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Original Article

Diverse *VacA* Allelic Types of *Helicobacter pylori* in Korea and Clinical Correlation

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Helicobacter pylori has a diversity of *vacA* allelic types. The purpose of this study was to correlate the *vacA* status and the clinical outcome. After constructing specific primers for the *vacA* signal sequence, *H. pylori*-positive antral biopsy specimens were examined for the *vacA* status in 25 gastric ulcers, 31 duodenal ulcers, 22 gastric cancers, 42 chronic gastritis, and 8 gastroduodenal ulcers. The relationship between the *vacA* allele and the clinical disease was examined. The *vacA* genotype s1c/m1 is predominant in Korea (71/128, 55.5%). Other strains including s1b or s2 were not found in this study. s1c/m1 was more prominent in duodenal ulcers, than in gastric ulcers (p=0.041) and cancer (p=0.029). Seven out of 8 patients with gastric and coexistent duodenal ulcers had the s1c/m1 allele. No statistical differences in the positive rates of the s1a/m1, s1a/m2, and s1c/m2 alleles among the disease groups were found. In conclusion, s1c/m1 is the main *vacA* allele in Korea and it is particularly associated with duodenal ulcers.

Key Words: *Helicobacter pylori*, *vacA*, allele, signal sequence, mid-region

INTRODUCTION

Helicobacter pylori is extremely diverse as a species,[1-4] and two distinct genetic loci have been identified during the past decade; the polymorphic gene encoding a cytotoxin (*vacA*), and the cytotoxin-associated gene (*cag*) pathogenicity island, which is often detected by tests for *cagA*, one of its component genes.[5] *vacA* encodes a vacuolating toxin that is excreted by *H. pylori* and damages the epithelial cells.[6,7] The gene consists of two variable parts.[8,9] The s region (signal sequence) encodes the signal peptide and exists as an s1 or s2 allele. s1 has three subtypes: s1a, s1b, and s1c.[10] The m-region (mid-region) occurs as an m1 or m2 allele. The m1 strains are almost always toxigenic, whereas those with an m2 mid-region are rarely toxigenic.[3] The weakly toxigenic m2 strains all have an s1a or s1b signal sequence; the s2/m2 strains have not been found to produce measurable vacuolating activity. Among the m1 strains, those with an s1a signal sequence produce higher vacuolating activity levels than the s1b strains. The s1a/m1 strains are the type found in all toxigenic strains. *cagA* is considered as a marker for the presence of a pathogenicity island of approximately 35 kilobase pairs.[11] Strains that do not produce the *CagA* protein generally lack the entire cag pathogenicity island. van Doorn et al. reported that *cagA* positivity and the *vacA* s1 genotype are associated with peptic ulcer disease.[11] However, data relating *cagA* and/or *vacA* strains to gastroduodenal disease are mainly obtained from North American and Western European studies.[12-14] In Japan, the main genotype is *cagA* positive *vacA* genotype s1/m1 irrespective of the clinical outcome.[15-17] Data from China indicates a high prevalence of *cagA*-positive *H. pylori* populations in Chinese patients with peptic ulcers

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and chronic gastritis,[18] and no association between any *vacA* allele and these diseases was reported.[19]

Most studies in East Asia have used the PCR primers previously made in Western countries, which means that these studies were based on the assumption that the primers for the *vacA* subtypes are the same all over the world. van Doorn et al. investigated the alignment of the partial nucleotide sequences of the *vacA* s region, and reported differences between s2, s1a, s1b, and s1c subtypes between countries.[10] In other words, a certain part of the nucleotide in primers used in such studies might be different from the nucleotide sequence of the indigenous species. Therefore, new s allele primers were constructed and used to determine the correlation between the *vacA* status and the clinical outcome.

MATERIALS AND METHODS

Subjects I

Fifty patients who underwent a gastroduodenoscopy and were diagnosed with a *H. pylori* infection were enrolled in this study. *H. pylori* positivity was defined as a positive CLO test (Delta West, Perth, Australia) plus the identification of the microorganisms by the Giemsa stain. The patients' ages ranged from ten to 18 years.

DNA isolation from gastric biopsy specimens

DNA was isolated from the antral biopsy specimens, which were stored at -70℃. The biopsy specimens were homogenized in 400 μL of a proteinase K solution (10 mmol/L Tris-HCL [pH 8.5], 10 mmol/L ethylenediaminetetraacetic acid, 0.5% sodium dodecyl sulfate, and 0.5 mg/mL proteinase K) and incubated at 55℃ for 1 hour. The DNA was isolated from the homogenate by phenol/chloroform extraction and ethanol precipitation.

PCR amplification

PCR amplification was performed using s region primers described by Atherton;[9] s1, VA1-F 5′ ATGGAAATACAACAAACACAC 3′ and VA1-R 5′ CTGCTTGAATGCGCCAAAC 3′ (259 bp), s2, VA1-F and VA1-R (286 bp), s1a, SS1-F 5′ GTC AGCATCACACCGCAAC 3′ and VA1-R (190 bp), and s1b, SS3-F 5′ AGCGCCATACCGCAAGAG 3′ and VA1-R (187 bp). The PCR reactions were performed using a GeneAmp PCR system 9600 (Perkin Elmer, Norwalk, CT, USA) in a 25 μL volume containing 25 mmol/L *N*-tris [Hydroxymethyl] methyl-3-aminopropanesulfonic acid (pH 9.3 at 25℃), 50 mmol/L KCl, 2.0 mmol/L $MgCl_2$, 200 μmol/L deoxynucleoside triphosphase, 0.25 U of TaKaRa Ex Taq polymerase (TaKaRa Shuzo Co., Shiga, Japan), and 10 pmol of both the forward and reverse primers. The incubation conditions with the primers were as follows: 5-minutes preincubation at 94℃, followed by 30 cycles of 1 minute at 94℃, 1 minute at 59℃, 60 seconds at 72℃, and a final 5-minute incubation at 72℃. Thirty-four patients were found to have the s region.

DNA sequence analysis and new primers

The DNA sequences were determined by the dideoxynucleotide chain-termination method using an ABI PRISM 310 Genetic Analyzer (Perkin Elmer, Norwalk, CT, USA) and a BigDye™ Terminator Cycle Sequencing Kit. The nucleotide and protein sequences of the s regions from the Korean *H. pylori* strains were compared with *H. pylori* U29401, NL3964, U07145, CH3, AU10, PE9012, PO76, PO70, HK43, and JA3, which were described in van Doorn's study.[10] From the comparisons made, the Korean *H. pylori* strains have common, unique parts of the nucleotide sequences, which discriminate them from those of other countries (Fig. 1). New primers (VAS_K-F, VAS_K-R, $S1A_K$-F, and $S1C_K$-F) were designed using these specific parts of the sequences. These are shown in Table 1. The s1b and s2 primers were not made, because neither the s1b nor the s2 strains were found during the course of this study.

Subjects II

One hundred and twenty eight antral biopsy specimens were examined. These were confirmed to be *H. pylori*-positive by two biopsy-related

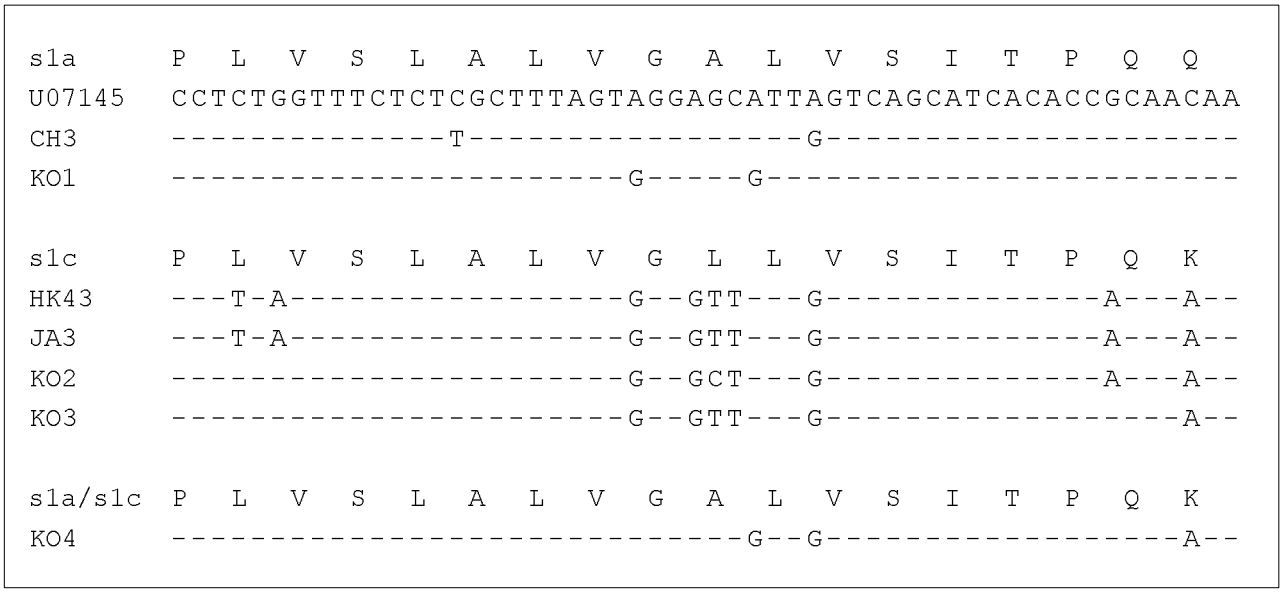

Fig. 1. Divergence of the sequences in the *vacA* s region, showing differences between the s1a, and s1c subtypes. Each sequences begins at position 37 of the *vacA* open reading frame (U29401). KO means Korean strain.

methods; an assay for urease activity and a histological examination. The patients' ages ranged from 9 to 83 years. The clinical status was as follows: 25 with a gastric ulcer, 31 with a duodenal ulcer, 22 with gastric cancer, 42 with chronic gastritis, and 8 with both a gastric and a duodenal ulcer. Gastritis was defined as histological gastritis with no peptic ulcers, or gastric cancer.[20] No subjects had received treatment for *H. pylori* eradication. Informed consent was obtained from all patients or parents. The DNA was isolated from the antral biopsy specimens and PCR amplification was performed using the new s region primers. The DNA sequences were determined using the above method for all 128 samples, and the subtypes of the s region alleles were reconfirmed.

For the mid-region, VAG-F and VAG-R primers, which were described by Yamaoka,[21] were used. CAGF1 and CAGB1 primers were used for *cagA*, which were described by de Jong[22] (Table 1). These are the primers that are used universally for subtyping the mid-region and *cagA* of the Korean *H. pylori* strains.

Statistical analyses

A Chi square test and Fisher's Exact test were used to assess the association between the *vacA* s/m allele types and the clinical groups. Unless stated otherwise, a p value ≤ 0.05 was taken to be significant. All statistical analyses were performed using the SAS statistics software (version 6.12).

RESULTS

The relationship between both the *vacA* alleles and *cagA* status, and the clinical outcome is shown in Table 2. The *vacA* genotype s1c/m1 proved to be predominant in Korea (71/128, 55.5%). s1a/m1 was found in 21.9%, the s hybrid mixed with s1a and s1c were found in 9.4%, s1c/m2 in 7.8%, and s1a/m2 in 5.4%. Strains including s1b or s2 were not found in this study. s1c/m1 was more prevalent in duodenal ulcer patients, than in either gastric ulcer (p=0.041) or cancer patients (p=0.029) (Fig. 2). The prevalence of s1c/m1 in the duodenal ulcer group was higher than that in gastritis group, but this was not statistically significant. In the cancer group, s1c/m1 was found in 40.9%, s1c/m2 in 18.2%, and the s hybrid in 18.2%. Seven out of 8 patients with gastric and coexistent duodenal ulcers had the s1c/m1 allele. No statistical differences in the positive rates of the s1a/m1, s1a/m2, and s1c/m2

alleles were found among the disease groups. The positive rates of *cagA* were found to be high in all disease groups, varying from 84% to 100%. The mean *cagA* prevalence rate was 91.4%.

DISCUSSION

This study showed that s1c/m1 is the major type of *vacA* allele in Korea and that the s1c/m1

Table 1. PCR Primers Used for Typing and Amplifying the *H. pylori vacA* and *cagA* Sequences

Region amplified	Primer	Primer sequence	Size (bp) of PCR product
vacA s1	VAS_K-F	5′ MTKRTTTCTCTCGCTTT 3′	271 (445-715[a])
	VAS_K-R	5′ GGGATYTGTATAAGTCGTATT 3′	[s2, 298 (385-682[b])]
vacA s1a	$S1A_K$-F	5′ TCTYGCTTTAGTAGGAGC 3′	212 (453-664[a])
	VA1-R	5′ CTGCTTGAATGCGCCAAAC 3′	
vacA s1c	$S1C_K$-F	5′ TTAGTTTCTCTCGCTTTAGTRGGGYT 3′	220[c]
	VA1-R	5′ CTGCTTGAATGCGCCAAAC 3′	
vacA m1	VAG-F	5′ CAATCTGTCCAATCAAGCGAG 3′	570 (2071-2640[d])
	VAG-R	5′ GCGTCTAAATAATTCCAAGG 3′	
vacA m2	VAG-F	5′ CAATCTGTCCAATCAAGCGAG 3′	645 (639-1283[e])
	VAG-R	5′ GCGTCTAAATAATTCCAAGG 3′	
cagA	CAGF1	5′ GATAACAGGCAAGCTTTTGAGG 3′	349 (1228-1576[f])
	CAGB1	5′ CTGCAAAAGATTGTTTGGCAGA 3′	

M is A or C, K is G or T, R is A or G, and Y is C or T.
[a]Corresponding nucleotide positions in the *vacA* of *H. pylori* U07145.
[b]Corresponding nucleotide positions in the *vacA* of *H. pylori* U29401.
[c]No published coordinates for genes in strains of these types.
[d]Nucleotide positions in the *vacA* of *H. pylori* 60190.
[e]Nucleotide positions in the *vacA* of *H. pylori* Tx30a.
[f]Nucleotide positions in the *vacA* of *H. pylori* ATCC 53726.

Table 2. Relationship between the *vacA* Alleles and *cagA* Status, and the Clinical Outcome

	No.(%) of strains with the following genotype									Total
	s1a/m1	s1b/m1	s1c/m1	s1a/m2	s1b/m2	s1c/m2	s2/m2	s hybrid	*cagA*	
GU	8	0	11	0	0	3	0	3	21	25
	(32.0)		(44.0)			(12.0)		(12.0)	(84.0)	
DU	3	0	22	2	0	1	0	3	30	31
	(9.7)		(71.0)	(6.4)		(3.2)		(9.7)	(96.8)	
Cancer	5	0	9	0	0	4	0	4	19	22
	(22.7)		(40.9)			(18.2)		(18.2)	(86.4)	
Gastritis	12	0	22	4	0	2	0	2	39	42
	(28.5)		(52.4)	(9.5)		(4.8)		(4.8)	(92.9)	
GU+DU	0	0	7	1	0	0	0	0	8	8
			(87.5)	(12.5)					(100)	

GU, gastric ulcer; DU, duodenal ulcer.

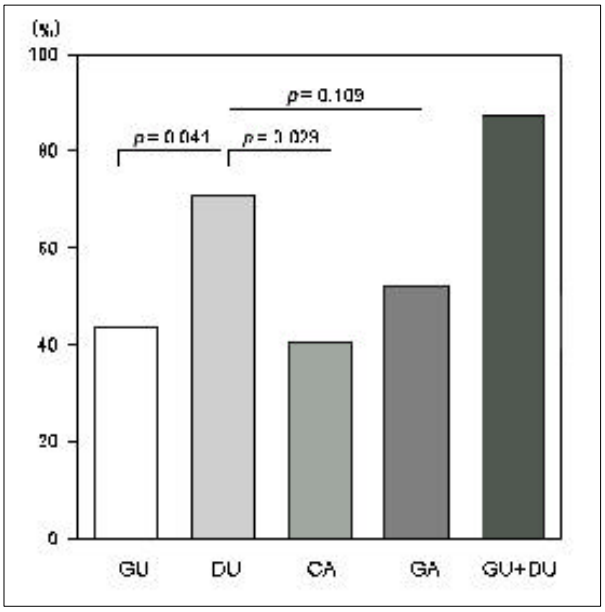

Fig. 2. s1c/m1 allele status in the clinical disease groups. GU, gastric ulcer; DU, duodenal ulcer; CA, gastric cancer; GA, gastritis.

allele is particularly associated with a duodenal ulcer when the new primers for the *vacA* s region alleles were used. Recent reports showed that the *vacA* genotype does not predict the clinical outcome.[15,16,19,21,23] However, these results were somewhat different. In the cancer group, the prevalence of the s1c/m1 (40.9%) was relatively low in comparison with that of a duodenal ulcer (71.0%), and the prevalence of both s1c/m2 (18.2%) and the s hybrid (18.2%) were relatively high, compared to that of a duodenal ulcer (3.2% and 9.7%, respectively). This difference might have arisen from the limited sample number because the results reflect data from only one university hospital in one region in Korea. A further large-scale study including multi-center data will be needed in order to confirm these results.

Initially, 34 (68%) out of 50 samples were signal sequence-positive using the known Western primers. The signal sequence was not detected in 32% of patients, although they actually contained the s region. After the new primers for the signal sequence were designed on the basis of the nucleotide sequences that could be discriminated from those in other countries, they were validated using the initial 50 samples. All tested positive for the *vacA* s region. Yamaoka's study[21] showing new primers were not observed until the our new primers were designed. In comparing our primers to Yamaoka's, some similarity was found. Therefore, it is believed that updated and reliable s region primers were used in our study.

Neither s1b nor s2 was found in this study. A recent report dealing with Korean *H. pylori* strains also revealed no s1b or s2 strain.[21] s1b is the main type in North, Central, and South America, France, Italy, Portugal, and Spain.[23] However, in Colombia, and Northern and Eastern Europe, s1a is reported to be the predominant signal sequence type.[21,23] The major signal sequence type in East Asia, including China, Hong Kong, and Japan was identified as s1c as reported in this study.[23]

Seven out of 8 patients with gastric and coexistent duodenal ulcers also had the s1c/m1 allele type. We do not believe that this finding is significant, because the number of patients studied in this group was too small. Therefore, comparisons between other groups and these 8 subjects were not performed.

In conclusion, new primers for the *vacA* signal sequence were designed, and the association between the identified *vacA* alleles and the clinical diseases were examined. s1c/m1 was found to be the predominant type of *vacA* allele in Korea and that it is particularly associated with a duodenal ulcer. This study suggests a possible relation between specific *vacA* genotypes and specific diseases.

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