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Research paper

Genetic diversity and functional analysis of *oipA* gene in association with other virulence factors among *Helicobacter pylori* isolates from Iranian patients with different gastric diseases

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ABSTRACT

Helicobacter pylori (*H. pylori*) is one of the most genetically diverse bacterial pathogens that persistently colonizes the human gastric epithelium. This remarkable genomic plasticity may act as a driving force for successful adaptation and persistence of the bacteria in the harsh gastric environment. Outer inflammatory protein A (OipA) encoded by *oipA* gene (*HP0638/hopH*) is a member of the outer membrane proteins (OMPs) of *H. pylori* involved in induction of IL-8 secretion and is associated with development of peptic ulcer and gastric cancer. Expression of OipA is regulated by phase variation within a CT dinucleotide repeat motif of the *oipA* gene. In this study we carried out direct DNA sequence analysis of 53 amplified fragments to investigate the *oipA* "On/Off" status among Iranian *H. pylori* isolates from patients with various gastric diseases. The prevalence of *cagL*, *cagA*, EPIYA motifs, *vacA* alleles, *babA2* and *sabA* genotypes as well as *cag*PAI integrity of the isolates were determined by PCR. Our results demonstrated a high prevalence of strains with functional *oipA* status (79%) and significant associations were found between functional *oipA* and *cagA* ($P = 0.027$) and *vacA* s1m1 ($P = 0.022$) genotypes. The *vacA* s1m2 genotype was also found to be statistically associated with PUD ($P = 0.0001$). Interestingly, we showed that *H. pylori* strains with intact *cag*PAI co-expressed *oipA* gene in a significant synergistic relationship ($P < 0.01$). However, no significant association was observed between the functional *oipA* status and clinical outcomes ($P > 0.05$). In conclusion, our findings denotes great diversity in the number and pattern of CT dinucleotide repeats of *oipA* among Iranian *H. pylori* strains. The synergistic link between functional *oipA* and other important virulence factors is proposed to be critical in the pathogenesis of *H. pylori*, which needs further studies with a larger number of samples.

1. Introduction

Helicobacter pylori (*H. pylori*) is one of the most common bacterial agents that persistently colonizes the human gastric mucus. This pathogen is formally recognized as the main cause of chronic active gastritis, peptic ulcer disease (PUD), gastric cancer, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma (Kao et al., 2016; Peek and Blaser, 2002; Polk and Peek, 2010). The severity of *H. pylori*-associated diseases depends on a number of factors, including host genetic predisposition, induced inflammatory responses of host cells, and more importantly the virulence capability of *H. pylori* strains (Atherton and Blaser, 2009; Miftahussurur et al., 2015; Yamaoka and Graham, 2014).

The genome of *H. pylori* seems to be one of the most genetically diverse among other bacterial pathogens, which is characterized by high mutational frequency, elevated recombination rate, and great exchange of genetic elements especially during the mixed infection with multiple unrelated strains (Farzi et al., 2015; Gunaletchumy et al., 2014; Herrera et al., 2008). This remarkable genomic plasticity was

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Table 1
Demographic characteristics of 68 *H. pylori*-positive patients in different clinical outcome groups.

Parameter	Chronic gastritis	Gastric erosion	Peptic ulcer disease	Intestinal metaplasia	Gastric cancer
No. of strains (%)	35 (51.4)	10 (14.7)	11 (16.2)	7 (10.3)	5 (7.3)
Mean age ± SD (yr)	45.2 ± 14.1	45 ± 10.8	51.4 ± 13.2	40.7 ± 9.3	57.4 ± 7.7
Age range	14–73	25–60	25–75	28–54	50–68
Sex (F/M)	24/11	7/3	7/4	3/4	4/1
Ethnicity					
Persians	25 (71.4)	6 (60)	8 (72.7)	7 (100)	3 (60)
Turks	7 (20)	2 (20)	2 (18.1)	0	1 (20)
Kurds	1 (2.8)	1 (10)	0	0	1 (20)
Lurs	2 (5.7)	1 (50)	1 (9.1)	0	0
Smoking	1 (2.8)	1 (10)	2 (18.8)	2 (33.3)	1 (20)
Drinking	3 (8.5)	1 (10)	1 (9.1)	1 (14.2)	2 (40)
Abdominal pain	18 (51.4)	3 (33.3)	5 (45.4)	2 (28.5)	3 (60)

found to affect the persistence, antigenicity and pathogenicity of these organisms, and also may act as a driving force for successful adaptation to harsh gastric epithelium of the infected hosts (Algood and Cover, 2006; Blaser and Berg, 2001; Nilsson et al., 2003).

Several virulence factors have been identified in the *H. pylori* genome such as proteins involving in attachment, colonization, chronic persistence and ultimately cytotoxic damage to host cells (Yamaoka, 2010). To date, CagA oncoprotein encoded by cytotoxin-associated gene A (*cagA*) is the best characterized virulence marker associated with increased risk of peptic ulceration and gastric carcinoma (Kauser et al., 2004). The *cagA* gene is located at the end of the *cag* pathogenicity island (*cag*PAI), when intact, encodes a functional type IV secretion system (T4SS) that is responsible for translocating bacterial effectors including CagA and peptidoglycan fragments into host cytoplasm (Backert and Tegtmeyer, 2017). Following delivery, CagA is tyrosine-phosphorylated at the carboxy-terminal Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs by host cell kinases and triggers manipulation of cell signaling pathways and also induction of the proinflammatory cytokines specifically interleukin (IL-8) (Nishikawa and Hatakeyama, 2017). Moreover, CagL protein that is encoded by the *cagL* gene present on the *cag*PAI, is a small pilus component of *H. pylori* T4SS contributed to induction of host inflammatory responses through the interaction with integrin $\alpha_5\beta_1$ receptors (Yadegar et al., 2014). It has also been reported that presence of an intact *cag*PAI region is frequently associated with the severity of gastric diseases like duodenal ulcer, gastric atrophy, and gastric cancer (Nguyen et al., 2010). However, *cag*PAI is usually exposed to genetic alterations, suggesting that *H. pylori* strains possessing *cag*PAI may be further grouped into intact and partial ones (Kauser et al., 2004; Yadegar et al., 2015).

The vacuolating cytotoxin A (*vacA*) is present in all strains and is considered to be linked to an increased risk for promotion of peptic ulcers and gastric cancer. Specific allelic types in the *vac*A gene are found in its signal (s1, s2) and the middle regions (m1, m2) due to sequence heterogeneity, leading to considerable variation in cytotoxic activities among different strains (Cover and Blanke, 2005; Palframan et al., 2012). In addition to the over mentioned virulence factors, *H. pylori* has several well-characterized adhesins, including *babA2* and *sabA*, which binds to the blood group antigens and were shown to be associated with an increased risk of peptic ulcers and gastric carcinoma (Bäckström et al., 2004; Olfat et al., 2005; Rad et al., 2002; Yamaoka et al., 2006).

Adherence of *H. pylori* to the gastric mucosa is believed to play an important role in the inflammatory response to the organism. Outer inflammatory protein A (OipA) encoded by *oipA* gene (*HP0638/hopH*) that is located approximately 100 kb from the *cag*PAI on the *H. pylori* chromosome and is a member of the outer membrane proteins (OMPs) involved in adhesion (Dossumbekova et al., 2006). OipA protein seems to be important in induction of IL-8 secretion and gastric colonization, and it is associated with the elevated risks of PUD and gastric cancer (Kauser et al., 2005b; Yamaoka et al., 2002). Expression of OipA is regulated by slipped-strand mispairing mechanism (SSM) within a CT dinucleotide repeat motif located in the 5′ region of the gene. Therefore, the functional status of *oipA* and its gene switch (on/off status) depends on the variable number of CT repeats in the signal-peptide sequence that determine whether the complete open reading frame is in-frame (Liu et al., 2013).

Our previous studies have revealed that Iran is a country with high prevalence of *H. pylori* infection, particularly with hypervirulent strains (Farzi et al., 2015; Vaziri et al., 2013; Yadegar et al., 2015; Yadegar et al., 2014). Since the prevalence and significance of *oipA* functional (on/off) status in *H. pylori* strains from Iran are still unclear, the main goal of this study was to investigate the genetic diversity of *oipA* genotypes by using a PCR-based sequencing method of the signal peptide coding region of *oipA* gene. We also evaluated the association of *oipA* functionality with other virulence characteristics and clinical outcomes among Iranian *H. pylori* strains.

2. Materials and methods

2.1. Patients and biopsy samples

A total of 68 clinical strains of *H. pylori* isolated from 133 patients who underwent upper gastroduodenal endoscopy at Taleghani Hospital were enrolled in this study. The mean age of *H. pylori*-positive patients was 47 ± 13.1, ranged from 14 to upper 70 years old. Regarding endoscopic and pathological findings, patients were distributed into chronic gastritis (CG; 35), peptic ulcer disease (PUD; 11), gastric erosion (GE; 10), intestinal metaplasia (IM; 7), and gastric cancer (GC; 5). The characteristics of the study population are summarized in Table 1.

The antral biopsies for culture from each patient were immediately kept in transport medium consisting of thioglycolate with 1.3 g/l agar (Merck, Germany) and 3% yeast extract (Oxoid Ltd., Basingstoke, UK). Written informed consent was obtained from all patients under a protocol approved by the Ethical Review Committee of the Gastroenterology and Liver Diseases Research Center at Shahid Beheshti University of Medical Sciences.

2.2. H. pylori culture and DNA preparation

The biopsy specimens were cut into small pieces, homogenized and were smeared on Brucella agar (Merck, Germany) plates containing 7% horse blood (v/v), 10% fetal calf serum (FCS) and *Campylobacter*-selective supplement (vancomycin 2.0 mg, polymyxin B 0.05 mg, trimethoprim 1.0 mg) and amphotericin B (2.5 mg/l). The cultured plates were incubated in a CO_2 incubator (Innova® CO-170; New Brunswick Scientific, USA) for 3–7 days. The *H. pylori* growth was observed by small grey translucent colonies and confirmed by urease, catalase, and oxidase tests. Molecular identification was also performed by previously delineated assays (Farzi et al., 2015; Yadegar et al., 2015). Subcultures of the single colonies were prepared and a lawn from each