Clinical relevance of *Helicobacter pylori* virulence factors in *Iranian* patients with gastrointestinal diseases

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ABSTRACT

Helicobacter pylori (*H. pylori*) usually colonizes the gastric mucosa of more than 50% of the human population, causing an infection that may appear in early childhood and can persist for life. *H. pylori* is suggested as the main cause of peptic ulcer and chronic gastritis. It is also associated with gastric cancer. Its severity and symptoms depend on environmental factors, host susceptibility and bacterial components, which allow *H. pylori* to switch between commensalism and pathogenicity. *H. pylori* is genetically highly variable, and the variability which affects *H. pylori* virulence factors might be useful in identifying the strains with different degrees of pathogenicity. The geographic distribution of distinct *H. pylori* genotypes is largely unknown and should be established. The prevalence of more pathogenic genotypes in certain areas may have important epidemiological consequences. It also might be associated with the severity of *H. pylori* related diseases in such regions. Given that Iran is located in the Middle East and Asian populations have revealed high levels of gastric cancer, it is of clinical interest to clarify the potential of *H. pylori* virulence markers in predicting the associated clinical outcomes. In this review, clinical relevance of adhesion molecules and significant virulence factors of *H. pylori* in Iranian patients with gastrointestinal diseases are discussed in comparison to other countries.

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1. Introduction

Helicobacter pylori (*H. pylori*) is a flagellated, microaerophilic gram-negative bacillus with spiral-shape that colonizes the gastric mucosa, causing gastroduodenal diseases such as peptic ulcer, chronic gastritis, atrophic gastritis as well as gastric cancer [1—3]. More than 50% of the world's population is infected with *H. pylori*, and most of colonized individuals develop chronic inflammation. In the majority of infected patients, *H. pylori* colonization does not cause any symptoms [4]. However, long duration of infection with *H. pylori* increases the risk of developing site-specific diseases. About 0.1% of the infected patients develop mucosa-associated lymphoid tissue (MALT) lymphoma, 1—3% develop gastric adeno-carcinoma and 10% develop peptic ulcer disease [5]. Indeed, the clinical consequences of *H. pylori* infection are determined by multiple factors, including environmental factors (such as smoking, high salt intake, malnutrition, vitamin and antioxidants deficiency), genetic predisposition of the host, especially regarding certain cy-tokines, receptor gene polymorphisms such as IL-1β, IL-8, IL-10, IL-17A and TNF-α and gene regulation [6—12]. In addition to envi-ronmental and host factors, *H. pylori* strain-specific factors play a role in different clinical expression, too [13,14]. *H. pylori* are genetically highly diverse bacteria, mostly due to high mutational rates, that include large insertions or deletions and chromosomal rearrangements that affect housekeeping and virulence genes [15]. Within the *H. pylori* virulence factors, genetic variation may ac-count for differences in the pathogenic properties of strains, and thus may help explain the discrepancies between the number of infected individuals and those that end up developing gastric car-cinoma [13,16]. In this review, we tried to explain the contribution of *H. pylori* virulence factors to different clinical expressions. We will mainly focus on *cagA*, *vacA*, adhesins and outer membrane proteins [17]. These are virulence factors that show different fre-quency and genetic variation between strains and that have been more extensively studied, thus gaining clinical interest as putative markers in different clinical expressions.

2. The *vacuolating cytotoxin A* (*vacA*)

The *vacuolating cytotoxin A* (*vacA*) gene, which is an important virulence factor of *H. pylori* and is present in all strains, encodes an 87 kD protein. The initial studies on *vacA* detected two main polymorphic regions including the signal sequence (s1 and s2) and two types of mid region (m1 and m2). However, more recent studies have identified an intermediate (i1 and i2) region which is located between the s and m regions [18,19]. The mosaic combi-nation of s and m region allelic types determines the production of the cytotoxin and is associated with pathogenicity of the bacteria [20]. VacA is considered as a *H. pylori* toxin with multiple cellular effects in various host cell types. The toxin inserts into the cell membrane and forms anion-conducting channels. This seems to be important for the modulation of other VacA activities including the ability to induce large acidic vacuoles, which was the first epithelial cell phenotype attributed to the toxin [21]. VacA toxin interferes with autophagy pathways of gastric cells, causes gastric epithelial erosions in mice, induces cell apoptosis and causes cell necrosis. It also has immunomodulatory properties by inhibiting T and B-lymphocyte proliferation and activation [22,23]. VacA with an

unknown mechanism has an indirect effect on T cells, too. It can induce dendritic cells tolerance and regulatory T cell induction; however this effect has not been yet documented in human cells [24,25]. It also induces a proinflammatory effect on T cells which is mediated by activation of *NF-kB* and leads to upregulation of IL-8 [25].

The mosaic combination of the *vacA* s and m region alleles can give rise to s1/m1, s1/m2, s2/m1 and s2/m2 type strains. There is typically no vacuolating activity in s2/m2 strains, however, *vacA* s1/m1 chimeric strains induce greater vacuolation than do s1/m2 strains [20,26,27]. The i region plays a functional role in vacuolating activity, since *vacA* s1/i1/m2 strains are vacuolating types and *vacA* s1/i2/m2 strains do not induce vacuolation. All *vacA* s1/m1 alleles are of type i1, all s2/m2 alleles are of type i2, and s1/m2 alleles can be either i1 or i2 [20].

Recently, a deletion of 81 bp between the i region and m region was identified which was termed d region. D1 strains have no deletion, however, the strains of the d2 type contain a 69- to 81-bp deletion for a small number of Western strains, but not East *Asian* strains [28].

2.1. Clinical relevance of vacA genotypes in different clinical expression

The first studies evaluating the role of *vacA* genotypes in gastric disease showed an association between the *vacA* s1 strains and enhanced inflammation of the mucosa [29—31]. However, more detailed analyses of gastritis parameters in the context of *H. pylori* infection showed that *vacA* s1 and *vacA* m1 genotypes were asso-ciated with higher levels of neutrophilic and lymphocytic in-filtrates, gastric atrophy, epithelial damage and intestinal metaplasia [31]. Furthermore, studies including Brazilian, Portu-guese, South African, Italian and German patients showed that *H. pylori vacA* s1 and m1 strains were more frequently observed in gastric carcinoma in comparison to chronic gastritis patients [32—35]. In Western populations, the *vacA* s1/m1 allele is strongly associated with duodenal and gastric ulcer disease as well as with gastric cancer [32,36]. The evaluation of 135 chronic superficial gastritis and 130 gastric carcinoma patients from Portugal, showed that patients infected with *vacA* s1 and m1 strains had respectively 17- and 6.7-fold increased risk for gastric carcinoma compared with those infected with *vacA* s2 and m2 strains [33]. The study of Kamali-Sarvestani and colleagues revealed a significant difference in *vacA* genotype distribution between gastric cancer and gastritis or peptic ulcer patients.

The significant increased level of s1m1 genotype in gastric cancer patients in comparison with those in patients with peptic ulcer or gastritis confirms the pathogenic role of this virulence determinant in Iranian patients [37]. Another study in Iranian pa-tients demonstrated that the presence of m1 allele was associated with duodenal and gastric ulcer, whereas all patients with gastritis had m2 allele [38]. It has been reported that association of allele m1 and histological alterations has related to an increase in epithelial damage (microscopic erosions, epithelial degeneration and mucus depletion) in contrast to reports on allele m2 [39].

Several studies in Iranian patients revealed that there was not any association between *vacA* status and different clinical outcomes including duodenal ulcer, gastric ulcer, gastritis, and non-ulcer

dyspepsia in Iranian patients [40–43] (Table 1). Study in Iranian patients indicated that vacA i1, s1 and m1 strains were strongly associated with gastric cancer [20,29]. This result was further supported by Basso et al. [31] in an Italian population. Among H. pylori strains isolated from patients in Iraq and Italy, vacA i1 strains were associated with gastric ulcer disease [29,31,44]. However, in East Asian and Southeast Asian populations, where the incidence of gastric cancer is high, vacA i-region subtype is not associated with risk of disease [45]. Other studies from Iran revealed that s1 allele is associated with peptic ulcer disease including duodenal ulcer and gastric ulcer and also s1 and s1m2 strain is dominant genotype among infected Iranian patients [30,37,42,44,46–49]. Similarly, s1m2 genotype was shown to be predominant in Turks and in Western countries [50]. However, the vacA s1m1 genotype is more predominant in Afghani and Indian strains [42,51,52]. Similar to other reports [30], Molaei et al. also found that cagA and vacA s1 were highly associated with each other and vacA s2 genotype was more frequent in cagA negative patients. Moreover, patients with type s1 strains had more severe degree of mononuclear cell infiltration in comparison to type s2 strains [53]. The vacA d-region remains poorly studied. The only study in Western countries revealed that infection with vacA d1 strains significantly increased the risk for gastric carcinoma when compared with infection with d2 strains, and was associated with increased neutrophil infiltration and gastric atrophy, independent of the vacA s-, m-, and i-region genotypes. In contrast, in East Asian countries, there was no relationship between the vacA d-genotypes and clinical outcome or histopathological changes in the gastric mucosa [28].

3. The cytotoxin associated gene A (cagA)

H. pylori strains are divided into two main subpopulations based on their capability to produce a 120–145-kDa immunodominant protein called cytotoxin-associated gene A (CagA) antigen. The cagA gene that encodes cagA is localized at one end of the cag PAI, a 40 kb DNA segment that was most likely incorporated into the H. pylori genome by a process of horizontal transfer state [56]. The cagA gene is present in 44%–91% of H. pylori strains in Iranian patients [37,40,48,57]. This result is different from the reports from South and East Asian where the presence of cagA strain and its association with clinical outcomes is more than 90% [46,58]. It has been shown that the prevalence of cagA-positive strains in USA and Europe is 60–70% [59] which is more similar to our findings than in different places of Asia. Prevalence of cagA in different geographical areas of Iran is still under debate.

The cagPAI DNA segment possesses 31 putative genes, including cagA and those encoding components of a molecular 'syringe' termed the type IV secretion system (T4SS), through which macromolecules are delivered from the inside to the outside of the bacterium [59]. The cagL gene, which is also located in the cagPAI, encodes the CagL protein which is expressed on the surface of H. pylori in a T4SS-dependent manner [60]. Upon the attachment of cagA-positive H. pylori to the gastric epithelial cell, the CagA protein is injected directly into the cell [61]. Upon injection, unphosphorylated CagA interacts with host cell proteins. This causes dysregulation of epithelial structure, integrity through its effect on host cell signaling and induction of pro-inflammatory, as well as mitogenic responses [62,63]. CagA is not only injected into gastric

Table 1
Prevalence of vacA genotype of H. pylori in Iranian patients with gastrointestinal diseases.

Group [n]		Allelic variants	Disease group					Results	References
			Gastritis	Peptic ulcer		Non-ulcer patients	Gastric cancer		
				Gastric ulcer	Duodenal ulcer				
			No. of patients (%)	No. of patients (%)	No. of patients (%)	No. of patients (%)	No. of patients (%)		
shiraz (Iran 1)	Gastritis = 199 Gastric Ulcer = 12 Duodenal Ulcer = 67 Gastric Cancer = 20	s1m1 s1m2 s2m1 s2m2	48 (26.5) 74 (40.9) Not seen 59 (32.6)	21 (32.3) 33 (50.8) Not seen 11 (16.9)		—	12 (66.7) 3 (16.7) Not seen 3 (16.7)	Association with the type of disease	[37]
Guilan (Iran 2)	Gastritis = 29 Gastric Ulcer = 13 Duodenal Ulcer = 35	s1 s2 m1 m2	16 (29) 0 (0) 29 (100)	10 (77) 8 (61)	29 (83) 23 (66)	—	—	s1 and m1 allele was associated with duodenal and gastric ulcer.	[38]
Tehran (Iran 3)	Gastritis = 32 Gastric Ulcer = 30 Duodenal Ulcer = 43 Non-Ulcer Dyspepsia = 35	s1 s2 m1 m2 s1m1 s1m2 s2m1 s2m2	19 (59.4) 13 (40.6) 7 (15.7) 25 (84.3) 5 (15.6) 12 (37.5) 5 (15.6) 10 (31.3)	22 (73.4) 8 (26.6) 12 (40) 18 (60) 5 (16.7) 15 (50) 2 (6.6) 8 (26.7)	35 (81.4) 8 (18.6) 20 (46.6) 23 (53.4) 11 (25.6) 22 (51.2) 4 (9.2) 6 (16)	21 (60) 14 (40) 47 (33.6) 27 (77.1) 8 (22.8) 64 (45.8) 14 (10) 9 (25.7)	—	No association with the type of disease	[40]
Tehran (Iran 4)	Gastric Ulcer = 25 Duodenal Ulcer = 25	s1 s2 m1 m2	—	25 (100) 0 (0) 14 (56) 11 (44)	25 (100) 0 (0) 17 (68) 8 (32)	—	—	vacA allele s1 of this bacterium is associated with ulcer	[54]
Tehran (Iran 5)	Gastritis = 12 Peptic Ulcer Disease = 8 Non-Ulcer Dyspepsia = 36 Gastric Cancer = 5	s1 s2 m1 m2 s1m1 s1m2 s2m1 s2m2	9 (75) 3 (25) 4 (33.3) 8 (66.7) 3 (25) 5 (41.6) 0 (0) 4 (33.3)	8 (100) 0 (0) 2 (25) 6 (75) 2 (25) 6 (75) 0 (0) 0 (0)		25 (69.4) 11 (30.6) 11 (30.6) 25 (69.4) 12 (33.3) 14 (38.8) 0 (0) 10 (27.7)	4 (80) 1 (20) 1 (20) 4 (80) 1 (20) 3 (60) 0 (0) 1 (20)	No association with the type of disease	[55]

epithelial cells, but it can be also injected into B lymphoid cells and murine, as well as human dendritic cells (DCs) [64—66]. Remarkably, CagA translocation into DCs suppresses host immune response by reducing the secretion of pro-inflammatory cytokines as IL-12p40 and enhancing the expression of the suppressive cytokine IL-10, indicating a dual pro- and anti-inflammatory role for CagA during *H. pylori* infection dependent on the cellular context [66,67]. The *cagA* gene contains a 5′ end which is highly conserved and a 3′ end which is variable. The 3'-variable region contains several repeat sequences, each of which contains an EPIYA motif; the size variation in *cagA* correlates with the number of repeat sequences located in this region. Once translocated into the host cells, *cagA* is tyrosine phosphorylated on EPIYA motifs by ABL and SRC family kinases [68,69]. The tyrosine phosphorylation site of *cagA* is characterized by the presence of a unique Glu-Pro-Ile-Tyr-Ala (EPIYA) motif, which is present in multiple numbers in the carboxyterminal region of the protein [70]. From the sequences flanking these EPIYA motifs, four distinct EPIYA segments, EPIYA-A, B, C and D, each of which contains a single EPIYA motif, have been identified in the CagA protein [71]. EPIYA motifs in Western *H. pylori* isolates such as those from Australia, North America and Europe, are classified as EPIYA-A, EPIYA-B, and EPIYA-C and are defined by the amino acid sequences surrounding the EPIYA motifs. It has been suggested that the number of EPIYA-C sites directly correlates with the level of tyrosine phosphorylation, SHP-2 binding activity, and cell damage. CagA proteins in East Asian *H. pylori* isolates such as Japan, Korea and China possess EPIYA-A, EPIYA-B, and EPIYA-D motifs; the EPIYA-D motif has a higher affinity for SHP-2 than does the Western EPIYA-C motif and appears to induce morphological changes that involve cell-shape modulation and that result in cell elongation [72,73]. The tyrosine residue which constitutes the EPIYA-C site seems to be the main site of tyrosine phosphorylation in Western *cagA* by SFK in gastric epithelial cells. However, those present in the EPIYA-A and EPIYA-B segments are porly phosphorylated in the cells [59,70]. Furthermore, the experiments on transgenic mice models also provided evidence that the East Asian-type *cagA* is more carcinogenic than the Western-type *cagA* [74].

3.1. Clinical relevance of cagA genotypes in different clinical expression

The CagA protein has oncogenic potential. Infection with *cagA*-positive strains is associated with higher degrees of epithelial damage, chronic inflammation and neutrophilic activity in the gastric mucosa, as well as increased risk of gastric atrophy and intestinal metaplasia, compared to infection with *cagA*-negative strains [33,75—77]. Both intensity of inflammation and epithelial damage might be involved in the pathogenesis of peptic ulceration [78]. In a study conducted in Spain, patients infected with *cagA*-positive strains had 2.3-fold increased risk of progression of gastric precancerous lesions in comparison with those infected with *cagA*-negative strains [79]. In a meta-analysis study which included the cross-sectional and case-control trials with *cagA* seroprevalence or *cagA* detection by PCR, the odds ratios for gastric carcinoma in *cagA*-positive infections were respectively 2.1 (95% CI, 1.5—2.9) and 2.4 (95% CI, 1.3—4.7), compared with normal controls, and with gastritis controls, [80]. Several studies in Iranian patients demonstrated that there were no associations between *cagA* and different clinical outcomes including gastric cancer and gastritis or peptic ulcer, which is in accordance with the results of other Asian countries [37,40—42] (Table 2). A low prevalence of *cagA* has been reported among MALT lymphoma and gastric cancer patients, and there is no correlation between *cagA* gene and MALT lymphoma and gastric cancer patients [81]. Several studies from the northern

areas of Iran indicated that there was a stronger correlation between *cagA* gene and severe gastroduodenal complications such as gastric cancer [82,83]. Salehi and Jafarzadeh reported that there was a stronger correlation between *cagA* gene and duodenal ulcer disease than with gastritis. In the gastric ulcer group, the prevalence of *cagA* positivity was 77% [38,78,84,85]. Study of Yadegar et al. in Iranian patients indicated that there was a significant relationship between both *cagL* and *cagA* positivity and *H. pylori* density in the antrum [55]. Only one study in Iranian patients showed that the most frequent type of *cagA* 3′ region was type A (74%) and they did not find the type C reported by Yamaoka et al. [52], but they observed the subtypes of B or D (26%). Moreover their study did not support the view that the subtypes of 3′ region of *cagA* gene in *H. pylori* isolated from Iran are correlated with the clinical outcomes of *H. pylori* [78]. Unlike the previous studies, Shokrzadeh et al. [86] revealed that there were no strains with EPIYA-D segments, in agreement with the studies examining Iranian strains [44,87,88]. The structure of the 3′ region of the *cagA* gene in Iranian strains was Western type. Furthermore, their study did not support differences between EPIYA types and clinical outcomes.

4. Adhesins and OMPs

Adherence of *H. pylori* to the gastric epithelium facilitates persistence of infection, initial colonization and delivery of virulence factors to host epithelial cells. The adherence of *H. pylori* to the gastric mucosa is important for protection from mechanisms such as mucus, acidic pH, and exfoliation [89,90]. *H. pylori* adhesins which are considered as bacterial virulence factors are involved in numerous processes during early and chronic phases of infection. They also contribute to the differential outcome in infected patients by triggering disease development. Approximately 4% of the *H. pylori* genome is predicted to encode outer membrane proteins [17], which is significantly more than that for other known bacterial species [91].

4.1. OipA

The outer inflammatory protein A (Oip A) is a 35 kDa pro-inflammatory protein which is an inflammation related outer membrane protein. The exact role of OipA is still not clear. OipA expression is linked to increased IL-8 production *in vitro* [92]. Recent work using Mongolian gerbils infected with wild-type *H. pylori* and an isogenic *oipA* mutant strain demonstrated a role for *oipA* in induction of the mucosal cytokines IL-1, IL-17, and tumor necrosis factor alpha (TNF-a) and in gastric mucosal inflammation [93]. Also *oipA* is involved in upregulation of matrix metalloproteinase 1 (MMP-1) [94].

4.2. BabA

Blood group antigen binding adhesin (BabA) is a 75-kDa adhesion molecule that mediates the attachment of *H. pylori* to Lewis[b] blood group antigens on human gastric epithelial cell. Three *bab* alleles namely *babA1*, *babA2*, and *babB* have been identified. *BabA1* and *babA2* are identical alleles except that *babA1* has a 10-bp deletion of the translational initiation codon. Only the *babA2* gene product is necessary for Lewis[b] binding activity [95]. About 70% of *H. pylori* strains in Western countries were typed as *babA2*, which was associated with increased virulence [96].

4.3. DupA

Recently, Lu et al. described a new putative *H. pylori* virulence marker located in the "plasticity region" of the *H. pylori* genome. The authors suggested the name *dupA* (duodenal ulcer-promoting

Table 2
Prevalence of *cagA* genotype of *H. pylori* in Iranian patients with gastrointestinal diseases.

	Genotype	Disease group						Results	References
		All patients	Gastritis	Peptic ulcer		Non-ulcer patients	Gastric cancer		
				Gastric ulcer	Duodenal ulcer				
		No. of patients (%)	No. of patients (%)	No. of patients (%)	No. of patients (%)	No. of patients (%)	No. of patients (%)		
shiraz (Iran 1)	cagA-Positive	219 (76.5)	148 (74.4)	54 (80.6)		–	17 (85)	No association with the type of disease	[37]
	cagA-Negative	67 (23.5)	51 (25.6)	13 (19.4)			3 (15)		
Iran 2 (Guilan)	cagA-Positive	52 (67.5)	14 (48)	10 (77)	28 (80)	–	–	Association with peptic ulcer disease	[38]
	cagA-Negative	25 (32.5)	15 (52)	13 (33)	7 (20)				
Guilan (Iran 3)	cagA-Positive	76 (71)	11 (46)	33 (77)	32 (80)	–	–	Association with duodenal ulcer	[78]
	cagA-Negative	31 (32.5)	13 (54)	10 (33)	8 (20)				
Tehran (Iran 4)	cagA-Positive	98 (70)	18 (56.3)	21 (70)	35 (81.4)	24 (68.6)	–	No association with the type of disease	[40]
	cagA-Negative	42 (30)	14 (43.7)	9 (30)	8 (18.6)	11 (31.4)			
Tehran (Iran 5)	cagA-Positive	52 (85.2)	10 (83.3)	7 (87.5)		31 (86.1)	4 (80)	No association with the type of disease	[55]
	cagA-Negative	9 (14.8)	2 (16.6)	1 (12.5)		5 (13.9)	1 (20)		
Tehran (Iran 6)	cagA-Positive	45 (76)	–	13 (76.4)		32 (76)	–	No association with the type of disease	[44]
	cagA-Negative	14 (24)		4 (23.6)		10 (24)			
Sari (Iran 9)	cagA-Positive	84 (65.6)	63 (85.1)	–		–	16 (57.1)	No association with the type of disease	[81]
	cagA-Negative	44 (34.4)	11 (14.9)				12 (42.9)		
Tehran (Iran 7)	cagA-Positive	92 (73.6)	–	0		3	0	No association with the type of disease	[86]
	cagA-Negative	49 (26.4)		10		73	3		
	EPIYA-AB	3 (3.3)		1		1	1		
	EPIYA-ABC	86 (93.5)							
	EPIYA-ABCC	3 (3.3)							
Tehran (Iran 8)	EPIYA-ABC	30 (68)	–					EPIYA-ABCC association with duodenitis	[88]
	EPIYA-ABCC	4 (9)							
	EPIYA-ABCCC	1 (2)							
	Mixed Types	6 (14)							
	Undefined Type	3 (7)							
Iraqi (Dohuk)	cagA-Positive	35 (71)	–	19 (95)	16 (55)	–		Association with peptic ulcer disease	[44]
	cagA-Negative	14 (29)		1 (5)	14 (45)				

A) for this gene due to an association with increased risk for duodenal ulcer and protection against gastric carcinoma, gastric atrophy and intestinal metaplasia in Korea and Japan. An association between *dupA* and increased expression levels of IL-8 has been reported in the gastric mucosa of *H. pylori*-infected subjects [97–99], but neither *dupA1* nor *dupA2* were found to induce IL-8 secretion by gastric epithelial cells. However, *DupA1* was found to increase pro-inflammatory cytokine expression, most markedly IL-12p40, IL-12p70, and IL-23 by CD14$^+$ mononuclear cells, which may explain how *dupA1* contributes to gastric inflammation [98].

4.4. iceA1/2

A novel gene has recently been discovered, designated *iceA* (induced by contact with epithelium). There are two main allelic variants of the gene: *iceA1* and *iceA2*. The expression of *iceA1* is up-regulated by contact between *H. pylori* and human epithelial cells [17].

4.5. Clinical relevance of adhesins and OMPs in different clinical expression

The prevalence of *dupA* is significantly higher in strains of subjects having duodenal ulcer but lower in patients with gastric cancer (42% vs 9% on average), regardless of nationality (Japan, Korea and Colombia) [100,101]. A review study reported that the prevalence of *dupA* in subjects with gastritis worldwide was 44.88% and differed significantly among nationalities and ethnicities [102]. The correlation between *dupA* status and disease development can be found in several Asian countries. A meta-analysis study including 17 trials with a total of 2466 patients was performed to confirm the association between the *dupA* and clinical outcomes, and we revealed that the overall prevalence of the *dupA* gene was 31.0% (496/1600) in Asian countries and 64.1% (526/820) in Western countries. Infection with *dupA*-positive *H. pylori* increased the risk of duodenal ulcer (OR 1.41, 95% confidence interval [CI] 1.12–1.76) in Asian countries (OR 1.57, 95% CI 1.19–2.06) but not in Western countries (OR 1.09, 95% CI 0.73–1.62) [103]. However, there was no association between the presence of *dupA* and gastric cancer or gastric ulcer. Furthermore, studies have reported that the presence of *dupA* was significantly associated with eradication failure [104]. Studies in Iranian patients revealed that possession of *dupA* was inversely correlated with dysplasia such that 83.3% of dysplasia-positive patients were colonized with *dupA*-negative strains however; only 30.2% of dysplasia-negative patients were colonized with *dupA*-positive. The presence of lymphoid follicles was also inversely associated with *dupA*-positive strains, taking into account that 81.3% of patients with no lymphoid follicles possessed *dupA*-positive strains whereas only 52.1% of patients with lymphoid follicles were infected with these strains. Also the presence of the

dupA gene was not related to chronic gastritis, gastric ulcer, duodenal ulcer, atrophy, intestinal metaplasia or the presence of *H. pylori* [105] (Table 3). These findings are in accordance with the results of Argent et al. [106], who demonstrated a lack of association between the presence of *dupA* and duodenal ulcer in strains isolated from four different geographical regions of the United States, South Africa, Belgium and China. Furthermore, Gomes et al. [107] also reported the lack of association of *dupA* with duodenal ulcer as well as gastric cancer in both Brazilian adults and paediatric patients. In contrast, Arachchi et al. [101] confirmed the association of *dupA* with DU as an informative virulence marker. *OipA* was more frequently detected in duodenal ulcer and gastric cancer, but significant effect on gastroduodenal diseases was not found. Previous reports showed that *oipA*-positive status related with the development of duodenal ulcer and gastric cancer [108,109]. A recently published study in Iranian patients revealed that *oipA*-positive patients had higher neutrophil activity and *H. pylori* density in gastric biopsy specimens [30]. *OipA* expression was reported to be linked to severe inflammation and the presence of a functional gene is significantly associated with the presence of gastric cancer, duodenal ulcer, and increased neutrophil infiltration [108,110]. A study on Iranian patients demonstrated that the *iceA1* allele was predominant [30,55]. This is in agreement with previous reports from Turkey, Venezuela, Japan and Hong Kong, but different from the strains in the Brazil and USA. Studies among South African showed that *iceA2* was predominant, however, *iceA* positivity was not associated with any specific disease outcome [52,111,112]. Indeed, multiple *iceA* (*iceA1+iceA2*) genotypes which show mixed infection with two or more different strains of *H. pylori*, were found in Iranian patients of our isolates [30,55]. The prevalence of mixed infections regarding multiple *iceA* genotypes was frequently reported from other countries [113]. A meta-analysis including 50 studies with a total of 5357 patients was performed to confirm the relationship between the *iceA* allelic type and clinical outcomes, and we revealed that the overall prevalence of iceA1 was significantly higher in Asian countries than in Western countries (64.6% vs 42.1%), whereas iceA2 was more prevalent in Western countries than in Asian countries (45.1% vs 25.8%). Sensitivity analysis in Western countries showed that the presence of iceA1 was significantly associated with peptic ulcer (OR 1.25, 95% CI 1.08−1.44). On the other hand, the presence of iceA2 was inversely correlated with peptic ulcer (OR 0.76, 95% CI 0.65−0.89) [113]. The binding specificities of *H. pylori* strains worldwide suggest that the BabA adhesin has evolved in response to host mucosal glycosylation patterns to permit *H. pylori* to adapt to its host and to maintain persistent colonization [114]. The presence of *babA2* is associated with duodenal ulcer disease and gastric cancer, and when found in conjunction with *cagA* and *vacA* s1 alleles, it is associated with an even greater risk of developing more severe disease [96]. More recent analyses of babA2 as a virulence marker have shown conflicting data on the usefulness of *babA2* expression in predicting clinical outcome, which is most likely dependent on the geographic origin of the *H. pylori* strains. In Thai and Portuguese populations, *babA2* has not shown to be a biomarker for peptic ulcer disease or gastric cancer [58,115]. However, for strains isolated from northern Portugal, Turkey, and Germany *babA2* expression is associated with the severity of gastric disease [96,116,117].

5. Conclusion

H. pylori plays a central role in the pathogenesis gastrointestinal disorder. The complex combination of environmental, host, and bacterial factors determines the susceptibility and severity of outcome of *H. pylori* infection and related pathology in the subset of individuals. Iran is located in the Middle East between Europe and East Asian countries, and has close economic and cultural relationships with both regions. Although in Iran, the patterns of the prevalence of *vacA* genotypes is similar to those in European countries [20,37,47,118], it is unclear whether *cagA* EPIYA sequences in Iranian strains are similar to the Western type or not. The number and/or kind of EPIYA motifs/segments in Iranian strains was not associated with gastro-duodenal diseases, due to few previous information about the patterns of EPIYA motifs in the Iranian population [44,78]. Besides, the presence of the *dupA* gene in Iranian patients was not similar to Asian countries, and has no association with gastro-duodenal diseases. But Western countries

Table 3
Prevalence of other virulence factor of *H. pylori* in Iranian patients with gastrointestinal diseases.

	Genotype	Disease group						Results	References
		All patients	Gastritis	Peptic ulcer		Non-ulcer patients	Gastric cancer		
				Gastric ulcer	Duodenal ulcer				
		No. of patients (%)	No. of patients (%)	No. of patients (%)	No. of patients (%)	No. of patients (%)	No. of patients (%)		
Tehran (Iran 1)	dupA-Positive	23 (39)	—	6 (35)		17 (40)	—	No association with the type of disease	[44]
	dupA-Negative	36 (61)		11 (65)		25 (60)			
Tehran (Iran 2)	dupA-Positive	78 (49.7)	34 (50)	9 (39.1)	15 (50)	—	20 (55.6)	No association with the type of disease	
	dupA-Negative	79 (50.3)	34 (50)	14 (60.9)	15 (50)		16 (44.4)		
Iraqi (Dohuk)	dupA-Positive	16 (32.7)	—	11 (55)		5 (17)	—	Association with peptic ulcer disease	[44]
	dupA-Negative	33 (67.3)		9 (45)		24 (83)			
Tehran (Iran 3)	babA2-Positive	59 (96.7)	12 (100)	8 (100)		34 (94.4)	5 (100)	No association with the type of disease	[55]
	babA2-Negative	2 (3.3)	0 (0)	0 (0)		2 (5.5)	0 (0)		
Tehran (Iran 5)	iceA1-Positive	26 (42.6)	7 (58.3)	3 (37.5)		13 (36.1)	3 (60)	No association with the type of disease	[55]
	iceA2-Positive	14 (23)	3 (25)	3 (37.5)		7 (19.4)	1 (20)		
	iceA1/iceA2 mixed	21 (34.4)	2 (16.6)	2 (25)		16 (44.4)	1 (20)		

have similar results in this virulence factor. A better understanding of the molecular mechanism for *H. pylori*-related inflammation and gastric carcinogenesis would allow the identification of more effective therapeutic targets for gastric cancer. Further research into Iranian host and *H. pylori* genetics would be warranted to draw more far-reaching conclusions.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' contribution

Nader Bagheri and Fatemeh Azadegan-Dehkordi contributed equally in preparation of this paper. All authors contributed in preparation of the first draft and confirmed the last version. Mahmoud Rafieian-Kopaei edited the last version.

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