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Patterns of EPIYA motifs among *cagA*-positive *Helicobacter pylori* strains: a case–control study in a Turkish population with Eurasian geographical features

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Geographical variation in the frequency of various gastroduodenal pathologies was shown to be related to the geographical diversity of *H. pylori* CagA Glu-Pro-Ile-Tyr-Ala (EPIYA) patterns. We examined the EPIYA patterns of *H. pylori* and the association of EPIYA patterns with gastric cancer (GC) for the first time, to the best of our knowledge, in Turkey. The patient group (PG) contained 60 patients [38 GC and 22 duodenal ulcer (DU) patients]. The control group (CG) was 110 individuals [94 gastritis patients and 16 persons with a normal gastrointestinal system (NGIS)]. Specific primers were used for the detection of *cagA* including empty-site-positive and EPIYA-A, -B, -C, -D PCR. Bands of EPIYA-A, -B, -C were confirmed by DNA sequencing. One hundred and forty-two (83.5 %) strains [60 in the PG (38 GC, 22 DU), 82 in the CG (72 gastritis, 10 NGIS)] were positive for the *cagA* gene. EPIYA-C with multiple repeats was detected in 34 (23.9 %) strains, and 22 (64.7 %) were from GC patients. EPIYA-C with one repeat was detected in 89 (62.7 %) strains, and 54 (60.7 %) were from gastritis patients. EPIYT was detected in 10 strains, and EPIYA-D was not detected. The number of EPIYA-C with multiple repeats was significantly higher for the PG than for the CG ($P<0.0001$). In GC patients, the number of EPIYA-C with multiple repeats was significantly higher than one repeat ($P<0.0001$). In conclusion, our study showed that multiple EPIYA-C repeats increases the GC risk by 30.6-fold and the DU risk by 8.9-fold versus the CG. This indicates that Western-type *H. pylori* strains in Turkey have similar EPIYA motifs to those of neighbouring countries and Western populations.

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INTRODUCTION

Helicobacter pylori is the principal cause of gastritis and gastric or duodenal ulcer (DU) diseases and is involved in the development of gastric cancer (GC) and mucosa-associated lymphoid tissue lymphoma (Atherton, 2006). *H. pylori* is a class 1 carcinogen identified by the International Agency for Research on Cancer for chronic and persistent infections. It is resistant to antibacterial agents in spite of innate and adaptive immunity (International Agency for Research on Cancer, 1994). Depending on virulence factors such as cytotoxin-associated gene A (CagA), blood group antigen-binding adhesion (BabA2) and vacuolating cytotoxin gene A (VacA), different genotypes cause different pathological and clinical outcomes and geographical regional differences (Atherton *et al.*, 1995; Basso *et al.*, 2008). Recently, the Glu-Pro-Ile-Tyr-Ala (EPIYA)

Abbreviations: cagPAI, *cag* pathogenicity island; CG, control group; CI, confidence interval; DU, duodenal ulcer; EPIYA, Glu-Pro-Ile-Tyr-Ala; CagA, cytotoxin-associated gene A; GC, gastric cancer; NGIS, normal gastrointestinal system; PG, patient group; OR, odds ratio.

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pattern of CagA was suggested to be involved in the pathogenesis of GC, as this pattern manifests important variations depending on the region (Argent *et al.*, 2004, 2005).

The CagA protein is injected into host epithelial cells with its peptidoglycan by a type IV secretion system encoded by the *cag* pathogenicity island (*cagPAI*). The *cagA* gene has a C-terminal region with a motif of five amino acid residues – glutamic acid-proline-isoleucine-tyrosine-alanine – which is designated the EPIYA motif. The tyrosine residue is the target of phosphorylation. The EPIYA motif plays an important role in the relationships of CagA with membrane and tyrosine phosphorylation (Akopyants *et al.*, 1998; Higashi *et al.*, 2002). There are four types of EPIYA segments: EPIYA-A,-B,-C and -D. Based on amino acid sequences, the EPIYA-C segment is hypothesized to be characteristic of CagA of *H. pylori* in Western countries, while the EPIYA-D segment is specific to CagA of *H. pylori* in Eastern countries (Panayotopoulou *et al.*, 2007). Several researchers have indicated that *H. pylori* strains with EPIYA-C and -D repeats are significantly associated with GC and atrophic gastritis (Yamaoka *et al.*, 1998; Azuma *et al.*, 2002; Batista *et al.*, 2011).

In Turkey, the GC incidence was reported as 21.6 and 8.6 per 10 000 of the population among men and women, respectively (Basara *et al.*, 2013). Unfortunately, we do not have any data about the increased GC risk due to EPIYA patterns in Turkey. In two Turkish studies, Kucukoner *et al.* (2013) and Celik *et al.* (2015) found 53.9 and 72.1 % *H. pylori* positivity for GC and gastric cardia tumours, respectively.

In this study, our goal was to determine the EPIYA patterns of *cagA*-positive *H. pylori* strains isolated from patients and controls with different endoscopic diagnoses living in Istanbul and to evaluate the association between the patterns of EPIYA motifs and these endoscopic diagnoses. The present study is of utmost importance for investigating the *H. pylori* EPIYA patterns and their clinical outcomes in Turkey, especially for the first time, to the best of our knowledge, for GC.

METHODS

Study design and patients. This case–control study was conducted between November 2011 and December 2012. The patient group (PG), comprising a total of 60 patients (38 GC and 22 DU patients; 34 male, 26 female; mean age 51.7 years; age range 29–81 years), and a control group (CG), comprising a total of 110 individuals [94 gastritis patients and 16 persons with normal gastrointestinal system (NGIS); 59 male, 51 female; mean age 49.9 years; age range 18–78 years] were enrolled in this study. The CG was matched with the PG according to the age and gender distribution of the PG ($P>0.05$). In the CG, 110 *H. pylori* strains (from gastritis patients and persons with NGIS) were isolated from the antrum and corpus biopsy specimens. For the PG, paraffin blocks of 38 cases with GC and 22 cases with DU with positive *H. pylori* were obtained by screening the archives. We excluded patients who were under 18 years, had had previous gastric surgery and *H. pylori* eradication treatment, or had a history of therapy with antibiotics, antisecretory drugs, bismuth salts or sucralfate in the month prior to sampling.

The study was approved by the Clinical Research Ethics Board of Istanbul University, Cerrahpasa Faculty of Medicine (Ethical approval; 4548/9 February 2011). All patients gave informed consent to participate in the study.

PCR

H. pylori DNA extraction from strains and paraffin blocks

Genomic DNA extraction (Real Genomics Quality Nucleic Acid/Purification system; RBC Bioscience Laboratories) and QIAamp DNA mini prep kits (Qiagen) were used according to the manufacturer's instructions for DNA extractions from *H. pylori* strains and paraffin blocks, respectively.

ureC gene detection in *H. pylori*

An *H. pylori*-QLS 1.0 kit (Fluorion) was used to detect 156 bp of the *ureC* gene in DNA extractions of *H. pylori* strains and paraffin

Table 1. PCR primers used in this study

All PCRs were performed in a 50 µl final volume with 1.25 U *Taq* polymerase (Thermo Scientific), 1 × PCR buffer, 3 µl 250 mM $MgCl_2$ and 1 µl dNTP mix (10 mM each) with the described primers using the thermal profiles detailed.

Primer	Primer sequence (5′→3′)	References
cagA-F	GATAACAGGCAAGCTTTTGAGG*	Erzin *et al.* (2006); van Doorn *et al.* (1998)
cagA-R	CTGCAAAAGATTGTTTGGCAGA*	
cagA28F	TTCTCAAAGGAGCAATTGGC†	Argent *et al.* (2005)
cagA-P1C	GTCCTGCTTTCTTTTTATTAACTTKAGC†	
cagA-P2CG	TTTAGCAACTTGAGCGTAAATGGG†	
cagA-P2TA	TTTAGCAACTTGAGTATAAATGGG†	
cagA-P3E	ATCAATTGTAGCGTAAATGGG†	
cag empty PCR	GCTTGCTTGTATTGGCCTTG/GCATGCACATTCCCTAAAGT‡	Occhialini *et al.* (2001)

*95 °C for 2 min initial denaturation, followed by 45 cycles of 30 s at 95 °C, 45 s at 53 °C and 45 s at 72 °C, with a final elongation step performed for 5 min at 72 °C.

†95 °C for 2 min initial denaturation, followed by 50 cycles of 30 s at 95 °C, 45 s at 57 °C and 35 s at 72 °C, with a final elongation step performed for 5 min at 72 °C.

‡95 °C for 2 min initial denaturation, followed by 40 cycles of 30 s at 95 °C, 30 s at 57 °C, 20 s at 72 °C, with a final elongation step performed for 5 min at 72 °C.

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blocks according to the manufacturer's instructions (He *et al.*, 2002).

Amplification of the *H. pylori cagA* gene

Previously described primers were used to detect the *H. pylori cagA* gene (349 bp) (Table 1) (van Doorn *et al.*, 1998; Erzin *et al.*, 2006). For this, the protocol was as follows: initial denaturation at 95 °C for 2 min, followed by 45 cycles of 95 °C for 30 s, 45 s at 53 °C and 45 s at 72 °C. The final elongation was at 5 min at 72 °C.

Amplification and typing of EPIYA motifs in the *cagA* 3′ variable region

Previously described primers [forward (cagA28F) and reverse (cagA-P1C, cagA-P2CG, cagA-P2TA and cagA-P3E)] were used to amplify DNA for EPIYA-A, -B, -C and –D, respectively (Table 1) (Argent *et al.*, 2005). For this, the PCR protocol was as follows: initial denaturation at 95 °C for 2 min, followed by 50 cycles of 30 s at 95 °C, 45 s at 57 °C and 35 s at 72 °C. The final elongation step was 5 min at 72 °C. After the PCR amplification, PCR products were sequenced bidirectionally using a Sequence Reagent Mix kit (DYEnamic ET Terminator Cycle Sequencing kit; GE Healthcare) with an ABI 310 (Applied Biosystems) automatic sequencing machine.

Empty-site PCR

All strains negative for EPIYA PCR were confirmed as being true CagA negative by performing an empty-site-positive PCR assay (Occhialini *et al.*, 2001). To confirm *cagPAI* in all of the strains, amplification was performed with two primers (forward 468 HP519 and reverse 496 HP549) located in the two genes flanking the *cagPAI* in the reference strains (HP519 and HP549) (Table 1, cag empty PCR). The PCR protocol was as follows: initial denaturation at 95 °C for 2 min followed by 40 cycles of 30 s at 95 °C, 30 s at 57 °C, and 20 s at 72 °C. The final elongation step was performed for 5 min at 72 °C.

Statistical analyses. The Pearson χ^2 test was used to compare the PG and CG for the presence of one or more EPIYA-C repeats. The Pearson χ^2 test and Fisher's exact test were used to compare one or more EPIYA-C repeats for GC and DU cases of the PG, respectively. In our study, the risk factor determination of the PG (GC and DU cases) was performed according to the multivariate analysis. All analyses were performed using the SPSS 21.0 (SPSS) package program. The gender, age and one or more EPIYA-C repeats were included as independent variables in multivariate analysis by logistic regression test. The odds ratio (OR) operation was calculated to evaluate the increase in the risk of DU or GC in terms of EPIYA-C repeats. Significance values were defined as $P<0.05$; $P<0.001$ was considered highly statistically significant.

RESULTS

All observed *H. pylori* strains ($n=170$) were positive for the *ureC* gene. There were 142 (83.5 %) of the 170 *H. pylori* strains that were positive for *cagA*. The EPIYA patterns of the 142 *cagA*-positive *H. pylori* strains were evaluated in the 38 (26.8 %) GC and 22 (15.5 %) DU cases of the PG and the 72 (50.7 %) gastritis and 10 (7 %) NGIS cases of the CG.

Table 2. Distribution of the patterns of EPIYA motifs according to endoscopic diagnosis

EPIYA motif pattern	PG		HG		Total ($n=142$)
	GC [*n* (%)]	DU [*n* (%)]	Gastritis [*n* (%)]	NGIS [*n* (%)]	
ABC	9 (23.7)	14 (63.7)	50 (69.5)	10 (100)	83
AC	1 (2.6)	–	4 (5.5)	–	5
BC	1 (2.6)	–	–	–	1
ABCC	15 (39.5)	5 (22.8)	4 (5.5)	–	24
BCC	2 (5.3)	1 (4.5)	–	–	3
ACCC	1 (2.6)	1 (4.5)	–	–	2
ABCCC	4 (10.5)	1 (4.5)	–	–	5
AB	3 (7.9)	–	14 (19.5)	–	17
A	2 (5.3)	–	–	–	2
D	–	–	–	–	–
Total	38 (100)	22 (100)	72 (100)	10 (100)	142

Different EPIYA-A, -B, and -C motifs were found in all 142 (100 %) cases; no EPIYA-D was found. In 28 (16.5 %) patients, no EPIYA motif was detected. Using the empty-site-positive PCR assay, we found that all 28 strains were truly *cagA* negative. EPIYA-C with multiple repeats was detected in 30 (88.2 %) and four (11.8 %) of the PG and CG cases, respectively. On the other hand, EPIYA-C with one repeat was detected in 25 (28 %) and 34 (23.9 %) of PG and CG cases, respectively. The most frequently detected EPIYA pattern was EPIYA-ABC in 83 cases. The distribution of the 83 EPIYA-ABC occurrences was 23 (27.7 %) and 60 (72.2 %) for the PG and CG respectively (Table 2). EPIYA-ABCC and EPIYA-ABCCC were detected in 15 (39.5 %) and 4 (10.5 %) cases with GC, respectively (Table 2).

In the distribution of EPIYA-C with one or multiple repeats for the the PG and CG cases, EPIYA-C with one repeat was detected in 25 (28 %) and 64 (72 %) of the PG and CG cases, respectively. The difference for EPIYA-C with one repeat was statistically significant ($P=0.039$) for the CG cases compared with the PG cases. On the other hand, EPIYA-C with multiple repeats was detected

Table 3. Comparison of the PG and CG for EPIYA-C repeat patterns

EPIYA-C repeat patterns	PG (GC and DU) [*n* (%)]	CG (gastritis and NGIS) [*n* (%)]	OR	95 % CI	*P* value
One repeat of EPIYA-C ($n=89$)	25 (28 %)	64 (72 %)	0.513	0.271–0.972	0.039
Multiple repeats of EPIYA-C ($n=34$)	30 (88.2 %)	4 (11.8 %)	26.500	8.653–81.157	<0.0001

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Table 4. Comparison of EPIYA repeat patterns (one and multipl repeats) in the PG

Endoscopic diagnosis	One repeat of EPIYA-C [*n* (%)]	Multiple repeats of EPIYA-C [*n* (%)]	OR	95 % CI	*P* value
GC	11 (28.9)	22 (57.8)	32.000	9.236–110.875	<0.0001
DU	14 (63.6)	8 (36.3)	9.143	2.413–34.648	0.001

in 30 (88.2 %) and four (11.8 %) of the PG and CG cases, respectively. The difference for EPIYA-C with multiple repeats was statistically significant for the PG cases compared with the CG cases [$P<0.0001$, OR 26.5, 95 % confidence interval (CI) 8.653– 81.157] (Table 3).

In the PG, EPIYA-C with multiple repeats was compared for the GC and DU cases (Table 4). EPIYA-C with one repeat and multiple repeats were detected in 11 (28.9 %) and 22 (57.8 %) of the GC cases, respectively. In the GC cases of the PG, a statistically higher significant difference was detected for EPIYA-C with multiple repeats than for EPIYA-C with one repeat ($P<0.0001$, OR 32.0, 95 % CI 9.236–110.875). In the DU cases of the PG, the numbers of EPIYA-C with one repeat and multiple repeats were 14 (63.6 %) and 8 (36.3 %), respectively. In the DU cases, a statistically significant difference was detected for EPIYA-C with one repeat compared with EPIYA-C with multiple repeats ($P=0.001$, OR, 9.143, 95 % CI 2.413–34.648). Also, when EPIYA-C with exactly two and more than one occurrence were compared for the GC and DU cases of the PG, no significant difference was detected between them ($P>0.05$).

A multivariate analysis by logistic regression test of the PG composed of GC and DU cases was carried out with gender, age and one or multiple EPIYA-C repeats included as independent variables (Table 5). Having multiple EPIYA-C repeats was a risk factor for GC cases and increased the GC risk by 30.6-fold ($P<0.0001$, OR 30.617, 95 % CI 8.574–109.326). The DU risk was increased by 8.9-fold ($P=0.001$, OR 8.902, 95 % CI 2.323–34.116).

DISCUSSION

H. pylori infections play a role in the aetiology of gastroduodenal diseases including gastritis, peptic ulcer and GC (Suzuki & Moayyedi, 2013). However, the high prevalence of *H. pylori* infection does not explain the different incidences of GC; for example, the high *H. pylori* prevalence does not match the lower GC incidence in India (known as the Asian enigma) (Malaty, 2007). This may be explained by the difference in *H. pylori* virulence factors rather than *H. pylori* infection rate (Yamaoka, 2010). There is strong evidence that CagA functions as a bacterial oncoprotein in mammals (Wroblewski *et al.*, 2010), and CagA-positive *H. pylori* strains have been suggested to increase the risk of developing atrophic gastritis and GC by several researchers (Huang *et al.*, 2003; Sahara *et al.*, 2012; Ajami *et al.*, 2013). As indicated by several studies, phosphorylated CagA–SHP-2 interactions contribute to cytoskeletal rearrangements and cell elongation by stimulating the ERK–MAP kinase signalling pathway. This is called the 'hummingbird phenotype' in epithelial cells (Higashi *et al.*, 2002; Tsutsumi *et al.*, 2003; Lima *et al.*, 2011).

EPIYA-C and -D have a role in the interaction between CagA and SHP-2, which causes a characteristic pathology

Table 5. Results of logistic regressions according to the variables in GC and DU cases of the PG

B, beta regression coefficient; Wald, test statistics used for the determination of the meaning of variables; d.f., degrees of freedom; exp(B), exponential. Variables included in the logistic regression model were gender, age, EPIYA-C with one repeat and EPIYA-C with multiple repeats.

Variable	B	SEM	Wald	df	*P* value	Exp(B)	95 % CI for exp(B)	
GC								
Gender	0.188	0.576	0.106	1	0.744	1.207	0.390	3.730
Age	0.071	0.042	2.795	1	0.095	1.073	0.988	1.166
One repeat of EPIYA-C	−3.422	0.649	27.760	1	0.000	0.033	0.009	0.117
Multiple repeats of EPIYA-C	3.422	0.649	27.760	1	0.000	30.617	8.574	109.326
Constant	−3.552	2.142	2.749	1	0.097	0.029		
DU								
Gender	0.159	0.543	0.086	1	0.769	1.173	0.405	3.399
Age	0.051	0.035	2.065	1	0.151	1.052	0.982	1.128
One repeat of EPIYA-C	−2.186	0.685	10.174	1	0.001	0.112	0.029	0.430
Multiple repeats of EPIYA-C	2.186	0.685	10.174	1	0.001	8.902	2.323	34.116
Constant	−2.939	1.805	2.649	1	0.104	0.053		

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in the gastric epithelium. It is suggested that oncogenic potential is increased with cytoskeletal disruption and that excessive activation of signal transduction pathways will increase IL-8 induction by increasing the CagA phosphorylation level (Hatakeyama, 2004; Cendron *et al.*, 2009; Papadakos *et al.*, 2013).

In various studies, different Cag A positivity rates were reported. For example, Yamaoka et al. (1999, 2002) stated that more than 90 % of *H. pylori* strains isolated from East Asian populations had the *cagA* gene but 40 % of Western strains did not have the *cagA* gene. Higher rates of CagA positivity have been reported: 52.1–94.3 % in Turkish studies from 2006 to 2013 (Erzin *et al.*, 2006; Nagiyev *et al.*, 2009; Kolaylı *et al.*, 2012; Ozbey *et al.*, 2013; Yula *et al.*, 2013). Similarly, our Cag A positivity rate (83.5 %) for *H. pylori* strains was in accordance with previous Turkish studies. In other Turkish studies related to EPIYA-C repeats, Salih *et al.* (2010) indicated that the *H. pylori*-positive patients with gastritis had 87.5 % EPIYA-ABC and 12.5 % multiple EPIYA-C repeats, whereas DU patients had 46.4 % EPIYA-ABC and 53.6 % multiple EPIYA-C repeats. In another study, Salih *et al.* (2014) reported that gastritis patients had 76.9 % EPIYA-ABC and 23.1 % EPIYA-ABCC; DU patients had 30 % EPIYA-ABC, and 70 % had multiple EPIYA-C repeats. In a study by Gücin *et al.* (2013), EPIYA-ABC was detected in 93.3 % of gastritis patients, and EPIYA-ABCC was detected in 6.7 % of gastritis patients. The rate of EPIYA-ABC reported by Salih *et al.* (2010, 2014) and Gücin *et al.* (2013) in gastritis cases was higher than our 50 (69.5 %) EPIYA-ABC gastritis cases; this is different to the number of EPIYA-ABC gastritis cases in our CG sample cohort. The EPIYA-ABC rates reported for DU cases were lower than 14 (63.7 %) EPIYA-ABC DU cases in our PG. Thus, our EPIYA-ABC rates were different from those obtained in previous Turkish studies regarding patients with gastritis of the CG or cases with DU of the PG. We suggest that this could be due to a limited number of enrolled subjects in other studies. Unfortunately, we do not have any data about the increased GC risk due to EPIYA patterns in Turkey.

While some studies have shown an association between EPIYA-C repeat numbers and *H. pylori*-related diseases, other studies did not. Similar to our study, Yamaoka *et al.* (1998), Azuma *et al.* (2002) and Batista *et al.* (2011) showed that *H. pylori* strains with EPIYA-C repeats were significantly associated with GC and atrophic gastritis. Additionally, Yamaoka *et al.* (1999) reported that seven out of eight *H. pylori* Colombian strains (87.5 %) had more than three EPIYA-C repeats in GC patients. Similarly, Sicinschi *et al.* (2010) indicated that two EPIYA-C (34.3 %) and three EPIYA-C (1.5 %) repeats were found in patients with GC and suggested a significant association between the multiple EPIYA-C repeats and more severe lesions. Qadri *et al.* (2014) found that 70 % of CagA-positive GC cases had more than one EPIYA-C repeat. Ajami *et al.* (2013) found EPIYA-ABCC in 36.2 % of the patients with GC, and patients with EPIYA-C had a 2.27-fold increase in GC development versus patients with DU. Again, Kalaf *et al.* (2013) indicated that more than one EPIYA-C repeat occurred in 66.6 % of GC patients. Similar to our study, Kalaf *et al.* (2013) found a significant association between multiple EPIYA-C repeats and GC ($P \leq 0.01$). Vaziri *et al.* (2013) studied EPIYA-ABCC and EPIYA-A-B/C and showed associations with GC and duodenitis, respectively. Silva *et al.* (2014) stated that the risk of GC was approximately fourfold higher among patients infected with strains carrying two or three EPIYA-C. The subtypes and distribution of EPIYA-C in our study were consistent with previous studies; the rate of EPIYA-C with multiple repeats in GC cases of our PG was significantly higher than the rate of EPIYA-C with one repeat ($P=0.039$). We detected a higher significant difference between the PG and CG for the presence of multiple EPIYA repeats ($P<0.0001$, OR 26.5, 95 % CI 8.653–81.157). Recently, Vaziri *et al.* (2015) studied the eukaryotic vector carrying the *cagA* gene (ABC and ABCCC types) and identified 42 key signal transduction genes involved in GC. They suggested that the ABCCC type could induce intestinal metaplasia. Their results may support our findings that multiple EPIYA-C repeats increased the GC risk by 30.6-fold by logistic regression analysis ($P<0.0001$, OR 30.617, 95 % CI 8.574–109.326).

In contrast, Batista *et al.* (2011) and Torres *et al.* (2012) did not find any association between ulcers and the number of EPIYA-C repeats. Acosta *et al.* (2010) and Shokrzadeh *et al.* (2010) could not find differences between EPIYA patterns and clinical outcomes. The number of EPIYA-C with multiple repeats was observed less frequently in cases with gastritis (CG) and DU cases (PG). This may be because of the association of *H. pylori* strains with EPIYA-C with more than one repeat in the 3′ region of the *cagA* gene with enhanced histological injury and reduced survival in acidic conditions (Yamaoka *et al.*, 1999); however, the number of strains with EPIYA-C with multiple repeats was higher in GC cases. This difference may be explained by the following hypothesis.

Yamaoka *et al.* (1999) reported that *H. pylori* strains with multiple EPIYA-C were less resistant to gastric acid and can survive only in the presence of advanced atrophic gastritis (such as in GC) in which gastric acid secretion is low. Yamaoka (2010) concluded that multiple EPIYA-C repeats may have developed after the onset of atrophy, and are not the cause, but rather the effect, of atrophy. This hypothesis may partly explain the contradictory results. A combination of bacterial, host and environmental factors may contribute to the development of GC.

The polymorphisms in the EPIYA motif pattern vary according to the geographical distribution (Jones *et al.*, 2009; Acosta *et al.*, 2010). While the Western type of EPIYA-C is common in Western communities, EPIYA-D is frequently isolated in East Asian communities (Higashi *et al.*, 2002). Jones *et al.* (2009) identified EPIYA-ABD in 100 % of GC patients. This relationship between the Eastern type of the *cagA* genotype and GC may be linked to the Eastern CagA's higher affinity for SHP-2 (Higashi *et al.*, 2002). However, we did not detect any EPIYA-D.

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In conclusion, we found that Western-type *H. pylori* strains in Turkey have similar patterns of EPIYA motifs to those of neighbouring countries with Western populations. Crucially, our study revealed that having a multiple EPIYA-C repeat increased the GC risk by 30.6-fold and the DU risk by 8.9-fold compared with the CG (gastritis and NGIS).

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