Is there a relationship between *Helicobacter pylori vacA* i1 or i2 alleles and development into peptic ulcer and gastric cancer? A meta-analysis study on an Iranian population

M. Keikha[1,2,3]

1) *Antimicrobial Resistance Research Centre,* 2) *Department of Microbiology and Virology, Faculty of Medicine and* 3) *Student Research Committee, Mashhad University of Medical Sciences, Mashhad, Iran*

Abstract

Helicobacter pylori has several virulance factor i.e. *VacA*, *CagA*, *BabA*, *SabA*, *AlpA*, *AlpB* and etc. *VacA* has several polymorphic region in the nucleotide sequence such as s,m,i,d and, c. It has been suggested that each variation in these polymorphic region has been influenced the toxicity of *VacA* toxin. We performed a comprehensive meta-analysis to determine the main role of *VacA*i1/i2 in development into peptic ulcer and gastric cancer in an Iranian population.

Keywords: Gastric cancer, *Helicobacter pylori*, Iran, peptic ulcer, vacA

Original Submission: 21 April 2020; **Revised Submission:** 29 June 2020; **Accepted:** 30 June 2020

Article published online: 3 July 2020

Corresponding author: M. Keikha, Department of Microbiology and Virology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

E-mail: masoud.keykha90@gmail.com

To the Editor,

Helicobacter pylori is the most highlighted and prominent pathogenic bacterium of the human gastric submucosa; it colonizes the stomachs of half of the world's population [1]. Most of the populations living in developing countries are predominantly infected during childhood and the amount of infection in these areas is nearly 100% [2]. With the introduction of *H. pylori* as a first-class factor for gastric cancer (GC) by the International Agency for Research on Cancer in 1994, extensive attention was paid by gastroenterologists to this bacterium. *Helicobacter pylori* has been identified as the aetiological cause of chronic gastritis, peptic ulcer diseases (PUD), gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma and some extra-gastrointestinal disorders [3–5]. A significant number of *H. pylori*-infected individuals remain asymptomatic and PUD is observed in only 15%–20% and GC in 1%–2% of individuals, so the question arises as to why the clinical symptoms are observed in only a small part of the population with the rest remaining as asymptomatic carriers. Based on available evidence, genetic characteristics and *H. pylori* strain virulence factors, host genome and its epigenetic events, and the environmental conditions can play decisive roles in the incidence of *H. pylori*-related gastrointestinal outcomes [1,5–7].

The most famous *H. pylori* virulence factors include *vacA* and *cagA*. The *cagA* (cytotoxin-associated gene A) encodes a toxin of 120–140 kDa, which is phosphorylated at the site of its own EPIYA motif (tyrosine residue) by Src kinase family proteins of the host and, subsequent to phosphorylation, *cagA*-P induces the 'hummingbird phenotype' and incidence of GC through alteration in cytoskeletal rearrangements, cell survival and proliferation, and changes in polarity [8]. According to the previous studies, nearly 100% of *H. pylori* isolates in the Japanese population produce and express *cagA* [9]. Iran is the fourth country in terms of GC prevalence in Asia, and nearly 69% of *H. pylori* isolates in Iranian patients harbour (containing, transporting) *cagA* [10]. The *vacA* (vacuolating cytotoxin gene A) is another virulence factor, which releases cytochrome c from the mitochondria by entering into the gastric epithelium, induces apoptosis, destroys tight junctions and intercellular connections, and produces vacuole in the host cells [11]. The *vacA* consists of five polymorphic regions including signal sequence (s1/s2), middle (m1/m2), intermediate (i1/i2), deletion (d1/d2) and c (c1/c2). Only about 50% of the *H. pylori* strains encode the *vacA* [12,13]. Also, the combination of each region determines the vacuolating formation strength. For example, the vacA s1m1 strains express a large amount of toxin with high vacuolating formation strength, whereas s1m2 strains produce a moderate amount of toxin and s2m2 strains are not, or are rarely, toxic [14]. There are controversial results regarding the relationship between i1/i2 segments [15,16]. In this study, for the first time in Iran, we conducted a meta-analysis study to assess the possible role and relationship of *vacA* i1/i2 in the Iranian population.

https://doi.org/10.1016/j.nmni.2020.100726

Potentially supported documents were collected by searching the PubMed, Scopus, Embase, Elton B. Stephens Company (EBSCO), Google scholar, Scientific Information Database (SID), Islamic Science Citation Database (ISC), and magiran databases. The documents were investigated using the keywords '*Helicobacter pylori*', 'Peptic Ulcer Disease', 'Gastric Cancer', '*VacA* protein' and 'Iran'. Studies were collected up to April 2020, without language and time constraints, and all the original articles (including case–control, cohort, prospective, retrospective), letters to the editor and congress abstracts that were about determining the frequency of *vacA* i1/i2 genotypes in the Iranian PUD and GC patients were studied; information including the first author, publication year, location, distribution of gender and age, number of *H. pylori* strains and distribution of *vacA* i1/i2 genotypes are listed in Table 1.

The relationship of the infection with *vacA* i1/2 strains and development of GC lesions was calculated using the odds ratio index with 95% CI. The random effects model (Dersimonian and Laird method) was used in cases of high heterogeneity including I^2 index >25% and Cochran's *Q*-test p < 0.005; the statistical analysis was performed using Comprehensive Meta-Analysis (CMA v. 2.0) software. The publication bias was also measured according to Egger's regression method [4].

The eight studies that met our criteria were entered into the present analysis [12,17–21]. The studies were conducted in the period 2009–2019, five studies were performed in Ardabil, two in Tehran and one in Tabriz. In this study, the data from 1746 patients with an average age of about 48 ± 4 years were evaluated; about 41.9% of the patients were women and the rest were men. In general, 1414 strains of *H. pylori* were isolated from the patients in the present study. The frequency of *vacA* i1 in the present study was about 45.61% and the frequency of *vacA* i2 was about 43.21%. Based on the results of statistical analysis, we did not observe any significant relationship between infection acquisition with *H. pylori vacA* i1 or i2 and development of PUD or GC in the Iranian patients (Fig. 1).

In the next step, we evaluated the role of infection with the *H. pylori* strains *cagA* + *vacA* i1/2 with PUD and GC, although no significant relationship was obtained for this. Moreover, because of insufficient information and the ambiguity of some results, we could not determine the role of *cagA*+/i1 or i2/s1m1 strains or *vacA* i1 or i2/s1m1 strains in the formation and development of PUD and GC. According to Egger's regression intercept (bias studies) no publication bias was observed in the included studies. It seems that *vacA* i1 or i2 has no role in the formation and onset of PUD and GC in the Iranian population.

The i locus is located between regions s and m and plays a functional role in the activity and formation of vacuole, such that the strains containing *vacA* i1 have the ability of vacuolating formation, while *vacA* i2 strains are unable to produce vacuolation [22]. Our data on the role of constellation in *vacA* polymorphic regions and the synergistic effects between *vacA* i1/2 alleles and *cagA* in the occurrence of PUD and GC are limited (Fig. 2).

In this study, we showed that infection with *vacA* i1/2 strains had no effect on the development and severity of PUD and GC in the Iranian population. We also demonstrated that the combination of *vacA* i1/2 and *cagA* played no roles in the formation of *H. pylori*-related gastrointestinal issues.

In a study of strains isolated from Iraqi and Iranian patients with gastric ulcer, the *vacA* i1 genotype was only associated with gastric ulcer in the Iraqi patients [23]. However, studies in East Asia have found a significant relationship between the *vacA* i1 allele as well as PUD and GC, such that i1 is considered a biomarker for the *H. pylori*-related gastrointestinal disease. Bagheri et al. revealed the high abundance of *vacA* i1 allele in Iranian patients [24–26].

In their study on the Swedish population, Karlsson et al. found no significant relationship between *vacA* i1 and PUD or GC development [27]. González-Rivera et al. found that by inhibiting the nuclear factor of activated T cells, i1 suppresses interleukin-2 production and prevents the formation of chronic inflammation and PUD [28]. However, Memon et al.

TABLE 1. Characteristics of included study

Ref.	Publication year	Location	Female/male; age	*H. pylori* strains	*vacA* i1/2 genotype					
					Total		PU		GC	
					i1	i2	i1	i2	i1	i2
Bakhti et al. [17]	2019	Ardabil	NA; 46.52	290	112	120	34	23	32	17
Mottaghi et al. [18]	2014	Tabriz	45/44; NA	89	46	43	6	11	21	3
Bakhti et al. [19]	2015	Ardabil	77/100; 50	177	76	98	34	23	NA	NA
Bakhti et al. [SID]	2014	Ardabil	NA; NA	160	77	90	NA	NA	37	NA
Bakhti et al. [SID]	2014	Ardabil	NA; NA	171	33	33	33	NA	NA	NA
Abdi et al. [20]	2017	Ardabil	44/85; 53.57	103	40	27	NA	NA	24	9
Bakhti et al. [21]	2015	Tehran	NA; 45.34	217	102	106	34	23	29	9
Douraghi et al. [21]	2009	Tehran	91/116; 44.8 ± 16	207	159	94	20	NA	30	NA

SID: Scientific Information Database.

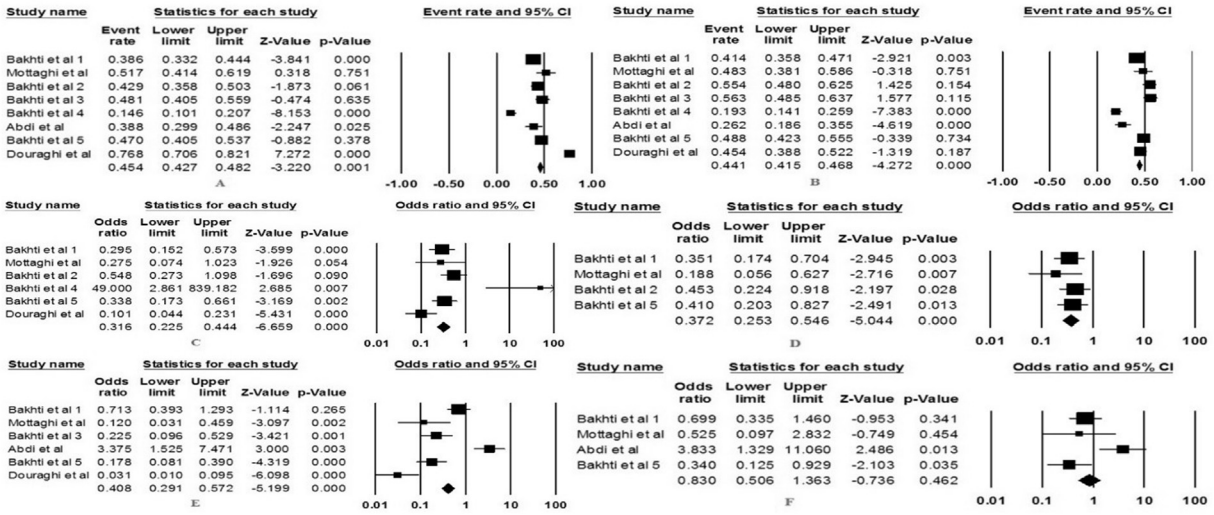

FIG. 1. Forrest plots of the *vacA* i1/i2 association with *Helicobacter pylori*-related gastrointestinal diseases. (a) *vacA* i1 in peptic ulcer disease (PUD) and gastric cancer (GC) development; (b) *vacA* i2 in PUD and GC development; (c) *vacA* i1 in PUD development; (d) *vacA* i2 in PUD development; (e) *vacA* i1 in GC development; (f) *vacA* i2 in GC development.

observed a significant relationship between i1 genotype and duodenal ulcer [29].

In their meta-analysis, Liu et al. stated that infection with the *vacA* i1 genotype was significantly related to gastric cancer. They stated that the *vacA* i1 genotype had a significant relationship with GC, particularly in Asian countries (OR 10.89; 95% CI 4.11–20.88) [30]. Sugimoto et al. also showed that there is a significant relationship between *vacA* i1 genotype and GC development in Asian countries [14]. However, they noted in their study that a great number of s1, m1 and s1m1 strains contained i1. Hence, it is not yet possible to make a definite decision as to whether the *vacA* i1 genotype causes

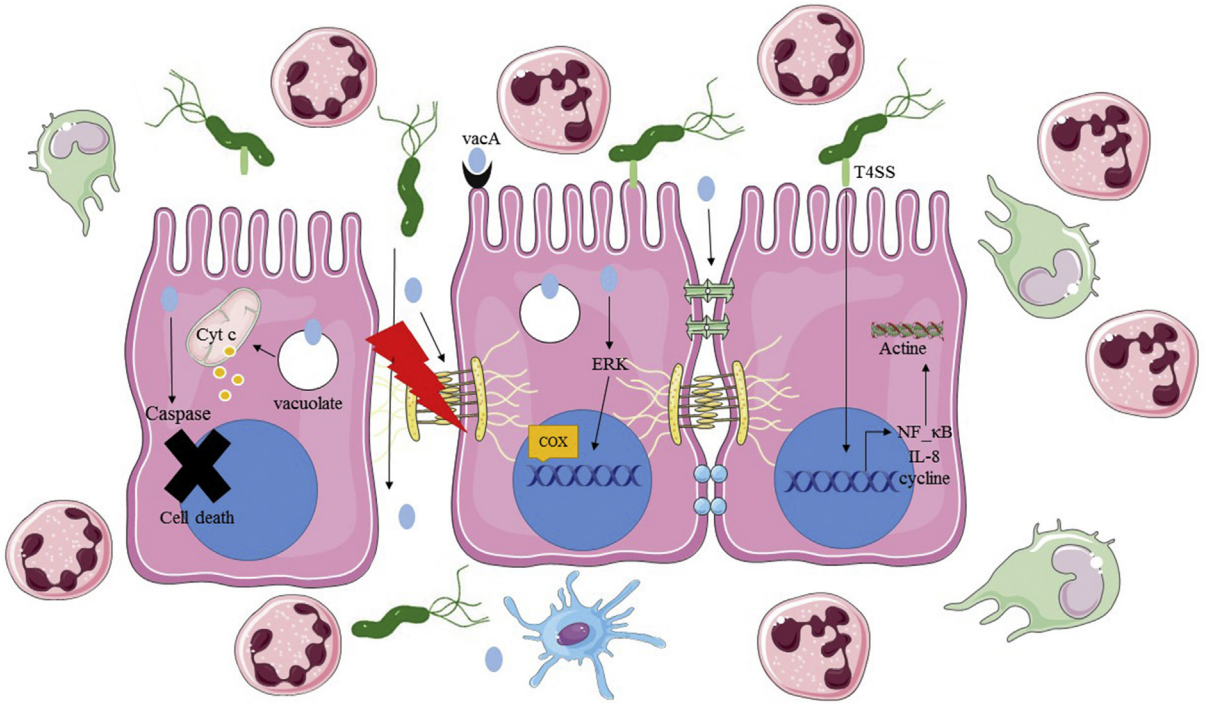

FIG. 2. The schematic of the vacA role in *Helicobacter pylori* pathogenesis.

GC [14]. Numerous studies have also shown that the frequency of the *vacA* i1 genotype is high in *vacA* s1m1 strains (based on the results of many studies, these strains increase the risk of PUD and GC), which could alter the results and lead to false negatives [22,23,31,32]. In their meta-analysis, Pourmohammadi et al. showed that the *vacA* i1 genotype has no role in the GC development. They stated that *cagA* and *vacA* s1m1 have a significant relationship with the GC development [33].

Hence, the role of host genetics and environmental factors in the occurrence of PUD and GC, as well as the high frequency of *cagA*+ and *vacA* s1m1 strains in individuals with PUD and GC, cannot be definitely decided through the impact of *vacA* i1/2 alleles in PUD and GC development because of the diversity of the strains and differences in the frequency of *vacA* i1/2 in strains of different regions of the world. We need to plan and implement more and larger studies.

Conflict of interest

There is no conflict of interest.

References

[1] Yousefi B, Mohammadlou M, Abdollahi M, Salek Farrokhi A, Karbalaei M, Keikha M, et al. Epigenetic changes in gastric cancer induction by *Helicobacter pylori*. J Cell Physiol 2019;234:21770–84.

[2] Segal I, Ally R, Sitas F, Walker A. *Helicobacter pylori*: the African enigma. Gut 1998;43:300.

[3] Takahashi-Kanemitsu A, Knight CT, Hatakeyama M. Molecular anatomy and pathogenic actions of *Helicobacter pylori CagA* that underpin gastric carcinogenesis. Cell Mol Immunol 2020;17:50–63.

[4] Youssefi M, Ghazvini K, Farsiani H, Tafaghoudi M, Keikha M. A systematic review and meta-analysis of outcomes of infection with *Helicobacter pylori* dupA+ strains in Iranian patients. Gene Rep 2020: 100650.

[5] Keikha M, Eslami M, Yousefi B, Ghasemian A, Karbalaei M. Potential antigen candidates for subunit vaccine development against *Helicobacter pylori* infection. J Cell Physiol 2019;234:21460–70.

[6] Kusters JG, van Vliet AH, Kuipers EJ. Pathogenesis of *Helicobacter pylori* infection. Clin Microbiol Rev 2006;19:449–90.

[7] Yamaoka Y. Mechanisms of disease: *Helicobacter pylori* virulence factors. Nat Rev Gastroenterol Hepatol 2010;7:629.

[8] Nagase L, Hayashi T, Senda T, Hatakeyama M. Dramatic increase in SHP2 binding activity of *Helicobacter pylori* Western CagA by EPIYA-C duplication: its implications in gastric carcinogenesis. Sci Rep 2015;5: 15749.

[9] Shiota S, Murakawi K, Suzuki R, Fujioka T, Yamaoka Y. *Helicobacter pylori* infection in Japan. Exp Rev Gastroenterol Hepatol 2013;7:35–40.

[10] Nouraie M, Latifi-Navid S, Rezvan H, Radmard AR, Maghsudlu M, Zaer-Rezaii H, et al. Childhood hygienic practice and family education status determine the prevalence of *Helicobacter pylori* infection in Iran. Helicobacter 2009;14:40–6.

[11] Palframan SL, Kwok T, Gabriel K. Vacuolating cytotoxin A (VacA), a key toxin for *Helicobacter pylori* pathogenesis. Front Cell Infect Microbiol 2012;2:92.

[12] Bakhti SZ, Latifi-Navid S, Mohammadi S, Zahri S, Bakhti FS, Feizi F, et al. Relevance of *Helicobacter pylori* vacA 3′-end region polymorphism to gastric cancer. Helicobacter 2016;21:305–16.

[13] Ito Y, Azuma T, Ito S, Miyaji H, Hirai M, Yamazaki Y, et al. Analysis and typing of the vacA gene from cagA-positive strains of *Helicobacter pylori* isolated in Japan. J Clin Microbiol 1997;35:1710–4.

[14] Sugimoto M, Zali MR, Yamaoka Y. The association of vacA genotypes and *Helicobacter pylori*-related gastroduodenal diseases in the Middle East. Eur J Clin Microbiol Infect Dis 2009;28:1227–36.

[15] Kim JY, Kim N, Nam RH, Suh JH, Chang H, Lee JW, et al. Association of polymorphisms in virulence factor of *Helicobacter pylori* and gastroduodenal diseases in South Korea. J Gastroenterol Hepatol 2014;29:984–91.

[16] Yakoob J, Abid S, Abbas Z, Jafri W, Ahmad Z, Ahmed R, et al. Distribution of *Helicobacter pylori* virulence markers in patients with gastroduodenal diseases in Pakistan. BMC Gastroenterol 2009;9:87.

[17] Bakhti SZ, Latifi-Navid S, Zahri S. Unique constellations of five polymorphic sites of *Helicobacter pylori* vacA and cagA status associated with risk of gastric cancer. Infect Genet Evol 2020;79:104167.

[18] Mottaghi B, Safaralizadeh R, Bonyadi MJ, Latifi-Navid S, Somi MH, Mahdavi M, et al. Relationship of *Helicobacter pylori* vacA i1 and i2 alleles with gastric cancer risk, Iran: a brief report. Tehran Univ Med J 2014;72(9).

[19] Bakhti SZ, Latifi-Navid S, Zahri S, Yazdanbod A. Relationship between new allelic types of *Helicobacter pylori* vacA Gene and cagA status and risk of GU or DU in Iran. J Ardabil Univ Med Sci 2015;15:246–54.

[20] Abdi E, Latifi-Navid S, Zahri S, Yazdanbod A, Safaralizadeh R. *Helicobacter pylori* genotypes determine risk of non-cardia gastric cancer and intestinal-or diffuse-type GC in Ardabil: a very high-risk area in Northwestern Iran. Microb Pathog 2017;107:287–92.

[21] Douraghi M, Talebkhan Y, Zeraati H, Ebrahimzadeh F, Nahvijoo A, Morakabati A, et al. Multiple gene status in *Helicobacter pylori* strains and risk of gastric cancer development. Digestion 2009;80:200–7.

[22] Rhead JL, Letley DP, Mohammadi M, Hussein N, Mohagheghi MA, Hosseini ME, et al. A new *Helicobacter pylori* vacuolating cytotoxin determinant, the intermediate region, is associated with gastric cancer. Gastroenterology 2007;133:926–36.

[23] Hussein NR, Mohammadi M, Talebkhan Y, Doraghi M, Letley DP, Muhammad MK, et al. Differences in virulence markers between *Helicobacter pylori* strains from Iraq and those from Iran: potential importance of regional differences in *H. pylori*-associated disease. J Clin Microbiol 2008;46:1774–9.

[24] Jang S, Jones KR, Olsen CH, Joo YM, Yoo YJ, Chung IS, et al. Epidemiological link between gastric disease and polymorphisms in VacA and CagA. J Clin Microbiol 2010;48:559–67.

[25] Lui SY, Chuah SW, Goh HL, Lee KY, Lee VS, Ho B, et al. Different CagA and VacA polymorphisms are found in the Chinese versus the Malay and Indian populations: an analysis of *Helicobacter pylori* virulence genes in Singapore. Proc Sing Healthc 2010;19:12–8.

[26] Bagheri N, Azadegan-Dehkordi F, Rafieian-Kopaei M, Rahimian G, Asadi-Samani M, Shirzad H, et al. Clinical relevance of *Helicobacter pylori* virulence factors in Iranian patients with gastrointestinal diseases. Microb Pathog 2016;100:154–62.

[27] Karlsson A, Ryberg A, Dehnoei MN, Borch K, Monstein HJ. Association between cagA and vacA genotypes and pathogenesis in a *Helicobacter pylori* infected population from South-eastern Sweden. BMC Microbiol 2012;12:129.

[28] González-Rivera C, Algood HM, Radin JN, McClain MS, Cover TL. The intermediate region of *Helicobacter pylori* VacA is a determinant of toxin potency in a Jurkat T cell assay. Infect Immun 2012;80:2578–88.

[29] Memon AA, Hussein NR, Deyi VY, Burette A, Atherton JC. Vacuolating cytotoxin genotypes are strong markers of gastric cancer and

duodenal ulcer-associated *Helicobacter pylori* strains: a matched case–control study. J Clin Microbiol 2014;52:2984–9.

[30] Liu X, He B, Cho WC, Pan Y, Chen J, Ying H, et al. A systematic review on the association between the *Helicobacter pylori* vacA i genotype and gastric disease. FEBS Open Bio 2016;6:409–17.

[31] Basso D, Zambon CF, Letley DP, Stranges A, Marchet A, Rhead JL, et al. Clinical relevance of *Helicobacter pylori* cagA and vacA gene polymorphisms. Gastroenterology 2008;135:91–9.

[32] Ogiwara H, Graham DY, Yamaoka Y. vacA i-region subtyping. Gastroenterology 2008;134:1267.

[33] Pourmohammadi A, Ghotaslou R, Leylabadlo HE, Nasiri MJ, Dabiri H, Hashemi A, et al. Risk of gastric cancer in association with *Helicobacter pylori* different virulence factors: a systematic review and meta-analysis. Microb Pathog 2018;118:214–9.