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Prevalence of *Helicobacter pylori vac*A, *cag*A, *dup*A and *oip*A Genotypes in Patients with Gastric Disease

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Abstract

Gastric diseases such as chronic gastritis and gastric cancer are most commonly caused by virulence factors of *Helicobacter pylori* (*H. pylori*), such as the *vac*A, *cag*A, *dup*A and *oip*A genes. Therefore, this study investigated the prevalence and the combination of these virulence factors from patients with gastric diseases. The endoscopic biopsies were obtained from 516 patients with gastric symptoms, 101 of which were from patients with normal gastric tissue, 365 of which were from patients with chronic gastritis, and 50 of which were from patients with gastric cancer. *H. pylori* and the virulence factors were detected by PCR. The *oip*A gene exhibited an increased risk for chronic gastritis ($p = 0.0296$), and the vacA gene demonstrated a risk for gastric cancer from chronic gastritis ($p = 0.0002$). Based on the combination of the virulence factors, *cag*A, *vac*A, *dup*A and *oip*A genes exhibited a high prevalence in patients with chronic gastritis and gastric cancer. The *cag*A+/*dup*A+ genotype demonstrated a significant correlation in patients with normal gastric mucosa ($p = 0.0278$). In the chronic gastritis group, a significant association was observed between the *cag*A+ and the *vac*A s1m1 genotypes ($p < 0.0001$), the *cag*A+/*dup*A+ genotypes ($p = 0.0183$), the *dup*A+/*oip*A+ genotypes ($p < 0.0001$), and the *dup*A+/*vac*A s1m1 genotypes ($p = 0.0008$) genotypes. This study revealed a high prevalence of the combination of *cag*A, *vac*A, *dup*A, and *oip*A genes, which contributed to the risk of developing gastroduodenal diseases. Furthermore, this is the first study to reveal a high prevalence of the *oip*A gene in *H. pylori* isolates in Brazil.

Keywords

Helicobacter pylori, Virulence Factors, *cag*A, *dup*A, *oip*A, *vac*A

1. Introduction

Helicobacter pylori (*H. pylori*) is a gram-negative and microaerophilic bacterium that infects approximately 50% of the world's population. However, the prevalence of the infection varies between developed and developing countries. A higher prevalence of *H. pylori* infection (75% - 83%) was found in Latin American countries than in Japan (40%) and other developed countries [1] [2].

H. pylori infection is the main cause of peptic or duodenal ulcers, gastritis, and gastric cancer. The bacterium colonizes the gastric mucosa by using urease activity to resist the hostile acidic condition of the stomach. After surviving in the stomach environment, flagellar motility is necessary to move the bacterium through the gastric epithelium cells. The interaction of *H. pylori* adhesins and cellular receptors supports gastric mucosa infection, and it is followed by the secretion of virulence factors (such as *vac*A, *cag*A, *dup*A and *oip*A genes) which cause tissue damage [1] [3] [4].

The *vac*A gene encodes the vacuolating cytotoxin A, which may participate in several cellular activities, including the inhibition of T-cell proliferation and activation and the onset of proinflammatory response. These activities depend on differences in the gene's structure, the signal region, or s (s1 and s2), and the middle region, or m (m1 and m2). Strains containing s1/m1 are more cytotoxic than those containing s1/m2, and they also increase the risk for developing gastric cancer. Meanwhile, s2/m2 does not exhibit cytotoxic activity [5] [6] [7].

*cag*A (cytotoxin-associated gene A) is the most frequently studied virulence factor encoded by the *cag* pathogenicity island (PAI). This protein is injected into gastric cells, causing oncogenic activity and modification to normal cell differentiation, such as cell polarity and cell adhesion. This gene is associated with increased inflammatory response and risk of gastric cancer and peptic ulcers [7] [8].

The *dup*A (duodenal ulcer promoter gene A) gene was identified as the first disease-specific virulence factor in 2005, for its ability to increase the risk for duodenal ulcers. It was also reported that *dup*A had a suppressive effect on gastric cancer. Recent studies have reported an association between the *dup*A gene and elevated IL-8 production from gastric epithelial cells *in vivo*. However, the presence of *dup*A and the increased IL-8 production *in vitro* is still under discussion [6] [9].

*oip*A (outer inflammatory protein A) was identified in 2000 by Yamaoka, *et al.* [10] as one of the outer membrane proteins. The *oip*A gene has two functional statuses: "on", which reveals the functional gene; and "off", which reveals the non-functional gene. This condition is regulated by a repair mechanism known as slipped strand mispairing, which is based on the number of CT dinucleotide repeats in the 5' region of the *oip*A gene [7] [8]. The presence of *oip*A and *cag*A and/or *cag* PAI seems to be associated with β-catenin signaling, which promotes the opening of cell-cell junctions [6]. Furthermore, the *oip*A's "on" status is associated with higher risks of gastric cancer and peptic ulcers than it is with gastritis and functional dyspepsia [11].

These virulence factors may be related by acting synergistically with each other, thus increasing the risks of several gastric diseases [6]. Therefore, more studies on the clinical importance of *H. pylori* virulence markers are necessary, particularly research that considers the recent *dup*A and *oip*A genes, the functions of which have not yet been

clarified.

Considering the unclear epidemiological aspects of *H. pylori*, the aim of present study was to detect status of *cag*A, *vac*A, *oip*A and *dup*A genotypes in *H. pylori* isolated from patients with chronic gastritis and gastric cancer.

2. Materials and Methods

2.1. Study Populations

Five hundred and sixteen gastric mucosa biopsy samples were collected from patients with gastric symptoms who had undergone an endoscopic investigation at the Bauru State Hospital (HEB), the Gastrointerology Department of the Marilia School of Medicine (FAMEMA), and the Federal University of São Paulo (UNIFESP). Informed consent was obtained from all patients, and the study was approved by the Research Ethics Committee of Universidade do Sagrado Coração (USC) in Bauru, São Paulo, Brazil (No. 1.045.181).

The macroscopic aspects of the mucosa and histological routine results were considered. The study analyzed 101 participants (32 ♂/69 ♀, mean age 54 ± 14.6 years) with normal gastric tissue (Control-C), 365 patients (175 ♂/190 ♀, mean age 53 ± 14.4 years) with chronic gastritis (CG), and 50 patients (30 ♂/20 ♀, mean age 59 ± 13.8 years) with gastric cancer (GC). Patients who received antimicrobial therapy, anti-cholinergic therapy, chemotherapy and anti-inflammatory agents or proton pump inhibitors within 30 days of the investigation were excluded from the study.

2.2. DNA Extraction and *H. pylori* Detection

Genomic DNA from the gastric biopsy samples was extracted using the QIA amp® tissue kit (Qiagen, Germany) according to the manufacturer's instructions. Polymerase chain reaction (PCR) was used to detect the presence of *Helicobacter pylori* and virulence factors from the *vac*A, *cag*A, *dup*A and *oip*A genes. All samples were submitted to diagnosis of *H. pylori* but just the positive samples were submitted to detection of virulence factors. PCR and the primer information from each amplicon are summarized in Table 1.

2.3. Statistical Analysis

To estimate the statistical differences between the diseases and the various genotypes, the Chi square test and Fischer's exact test were used. The risk was estimated using an odds ratio (OR) and a 95% confidence interval (CI). A p value of 0.05 was considered statistically significant.

3. Results

Helicobacter pylori was detected in 256/516 (49.6%) of the samples. The prevalence of *H. pylori* and its virulence factors in each group of patients (the normal group, the chronic gastritis group, and the gastric cancer group) are detailed in Table 2, as are the ORs, the 95% CI, and the p value.

The results suggest a significant association between gastric diseases and the presence of *H. pylori*. Likewise, the *oip*A gene was found to be associated with an increased risk

of chronic gastritis. In the comparison between the groups of patients with chronic gastritis and the group with gastric cancer, the OR (2.46), the 95% CI (1.244-4.861), and the *p* value (0.0089) of the bacterium's presence also indicated a significant association. After the detection of the virulence factors in this comparison (Gastritis and Gastric Cancer groups), only the *vac*A gene was found to be statistically significant (OR: 7.314; 95% CI: 2.157-24.8; $p = 0.0002$).

Based on the combination of the *cag*A gene with the other virulence factors represented in Table 3, a significant correlation was observed between the *cag*A and the *dup*A gene in the normal group (OR: 24.2; 95% CI: 0.9298-629.8; $p = 0.0278$). In the

Table 1. PCR conditions and primers used to detect *H. pylori*, *vac*A, *cag*A, *dup*A, and *oip*A.

Genes	Primers	Primer sequence (5'-3')	PCR conditions	Amplicons	References
16SrRNA	Hpx1[a]	CTGGAGARACTAAGYCCTCC	94˚C, 1 min; 59˚C, 1 min; 72˚C, 1 min (40 cycles)	150 bp	Scholte *et al.*, 1997
	Hpx2	GAGGAATACTCATTGCGAAGGCGA			
vacA s1/s2	SA	ATGGAAATACAACAAACACAC	94˚C, 45 s; 54˚C, 45 s; 72˚C, 45 s (40 cycles)	s1: 176 bp s2: 203 bp	Atherton *et al.*, 1995 Van Doorn *et al.*, 1998
	SC[a]	CCTGARACCGTTCCTACAGC			
vacA m1/m2	MA[a]	CACAGCCACTTTYAATAACGA	94˚C, 45 s; 55˚C, 45 s; 72˚C, 1 min (35 cycles)	m1: 400 bp m2: 475 bp	Van Doorn *et al.*, 1998
	MB	CGTCAAAATAATTCCAAGGG			
*cag*A	Cag1	ATGACTAACGAAACTATTGATC	94˚C, 1 min; 53˚C, 1 min; 72˚C, 1 min (40 cycles)	232 bp	Rasmussen *et al.*, 2010
	Cag2	CAGGATTTTTGATCGCTTTATT			
*dup*A	Dupa1	CGTGATCAATATGGATGCTT	94˚C, 45 s; 52˚C, 45 s; 72˚C, 45 s (35 cycles)	197 bp	Gomes *et al.*, 2008
	Dupa2	TCTTTCTAGCTTGAGCGA			
*oip*A	HPO638F	GTTTTTGATGCATGGGATTT	94˚C, 45 s; 57˚C, 45 s; 72˚C, 45 s (35 cycles)	401 bp	Ben Mansour *et al.*, (2010)
	HPO638R	GTGCATCTCTTATGGCTTTG			

Alleles s1 and m1 of *vac*A gene, *H. pylori* strain 60190 (GeneBank U05676); Alleles s2 and m2 of *vac*A gene, *H. pylori* strain Tx30a (GeneBank U29401); a R = A or G and Y = C or T.

Table 2. Distribution of the *Helicobacter pylori* genotypes and the risk of developing chronic gastritis and gastric cancer Comparisons between the normal group and the chronic gastritis group and the normal group versus the gastric cancer group.

	Normal (n = 101)	Chronic Gastritis (n = 365)	OR (95% CI), *p*	Gastric Cancer (n = 50)	OR (95% CI), *p*
*vac*A	*Hp*+ (n = 12)	*Hp*+ (n = 206)	9.55 (5.05 - 18.06), <0.0001*	*Hp*+ (n = 38)	23.49 (9.684 - 56.96), <0.0001*
s1/m1	07 (58.3%)	105 (50.9%)	0.62 (0.16-2.50), 0.7422	32 (84.2%)	4.57 (0.7575-27.59), 0.1129
s2/m2	03 (25%)	72 (34.9%)		03 (7.8%)	
others	02 (16.6%)	29 (14%)		03 (7.8%)	
*cag*A	05 (41.6%)	101 (49%)	1.34 (0.41-4.38), 0.7691	19 (50%)	1.4 (0.3769-5.20), 0.7447
*dup*A	07 (58.3%)	109 (52.9%)	0.80 (0.25-2.61), 0.7739	18 (47.3%)	0.64 (0.1730-2.39), 0.7416
*oip*A	06 (50%)	163 (79.1%)	3.79 (1.16-12.35), 0.0296*	26 (68.4%)	2.17 (0.5773-8.13), 0.3088

Hp: *Helicobacter pylori*; n: number of samples. *statistically significant.

Table 3. Association between the *cag*A gene and the *vac*A, *dup*A, and *oip*A genes.

Virulence Factor		*n* (%)	*vac*A *n* (%)			*dup*A *n* (%)		*oip*A *n* (%)	
			*s*1*m*1	*s*2*m*2	*outros*	*pos*	*neg*	*pos*	*neg*
Normal									
*cag*A	*pos*	5 (100)	4 (80)	0	1 (20)	5 (100)*	0	4 (80)	1 (20)
	neg	7 (100)	3 (42.85)	3 (42.85)	1 (14.3)	2 (28.6)	5 (71.4)	2 (28.6)	5 (71.4)
	Total	12 (100)	7 (58.3)	3 (25)	2 (16.7)	7 (58.3)	5 (41.7)	6 (50)	6 (50)
Chronic Gastritis									
*cag*A	*pos*	101 (100)	78 (77.2)*	11 (10.9)	12 (11.9)	62 (61.4)*	39 (38.6)	77 (76.2)	24 (23.8)
	neg	105 (100)	27 (25.7)	61 (58.1)	17 (16.2)	47 (44.8)	58 (55.2)	86 (81.9)	19 (18.1)
	Total	206 (100)	105 (51)	72 (34.9)	29 (14.1)	109 (52.9)	97 (47.1)	163 (79.1)	43 (20.9)
Gastric Cancer									
*cag*A	*pos*	19 (100)	18 (94.7)	0	1 (5.3)	10 (52.6)	9 (47.4)	16 (84.2)	3 (15.8)
	neg	19 (100)	14 (73.7)	3 (15.8)	2 (10.5)	8 (42.1)	11 (57.9)	10 (52.6)	9 (47.4)
	Total	38 (100)	32 (84.2)	3 (7.9)	3 (7.9)	18 (47.4)	20 (52.6)	26 (68.4)	12 (31.6)

n: number of samples. *association statistically significant.

chronic gastritis group, two associations were found to be statistically significant. The first relationship was between the *cag*A and the *vac*A s1m1 genes (OR: 16.02; 95% CI: 7.364-34.85; $p < 0.0001$), and the second was between the *cag*A and the *dup*A genes (OR: 1.962; 95% CI: 1.126-3.419; $p = 0.0183$). However, no significant correlations werefound between the virulence factors in the gastric cancer group.

Moreover, an analysis of the combinations between the virulence factors, as outlined in **Figure 1**, revealed interesting correlations. Among the strains isolated from the patients with chronic gastritis, the statistically significant genotypes were *cag*A+/*vac*A s1m1 (data shown previously), *cag*A+/*dup*A+ (data shown previously), *dup*A+/*oip*A+ (OR: 5.105; 95% CI: 2.353-11.07; $p < 0.0001$), and *dup*A+/ *vac*A s1m1 (OR: 9.185; 95% CI: 2.582-32.67; $p = 0.0008$).There were no statistical associations among the strains from the patients with gastric cancer.

4. Discussion

The association between *Helicobacter pylori* and the development of duodenal and peptic ulcers, chronic gastritis, and an increased risk for gastric cancer is now a consensus [12]. Thus, our results demonstrate a significant relationship between the presence of *H. pylori* and the increased risks for gastric diseases. The results are in accordance with many studies that have been published since the discovery of *H. pylori* [12] [13] [14] [15] [16]. These clinical outcomes vary according to the host and *H. pylori* factors, such as the *vac*A, *cag*A, *dup*A, and *oip*A genes.

*Vac*A activity depends on the structure of the gene. Studies have found that in Western countries, strains containing the s1m1 genotype are associated with a higher risk of gastric disease, as well as with enhanced proinflammatory response [17]. Similar to our results, Asrat, *et al.* [18] and Suzuki, *et al.* [19] also found s1m1 to be the most common

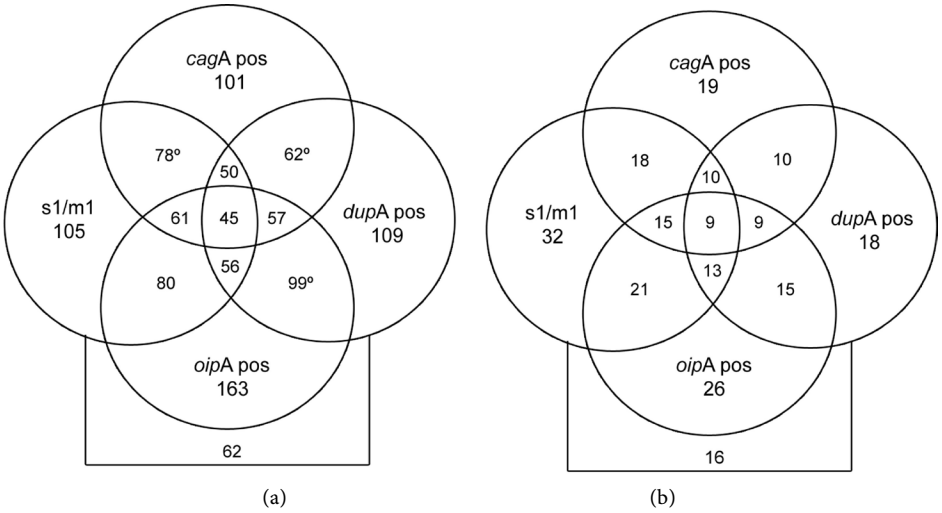

Figure 1. Combination among *cag*A, *vac*A, *dup*A, and *oip*A genotypes. (a) Chronic gastritis group; (b) Gastric cancer group. *Statistically significant association.

*vac*A genotype. Interestingly, all of these virulence factors have a mutual relationship; for example, *cag*A-positive strains often have the s1m1 genotype, and several studies have provided evidence of an association between the *cag*A+/*vac*A s1m1 genotype and the risk of gastric disease, which was also found in our study [20] [21] [22]. However, the significant association between this genotype and gastric diseases was only observed in the chronic gastritis group ($p < 0.0001$). No associations were found in the gastric cancer group, a result which conflicts with the data provided by Suzuki, *et al.* [21].

When the oncoprotein *cag*A is present in host cells, the risks for peptic ulcer and gastric cancer have been found to increase in Western countries when compared to *cag*A-negative strains [16] [19]. This data was found to be inconsistent with our study, because the *cag*A gene alone was not found to be associated with any clinical outcome; however, our report is consistent with the results found by Kim, *et al.* [23].

The prevalence of the *cag*A gene may vary according to the region. In Eastern countries, *cag*A-positive strains reach almost 100%, while in some Western countries the prevalence is less than 50% [6]. The present study detected 50% or fewer *cag*A-positive strains among a cohort of Brazilian patients. This number is similar to what Sasaki, *et al.* [24] observed in Ecuador (45.9%) and Panama (20%), though it is not in agreement with what Yamaoka, *et al.* [25] found in Japan or what Watada, *et al.* [22] found in Colombia.

In our report, the *cag*A gene also exhibited a significant association with another virulence factor, the *dup*A gene, which is a protecting factor against gastric cancer and which is associated with an increased risk for duodenal ulcer [26] Gomes, *et al.* [27] also found a correlation between *cag*A-positive and *dup*A-positive strains, but the association was in patients with duodenal ulcers. In a meta-analysis, Shiota, *et al.* [26] reported a higher prevalence of *dup*A in Western countries than in Eastern countries. The study performed by Gomes, *et al.* [27] revealed an increased prevalence of *dup*A in Brazilian patients with chronic gastritis (92.3%) and gastric cancer (87.6%), a finding which differed from that provided by Lu *et al.* (2005) in a study on strains from Colombia. It also differs from our findings. This difference likely occurred because the pa-

tients were from different regions in Brazil.

One interesting point about the *dup*A gene was that we observed a statistically significant association between it and the *vac*A s1m1 genotype. This fact has not been described previously. The only report on this association was provided by Arachchi *et al.* (2007), who observed a correlation between s1 and m1 alone. Interestingly, the *dup*A gene also exhibited a significant association with the *oip*A gene in our study; both genes are involved in the induction of IL-8 secretion [7]. However, our findings differ from those of Souod, *et al.* [28], who reported a statistically significant association with the *dup*A+/*oip*A- genotype, and with the same p value as ours ($p < 0.0001$). Though the clinical outcomes resulting from the presence of the *oip*A gene are still unclear, Souod, *et al.* [28] similarly observed a significant correlation between the *oip*A gene and chronic gastritis. In addition, they found no relationship with gastric cancer, as Yamaoka, *et al.* [29] also reported.

5. Conclusion

Taken together, our results suggest that the high prevalence of virulent factors contributes to the risk of developing gastroduodenal diseases. We hypothesize that the more virulent combination may be a trigger for chronic gastritis, a precursor lesion of gastric cancer. This is the first study to reveal a high prevalence of the *oip*A gene in *H. pylori* isolates in Brazil. Furthermore, this study reveals a high prevalence of the combination of *cag*A, *vac*A, *dup*A, and *oip*A genes. Our findings reinforce the importance of understanding the correlation between the virulence of *H. pylori* and host susceptibility.

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References

[1] Kao, C.Y., Sheu, B.S. and Wu, J.J. (2016) *Helicobacter pylori* Infection: An Overview of Bacterial Virulence Factors and Pathogenesis. *Biomedical Journal*, **39**, 14-23. https://doi.org/10.1016/j.bj.2015.06.002

[2] Safavi, M., Sabourian, R. and Foroumadi, A. (2016) Treatment of *Helicobacter pylori* Infection: Current and Future Insights. *World Journal of Clinical Cases*, **4**, 5-19. https://doi.org/10.12998/wjcc.v4.i1.5

[3] Shiota, S. and Yamaoka, Y. (2014) Biomarkers for *Helicobacter pylori* Infection and Gastroduodenal Diseases. *Biomarkers in Medicine*, **8**, 1127-1137. https://doi.org/10.2217/bmm.14.72

[4] Vinagre, I.D., Queiroz, A.L., Silva Jr., M.R., Vinagre, R.M. and Martins, L.C. (2015) *Helicobacter pylori* Infection in Patients with Different Gastrointestinal Diseases from Northern Brazil. *Arquivos de Gastroenterologia*, **52**, 266-271. https://doi.org/10.1590/S0004-28032015000400004

[5] Malfertheiner, P., Bornschein, J. and Selgrad, M. (2010) Role of *Helicobacter pylori* Infection in Gastric Cancer Pathogenesis: A Chance for Prevention. *Journal of Digestive Diseases*, **11**, 2-11. https://doi.org/10.1111/j.1751-2980.2009.00408.x

[6] Yamaoka, Y. (2010) Mechanisms of Disease: *Helicobacter pylori* Virulence Factors. *Nature Reviews Gastroenterology and Hepatology*, **7**, 629-641.

https://doi.org/10.1038/nrgastro.2010.154

[7] Yamaoka, Y. and Graham, D.Y. (2014) *Helicobacter pylori* Virulence and Cancer Pathogenesis. *Future Oncology*, **10**, 1487-1500. https://doi.org/10.2217/fon.14.29

[8] Shiota, S., Suzuki, R. and Yamaoka, Y. (2013) The Significance of Virulence Factors in *Helicobacter pylori*. *Journal of Digestive Diseases*, **14**, 341-349. https://doi.org/10.1111/1751-2980.12054

[9] Lu, H., Hsu, P.I., Graham, D.Y. and Yamaoka, Y. (2005) Duodenal Ulcer Promoting Gene of *Helicobacter pylori*. *Gastroenterology*, **128**, 833-848. https://doi.org/10.1053/j.gastro.2005.01.009

[10] Yamaoka, Y., Kwon, D.H. and Graham, D.Y. (2000) A M_r 34,000 Proinflammatory Outer Membrane Protein (*oip*A) of *Helicobacter pylori*. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 7533-7538. https://doi.org/10.1073/pnas.130079797

[11] Liu, J., He, C., Chen, M., Wang, Z., Xing, C. and Yuan, Y. (2013) Association of Presence/Absence and on/off Patterns of *Helicobacter pylori oip*A Gene with Peptic Ulcer Disease and Gastric Cancer Risks: A Meta-Analysis. *BMC Infectious Diseases*, **13**, 555. https://doi.org/10.1186/1471-2334-13-555

[12] Sugano, K., Tack, J., Kuipers, E.J., Graham, D.Y., El-Omar, E.M., Miura, S., *et al.* (2015) Kyoto Global Consensus Report on *Helicobacter pylori* Gastritis. *Gut*, **64**, 1353-1367. https://doi.org/10.1136/gutjnl-2015-309252

[13] Marshall, B.J. and Warren, J.R. (1984) Unidentified Curved Bacilli in the Stomach of Patients with Gastritis and Peptic Ulceration. *The Lancet*, **323**, 1311-1315. https://doi.org/10.1016/S0140-6736(84)91816-6

[14] Huang, J.Q., Sridhar, S., Chen, Y. and Hunt, R.H. (1998) Meta-Analysis of the Relationship between *Helicobacter pylori* Seropositivity and Gastric Cancer. *Gastroenterology*, **114**, 1169-1179. https://doi.org/10.1016/S0016-5085(98)70422-6

[15] Suerbaum, S. and Michetti, P. (2002) *Helicobacter pylori* Infection. *The New England Journal of Medicine*, **347**, 1175-1186. https://doi.org/10.1056/NEJMra020542

[16] Ahn, H.J. and Lee, D.S. (2015) *Helicobacter pylori* in Gastric Carcinogenesis. *World Journal of Gastrointestinal Oncology*, **7**, 455-465.

[17] Ribeiro, R.B., Martins, H.S., Dos Santos, V.A., El Khouri, M., Duarte, L.S., Burattini, M.N., *et al.* (2010) Evaluation of *Helicobacter pylori* Colonization by Serologic Test (IgG) and Dyspepsia in Volunteers from the Countryside of Monte Negro, in the Brazilian Western Amazon Region. *Revista do Instituto de Medicina Tropical de São Paulo*, **52**, 203-206. https://doi.org/10.1590/S0036-46652010000400007

[18] Asrat, D., Nilsson, I., Mengistu, Y., Kassa, E., Ashenafi, S., Ayenew, K., *et al.* (2004) Prevalence of *Helicobacter pylori vac*A and *cag*A Genotypes in Ethiopian Dyspeptic Patients. *Journal of Clinical Microbiology*, **42**, 2682-2684. https://doi.org/10.1128/JCM.42.6.2682-2684.2004

[19] Suzuki, R., Shiota, S. and Yamaoka, Y. (2012) Molecular Epidemiology, Population Genetics, and Pathogenic Role of *Helicobacter pylori*. *Infection, Genetics and Evolution*, **12**, 203-213. https://doi.org/10.1016/j.meegid.2011.12.002

[20] Atherton, J.C., Cao, P., Peek Jr., R.M., Tummuru, M.K., Blaser, M.J. and Cover, T.L. (1995) Mosaicism in Vacuolating Cytotoxin Alleles of *Helicobacter pylori*. Association of Specific *vac*A Types with Cytotoxin Production and Peptic Ulceration. *The Journal of Biological Chemistry*, **270**, 17771-17777. https://doi.org/10.1074/jbc.270.30.17771

[21] Suzuki, H., Hibi, T. and Marshall, B.J. (2007) *Helicobacter pylori*: Present Status and Future Prospects in Japan. *Journal of Gastroenterology*, **42**, 1-15. https://doi.org/10.1007/s00535-006-1990-z

[22] Watada, M., Shiota, S., Matsunari, O., Suzuki, R., Murakami, K., Fujioka, T., *et al.* (2011) Association between *Helicobacter pylor*i *cag*A-Related Genes and Clinical Outcomes in Colombia and Japan. *BMC Gastroenterology*, **11**, 141. https://doi.org/10.1186/1471-230X-11-141

[23] Kim, J.Y., Kim, N., Nam, R.H., Suh, J.H., Chang, H., Lee, J.W., *et al.* (2014) Association of Polymorphisms in Virulence Factor of *Helicobacter pylori* and Gastroduodenal Diseases in South Korea. *Journal of Gastroenterology and Hepatology*, **29**, 984-991. https://doi.org/10.1111/jgh.12509

[24] Sasaki, T., Hirai, I. and Yamamoto, Y. (2009) Analysis of *Helicobacter pylori* Infection in a Healthy Panamanian Population. *Kansenshogaku Zasshi*, **83**, 127-132. https://doi.org/10.11150/kansenshogakuzasshi.83.127

[25] Yamaoka, Y., Kodama, T., Kita, M., Imanishi, J., Kashima, K. and Graham, D.Y. (1999) Relation between Clinical Presentation, *Helicobacter pylori* Density, Interleukin 1Beta and 8 Production, and *cag*A Status. *Gut*, **45**, 804-811. https://doi.org/10.1136/gut.45.6.804

[26] Shiota, S., Matsunari, O., Watada, M., Hanada, K. and Yamaoka, Y. (2010) Systematic Review and Meta-Analysis: The Relationship between the *Helicobacter pylori dup*A Gene and Clinical Outcomes. *Gut Pathogens*, **2**, 13. https://doi.org/10.1186/1757-4749-2-13

[27] Gomes, L.I., Rocha, G.A., Rocha, A.M., Soares, T.F., Oliveira, C.A., Bittencourt, P.F., *et al.* (2008) Lack of Association between *Helicobacter pylori* Infection with *dup*A-Positive Strains and Gastroduodenal Diseases in Brazilian Patients. *International Journal of Medical Microbiology*, **298**, 223-230. https://doi.org/10.1016/j.ijmm.2007.05.006

[28] Souod, N., Sarshar, M., Dabiri, H., Momtaz, H., Kargar, M., Mohammadzadeh, A., *et al.* (2015) The Study of the *oip*A and *dup*A Genes in *Helicobacter pylori* Strains and Their Relationship with Different Gastroduodenal Diseases. *Gastroenterology and Hepatology from Bed to Bench*, **8**, S47-S53.

[29] Yamaoka, Y., Kikuchi, S., El-Zimaity, H.M., Gutierrez, O., Osato, M.S. and Graham, D.Y. (2002) Importance of *Helicobacter pylori oip*A in Clinical Presentation, Gastric Inflammation, and Mucosal Interleukin 8 Production. *Gastroenterology*, **123**, 414-424. https://doi.org/10.1053/gast.2002.34781