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Prevalence and Genotypes of *Helicobacter pylori* in Gastric Biopsy Specimens from Patients with Gastroduodenal Pathologies in the Cukurova Region of Turkey▽

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The effects of *Helicobacter pylori* genotypes on clinical prognosis in the Cukurova region of Turkey were investigated by PCR. The prevalence of type I strains carrying the *s1c* allele, unlike in neighboring regions and countries, was found to be significantly higher in patients with gastritis and/or gastric ulcers ($P = 0.001$), and that of type I strains carrying the *s1a* allele was found to be significantly higher in patients with duodenal ulcers ($P < 0.001$). The *cagA* gene was strongly associated with the more virulent *vacA* genotypes ($P < 0.001$).

The effects of *vacA* allelic combinations of *Helicobacter pylori* on clinical prognosis show geographic differences in Western and Eastern populations. Although all strains of *H. pylori* contain the *vacA* gene, they vary in the ability to produce cytotoxin. Type *m1* strains demonstrate more toxin activity than *m2* strains, type *s1a* is more active than *s1b*, and type *s2* produces no detectable activity (2, 3). The *s1a* alleles are frequently observed in strains from northern and eastern Europe, while the *s1b* allelic types are frequent in such regions as Central and South America, Spain, Portugal, and South Africa. The *s1b* types are rare in other regions and less virulent than other *s1* subtypes (2, 3, 10, 19, 20, 22, 23). Furthermore, although the *s1c* allele has been stated to be more common in Asian strains and it may be associated with gastric cancers that are prevalent in that region, other studies have, in contrast, indicated that cancer development cannot be explained by *s1c* alleles alone (4, 12, 18–20, 23). Thus, the relationship between the genotypes of causative strains and clinical outcome should be considered in different geographic regions in order to make a true estimation of prognosis. There are very few studies on the relationship of *vacA* alleles with clinical outcomes in Turkey, and no such study has been carried out in our region (5, 6, 15). Therefore, we aimed to determine the prevalence of *H. pylori*, the effects of the *cagA* and *vacA* genes and *vacA* allelic types of the colonizing strains on clinical prognosis, and the relationship between the *cagA* and *vacA* genotypes in patients with gastritis and/or gastric ulcers (G/GU) and those with duodenal ulcers (DU) in the Cukurova region of Turkey.

(Part of this study was presented as a poster [Determination of the Prevalence and Genotypes of *H. pylori* in Gastric Biopsy Specimens from Patients with Gastroduodenal Pathologies] at the XXI International Workshop on *Helicobacter* and Related Bacteria in Chronic Digestive Inflammation and Gastric Cancer in Riga, Latvia, 18 to 20 September 2008, and an abstract was published [Helicobacter **13:**420, 2008].)

The subjects of this study were 231 previously untreated patients seen at outpatient clinics of the gastroenterology departments of two regional hospitals between January 2005 and January 2006, 158 of whom had endoscopically diagnosed G/GU and 73 of whom had DU. Antral gastric biopsy specimens were transported to the laboratory in brain heart infusion broth (Oxoid, Basingstoke, Hampshire, England) within 2 h and stored at −20°C.

Extraction of DNA was performed with a QIAamp DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The extracted DNA was measured in an Optimum-One UV-VIS spectrophotometer (Chebios, Rome, Italy) at a 260-nm wavelength for determination of DNA concentrations, which were confirmed to be sufficient for amplification (>3 μg/ml). Then, the DNA samples were stored at −20°C until the amplification of all of the gene regions to be investigated was completed.

These samples were first examined by *glmM* (formerly *ureC*) gene PCR, the most sensitive, specific, and reliable method for determination of the prevalence of *H. pylori* (11, 13). *H. pylori*-positive DNA samples were analyzed for the *cagA* and *vacA* genes, and *vacA*-positive DNA samples were further investigated for the detection of *vacA* alleles (*s1a*, *s1b*, *s1c*, and *s2* in the signal region and *m1a*, *m1b*, and *m2* in the cytotoxin-encoding middle region) by the PCR method. The primers used in this study are shown in Table 1. All amplification procedures were performed with a Thermal Cycler 2720 (Applied Biosystems, Foster City, CA) with a total volume of 50 μl of a PCR mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM each deoxynucleoside triphosphate, 25 pmol of each primer, 2.5 U of *Taq* polymerase, and 5 μl of each DNA sample. The PCR cycling conditions were as described previously (2, 13, 22, 23). Amplified PCR products were examined by electrophoresis on 2% agarose gels with ethidium bromide, and bands were analyzed under UV illumination.

Fisher's exact chi square test was used to analyze the data for

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TABLE 1. Oligonucleotide primers used in this study

Gene and allele	Primer	Sequence (5′–3′)[a]	Size (bp) of PCR product (location)	Reference(s)
glmM	glmM1-F glmM2-R	AAG CTT TTA GGG GTG TTA GGG GTT T AAG CTT ACT TTC TAA CAC TAA CGC	294 (784–1077)[b]	13
cagA	CagA-F1 CagA-B1	GAT AAC AGG CAA GCT TTT GAG G CTG CAA AAG ATT GTT TGG CAG A	349 (1228–1576)[c]	6, 22
vacA	VA1-F VA1-R	ATG GAA ATA CAA CAA ACA CAC CTG CTT GAA TGC GCC AAA C	259 (797–1055)[d] or 286 (284–569)[e]	2, 22
vacA s1a	SS1-F VA1-R	GTC AGC ATC ACA CCG CAA C CTG CTT GAA TGC GCC AAA C	190 (866–1055)[d]	2
vacA s1b	SS3-F VA1-R	AGC GCC ATA CCG CAA GAG CTG CTT GAA TGC GCC AAA C	187 (869–1055)[f]	2, 23
vacA s1c	SlC-F VA1-R	CTC TCG CTT TAG TGG GGY T CTG CTT GAA TGC GCC AAA C	213 (843–1055)[f]	23
vacA s2	SS2-F VA1-R	GCT AAC ACG CCA AAT GAT CC CTG CTT GAA TGC GCC AAA C	199 (371–569)[e]	2,
vacA m1a	VA3-F VA3-R	GGT CAA AAT GCG GTC ATG G CCA TTG GTA CCT GTA GAA AC	290 (2741–3030)[d]	2, 23
vacA m1b	VAm-F3 VAm-R3	GGC CCC AAT GCA GTC ATG GAT GCT GTT AGT GCC TAA AGA AGC AT	291 (2741–3031)[f]	23
vacA m2	VA4-F VA4-R	GGA GCC CCA GGA AAC ATT G CAT AAC TAG CGC CTT GCA C	352 (2284–2635)[e]	23

[a] Y = C or T.
[b] Nucleotide positions in the *glmM* gene of *H. pylori* 26695.
[c] Nucleotide positions in the *cagA* gene of *H. pylori* ATCC 53726 (GenBank accession no. L117714).
[d] Nucleotide positions in the *vacA* gene of *H. pylori* 60190 (GenBank accession no. U05676).
[e] Nucleotide positions in the *vacA* gene of *H. pylori* Tx30a (GenBank accession no. U29401).
[f] Corresponding to nucleotide positions of *H. pylori* 60190.

the different patient groups. A P value of <0.05 was accepted as statistically significant.

H. pylori was detected in 201 (87%) of a total of 231 biopsy specimens by the *glmM* PCR method. Our finding is similar to those reported by Saribasak et al. (15) and Bolek et al. (5), who also used the PCR method for diagnosis (89% and 84.6%, respectively), yet it is higher than the 75.4% found by Kantarceken et al. (8), who evaluated biopsy specimens histologically. This inconsistency is assumed to have been caused by the nature of PCR-based methods, which are accepted as being more sensitive and specific (11, 13).

The effects of *H. pylori* genotypes on gastric colonization were studied by many researchers in large groups of cases, and type I (*cagA*$^+$ *vacA*$^+$) strains were shown to be more virulent than type II (*cagA* mutant) strains (1, 17, 21). The effects of the *cagA* and *vacA* alleles on prognosis were also intensely discussed in subsequent studies (2, 3, 9, 20, 22, 23, 24). In this study, we investigated the *cagA* and *vacA* genes and *vacA* gene alleles. However, we did not examine the *cagA* gene variants due to a lack of the means to perform sequence analysis during our study, although the effects of EPIYA motifs located at the 3′ end of the *cagA* gene on prognosis were shown in various studies.

We identified 84.1% of our *H. pylori* strains as type I and 15.9% as type II. The prevalence of type I *H. pylori* in patients with DU was observed to be significantly higher than in patients with G/GU (94.3% and 78.6%, respectively; $P = 0.004$) (Table 2). In a great amount of research, the prevalence of type I *H. pylori* was reported to vary among different countries, regions, and patient groups, and it was reported as 68% in England (21), 78 to 80% in Turkey (5, 15), 80% in Belgium (11), 82% in Brazil (1), 82% in Japan (17), 93% in Nigeria (16), and 97% in China (24). The majority of those studies (1, 5, 11, 17, 21) indicated that in patients with DU, the *cagA* positivity rate is relatively higher than in patients with gastritis or GU and ranges from 80 to 100%. Our results revealed that, as in the rest of the world, *cagA*$^+$ *H. pylori* strains were especially associated with DU in our country. In the another study performed in Turkey by Saribasak et al., type I *H. pylori* preva-

TABLE 2. Distribution of *cagA* and *vacA* genotypes in 201 *H. pylori*-positive samples by *glmM* PCR

Genotype	No. (%) of samples		
	G/GU[a] (n = 131)	DU[b] (n = 70)	Overall (n= 201)
cagA$^+$ *vacA*$^+$	103 (78.6)	66 (94.3)	169 (84.1)
cagA mutant *vacA*$^+$	26 (19.9)	4 (5.7)	30 (14.9)
cagA mutant *vacA* mutant	2 (1.5)		2 (1.0)

[a] Specimens obtained from patients with G/GU via endoscopy.
[b] Specimens obtained from patients with DU via endoscopy.

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TABLE 3. Distribution of *vacA* allelic combinations in all of 199 *vacA*$^+$ and 169 type I (*cagA*$^+$ *vacA*$^+$) *H. pylori* strains from patients with G/GU and DU

vacA allelic combination	No. (%) of samples						
	G/GU[a]		DU[b]		Overall		
	All *vacA*$^+$ strains	Type I strains[c]	All *vacA*$^+$ strains	Type I stains	All *vacA*$^+$ strains	Type I strains	Type II strains[d]
s1a/*m1a*	21 (16.3)	20 (19.4)	19 (27.2)	19 (28.8)	40 (20.1)	39 (23.1)	1 (3.3)
s1a/*m1b*	22 (17.0)	20 (19.4)	27 (38.6)	27 (40.9)	49 (24.6)	47 (27.8)	2 (6.6)
s1b/*m1a*	8 (6.2)	3 (2.9)	1 (1.4)		9 (4.5)	3 (1.8)	6 (20.0)
s1b/*m1b*	5 (3.9)				5 (2.5)		5 (16.7)
s1c/*m1a*	26 (20.1)	26 (25.3)	8 (11.4)	8 (12.1)	34 (17.1)	34 (20.1)	
s1c/*m1b*	33 (25.6)	33 (32.0)	12 (17.1)	12 (18.2)	45 (22.6)	45 (26.6)	
s1/*m2*	9 (7.0)	1 (1.0)	3 (4.3)		12 (6.1)	1 (0.6)	11 (36.7)
s2/*m2*	5 (3.9)				5 (2.5)		5 (16.7)
Total	129	103	70	66	199	169	30

[a] Specimens obtained from patients with G/GU via endoscopy.
[b] Specimens obtained from patients with DU via endoscopy.
[c] *cagA*$^+$ *vacA*$^+$ strains.
[d] *cagA* mutant strains.

lences in patients with DU, GU, and gastritis were determined to be 89%, 100%, and 55%, respectively (15). In that study, the *cagA* positivity rate was accompanied by a severity of pathology similar to ours, but GU and gastritis were scored differently on the basis of clinical definitions. However, although in Nigeria, where a high *cagA* positivity rate was reported, this ratio was higher in patients with DU than in patients with nonulcer dyspepsia (95% and 91%, respectively), the overall evaluation makes the effects of the *cagA* profile on clinical outcome quite disputable (16).

We also analyzed a total of 199 *vacA*-positive samples in order to determine predominant *vacA* alleles in the Cukurova region and to detect the effects of the alleles on prognosis and their relationship with *cagA*. The most frequent *vacA* allelic combination was *s1a*/*m1b* in 49 (24.6%) strains, followed by *s1c*/*m1b* in 45 (22.6%) and *s1a*/*m1a* in 40 (20.1%) strains. No sign of the *s2*/*m1* genotype was detected in any of the strains. The most prevalent genotypes in patients with G/GU and DU were *s1c*/*m1b* (25.6%) and *s1a*/*m1b* (38.6%), respectively (Table 3). The relatively higher prevalence of the *s1a*/*m1b* genotype in patients with DU than in those with G/GU was determined to be statistically significant ($P = 0.001$). The predominant *vacA* genotype was *s1a*/*m1b* (27.8%) in type I strains and *s1*/*m2* (36.7%) in type II strains (Table 3).

In 59 (57.3%) and 40 (38.8%) of the 103 type I *H. pylori* strains from patients with G/GU, the *s1c* and *s1a* alleles were detected, respectively, while in 46 (69.7%) and 20 (30.3%) of the 66 type I *H. pylori* strains from patients with DU, the *s1a* and *s1c* alleles were found, respectively (Table 3). The relatively higher prevalence of the *s1c* allele in patients with G/GU and of the *s1a* allele in patients with DU was determined to be statistically significant ($P = 0.001$ and $P < 0.001$, respectively).

The findings of related studies on the distribution of *vacA* alleles in patient groups seem to be different, yet it is the common opinion that while the *s1a*, *s1b*, and *m1a* alleles have higher prevalences in European, African, and American strains, the *s1c* and *m1b* allelic types are endemic in East Asia (1, 9, 10, 19, 23). In a study conducted in Istanbul, Turkey, Bolek et al. reported that among type I strains, the most frequent *vacA* genotypes were *s1*/*m1* in patients with DU and GU and *s1*/*m2* in patients with gastritis, while among type II strains the most frequent *vacA* genotype was *s1*/*m2* in all groups (5). Nevertheless, those researchers did not investigate the subtypes of *s* and *m* alleles. We found that *s1*/*m2* was the most common genotype among type II strains in both groups, similar to those of Bolek et al., but except for the *s1*/*m2* genotype detected in one patient with G/GU, all type I strains were *s1*/*m1*.

In addition, we observed that the prevalences of the *s1a*/*m1a* genotype, known as the Western type (23), the *s1c*/*m1b* and *s1a*/*m1b* genotypes, known as the East Asian type (23), and the *s1c*/*m1a* genotype, which may be called the mixed type, which was not previously reported, were close to each other. However, in two studies of *s* allelic subtypes in Turkey, Erzin et al. (6) and Saribasak et al. (15) reported that the prevalence of the *s1a* allele was substantially high (83% in both studies), there was no *s1c* allele and the *m2* allele was more prevalent than the *m1* allele in the Istanbul region. The high prevalence of the *s1c* allelic types observed in our study but not in the above-mentioned two studies is noteworthy and is assumed be due to geographic and ethnic differences. In addition, this difference may be attributable to some technical discrepancies in the methodologies used, such as differences in the primers used and the researchers' levels of experience. The findings on the *s1b* and *s2* alleles in the above-mentioned studies, on the other hand, seem to be consistent with ours. Besides, Erzin et al. correlated type I strains with DU and the *s1a* allele (6) and Saribasak et al. correlated them with peptic ulcer disease and the *s1a* allele (15).

Hussein et al. have reported the *s1*/*m2* allelic type, unlike the ones in our study, to be the most frequent type in the neighboring countries Iran and Iraq, and they also indicated that the prevalence of the *s2*/*m2* genotype was higher in Iran (27%) and close to ours in Iraq (8%) (7). In a great number of studies conducted in different parts of the world, *s2* allelic types have been found to be less frequent and to be associated with nonulcerative dyspepsia or asymptomatic carriage (14, 16, 23). Thus, *s2* allelic types which we marked only in type II strains are consistent with those reported in related studies.

The *s2*/*m2* genotype was detected only in five (2.5%) pa-

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tients with G/GU, and all of these strains were *cagA* negative. While 165 (98.2%) of a total of 168 strains carrying the more virulent *s1a*/*m1a*, *s1a*/*m1b*, *s1c*/*m1a*, and *s1c*/*m1b* genotypes were *cagA* positive, only 4 (12.9%) of a total of 31 strains carrying the nonvirulent *s2*/*m2* genotype, the less virulent *s1*/*m2* genotype, or the *s1b*/*m1a* or *s1b*/*m1b* genotype, supposedly less virulent in our region, were *cagA* positive (Table 3). The association between the more virulent *vacA* genotypes and the *cagA* gene was found to be statistically significant ($P < 0.001$). Therefore, it is important that whether the association between the more virulent *vacA* genotypes and the *cagA* gene is a coincidence or the *cagA* gene prefers strains with more virulent *vacA* genotypes be discussed. We assume that our results strengthen the idea that the *cag* pathogenicity island, an unstable gene island carrying the *cagA* gene, might prefer the virulent *vacA* allelic types during insertion into the genome.

In conclusion, the prevalences of type I *H. pylori* strains in our region were determined to be 78.6% and 94.3% in patients with G/GU and DU, respectively. The most prevalent *vacA* genotype was *s1c*/*m1b* in patients with G/GU and *s1a*/*m1b* in patients with DU, and more-virulent *vacA* genotypes were strongly associated with the *cagA* gene. The results imply that gastric colonization caused by strains carrying the *s1c* allele, which is supposed to be a risk factor for gastric cancer in Asia, is also important in our region. Thus, it is suggested that not only *H. pylori* colonization of antral biopsy specimens obtained from patients with gastroduodenal complaints during endoscopic examination be demonstrated, but also the detection of genotypic and allelic combinations which can help in the diagnosis and eradication of infections caused by strains carrying the *s1c* allele, which may lead to gastric cancer, be considered in the Cukurova region of Turkey.

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