



Basic Study

## Reciprocal impact of host factors and *Helicobacter pylori* genotypes on gastric diseases

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### Abstract

**AIM:** To assess the impact of *Helicobacter pylori* (*H. pylori*) genotypes and patient age and sex on the development of gastric diseases.

**METHODS:** *H. pylori*-infected patients ( $n = 233$ ) referred to the endoscopy unit at Tehran University of Medical Sciences (Tehran, Iran) were diagnosed with chronic gastritis (CG), gastric ulcer (GU), or duodenal ulcer (DU). Brucella blood agar was used for biopsy cultures and *H. pylori* isolation under microaerobic conditions. *H. pylori* isolates were confirmed with biochemical tests and through amplification of the *16S* rRNA gene. DNA was extracted from fresh cultures of the *H. pylori* isolates and used for amplification of *vacA* alleles and the *cagA* gene. Statistical analysis was performed to determine the association between *H. pylori* genotypes, age (< 40 years vs > 40 years) and sex of the patient, and gastric diseases.

**RESULTS:** CG was the most prevalent gastric disease (113/233; 48.5%), compared to GU (64/233; 27.5%)

and DU (56/233; 24%). More patients were male, and gastric diseases were more frequent in patients > 40 years ( $P < 0.05$ ). The percentage of CG and GU patients that were male and female did not show a significant difference; however DU was more common in males ( $P < 0.05$ ). Interestingly, a diagnosis of CG in patients > 40 years was more common in females (18.5%) than males (11.6%) ( $P = 0.05$ ), whereas a diagnosis of GU or DU in patients > 40 years was more frequent in males (14.6% *vs* 10.7% and 12.4% *vs* 4.3%, respectively). Overall, genotyping of the *H. pylori* isolates revealed that the *vacA* s1 (82%), *vacA* m2 (70%), and *cagA*<sup>+</sup> (72.5%) alleles were more frequent than *vacA* s2 (18%), *vacA* m1 (29.2%), and *cagA*<sup>-</sup> (all  $P < 0.05$ ). The *vacA* s1m2*cagA*<sup>+</sup> genotype was the most prevalent within the three disease groups. *vacA* s1m2 frequency was 56.2% with a similar occurrence in all diagnoses, while *vacA* s1m1 appeared more often in DU patients (33.9%). A genotype of *vacA* s2m2 occurred in 15% of isolates and was more common in CG patients (21.2%); *vacA* s2m1 was the least common genotype (3%). The *vacA* s1 allele was found to be a risk factor for DU, *vacA* s2 for CG, and *vacA* s1 and *vacA* s2 for GU (all  $P < 0.05$ ). The *vacA* s2m2 genotype was associated with the development of CG and GU compared to DU ( $P < 0.05$ ). No correlation was found between *vacA* m or *cagA* and gastric diseases.

**CONCLUSION:** The outcome of *H. pylori* infection is the result of interaction between bacterial genotypes and the age and sex of infected individuals.

**Key words:** Age; Gastric disease; Gender; Genotype; *Helicobacter pylori*

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**Core tip:** *Helicobacter pylori* (*H. pylori*) genotype and host and environmental factors have emerged as the risk factors of *H. pylori*-associated diseases. However, controversies exist regarding the reciprocal interaction between these factors. Results of this study demonstrate that increased age is an important risk factor for gastric ulcers in both males and females, for chronic gastritis in females, and for duodenal ulcers in males. Genotypes *vacA* s1 and *vacA* s2m2 emerged as significant risk factors for duodenal ulcers, and chronic gastritis and gastric ulcers, respectively. No correlation was found between *vacA* m or *cagA* and gastric diseases.

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## INTRODUCTION

Analysis of the genetic composition of *Helicobacter pylori* (*H. pylori*) has revealed a remarkable heterogeneity in gene content and sequence<sup>[1]</sup>. This versatile gene reservoir appears to serve as a powerful tool for bacterial adaptation when encountering new conditions in different human hosts<sup>[2]</sup>. Establishment of *H. pylori* in gastric epithelium is associated with a persistent induction of inflammatory responses and tissue damage that could lead to development of more critical clinical diagnoses, including chronic gastritis (CG), peptic ulcers (PUs), or gastric cancer<sup>[3,4]</sup>. An interaction between *H. pylori* virulence factors, host genetics, and environmental factors is currently thought to determine the extent of tissue damage<sup>[5,6]</sup>. In this regard, the longevity of *H. pylori* infection and sex of infected individuals have been investigated as important factors in the development of gastric diseases<sup>[7-11]</sup>. Many investigators have studied *H. pylori* virulence factors and proposed several candidate proteins, including vacuolating cytotoxin A (VacA) and cytotoxin-associated gene A (CagA)<sup>[12]</sup>. In contrast, few studies have focused on host genetics, eating habits, and lifestyle, and the results remain controversial<sup>[13-19]</sup>.

VacA, which occurs in all strains of *H. pylori*, is regarded as a multifunctional toxin with the potential to insert into the endosomal membranes of epithelial cells, inducing the formation of large vacuoles, and inhibition of antigen presentation<sup>[20]</sup>. VacA also inserts into the mitochondrial membrane and causes apoptosis<sup>[21]</sup>. Cellular tight junctions are loosened by VacA, releasing nutrients, such as iron, nickel, sugars, and amino acids, needed for the establishment of *H. pylori*<sup>[22]</sup>. Furthermore, VacA inhibits the proliferation of T cells, which helps the bacterium to evade an immune response and establish a chronic infection<sup>[23,24]</sup>. Although VacA is crucial for colonization of all *H. pylori* strains, its toxicity is determined by the presence of different allelic types of the signal sequence (s1 and s2) and middle region (m1 and m2). It has been proposed that *H. pylori* strains carrying *vacA* s1m1 are highly toxigenic, increasing the risk of PUs or gastric cancer, those with *vacA* s1m2 less toxigenic, and *vacA* s2m2 nontoxigenic, while a genotype of *vacA* s2m1 rarely occurs<sup>[25,26]</sup>. CagA binds to epithelial cells and causes perturbation of tight junctions, cell polarity, and differentiation<sup>[27]</sup>. Interaction of CagA with E-cadherin and  $\beta$ -catenin causes interruption of the adhesion of epithelial cells, as well as formation of junctions and growth. Furthermore, CagA induces the production of interleukin-8, which leads to an inflammatory response and tissue damage<sup>[28]</sup>. These interactions of CagA with epithelial cells lead to destabilization and damage of gastric epithelium, and thus, contribute to *H. pylori* pathogenesis<sup>[22]</sup>.

Studies have shown that *cagA*<sup>+</sup> strains are often associated with a higher risk of PUs or gastric cancer,

compared to *cagA*<sup>-</sup> strains<sup>[29,30]</sup>. It has been suggested that the combination of an active VacA toxin with CagA constitutes an efficient system for generating an appropriate niche for long-term colonization of *H. pylori* in gastric epithelium. CagA contributes to changes in the gastric epithelium in several ways. It has been demonstrated that CagA protects epithelial cells against apoptotic events induced by VacA, but by inducing proinflammatory and antiapoptotic activities, also causes severe tissue damage, leading to a PU and even gastric cancer<sup>[31]</sup>. Furthermore, the antiapoptotic activity of CagA has been shown to reduce the rate of turnover of epithelial cells<sup>[32]</sup>, whereas VacA decreases CagA-induced cell scattering and motility<sup>[33]</sup>.

Cure of CG<sup>[34]</sup>, gastric ulcer (GU), and duodenal ulcer (DU)<sup>[35]</sup> with antimicrobial therapy against *H. pylori* demonstrates that the bacterium is an important risk factor for dyspeptic diseases. However, several studies have observed a correlation between *H. pylori*-associated gastric atrophy and smoking<sup>[13]</sup>, intake of salt<sup>[14]</sup>, alcohol<sup>[15]</sup>, low levels of dietary beta-carotene, and consumption of soybean products<sup>[16]</sup>. Furthermore, acid-suppression due to GU<sup>[17]</sup> and consumption of acid-suppressing drugs<sup>[36]</sup> have been found to be associated with corpus atrophy in *H. pylori*-infected patients. In contrast, no correlation between *H. pylori*-associated gastritis and sex, age, smoking, and coffee intake or between atrophy or intestinal metaplasia and smoking or drinking alcohol was observed in other reports<sup>[19]</sup>. Furthermore, it is well known that the incidence of *H. pylori* infection increases with age<sup>[7,8]</sup>, and that aging is an important risk factor for dyspeptic diseases<sup>[9,10]</sup>. However, there are discrepancies about the role of the sex of the patient in *H. pylori*-associated dyspeptic diseases<sup>[11]</sup>. Although reports indicate a reduced incidence of *H. pylori* infection in some regions of the world due to antimicrobial therapies against *H. pylori* or other infections<sup>[10,37-40]</sup>, a considerable number of patients in Iran are still referred for endoscopy, seeking relief from dyspeptic diseases. The reported frequency of *H. pylori* in the general population of Iran is approximately 69%<sup>[41]</sup>, but reaches up to 89% in the northwestern province of Ardabil<sup>[42]</sup>, where 90% of individuals over 40 years-old suffer from *H. pylori*-associated CG<sup>[43]</sup>. In this study, *H. pylori* isolates from 233 patients with CG, GU, or DU were genotyped for *vacA* alleles and *cagA* gene. The reciprocal impact of *H. pylori* genotypes and host age and sex on the development of dyspeptic diseases was assessed.

## MATERIALS AND METHODS

### Patients

The recruited patients ( $n = 233$ ; 129 men and 104 women) were randomly selected *H. pylori*-positive referrals to the endoscopy unit of Shariati Hospital (Tehran University of Medical Sciences, Tehran, Iran) due to complaint of dyspepsia. Patients were stratified

based on diagnosis and age: CG, GU, and DU, and < 40 years and > 40 years, respectively.

### *H. pylori* isolation and cultivation

Two antral biopsies were taken from each patient for a rapid urease test and *H. pylori* cultivation. Biopsies were cultured on selective Brucella agar (Pronadisa, Madrid, Spain) containing 5% defibrinated sheep blood and 10 mg/L vancomycin, 5 mg/L trimethoprim, and 2.5 IU/L polymyxin B (all from MP Biomedical, Santa Ana, CA, United States). Cultures were incubated at 37 °C under microaerobic conditions for 3-5 d. Bacterial isolates were identified as *H. pylori* on the basis of Gram-stained morphology, positive urease, catalase, and oxidase tests, and amplification of the *H. pylori*-specific 16S rRNA gene. The purified bacterial isolates were harvested in phosphate-buffered saline (PBS) and stored at -20 °C until further use.

### Genotyping of *H. pylori* isolates

DNA was extracted from fresh cultures of *H. pylori* isolates with phenol/chloroform as previously described<sup>[44]</sup>. Genotyping of *H. pylori* isolates was performed by polymerase chain reaction (PCR) amplification of the *cagA* gene, *vacA* signal sequences (s1 or s2), and middle regions (m1 or m2). The primers for amplification are listed in Table 1. *Escherichia coli* (DSM 0498) and previously PCR-confirmed *H. pylori* isolates were used as negative and positive controls, respectively. Amplification was carried out in a total volume of 25 µL containing 2.5 µL of 10 × PCR buffer (Sinaclon, Karaj, Iran), 1.5 mmol/L MgCl<sub>2</sub>, 125 µmol/L of each dNTP (Sinaclon), 1U of Taq DNA polymerase (Sinaclon), 0.5 µmol/L of each primer, and 25 ng of bacterial DNA. Cycling parameters were 94 °C (1 min), optimized annealing temperature for each genes/alleles (1 min), and 72 °C (1 min) for 33 cycles with a final extension at 72 °C (7 min). PCR products were electrophoresed and visualized with a UV transilluminator (UVP, Upland, CA, United States). Amplified fragments of all genes/alleles from the five isolates were purified and sequenced with both forward and reverse primers using BigDye technology, and sequencing reactions were run on an AB13700XL DNA sequencer (Life Technologies of Thermo Fisher Scientific, Waltham, MA, United States). The BLAST program (<http://www.ncbi.nlm.nih.gov>) was used to match the nucleotide sequences with published sequences in Genbank (data not shown). The size of the PCR products of all genes was similar to those generated from the control *H. pylori* strains, and sequences showed 99%-100% similarity with the corresponding sequences of the reference *H. pylori* strains in Genbank (Table 1).

### Statistical analysis

Statistical analysis was performed using Pearson's  $\chi^2$  and Fisher's exact probability tests. Kendall's Tau  $b$

**Table 1** Oligonucleotide primers used for polymerase chain reaction

Gene	Primer	Sequence (5'→3')	PCR products (bp)	Annealing temperature (°C)	Ref.
16S rRNA	HP1	GCAATCAGCGTCAGTAATGTT C	519	58.5	[85]
	HP2	GCTAAGAGATCAGCCTATGTC			
<i>vacA</i> (s1, s2)	VA1F	ATGGAATACAAGAAACACACC	s1: 259 s2: 286	56.0	[25]
	VA1R	CTGCTTGAATGCGCCAAACTTTAATC			
<i>vacA</i> (m1, m2)	VAG-F	CAATCTGTCCAATCAAGCGAG	m1: 570 m2: 645	58.5	[25]
	VAG-R	GCGTCAAATAATTCCAAGG			
<i>cagA</i>	D008	ATAATGCTAAATTAGACAACCTTGAGCGA	298	58.5	[86]
	R008	TTAGAATAATCAACAAACATCACGCCAT			

PCR: Polymerase chain reaction.

**Table 2** Distribution of genotypes in *Helicobacter pylori* isolates n (%)

Characteristic	CG	GU	DU	Total
No. of cases	113 (48.5)	64 (27.5)	56 (24.0)	233 (100)
Sex				
Female	62 (54.9)	27 (42.2)	15 (26.8)	104 (44.6)
Male	51 (45.1)	37 (57.8)	41 (73.2)	129 (55.4)
Age				
< 40 yr	43 (38.1)	5 (7.8)	17 (30.4)	65 (27.9)
> 40 yr	70 (61.9)	59 (92.2)	39 (69.6)	168 (72.1)
<i>vacA</i>				
s1	83 (73.5)	53 (82.8)	55 (98.2)	191 (82.0)
s2	30 (26.5)	11 (17.2)	1 (1.8)	42 (18.0)
m1	32 (28.3)	16 (25)	20 (35.7)	68 (29.2)
m2	81 (71.7)	48 (75)	34 (60.7)	163 (70.0)
s1m1	24 (21.2)	15 (23.4)	19 (33.9)	58 (24.9)
s1m2	59 (52.2)	38 (59.4)	34 (60.7)	131 (56.2)
s2m1	6 (5.3)	1 (1.6)	0	7 (3.0)
s2m2	24 (21.2)	10 (15.6)	1 (1.8)	35 (15.0)
<i>cagA</i>				
+	82 (72.6)	50 (78.1)	37 (66.1)	169 (72.5)
-	31 (27.4)	14 (21.9)	19 (33.9)	64 (27.5)
s1m1 <i>cagA</i>				
+	17 (15.0)	10 (15.6)	10 (17.9)	37 (15.9)
-	7 (6.2)	5 (7.8)	9 (16.1)	21 (9.0)
s1m2 <i>cagA</i>				
+	41 (36.3)	30 (46.9)	24 (42.9)	95 (40.8)
-	18 (15.9)	8 (12.5)	10 (17.9)	36 (15.5)
s2m1 <i>cagA</i>				
+	5 (4.4)	1 (1.6)	0	6 (2.6)
-	1 (0.9)	0	0	1 (0.4)
s2m2 <i>cagA</i>				
+	19 (16.8)	9 (14.1)	1 (1.8)	29 (12.4)
-	5 (4.4)	1 (1.6)	0	6 (2.6)
s1m1m2 <i>cagA</i>				
+	0	0	2 (3.6)	2 (0.8)
-	0	0	0	0

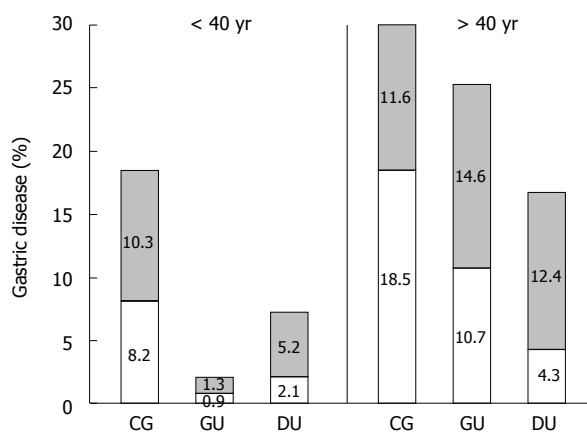
CG: Chronic gastritis; DU: Duodenal ulcer; GU: Gastric ulcer.

correlation coefficient was used to measure the strength of dependence between *H. pylori* genotypes, and age or sex and gastric disease. Logistic regression analysis was used to predict the outcome of gastric diseases based on age, sex, or *H. pylori* genotype (SPSS version 20, IBM Corp., Armonk, NY, United States). Statistical significance was defined as  $P \leq 0.05$ .

## RESULTS

### Classification of patients according to age, sex, and gastric disease

All patients ( $n = 233$ ) were *H. pylori* positive, but were diagnosed with one of three diseases, CG, DU, or GU. CG was the most prevalent gastric disease (113/233; 48.5%), compared to GU (64/233; 27.5%) and DU (56/233; 24%). The distribution of patients according



**Figure 1** Gastric disease cases are more prevalent in patients > 40 years of age. The percentages of the 233 *Helicobacter pylori*-positive patients are plotted according to disease diagnosis (CG: Chronic gastritis; GU: Gastric ulcer; and DU: Duodenal ulcer), age (< 40 years and > 40 years), and sex (male: White bars; female: Shaded bars).

to sex, age, and *H. pylori* genotype are presented in Table 2. A greater percentage of patients were > 40 years of age (168/233; 72.1%) and male (129/233; 55.4%). More patients were male in both age groups: < 40 years, 16.7% (39/233) vs 11.2% (26/233) females, and > 40 years, 38.6% (90/233) were male vs 33.5% (78/233) female. For all diagnoses, more patients were > 40 years (Figure 1, Table 2).

### Genotype frequencies within *H. pylori* isolates from CG, GU, and DU patients

In order to determine whether *H. pylori* isolates genetically differed among patients and/or disease, *vacA* and *cagA* genes were amplified from 233 *H. pylori* DNAs and sequenced. The most frequently detected alleles were *vacA* s1, *vacA* m2, and *cagA*<sup>+</sup>, at 82.0% (191/233), 70.0% (163/233), and 72.5% (169/233), respectively (Table 2). All *vacA* s/m genotypes were detected, with *vacA* s1m2 (131/233; 56.2%) as the most common and *vacA* s2m1 (7/233; 3.0%) the least common. Moreover, *vacA* s1m2 was equally prevalent among the three disease groups. For the remaining *vacA* s/m genotypes, *vacA* s1m1 was observed in 24.9% (58/233) of cases and most often associated with a diagnosis of DU (33.9%),

whereas *vacA* s2m2 was detected in 15% (35/233) of cases overall, but most often in CG patients (24/233; 21.2%). For all the alleles detected, *vacA* s1m2 *cagA*<sup>+</sup> (95/233; 40.8%) was the most common genotype observed in the cohort.

### **Sex, age, and *H. pylori* genotypes of CG, GU, and DU patients**

Statistical analysis was first performed on clinical characteristics of the patients, such as sex of the patient and age. In this part of statistical analysis, each individual disease group was considered separately. Whereas CG and GU were not associated with sex of the patient, In contrast, the increased proportion of male relative to female patients in the DU group was statistically significant (73.2% vs 26.8%,  $P = 0.001$ ). Increased age (> 40 years) was clearly associated with all diseases ( $P = 0.011$ , 0.000, and 0.003 for CG, GU, and DU, respectively).

The frequencies of specific virulence alleles were examined based on gastric disease diagnosis. In all diagnoses, the frequencies of the *vacA* s1 allele compared to *vacA* s2 and *vacA* m2 relative to *vacA* m1 were higher. The frequency of the *vacA* s1 allele was higher than *vacA* s2 (73.5% vs 26.5% and 82.8% vs 17.2%,  $P = 0.000$ ), and *vacA* m2 was higher than *vacA* m1 (71.7% vs 28.3% and 75.0% vs 25.0%,  $P = 0.000$ ) in CG and GU patients, respectively. In addition, in DU patients, the frequency of the *vacA* s1 allele was significantly higher than *vacA* s2 (98.2% vs 1.8%,  $P = 0.000$ ), and the *vacA* m2 allele was at a higher frequency than *vacA* m1 (60.7% vs 35.7%,  $P = 0.000$ ). Finally, the *vacA* s1m1m2 genotype was only detected in 2/56 (3.6%) female DU patients. *cagA* was detected in significantly more *H. pylori* strains derived from CG (72.6%;  $P = 0.000$ ) and DU (66.1%;  $P = 0.016$ ) patients. *H. pylori* isolates with *vacA* s1m2*cagA*<sup>±</sup> genotypes exhibited the highest frequency (56.3%) overall with similar prevalence among CG, GU, and DU patients (Table 2, Figure 2).

### **Reciprocal impact of host age and sex and *H. pylori* genotypes on the development of gastric diseases**

Clinical characteristics of patients were analyzed for associations with disease development. Increased age clearly emerged as a risk factor for all dyspeptic disease diagnoses. As indicated above, the number of patients in the three disease groups was significantly greater in patients > 40 years than those < 40 years. Increased age appeared as an important risk factor for GU in both males and females, compared to CG and DU ( $P = 0.000$ ). Furthermore, being male was correlated with DU and female with CG ( $P = 0.000$ ), whereas no significant correlation was found between the development of GU and being male or female.

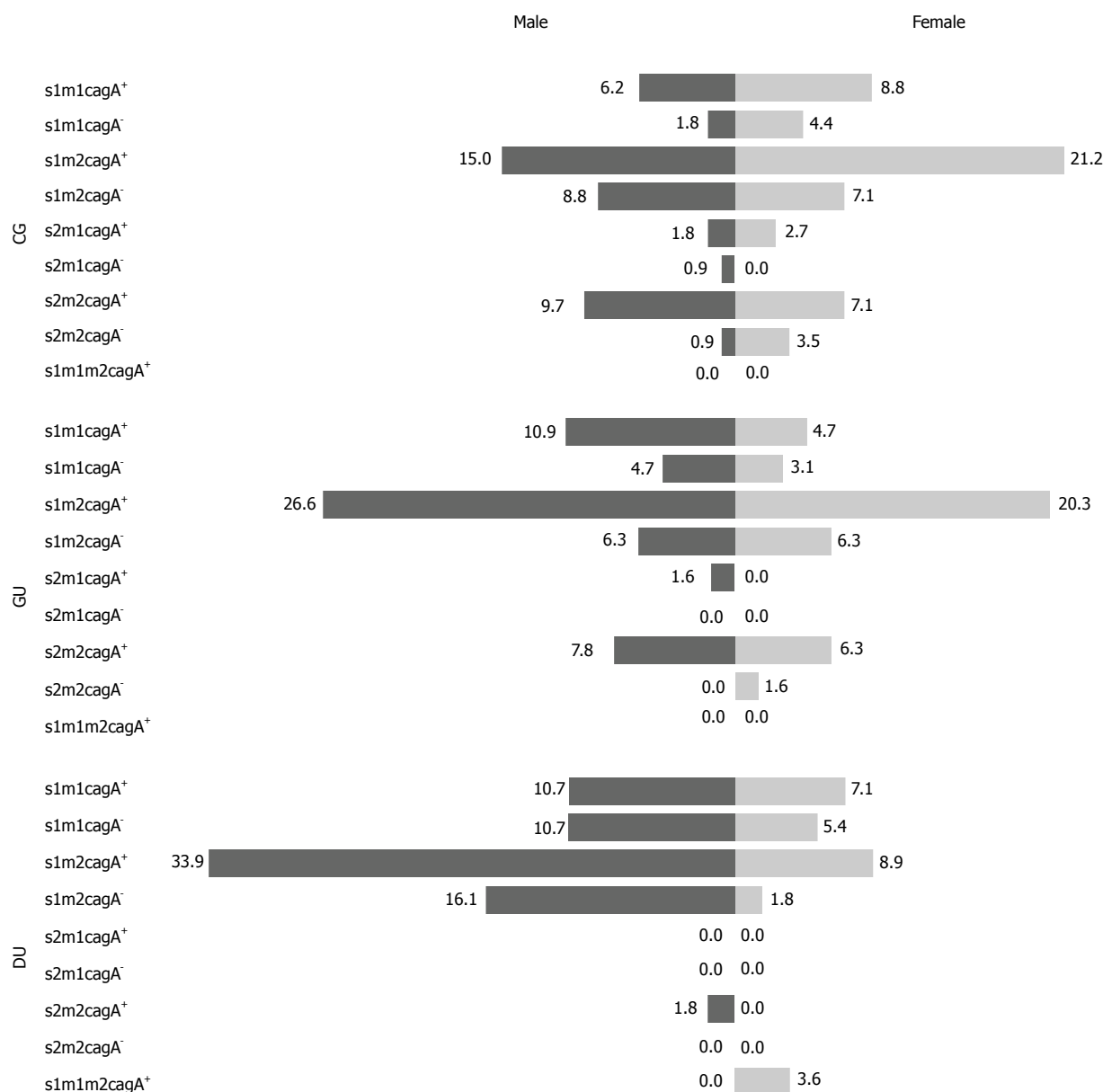
A strong correlation was found between specific *vacA* s genotypes and gastric disease diagnosis. The *vacA* s1 genotype was a risk factor for development

of DU and *vacA* s2 for CG ( $P = 0.000$ ); however, both of these genotypes were equally associated with the development of GU. There was no correlation between *vacA* m or *cagA*<sup>+</sup> alleles and gastric disease. For the combination of *vacA* s, *vacA* m, and *cagA* genotypes in gastric disease, only *vacA* s2m2 was found to have an association with the development of CG and GU compared to DU ( $P = 0.004$ ). The frequency of *vacA* s1m2*cagA*<sup>+</sup> and *vacA* s1m2*cagA*<sup>-</sup> strains was higher in male patients with DU compared to female patients ( $P = 0.004$  and 0.011, respectively). Statistically significant differences were not observed in CG and GU patients (Figures 1 and 2).

## **DISCUSSION**

Understanding the etiology of the development of gastric diseases will help to develop strategies for prevention and treatment. This study addressed age and sex of the patient and *H. pylori* bacterial genotype as major factors contributing to disease development in a cohort of Iranian patients. Among 233 patients, 48.5% were diagnosed with CG, 27.5% GU, and 24.0% DU. All three diseases were more common in patients > 40 years (72.1%). The most significant difference between patients < 40 and > 40 years was observed in the GU group. In addition, CG was found to be more frequent in females and DU in males; however, GU was similarly prevalent in males and females. Finally, genotyping of the *H. pylori* isolates indicated that the *vacA* s1 allele in combination with being male was a significant risk factor for DU, and that *vacA* s2m2 and being female were risk factors for CG and GU. However, no correlation was found between alleles of *vacA* m or *cagA* and dyspeptic diseases.

In developing countries, the prevalence of *H. pylori* infection reaches up to 80% before the age of 50 years and in developed countries, 50% of individuals older than 60 years are infected<sup>[45]</sup>. The reported *H. pylori*-infection rate in the adult population of Brazil ranged from 35.3%<sup>[46]</sup> to 97.9%<sup>[47]</sup>. In another study performed in Brazil, the incidence of *H. pylori*-related gastritis increased with age in women in their 50s and men in their 70s. Furthermore, the frequency of dyspepsia in patients over 70 years was twofold greater than in young adults, and two thirds of dyspeptic patients were women<sup>[10]</sup>. In Japan, the prevalence of *H. pylori* was considerably high (85%) and increased with age, from 26% in subjects 16-20 years up to 61% in those 50-64 years<sup>[48]</sup>. In Africa, an increased prevalence of *H. pylori* infection was detected in older patients<sup>[49]</sup>. In a study on 1391 Albanian subjects, *H. pylori* seropositivity was more prevalent in females > 40 years<sup>[50]</sup>. A cross-sectional study in the United Kingdom demonstrated a significant association between *H. pylori* seropositivity and males, shorter height, tobacco consumption, and lower socioeconomic



**Figure 2** Distribution of *Helicobacter pylori* genotypes according to sex. *vacA* s1m2 *cagA*<sup>+</sup> was the most common genotype in both males (dark bars) and females (light bars) of all three gastric diseases. CG: Chronic gastritis; GU: Gastric ulcer; DU: Duodenal ulcer.

status<sup>[45]</sup>. In a study from Brazil, the most prevalent gastric disease was CG (72.3%), with GU at 5.1% and DU at 6%. Gastroesophageal alterations were detected in 16.7% of these cases. Sex and age played no role in the development of CG; however, being male and older age were associated with GU, whereas being male alone was linked to the development of DU<sup>[51]</sup>. A similar prevalence of GU and DU and an association with being male was also observed in another study performed in Southern Brazil<sup>[11]</sup>. However, in a third study in Brazil, GU and DU were significantly more frequent in women<sup>[52]</sup>.

Reports indicate that males and females become similarly infected with *H. pylori*<sup>[53]</sup>. However, the clinical outcome depends on the longevity and severity of the inflammatory response to *H. pylori* infection in each

individual<sup>[54]</sup>. An increasing body of evidence indicates that the consequences of *H. pylori* infection are more severe in males; however, the contributing factors are currently unknown. Although the prevalence of *H. pylori* in males and females was found to be similar, as determined by the rapid urease test and stained biopsy smear examination, higher levels of IgG were observed in males<sup>[55]</sup>. Furthermore, being male, having polymorphism at the interleukin-1 $\beta$  promoter, and overexpression of interleukin-1 $\beta$  have all been associated with increasing the risk of atrophic gastritis and gastric adenocarcinoma in *H. pylori*-infected patients<sup>[56,57]</sup>. It has been demonstrated that gastrin, a hormone which stimulates the proliferation of epithelial cells<sup>[58]</sup>, can lead to gastric cancer if overexpressed, especially in the context of *H. pylori* infection<sup>[59]</sup>. In

Sweden, higher levels of antibodies against VacA and CagA in *H. pylori*-infected patients were associated with increased risk of the development of gastric cancer by twofold when compared with control patients without *H. pylori* infection<sup>[60]</sup>. *H. pylori* and aging have also been found to be strongly associated with an increased risk of atrophy and the development of intestinal metaplasia in gastric mucosa<sup>[19,61]</sup>. In Japan, intestinal metaplasia was evident in a considerable number of males (90%) over the age of 50 years compared to females in the same age group or younger individuals overall<sup>[62]</sup>. In the United States, the incidence of gastric cancer in males has been reported to be five times higher than in females<sup>[63]</sup>.

The frequencies of *vacA* s1 (82%), *vacA* s2 (18%), *vacA* m1 (29.2%), *vacA* m2 (70.8%), and *cagA* (72.5%) were within ranges reported by other studies performed on patients in Iran: *vacA* s1, 68%-80%; *vacA* s2, 20%-32%; *vacA* m1, 30%-70%; *vacA* m2, 27%-70%<sup>[64,65]</sup>; and *cagA*, 44%<sup>[66]</sup> to 91%<sup>[67]</sup>. *vacA* s1 was detected in 73.5%, 82.8%, and 98.2% of CG, GU, and DU patients, respectively, whereas *vacA* s2 was detected in 26.5%, 17.2%, and 1.8%. *vacA* m1 was found in 28.3% of CG, 25% of GU, and 35.7% of DU patients, and *vacA* m2 in 71.7% CG, 75% GU, and 60.7% of DU patients. The *cagA* gene was detected in most *H. pylori* isolates (66.1%-78.1%). The most frequent genotype among the 233 isolates was *vacA* s1m2*cagA*<sup>+</sup> (40.8%) followed by *vacA* s1m1*cagA*<sup>+</sup> (15.9%), and *vacA* s1m2*cagA*<sup>-</sup> (15.5%), with a similar distribution among gastric disease diagnoses. The frequency of *vacA* s2m2*cagA*<sup>+</sup> genotype was lower, at 12.4%. Reports indicate that the s1 genotype is very common in East Asian countries, but with no relationship to the clinical outcomes of infection, whereas *vacA* m1 is more frequent in North East Asia and *vacA* m2 in South Asia<sup>[30,68]</sup>. Several studies in Western countries have shown that individuals infected with *H. pylori* strains carrying *vacA* s1 or m1 alleles are at a higher risk of PU or gastric cancer when compared to those infected with *vacA* s2 or *vacA* m2-carrying strains<sup>[69,70]</sup>. In this cohort, genotypes *vacA* s1m1 and *vacA* s2m2 were detected at a high frequency. The *H. pylori vacA* s1m1 genotype is in fact common worldwide, ranging from 42% to 84%<sup>[71]</sup> around the globe, whereas *vacA* s2m2 varies from 0% to 57%<sup>[71,72]</sup>. The frequencies of the *vacA* s1m1 genotype within the isolates of this study exhibited no significant difference among gastric disease diagnoses (21.2%, 23.4%, and 33.9% for CG, GU, and DU patients, respectively). However, the frequencies of the *vacAs2m2* genotype were significantly higher in CG and GU patients compared to DU patients (21.2%, 15.6%, and 1.8%, respectively). In a study from Japan, *H. pylori* strains with the *vacA* s1m1 genotype were isolated from 59.2%, 79.2%, and 87.5% of CG, GU, and gastric cancer patients, respectively<sup>[73]</sup>. Furthermore, the *vacA* s1m2 genotype was found in 17.3%, 7.9%, and 27.2% of isolates from CG, GU, and

DU patients, respectively. The *vacA* s2m2 genotype was more common in *H. pylori* isolates from CG (22.4%) than GU (11.9%), DU (10.5%), and gastric cancer (4.2%) patients.

Although the frequency of *cagA* was high (72.5%) in our cohort, an association of *cagA*<sup>+</sup> genotypes with the development of CG, GU, and DU was not observed. The frequency of *cagA* has been reported to range from 50% in some Middle Eastern countries<sup>[74]</sup> to 88% in Europe and North America<sup>[75,76]</sup> and 99% in many East Asian countries<sup>[77,78]</sup>. Studies in Western countries have revealed a significant association of *cagA*<sup>+</sup> *H. pylori* strains with severe gastritis, PU, and gastric cancer<sup>[29,30,79]</sup>. However, such a relationship was not found between *cagA*<sup>+</sup> strains and PU, gastric cancer, and non-ulcer dyspepsia in Far Eastern countries<sup>[80]</sup>. In a study from Italy, 72% (132/193) of *H. pylori* isolates were *cagA*<sup>+</sup>, and *cagA* positivity was associated with PU and gastric cancer but not gastritis<sup>[81]</sup>. It has been proposed that the *vacA* s1m1 genotype is often linked to the presence of *cagA* and the *vacA* s2m2 genotype with its absence<sup>[25,82]</sup>. In Alaska, *cagA* was detected in 85% of *H. pylori* isolates; however, no correlation was found between the *cagA*<sup>+</sup> or *cagA*<sup>-</sup> genotype and development of gastric diseases. In the same study, 66% of *vacA* s2m2-carrying *H. pylori* strains contained the *cagA* gene<sup>[12]</sup>.

Results of this study demonstrate that gastric diseases are significantly more frequent in patients > 40 years. Being male and the *vacA* s1 genotype played an important role in the development of DU. Aging and the *vacA* s2m2 genotype were associated with a diagnosis of GU, and being female and the *vacA* s2m2 genotype with CG. However, no correlation was found between *vacA* m or *cagA* and gastric diseases. A large body of evidence indicates that the heterogeneity of *H. pylori* underlies the diversity of gastric diseases observed. This bacterial genetic diversity appears to be the result of recombination processes that evolved for the purpose of long-term colonization in humans, despite eliciting chronic inflammatory responses<sup>[83]</sup>. In this regard, investigators believe that VacA and CagA act together to stimulate signals in epithelial cells, affecting cell structure, differentiation and behavior<sup>[27]</sup>, and are balanced with the damage needed for long-term colonization<sup>[31,84]</sup>. Results of this study indicate that VacA and CagA are mainly involved in the colonization of *H. pylori* in the human stomach. However, the interplay between *H. pylori* genotypes and age and sex of the human hosts is likely to determine the severity of the gastric disease diagnosis.

## COMMENTS

### Background

*Helicobacter pylori* (*H. pylori*) infection has been regarded as a risk factor for gastric diseases, ranging from chronic gastritis to more severe outcomes, such as peptic ulcers, gastric cancer, and mucosa-associated lymphoid tissue lymphoma. *H. pylori* has a remarkable heterogeneous genetic reservoir which may enable efficient bacterial adaptation to the gastric niche in different

patients. Disease development is potentially the result of the interaction between *H. pylori* virulence factors, VacA, and CagA and the host, which leads to inflammation and tissue damage. Thus, underlying the clinical outcome of *H. pylori* infection may be the interplay between virulence factors, host genetics, and environmental factors. However, despite extensive research on *H. pylori*-related diseases, the impact of risk factors alone or in concert has not been thoroughly evaluated. Therefore, it remains possible that the age and sex of infected individuals play important roles in determining the outcome of *H. pylori* infection.

### Research frontiers

Reports on the risk factors involved in development of *H. pylori*-associated gastric diseases are controversial. *H. pylori*-associated gastric atrophy has been correlated to smoking, intake of salt, alcohol, or low beta-carotene, consumption of soybean products, and even acid-suppressing drugs. No correlation with age, sex, smoking, or coffee intake, however, has been observed in other studies. Currently, the relationship between bacterial, host, and environmental factors has only been examined in a few studies with larger numbers of patients. The incidence of *H. pylori* infection is considerably high in Iran (69%-80%); correspondingly, the frequency of referrals to endoscopy rooms due to complaint of dyspepsia is also high. Therefore, knowledge of the risk factors may contribute to the management and/or prevention of the more severe consequences of *H. pylori* infection in high-risk patients.

### Innovations and breakthroughs

The focus of this study was to assess the potential impact of individual factors, including host age and sex and *H. pylori* genotypes, on the development of *H. pylori*-associated chronic gastritis (CG), gastric ulcer (GU), and duodenal ulcer (DU). Results indicated that age and sex were associated with the development of gastric disease in the context of *H. pylori* infection, and specific *H. pylori* genotypes were differentially associated with the diagnosis of CG, GU, and DU.

### Applications

Increased age, being female, and the *vacA* s2m2 genotype were risk factors for CG, increased age in males and females and *vacA* s2m2 for GU, and increased age and *vacA* s1 for DU. Accordingly, for prevention and control of *H. pylori*-associated gastric diseases, results of this study might help to identify high-risk patients, particularly in the Iranian population.

### Terminology

GU is a defect in gastric mucosa that penetrates deep into the muscularis mucosa. The sensation of indigestion is described as burning and can be relieved by antacid. DU, the duodenal deformity caused by acid and pepsin from the duodenal mucosa, is often associated with pain in the upper stomach, vomiting, bleeding, perforation, and obstruction, and is also relieved by taking antacids. CG is the inflammation of gastric mucosa, mainly caused by *H. pylori* infection. CG usually has no definite symptoms, but the patient is susceptible to the development of GU.

### Peer-review

The relationship between *H. pylori* genotypes and host age and sex on the development of *H. pylori*-associated gastric diseases was investigated. Increased age (> 40 years) was found to be a risk factor for CG, GU, and DU. Furthermore, being female and *vacA* s2m2 were risk factors for CG, *vacA* s2m2 for GU, and *vacA* s1 for DU in males. No correlation between *H. pylori* alleles *vacA* m or *cagA* and gastric diseases was observed. Therefore, the disease outcome of *H. pylori* infection may be a direct result of the interaction of specific bacterial genotypes with the age and sex of infected individuals.

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