR amplification from *H. pylori* isolate

PCR amplifications for *cagA*, *vacA*, *iceA*, and *oipA* were performed in 50 μL solution containing 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 1.5-2.5 mmol/L MgCl2, 200 μmol/L deoxynucleoside triphosphatase, 0.25 U of AmpliTaq Gold, and 25 pmol of both forward and reverse primers, using previously described methods.[9, 10, 12, 16, 22, 23] PCR for the *cag* empty site was used to confirm the absence

of the entire *cag* PAI.[24] The primers used for PCR amplification and for direct sequencing of the entire coding regions of *cagA*, *vacA*, *iceA*, *oipA* and *dupA* are described in Table 2.

To determine the sensitivities of the PCR methods used, we processed and evaluated all specimen for the presence of *Helicobacter* genus-specific 16S ribosomal DNA (446 bp) (forward primer, 5'-CTGGAGAGACTAAGCCCTCC-3'; reverse primer, 5'-AGGATCAAGGTTTAAGGATT-3') and the *ureA* gene (411 bp) (forward primer, 5'-GCCAATGGTAAATTAGTT-3'; reverse primer, 5'-CTCCTTAATTGTTTTTAC-3') sequences by PCR, as described previously.[25] It was found that all specimens were positive for *Helicobacter* genus-specific 16S rDNA and the *ureA* gene. *Enterobacter coli* and *H. pylori*-negative subjects (negative for IgG anti-*H. pylori*, and negative by the CLO test and by histologic exam) were used as negative controls to ascertain the specificities of the PCR methods.[26] All primers were confirmed to be specific for *H. pylori*. To reduce false-positive results, separate pipettes were used for PCR work-ups and for post-PCR work, reagents were divided into aliquots, and disposable gloves were worn throughout.[27]

Table 2 PCR primers used to amplify the *cagA*, *vacA*, *iceA*, *oipA* and *dupA* sequences

Region amplified	Primer	Primer sequence(5'→3')[a]	Size (bp) of PCR product	Reference
cagA	CAGAF	GATAACAGGCAAGCTTTTGAGG	349 (1228-1576[b])	9
	CAGAR	CTGCAAAAGATTGTTTGGCAGA		
vacA s1	VA1-F	ATGGAAATACAACAAACACAC	259 (767-1055[c])	23
	VA1-R	CTGCTTGAATGCGCCAAAC		
vacA s2	VA1-F	ATGGAAATACAACAAACACAC	286 (284-569[d])	23
	VA1-R	CTGCTTGAATGCGCCAAAC		
vacA s1a	SAI-F[e]	TCTYGCTTTAGTAGGAGC	212 (844-1055[e])	9
vacA s1b	SS3-F[e]	AGCGCCATACCCCAAGAG	187[f]	40
vacA s1c	SIC-F[e]	CTYGCTTTAGTRGGGYTA	213[f]	9
vacA i1	VacF1	GTTGGGATTGGGGGAATGCCG	426 (1131-1151[c])	39
	C1R	TTAATTTAACGCTGTTTGAAG	426 (1536-1556[c])	
vacA i2	VacF1	GTTGGGATTGGGGGAATGCCG	432 (1131-1151[c])	39
	C2R	GATCAACGCTCTGATTTGA	432 (1120-1138[d])	
vacA m1	VAG-F	CAATCTGTCCAATCAAGCGAG	570 (2071-2640[c])	23
	VAG-R	GCGTCTAAATAATTCCAAGG		
vacA m2	VAG-F	CAA/TCTGTCCAATCAAGCGAG	645 (639-1283[d])	23
	VAG-R	GCGTCTAAATAATTCCAAGG		
*iceA*1	IceA1F	GTGTTTTTAACCAAAGTATC	247 (857-1103[g])	10
	IceA1R	CTATACCCASTYTCTTTGCA		
*iceA*2	IceA2F	GTTGGGTATATCACAATTTAT	229 or 334[f]	10
	IceA2R	TTRCCCTATTTTCTAGTAGGT		

oipA	OipA-F	CAAGCGCTTAGATAGGC	427 (45-882)	12
	OipA-R	AAGGCATTTTCTGCTGAA		
dupA 0917	JHP917(+)	TGGTTTCTACTGACAGAGCGC	307[f]	16
	JHP917(-)	AACACGCTGACAGGACAATCTCCC		
dupA 0918	JHP918(+)	CCTATATCGCTAACGCGCGCTC	276[f]	
	JHP918(-)	AAGCTGAAGCGTTTGTAACG		

[a]Y is C or T, M is A or C, S is C or G, and R is A or G.

[b]Nucleotide positions in the *cagA* gene of *H. pylori* ATCC 53726 (GenBank accession no. L117714).

[c]Nucleotide positions in the *vacA* gene of *H. pylori* 60190 (GenBank accession no. U05676).

[d]Nucleotide positions in the *vacA* gene of *H. pylori* Tx30a (GenBank accession no. U29401).

[e]Used with primer VA1-R.

[f]No published gene coordinates are available for these strains.

[g]Nucleotide positions in the *iceA* gene of *H. pylori* 60190 (GenBank accession no. U43917).

Statistical analysis

SPSS for Windows (version 18.0; SPSS, Chicago, IL, USA) was used for all statistical analyses. The χ2-test or Fisher's exact test were used for the analysis of categorical variables, such as gender, virulence factors. Continuous variables were analyzed using Student's *t*-test or one-way ANOVA, such as age. Univariate and multivariate analysis by multinomial logistic regression were used for the analysis of risk factors, and the results were expressed as odds ratios (ORs) and 95% confidence intervals (CI). Age, gender and the virulence factors with statistical significance in univariate analysis were analyzed for multivariate analysis. All results were considered statistically significant when *p*-values were < .05.

Results

Frequency of virulence factors

Positivity of *vacA*, *iceA*, *cagA*, *oipA* and *dupA* subtypes were described in Table 3. All colonies had *vacA* s1 (100.0%) in South Korea. According to the subtypes of *vacA* s1, s1a was detected more frequently in BGU (94.1%, $p = .041$) than control (82.1%). Most colonies had *vacA* m1 (total 92.7%, control 91.0%, BGU 98.5%, DU 85.6%, stomach cancer 96.6%, and dysplasia 93.2%) and it was not statistically different. *vacA* i1 was also dominant among all diseases (total 98.3%, control 96.2%, BGU 100.0%, DU 100.0%, stomach cancer 100.0%, and dysplasia 90.9%).

The *cagA* gene was detected around 85% among all diseases and there was no statistical significance among the diseases. Almost all patients with *cagA* had EPIYA-D. *iceA*1 was dominant among all diseases (total 95.6%), but *iceA*2 was detected significantly higher in stomach cancer (63.2%, $p = .034$) and dysplasia (70.5%, $p=.023$) than control (48.7%). *oipA* and *dupA* were also expressed in most strains. In colonies with DU (94.9%, $p = .003$) and stomach cancer (94.9%, $p = .002$), there were more frequent *oipA* expressions than control (80.8%). *dupA* was also significantly higher in BGU (98.4%, $p < .001$), DU (99.0%, $p < .001$) and stomach cancer (97.6%, $p < .001$) than control (68.8%).

Table 3 Positivity of *vacA, cagA, iceA, oipA* and *dupA* of *Helicobacter pylori* (*H. pylori)* in 413 colonies

	Control (n = 78*)	**BGU (n = 68*)**	**DU (n = 104*)**	**Stomach cancer (n = 119*)**	**Dysplasia (n = 44*)**	**Total (N = 413*)**	***p*-value**
vacA s1 (%)	78/78 (100.0)	68/68 (100.0)	104/104 (100.0)	119/119 (100.0)	44/44 (100.0)	413/413 (100.0)	.999
s1a	64/78 (82.1)	**64/68 (94.1)**†	85/101 (81.7)	106/119 (89.1)	39/44 (88.6)	358/413 (86.7)	.105
s1b	10/78 (12.8)	5/68 (7.4)	15/104 (14.4)	17/119 (14.3)	8/44 (18.2)	55/413 (13.3)	.522
s1c	70/78 (89.7)	55/68 (80.9)	85/104 (81.7)	111/119 (93.3)	38/44 (86.4)	359/413 (86.9)	.051
vacA m1	71/78 (91.0)	67/68 (98.5)	89/104 (85.6)	115/119 (96.6)	41/44 (93.2)	383/413 (92.7)	**.006**
vacA m2	5/78 (6.4)	1/68 (1.5)	13/104 (12.5)	4/119 (3.4)	3/44 (6.8)	26/413 (6.3)	**.024**
vacA i1	75/78 (96.2)	68/68 (100.0)	103/103 (100.0)	115/115 (100.0)	40/44 (90.9)	401/408 (98.3)	**<.001**
vacA i2	11/78 (14.1)	15/68 (22.1)	13/103 (12.6)	18/115 (15.7)	3/44 (6.8)	60/408 (14.7)	.233
cagA	41/49 (83.7)	42/53 (79.2)	65/76 (85.5)	81/87 (93.1)	21/26 (80.8)	250/291 (85.9)	.168
EPIYA-C	1/32 (3.1)	0/27 (0.0)	3/40 (7.5)	2/70 (2.9)	0/20 (0.0)	6/189 (3.2)	.406
EPIYA-D	31/32 (96.9)	27/27 (100.0)	37/40 (92.5)	69/71 (97.2)	20/20 (100.0)	184/190 (96.8)	.403
*iceA*1	75/76 (98.7)	65/68 (95.6)	96/102 (94.1)	111/117 (94.1)	41/43 (95.3)	388/406 (95.6)	.665
iceA2	38/78 (48.7)	28/68 (41.2)	48/104 (46.2)	**74/117 (63.2)**†	**31/44 (70.5)**†	219/411 (53.3)	**.002**
oipA	63/78 (80.8)	60/68 (88.2)	**97/102 (94.9)**†	**112/118 (94.9)**†	35/44 (79.5)	367/410 (89.5)	**.001**
dupA	44/64 (68.8)	**60/61 (98.4)**†	**99/100 (99.0)**†	**81/83 (97.6)**†	15/23 (65.2)	299/331 (90.3)	**<.001**

*Total number of each group

$^{\dagger}p < .05$, comparing with control group

Missing values are not included. Each number behind the dash is the total number of colonies which were analyzed.

BGU, benign gastric ulcer; DU, duodenal ulcer; *H. pylori*, *Helicobacter pylori*; EPIYA-C, Western-type *cagA*; EPIYA-D, East-Asian-type *cagA*

In the present study, multiple genotypes of virulence factors were found in some of the identical colonies as we identified the positivity of each factor. Hence we also checked subtypes of *vacA* and *iceA*. The most common subtype of *vacA* s1 was s1a-s1c among all diseases. s1a-s1c was significantly frequent in BGU (73.5%, $p = .024$) than control (59.0%). Most colonies had *vacA* m1 (total 92.7%) and *vacA* i1 (total 83.6%). There was no colony with only *vacA* i2 expression in South Korea. *vacA* s1i1m1 was detected in approximately 80% of the colonies and it was the most frequent genotype in South Korea. *vacA* s1i1m1 was significantly more frequent in colonies within stomach cancer (80.9%, $p = .038$) than control (74.4%). In case of *iceA* gene, most colonies showed *iceA*1 or *iceA*1 and *iceA*2 simultaneously, and only around 5% showed *iceA*2 itself (Table 4).

Table 4 *vacA* and *iceA* subtypes of *Helicobacter pylori* (*H. pylori)* in 413 colonies

	Control (**n = 78**[*])	**BGU** (**n = 68**[*])	**DU** (**n = 104**[*])	**Stomach cancer** (**n = 119**[*])	**Dysplasia** (**n = 44**[*])	**Total** (**N = 413**[*])
vacA s1 (%)						
a	8/78 (10.3)	9/68 (13.2)	12/104 (11.5)	6/119 (5.0)	3/44 (6.8)	38/413 (9.2)
b	-	-	-	-	-	-
c	14/78 (17.9)	**4/68 (5.9)**[†]	19/104 (18.3)	11/119 (9.2)	5/44 (11.4)	53/413 (12.8)
a-b	-	4/68 (5.9)	7/104 (6.7)	2/119 (1.7)	3/44 (6.8)	16/413 (3.9)
a-c	46/78 (59.0)	**50/68 (73.5)**[†]	58/104 (55.8)	85/119 (71.4)	28/44 (63.6)	267/413 (64.6)
others	10/78 (12.8)	1/68 (1.5)	8/104 (7.7)	15/119 (12.6)	5/44 (11.4)	39/413 (9.4)
vacA m						
m1	71/78 (91.0)	67/68 (98.5)	89/104 (85.6)	115/119 (96.6)	41/44 (93.2)	383/413 (92.7)
m2	5/78 (6.4)	1/68 (1.5)	13/104 (12.5)	4/119 (3.4)	3/44 (3.4)	26/413 (6.3)
m1&m2	-	-	-	-	-	-
None	2/78 (2.6)	-	2/104 (1.9)	-	-	4/413 (1.0)
vacA i						
i1	64/78 (82.1)	53/68 (77.9)	90/103 (87.4)	97/115 (84.3)	37/44 (84.1)	341/408 (83.6)
i2	-	-	-	-	-	-
i1&i2	11/78 (14.1)	15/68 (22.1)	13/103 (12.6)	18/115 (15.7)	3/44 (6.8)	60/408 (14.7)

None	3/78 (3.8)	-	-	-	4/44 (9.1)	7/408 (1.7)
vacA						
s1i1m1	58/78 (74.4)	53/68 (77.9)	80/104 (76.9)	**93/115 (80.9)**[†]	36/44 (81.8)	320/409 (78.2)
s1i1m2	4/78 (5.1)	-	9/104 (8.7)	4/115 (3.5)	1/44 (2.3)	18/409 (4.4)
s1i1&i2m1	9/78 (11.5)	**14/68 (20.6)**[†]	9/104 (8.7)	18/115 (15.7)	1/44 (2.3)	51/409 (12.5)
s1i1&i2m2	2/78 (2.6)	1/68 (1.5)	4/104 (3.8)	-	2/44 (4.5)	9/409 (2.2)
others	5/78 (6.4)	-	2/104 (1.9)	-	4/44 (9.1)	11/409 (2.7)
iceA						
1	38/76 (50.0)	40/68 (58.8)	54/102 (52.9)	42/115 (36.5)	**10/43 (23.3)**[†]	184/404 (45.5)
2	1/76 (1.3)	3/68 (4.4)	6/102 (5.9)	6/115 (5.2)	-	16/404 (4.0)
1&2	37/76 (48.7)	25/68 (36.8)	42/102 (41.2)	67/115 (58.3)	**32/43 (74.4)**[†]	203/404 (50.2)
none	-	-	-	-	1/43 (2.3)	1/404 (0.2)

[*]Total number of each group

[†]$p < .05$, comparing with control group

Missing values are not included. Each number behind the dash is the total number of colonies which were analyzed.

BGU, benign gastric ulcer; DU, duodenal ulcer; *H. pylori*, *Helicobacter pylori*

Association with virulence factors and clinical outcome

In Table 5, univariate analysis of risk factors for the development of gastroduodenal diseases was described. There were differences in age and gender among the groups, hence these variables were adjusted in multivariate analysis. Multivariate analysis showed that *vacA* s1a genotype increases risk of BGU (OR 3.89, 95% CI 1.03-14.66, $p = .044$). *dupA* was significantly associated with increased risk of BGU (OR 4.43, 95% CI 4.17-284.33 $p = .001$), DU (OR 36.99, 95% CI 4.56-300.10, $p = .001$) and stomach cancer (OR 16.28, 95% CI 3.44-77.02, $p < .001$) (Table 6).

Table 5 Univariate analysis of risk factors for development of gastroduodenal diseases

	Control (n = 78*)	BGU (n = 68*)			DU (n = 104*)			Stomach cancer (n = 119*)			Dysplasia (n = 44*)		
		n	OR (95% CI)	*p*	n	OR (95% CI)	*p*	n	OR (95% CI)	*p*	n	OR (95% CI)	*p*
Gender male	33/78 (42.3)	**55/68 (80.9)**	**5.77 (2.72-12.25)**	**.001**	**74/104 (71.2)**	**3.36 (1.81-6.24)**	**<.001**	**75/119 (63.0)**	**2.32 (1.30-4.16)**	**.005**	**31/44 (70.5)**	**3.25 (1.48-7.15)**	**.003**
Age	78/78 (100.0)	68/68 (100.0)	1.02 (0.99-1.04)	.254	**104/104 (100.0)**	**0.97 (0.95-1.00)**	**.023**	119/119 (100/0)	**1.04 (1.02-1.06)**	**.002**	**44/44 (100.0)**	**1.06 (1.02-1.09)**	**.001**
vacA s1	78/78 (100.0)	68/68 (100.0)	-	-	104/104 (100.0)	-	-	119/119 (100.0)	-	-	44/44 (100.0)	-	-
s1a	64/78 (82.1)	**64/68 (94.1)**	**3.50 (1.09-11.21)**	**.035**	85/101 (81.7)	0.98 (0.46-2.10)	.956	106/119 (89.1)	1.78 (0.79-4.03)	.165	39/44 (88.6)	1.71 (0.57-5.11)	.339
s1b	10/78 (12.8)	5/68 (7.4)	0.54 (0.18-1.67)	.283	15/104 (14.4)	1.15 (0.49-2.71)	.756	17/119 (14.3)	1.13 (0.49-2.62)	.770	8/44 (18.2)	1.51 (0.55-4.16)	.425
s1c	70/78 (89.7)	55/68 (80.9)	0.48 (0.19-1.25)	.133	85/104 (81.7)	0.51 (0.21-1.24)	.137	111/119 (93.3)	1.59 (0.57-4.42)	.378	38/44 (86.4)	0.72 (0.23-2.24)	.575
vacA m1	71/78 (91.0)	67/68 (98.5)	6.61 (0.79-55.13)	.081	89/104 (85.6)	0.59 (0.23-1.51)	.269	115/119 (96.6)	2.84 (0.80-10.03)	.106	41/44 (93.2)	1.35 (0.33-5.50)	.678
vacA m2	5/78 (6.4)	1/68 (1.5)	0.22 (0.03-1.91)	.169	13/104 (12.5)	2.09 (0.71-6.12)	.181	4/119 (3.4)	0.51 (0.13-1.95)	.324	3/44 (6.8)	1.07 (0.24-4.70)	.930
vacA i1	75/78 (96.2)	68/68 (100.0)	1.46E9	.999	103/103 (100.0)	2.22E9	.999	115/115 (100.0)	2.48E9	.999	40/44 (90.9)	0.40 (0.09-1.88)	.245
vacA i2	11/78 (14.1)	15/68 (22.1)	1.72 (0.73-4.06)	.213	13/103 (12.6)	0.88 (0.37-2.09)	.771	18/115 (15.7)	1.13 (0.50-2.55)	.768	3/44 (6.8)	0.45 (0.12-1.69)	.235
cagA	41/49 (83.7)	42/53 (79.2)	0.75 (0.27-2.04)	.567	65/76 (85.5)	1.15 (0.43-3.11)	.778	81/87 (93.1)	2.63 (0.86-8.10)	.091	21/26 (80.8)	0.82 (0.24-2.82)	.752
*iceA*1	75/76 (98.7)	65/68 (95.6)	0.29 (0.03-2.85)	.287	96/102 (94.1)	0.21 (0.03-1.81)	.157	111/117 (94.1)	0.25 (0.03-2.09)	.119	41/43 (95.3)	0.27 (0.02-3.11)	.296

*iceA*2	38/78 (48.7)	28/68 (41.2)	0.74 (0.38-1.42)	.362	48/104 (46.2)	0.90 (0.50-1.62)	.732	**74/117 (63.2)**	**1.81 (1.01-3.24)**	**.045**	**31/44 (70.5)**	**2.51 (1.15-5.51)**	**.022**
oipA	63/78 (80.8)	60/68 (88.2)	1.79 (0.71-4.52)	.221	**97/102 (94.9)**	**4.62 (1.60-13.34)**	**.005**	**112/118 (94.9)**	**4.44 (1.64-12.03)**	**.003**	35/44 (79.5)	0.93 (0.37-2.33)	.870
dupA	44/64 (68.8)	**60/61 (98.4)**	**27.27 (3.53-210.94)**	**.002**	**99/100 (99.0)**	**45.00 (5.85-345.91)**	**<.001**	**81/83 (97.6)**	**18.41 (4.11-82.44)**	**<.001**	15/23 (65.2)	0.85 (0.31-2.34)	.756

*Total number of each group

Missing values were not included. Each number behind the dash is the total number of colonies which were analyzed.

Bold style indicates statistical significance.

OR, odds ratio; CI, confidence interval; BGU, benign gastric ulcer; DU, duodenal ulcer

Table 6 Multivariate analysis of risk factors for development of gastroduodenal diseases

Disease	Factor	OR (95% CI)	*p*
BGU			
	***vacA* s1a**	**3.89 (1.03-14.66)**	**.044**
	dupA	**4.43 (4.17-284.33)**	**.001**
DU			
	dupA	**36.99 (4.56-300.10)**	**.001**
Stomach cancer			
	dupA	**16.28 (3.44-77.02)**	**<.001**

Age and gender were adjusted. The factors with $p < .05$ in univariate analysis were analyzed by multinomial logistic regression.

OR, odds ratio; CI, confidence interval; BGU, benign gastric ulcer; DU, duodenal ulcer; *H. pylori*, *Helicobacter pylori*

Discussion

The present study showed that *H. pylori* infection was strongly associated with gastroduodenal diseases and that highly virulent strains were dominant in South Korea. Considering these results, *H. pylori* infection is still important in the development of gastroduodenal diseases in South Korea. However, controversy exists in the association between the polymorphism of bacterial virulence factors and the clinical outcomes.

vacA s1 was expressed in all colonies in the present study. It was known that *vacA* s1 is more frequent in Asia than Western countries[28, 29] and some reports had shown that almost all patients had *vacA* s1 in Asia.[30, 31] In this study, *vacA* s1a-s1c was the most common subtype in South Korea, and considering the positivity of genotype, *vacA* s1c and s1a were the major genotypes. It was reported that *vcaA* s1c was the major subtype in East Asia, s1a in Europe, and s1b in America.[32] In the present study, wide diversity was observed in *vacA* s1 and this could possibly be a technical error because of the similar nature of PCR primers. And this might support that multiple strains could be infected in Korea.[33, 34] and other countries.[35] *vacA* s1a was expressed in around 85% of colonies, and its presence was associated with an increased risk of BGU. It was also known that *vacA* s1a was more associated with enhanced gastric inflammation and gastroduodenal diseases,[36] and there were some variations at several loci and amino acid sequences among s1a, s1b and s1c.[37] The present study was consistent with the previous study, but further studies will be required to clarify the diversity of the *vacA* s1 region.

vacA m1 was a dominant genotype as suggested in previous studies in South Korea.[38] *vacA* i1 was also dominant, but strains with *vacA* i2 only were not found in the present study. Strains which expressed *vacA* i1 and i2 simultaneously, were detected in about 15% of the colonies. This result corresponded to the previous

studies which identified a recombinant i1-i2 allele with a cluster specific combination.[29, 39] *vacA* m1 and *vacA* i1 strains are known for increasing the risk of gastroduodenal diseases in Western areas,[39, 40] but there was no such association in Asia.[22] In the present study, *vacA* m1 and *vacA* i1 were observed in almost all colonies, and their association with gastroduodenal diseases was not found. This result might be an account of the high positivity of *vacA* s1, m1 and i1. This needs to be interpreted with caution because there were no strains that expressed *vacA* i2 only, but there were strains with i1 and i2 simultaneously.

It was known that around 90% of patients with *H. pylori* infection have *cagA* in South Korea.[11,41] The prevalence of *cagA* gene was around 85% in the present study, which could suggest that *cagA* gene was not detected in some strains. Furthermore, the positivity of *cagA* was not associated with BGU, DU, or stomach cancer. Therefore, this result supports a number of previous reports found that *cagA* has no effect on gastroduodenal diseases in Asia.[38, 42]

The positivity of *iceA*1 detected was more than 95% and *iceA*2 was variable from 40% to 70% in this study. This was more frequent than the result of previous studies which only found approximately 15% in South Korea.[9, 33] In addition, strains expressed with *iceA*2 alone were only about 5% and most strains were detected with *iceA*1 along with *iceA*2. It was identified in previous studies that strains which carry both *iceA*1 and *iceA*2 genes simultaneously were 15 to 30%[43, 44] compared to this study which reported up to 70%.[45] This result could support the wide diversity of *H. pylori* infection in South Korea.

In the present study, *oipA* was significantly frequent in DU and SC and increasing the risk of those disease in univariate analysis, but there was no such relationship in multivariate analysis. Those result might be related with the high prevalence of *oipA* itself, almost 90%. The previous report showed that *oipA*

positive status plays a role in the development of DU[46] and stomach cancer.[8, 46]. But in Asia, It was reported that the strains appear to differ greatly from Western strains in outer membrane proteins and it actions.[47] Our result supports the previous study and it could be an evidence of different effect of virulence factor according to geographic difference.

dupA was found in almost all patients among BGU, DU and stomach cancer, and it was identified that *dupA* was associated with an increased risk of these diseases, in the present study. The prevalence of *dupA* itself (total 90.3%) was significantly higher than previous studies' results (20-70%).[48-50] Moreover, the prevalence of *dupA* in the control group (68.8%) was relatively higher than other countries (30-60%).[48-50] In Asia, the prevalence of *dupA* in control was low, and it was high in gastroduodenal diseases, especially in DU. Whereas, the prevalence of *dupA* was generally high in Western area.[18] In the present study, almost all colonies of BGU, DU and stomach cancer were expressed with *dupA* despite the high prevalence of *dupA* in control group. It was supposed that *dupA* might be a fundamental factor for developing gastroduodenal diseases in South Korea.

dupA was a well-known to be a risk factor of DU,[16, 18, 51] but some studies reported that there was no such relationship.[50, 52] A meta-analysis showed that the presence of the *dupA* gene was significantly associated with DU, especially in Asian countries.[18] The authors that explained this discrepancy may be due to the different prevalence of *dupA* gene between Asian and Western countries. Controversy exists in cases of BGU. Some studies reported that it increases the risk of BGU,[53] while some reported reversely[54] or no association.[18] Hence the effect of *dupA* on BGU seems to be unclear. In cases of stomach cancer, the effect of *dupA* was also controversial,[18, 53] but the risk of stomach cancer was decreased with *dupA* in many reports.[54-56] A previous study reported frame-shift mutations in some

dupA-positive samples.[57] Recently, it analyzed a panel of *dupA* polymorphisms and reported that the virulent type of *dupA* (*dupA1*) was linked to the mucosal inflammatory reaction.[17] These different relationships between *dupA* and gastroduodenal diseases in South Korea could be a result of polymorphism of *dupA*. There were only a few papers about the association between *dupA* and gastroduodenal diseases in South Korea, and this study might be important as the first relatively large study which evaluated the effect of *dupA* on diseases in South Korea. However, the effect of *dupA* gene could depend on geographic differences and further investigation about the pathophysiology of these diseases are necessary.

In conclusion, the major genotype of *H. pylori* contained highly virulent strains with *vacA* s1/i1/m1, *cagA* positive, *iceA*1, *oipA* positive and *dupA* positive. *dupA* were considered as strong risk factor of BGU, DU and stomach cancer. *dupA* were demonstrated as an important virulence factor in developing gastroduodenal diseases in South Korea. *vacA* s1a was shown to increased the risk of BGU These results support that there are geographic differences in virulence factors, and their pathogenesis could be different in the development of gastroduodenal diseases, when compared with those in Western areas.

References

1. Blaser MJ. Gastric Campylobacter-like organisms, gastritis, and peptic ulcer disease. *Gastroenterology* 1987;93:371-83.

2. Correa P, Piazuelo MB. Natural history of *Helicobacter pylori* infection. *Dig Liver Dis* 2008;40:490-6.

3. Sherman PM. Appropriate strategies for testing and treating *Helicobacter pylori* in children: when and how? *Am J Med* 2004;117 Suppl 5A:30S-35S.

4. Egan BJ, Marzio L, O'Connor H, O'Morain C. Treatment of *Helicobacter pylori* infection. *Helicobacter* 2008;13 Suppl 1:35-40.

5. Suerbaum S, Michetti P. *Helicobacter pylori* infection. *N Engl J Med* 2002;347:1175-86.

6. Sachs G, Scott DR. *Helicobacter pylori*: Eradication or Preservation. *F1000 Med Rep* 2012;4:7.

7. Yamaoka Y. Mechanisms of disease: *Helicobacter pylori* virulence factors. *Nat Rev Gastroenterol Hepatol* 2010;7:629-41.

8. Franco AT, Johnston E, Krishna U, et al. Regulation of gastric carcinogenesis by *Helicobacter pylori* virulence factors. *Cancer Res* 2008;68:379-87.

9. Yamaoka Y, Kodama T, Gutierrez O, Kim JG, Kashima K, Graham DY. Relationship between *Helicobacter pylori iceA*, *cagA*, and *vacA* status and clinical outcome: studies in four different countries. *J Clin Microbiol* 1999;37:2274-9.

10. Van Doorn LJ, Figueiredo C, Sanna R, et al. Clinical relevance of the *cagA*, *vacA*, and *iceA* status of *Helicobacter pylori*. *Gastroenterology* 1998;115:58-66.

11. Peek RM, Jr., Thompson SA, Donahue JP, et al. Adherence to gastric epithelial cells induces expression of a *Helicobacter pylori* gene, *iceA*, that is associated with clinical outcome. *Proc Assoc Am Physicians* 1998;110:531-44.

12. Yamaoka Y, Kwon DH, Graham DY. A M(r) 34,000 proinflammatory outer

membrane protein (*oipA*) of *Helicobacter pylori*. *Proc Natl Acad Sci U S A* 2000;97:7533-8.

13. Yamaoka Y, Kudo T, Lu H, Casola A, Brasier AR, Graham DY. Role of interferon-stimulated responsive element-like element in interleukin-8 promoter in *Helicobacter pylori* infection. *Gastroenterology* 2004;126:1030-43.

14. Tabassam FH, Graham DY, Yamaoka Y. *OipA* plays a role in *Helicobacter pylori*-induced focal adhesion kinase activation and cytoskeletal re-organization. *Cell Microbiol* 2008;10:1008-20.

15. Yamaoka Y, Kikuchi S, El-Zimaity HM, Gutierrez O, Osato MS, Graham DY. Importance of *Helicobacter pylori oipA* in clinical presentation, gastric inflammation, and mucosal interleukin 8 production. *Gastroenterology* 2002;123:414-24.

16. Lu H, Hsu PI, Graham DY, Yamaoka Y. Duodenal ulcer promoting gene of *Helicobacter pylori. Gastroenterology* 2005;128:833-48.

17. Hussein NR, Argent RH, Marx CK, Patel SR, Robinson K, Atherton JC. *Helicobacter pylori dupA* is polymorphic, and its active form induces proinflammatory cytokine secretion by mononuclear cells. *J Infect Dis* 2010;202:261-9.

18. Shiota S, Matsunari O, Watada M, Hanada K, Yamaoka Y. Systematic review and meta-analysis: the relationship between the *Helicobacter pylori dupA* gene and clinical outcomes. *Gut Pathog* 2010;2:13.

19. Shiota S, Nguyen LT, Murakami K, et al. Association of *Helicobacter pylori dupA* with the failure of primary eradication. *J Clin Gastroenterol* 2012;46:297-301.

20. Yim JY, Kim N, Choi SH, et al. Seroprevalence of *Helicobacter pylori* in South Korea. *Helicobacter* 2007;12:333-40.

21. Bertuccio P, Chatenoud L, Levi F, et al. Recent patterns in gastric cancer: a global overview. *Int J Cancer* 2009;125:666-73.

22. Ogiwara H, Graham DY, Yamaoka Y. *vacA* i-region subtyping. *Gastroenterology* 2008;134:1267; author reply 1268.

23. Yamaoka Y, Kodama T, Kita M, Imanishi J, Kashima K, Graham DY. Relationship of *vacA* genotypes of *Helicobacter pylori* to *cagA* status, cytotoxin production, and clinical outcome. *Helicobacter* 1998;3:241-53.

24. Mukhopadhyay AK, Kersulyte D, Jeong JY, et al. Distinctiveness of genotypes of *Helicobacter pylori* in Calcutta, India. *J Bacteriol* 2000;182:3219-27.

25. Clayton CL, Kleanthous H, Coates PJ, Morgan DD, Tabaqchali S. Sensitive detection of *Helicobacter pylori* by using polymerase chain reaction. *J Clin Microbiol* 1992;30:192-200.

26. Lu JJ, Perng CL, Shyu RY, Chen CH, Lou Q, Chong SK, Lee CH. Comparison of five PCR methods for detection of *Helicobacter pylori* DNA in gastric tissues. *J Clin Microbiol* 1999;37:772-4.

27. Kwok S, Higuchi R. Avoiding false positives with PCR. *Nature* 1989;339:237-8.

28. Basso D, Zambon CF, Letley DP, et al. Clinical relevance of *Helicobacter pylori cagA* and *vacA* gene polymorphisms. *Gastroenterology* 2008;135:91-9.

29. Chung C, Olivares A, Torres E, Yilmaz O, Cohen H, Perez-Perez G. Diversity of *vacA* intermediate region among *Helicobacter pylori* strains from several regions of the world. *J Clin Microbiol* 2010;48:690-6.

30. Park SM, Park J, Kim JG, Yoo BC. Relevance of *vacA* genotypes of *Helicobacter pylori* to *cagA* status and its clinical outcome. *Korean J Intern Med* 2001;16:8-13.

31. Chomvarin C, Namwat W, Chaicumpar K, et al. Prevalence of *Helicobacter*

pylori vacA, *cagA*, *cagE*, *iceA* and *babA*2 genotypes in Thai dyspeptic patients. *Int J Infect Dis* 2008;12:30-6.

32. Van Doorn LJ, Figueiredo C, Megraud F, et al. Geographic distribution of *vacA* allelic types of *Helicobacter pylori*. *Gastroenterology* 1999;116:823-30.

33. Kim YS, Kim N, Kim JM, et al. *Helicobacter pylori* genotyping findings from multiple cultured isolates and mucosal biopsy specimens: strain diversities of *Helicobacter pylori* isolates in individual hosts. *Eur J Gastroenterol Hepatol* 2009;21:522-8.

34. Han J, Yu E, Lee I, Lee Y. Diversity among clinical isolates of *Helicobacter pylori* in Korea. *Mol Cells* 1997;7:544-7.

35. Patra R, Chattopadhyay S, De R, et al. Multiple infection and microdiversity among *Helicobacter pylori* isolates in a single host in India. *PLoS One* 2012;7:e43370.

36. Atherton JC, Peek RM, Jr., Tham KT, Cover TL, Blaser MJ. Clinical and pathological importance of heterogeneity in *vacA*, the vacuolating cytotoxin gene of *Helicobacter pylori*. *Gastroenterology* 1997;112:92-9.

37. Van Doorn LJ, Figueiredo C, Sanna R, et al. Expanding allelic diversity of *Helicobacter pylori vacA*. *J Clin Microbiol* 1998;36:2597-603.

38. Kim SY, Woo CW, Lee YM, et al. Genotyping *cagA*, *vacA* subtype, *iceA*1, and *babA* of *Helicobacter pylori* isolates from Korean patients, and their association with gastroduodenal diseases. *J Korean Med Sci* 2001;16:579-84.

39. Rhead JL, Letley DP, Mohammadi M, et al. A new *Helicobacter pylori* vacuolating cytotoxin determinant, the intermediate region, is associated with gastric cancer. *Gastroenterology* 2007;133:926-36.

40. Atherton JC, Cao P, Peek RM, Jr., Tummuru MK, Blaser MJ, Cover TL. Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. Association of

specific *vacA* types with cytotoxin production and peptic ulceration. *J Biol Chem* 1995;270:17771-7.

41. Yang JJ, Cho LY, Ko KP, et al. Genetic susceptibility on *cagA*-interacting molecules and gene-environment interaction with phytoestrogens: a putative risk factor for gastric cancer. *PLoS One* 2012;7:e31020.

42. Miehlke S, Go MF, Kim JG, Graham DY, Figura N. Serologic detection of *Helicobacter pylori* infection with *cagA*-positive strains in duodenal ulcer, gastric cancer, and asymptomatic gastritis. *J Gastroenterol* 1998;33 Suppl 10:18-21.

43. Perng CL, Lin HJ, Sun IC, Tseng GY, Facg. *Helicobacter pylori cagA, iceA* and *vacA* status in Taiwanese patients with peptic ulcer and gastritis. *J Gastroenterol Hepatol* 2003;18:1244-9.

44. Wong BC, Yin Y, Berg DE, et al. Distribution of distinct *vacA*, *cagA* and *iceA* alleles in *Helicobacter pylori* in Hong Kong. *Helicobacter* 2001;6:317-24.

45. Gonzalez-Vazquez R, Herrera-Gonzalez S, Cordova-Espinoza MG, Zuniga G, Giono-Cerezo S, Hernandez-Hernandez JM, Leon-Avila G. *Helicobacter pylori*: Detection of *iceA*1 and *iceA*2 genes in the same strain in Mexican isolates. *Arch Med Res* 2012;43:339-46.

46. Yamaoka Y, Ojo O, Fujimoto S, et al. *Helicobacter pylori* outer membrane proteins and gastroduodenal disease. *Gut* 2006;55:775-81.

47. Kawai M, Furuta Y, Yahara K, et al. Evolution in an oncogenic bacterial species with extreme genome plasticity: *Helicobacter pylori* East Asian genomes. *BMC Microbiol* 2011;11:104.

48. Arachchi HS, Kalra V, Lal B, et al. Prevalence of duodenal ulcer-promoting gene (*dupA*) of *Helicobacter pylori* in patients with duodenal ulcer in North Indian population. *Helicobacter* 2007;12:591-7.

49. Schmidt HM, Andres S, Kaakoush NO, et al. The prevalence of the duodenal

ulcer promoting gene (*dupA*) in *Helicobacter pylori* isolates varies by ethnic group and is not universally associated with disease development: a case-control study. *Gut Pathog* 2009;1:5.

50. Argent RH, Burette A, Miendje Deyi VY, Atherton JC. The presence of *dupA* in *Helicobacter pylori* is not significantly associated with duodenal ulceration in Belgium, South Africa, China, or North America. *Clin Infect Dis* 2007;45:1204-6.

51. Alam J, Maiti S, Ghosh P, et al. Significant association of the *dupA* gene of *Helicobacter pylori* with duodenal ulcer development in a South-east Indian population. *J Med Microbiol* 2012;61:1295-302.

52. Nguyen LT, Uchida T, Tsukamoto Y, et al. *Helicobacter pylori dupA* gene is not associated with clinical outcomes in the Japanese population. *Clin Microbiol Infect* 2010;16:1264-9.

53. Hussein NR. The association of *dupA* and *Helicobacter pylori*-related gastroduodenal diseases. *Eur J Clin Microbiol Infect Dis* 2010;29:817-21.

54. Abadi AT, Taghvaei T, Wolfram L, Kusters JG. Infection with *Helicobacter pylori* strains lacking *dupA* is associated with an increased risk of gastric ulcer and gastric cancer development. *J Med Microbiol* 2012;61:23-30.

55. Queiroz DM, Rocha GA, Rocha AM, et al. *dupA* polymorphisms and risk of *Helicobacter pylori*-associated diseases. *Int J Med Microbiol* 2011;301:225-8.

56. Zhang Z, Zheng Q, Chen X, Xiao S, Liu W, Lu H. The *Helicobacter pylori* duodenal ulcer promoting gene, *dupA* in China. *BMC Gastroenterol* 2008;8:49.

57. Gomes LI, Rocha GA, Rocha AM, et al. Lack of association between *Helicobacter pylori* infection with *dupA*-positive strains and gastroduodenal diseases in Brazilian patients. *Int J Med Microbiol* 2008;298:223-30.

초 록

서론: *Helicobacter pylori* (H. pylori)는 여러 소화기 질병과 연관이 있다고 알려져왔다. *H. pylori* 감염의 임상 결과는 숙주 요인, 환경 요인에 더불어 독성 인자의 다양성에 의거하는 것으로 알려져 있다. 본 연구의 목적은 한국인에서 각 독성 인자의 유병율 확인하고, *H. pylori* 독성 인자의 유전적 다양성과 소화기 질병의 연관성에 대하여 연구하고자 하였다.

방법: 417개의 *H. pylori* 균락[대조군 47명의 78 균락, 양성 위궤양 환자(BGU) 37명의 68 균락, 십이지장 궤양 환자(DU) 55명의 104 균락, 위암 환자(SC) 75명의 119 균락, 이형성증 환자 35명의 44 균락]이 분석되었다. 위 점막 조직으로부터 배양된 *H. pylori*로부터 DNA를 추출하여 *vacA, cagA, iceA, oipA, dupA*에 대하여 PCR을 시행하였다.

결과: 대부분의 균락은 *vacA* s1 (100.0%), i1 (98.3%) and m1 (92.7%), *cagA* (85.9%), *iceA*1 (95.6%), *oipA* (89.5%), *dupA* (90.3%)의 유전자형을 보였다. *dupA* 는 BGU (98.4%)와 DU (99.0%), SC (97.6%)에서 control (68.8%)에 비하여 유의하게 많이 발현되었다(p<.001). *dupA* 가 발현된 *H. pylori* 균락에 감염된 경우 BGU (OR 4.43, 95% CI 4.17-284.33) 및 DU (OR 36.99, 95% CI 4.56-300.10), SC (OR 16.28, 95% CI 3.44-77.02)의 위험도가 증가하는 것으로 확인 되었다.

결론: 우리나라에서 *H. pylori* 감염은 주로 독성이 강한 균주[*vacA* s1/i1/m1, *cagA*(+), *iceA*1(+), *oipA*(+) and *dupA*(+)]에 의해 일어나는 것으로 생각된다. 특히 *dupA*는 한국인에서 소화기 궤양성 질환과 위암의 발생에 중요한 역할을 하는 것으로 판단된다.

주요어: *Helicobacter pylori*, 독성 인자, 유전적 다양성, 소화기 질병, *dupA*

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