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# Heterogeneity of *cag* genotypes of *Helicobacter pylori* in the esophageal mucosa of dyspeptic patients and its relation to histopathological outcomes

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SUMMARY

*Background:* A high prevalence of *Helicobacter pylori* in the esophageal mucosa of dyspeptic Venezuelan patients has been reported. We aimed to assess the genetic composition of the *cag* genotypes of *H. pylori* and its relation to histopathological outcomes in the gastroesophageal mucosa.
*Methods:* The presence of *cag*A, *cag*E, and *vir*B11 *cag* pathogenicity island (PAI) genes was detected by PCR in 80 of 150 *H. pylori*-positive dyspeptic patients in both mucosae. Alterations of the gastroesophageal mucosa were assessed by histological techniques.
*Results:* The frequency of intact, partial, and deleted *cag*-PAI genes in the stomach of dyspeptic patients was found to be 57.5%, 21.3%, and 21.3%, respectively, whereas in the esophagus, frequencies were 33.8%, 33.8%, and 32.5% respectively. The genetic composition in the stomach was 57.5% *cag*A-positive, 20.0% *cag*A-negative, 75.0% *cag*E, and 77.5% *vir*B11, whereas in the esophagus the distribution was 36.3% *cag*A-positive, 30.0% *cag*A-negative, 61.3% *cag*E, and 63.8% *vir*B11. The gene with the largest difference between the two mucosae was *cag*A, with 58.8% in the stomach and 37.5% in the esophagus; *cag*E and *vir*B11 were less variable. The correlation among single and/or mixed *cag* genotypes with histopathological outcomes in both mucosae from the same patient was higher for intact single *cag*-PAI genotypes, showing severe alterations.
*Conclusions: H. pylori* may coexist in similar proportions without dominance of one *cag* genotype, suggesting a heterogeneous distribution in the esophagus. The *cag*E and *vir*B11 genes can be used as markers of *cag*-PAI in the esophagus. The single *cag*-PAI genotype in both mucosae confers an increased risk of developing histological damage.


## 1. Introduction

*Helicobacter pylori* colonizes the gastric mucosa of humans, and if not treated, persists throughout life.[1,2] *H. pylori* infection has been implicated as the main cause of peptic ulceration, gastric lymphoma, and gastric adenocarcinoma, the second leading cause of death from cancer worldwide.[1] More than 50% of the world population carries *H. pylori*, with a prevalence as high as 90% in developing countries.[3] Although most infected persons remain asymptomatic, 15–20% of *H. pylori*-positive individuals will develop at least one of the associated diseases at some point in their lives.[4]

One of the disease-associated factors in *H. pylori* is the presence of the *cag* pathogenicity island (*cag*-PAI),[5] which confers an increased risk for peptic ulcer disease and gastric cancer.[2,4,6] This *cag*-PAI comprises 31 genes, encoding a functional type IV secretion system.[5] Three of these *cag* genes (*cag*A, *cag*E, and *vir*B11) have been recognized as markers of an intact *cag*-PAI.[2,4,6]

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*cag*A-positive and negative *H. pylori* strains may coexist in a single individual and even in a single gastric biopsy specimen.[7] Gastric colonization by multiple *H. pylori* strains is common in countries where *H. pylori* is highly prevalent, carrying virulence genotypes with genetic diversity, as has been demonstrated in infected patients in Venezuela.[8,9]

In the upper gastrointestinal tract, the esophagus is a potential site for the presence of a bacterial biota and may provide a niche for colonization by *H. pylori*.[10] This bacterium has been detected in esophagus mucosa biopsies from patients with symptoms of dyspepsia in the range of 34% to 73%.[11,12] However, its role in esophageal diseases, such as heterotopic gastric mucosa in the upper esophagus (inlet patch), gastroesophageal reflux diseases (GERD), Barrett's esophagus, and esophageal carcinoma has not yet been determined.[11,13] We have previously reported an *H. pylori* prevalence of 86% in esophageal mucosa biopsies from dyspeptic patients in Venezuela, and its presence was correlated with signs of inflammation.[14] In the present study, we further investigated the genetic composition of the *cag* genotype profiles of *H. pylori* through the detection of *cag*A, *cag*E, and *vir*B11 genes by PCR in the gastroesophageal mucosa, and its relation to histopathological outcomes at the individual level and among the population studied.

## 2. Methods

### 2.1. Clinical specimens

Patients consulting the Gastroenterology Unit of the Oncology Service Hospital of IVSS (Instituto Venezolano de los Seguros Sociales) with gastrointestinal symptoms of dyspepsia, requiring upper gastrointestinal endoscopy, were recruited into this study. All volunteers signed an informed consent form. The bioethics committees of the IVIC (Instituto Venezolano de Investigaciones Científicas) and IVSS approved the study. Patients who had previously received treatment were excluded. The study was performed on 150 volunteers aged 19–69 years (mean age 43 years).

Two paired biopsy sets from the gastric mucosa (antrum) and esophageal mucosa (lower third or within 2 cm of the Z-line), performed as described previously,[14] were collected from each patient. For each mucosa, one biopsy specimen was immediately placed in formalin for histopathology, and the other was submitted to PCR assays. Biopsies were taken with care to avoid inter-sample contamination.

### 2.2. DNA extraction and PCR

A total of 300 DNA samples from 150 patients were processed using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA). *Helicobacter spp* and *H. pylori* infection were detected by PCR for all three 16S rRNA, *glm*M, and *ure*A genes, using genus- and species-specific primers.[15–17] The presence of *cag*A, *cag*E, and *vir*B11 *cag*-PAI genes was investigated in 80 *H. pylori*-positive patients in both mucosae using specific primers described for each gene.[6,18]

PCRs were performed using the PCR Master Mix Kit (Promega Corp., Madison, WI, USA) and a thermal cycler (model GeneAMP PCR System 9700; Applied Biosystems, USA), as described previously.[14] According to the genetic composition of the *cag* island by PCR, we considered an intact *cag*-PAI to be one where all three genes (*cag*A, *cag*E, and *vir*B11) were present, a partial *cag* island to be one where at least one gene was lacking, and a deleted *cag* island to be one where all three genes were absent.

### 2.3. Histopathology

All gastric and esophageal biopsy specimens submitted for histopathology analysis were fixed in formalin and embedded in paraffin. Sections of 4 μm in thickness were obtained and adhered onto glass slides, dewaxed in xylene, and subsequently rehydrated in phosphate buffer solution at pH 7.4. The sections were assessed by conventional histological techniques. Histological classification was done according to the updated Sydney classification for the stomach (antrum), and the pattern of inflammation in the esophagus was determined as described by Contreras et al.[14]

### 2.4. Statistical analysis

The statistical relationship between different histopathological parameters and the detection of *cag*-PAI genotypes was performed using Spearman's rank correlation. $p$-Values of $<0.05$ were considered significant. The presence of a histopathological variable, grade-independent, was considered as positive (= 1).

## 3. Results

### 3.1. Detection of H. pylori and presence of cag-PAI genes: cagA, cagE, and virB11

The prevalence of *H. pylori* infection in the gastric and esophageal mucosae of 150 dyspeptic patients, as determined by amplification of 16S rRNA, *glm*M, and *ure*A genes, was 53.3% ($n = 80$) positive in both mucosae; 34.0% ($n = 51$) were negative in both mucosal samples, 11.3% ($n = 17$) were positive only in the gastric mucosa, and 1.3% ($n = 2$) were positive only in the esophageal mucosa. Thus, we studied the *cag*-PAI gene composition of the 80 *H. pylori*-positive patients.

An intact *cag*-PAI was detected by PCR in 57.5% of stomach biopsies and 33.8% of esophagus biopsies. Partial deletions of *cag*-PAI were present in 21.3% and 33.8%, whereas complete deletions of *cag*-PAI were found in 21.3% and 32.5%, respectively (Table 1). Analysis of the *cag* genotypes showed that the most frequent genotypes in the stomach were *cag*A-positive (57.5%) and *cag*A-negative (20.0%), with both of the other two genes studied present (*cag*E 75.0% and *vir*B11 77.5%). Similarly in the esophagus, frequencies of 36.3% and 30.0% were found for *cag*A-positive and negative genotypes, respectively, with 61.3% for *cag*E and 63.8% for *vir*B11 (Table 1).

When we examined the distribution of the *cag* genotype profiles in the gastroesophageal mucosa on a per-patient basis, we found 50% of patients to be carrying a single genotype with the same genetic composition of *cag*-PAI in stomach and esophagus; the other 50% carried mixed genotypes with different *cag*-PAI composition in the two mucosae (Table 2). The prevalence in the single *cag* genotype profile with an intact *cag*-PAI was 60.0% and with a deleted *cag* island was 32.5%. The gene with the largest difference between the two mucosae in the mixed genotype was *cag*A with 57.5% in stomach and 15.0% in esophagus, while *cag*E and *vir*B11 were less variable. The *cag*E and *vir*B11 genes in the

**Table 1**
Analyses of *cag* genotypes in the gastric and esophageal mucosa of 80 patients studied by PCR

| Genotypes | | | Gastroesophageal mucosa ($n = 80$) | |
|---|---|---|---|---|
| *cag*A | *cag*E | *vir*B11 | % of gastric biopsies ($n$) | % of esophageal biopsies ($n$) |
| Pos | Pos | Pos | 57.50 (46) | 33.75 (27) |
| Neg | Pos | Pos | 17.50 (14) | 27.50 (22) |
| Neg | Neg | Pos | 2.50 (2) | 2.50 (2) |
| Pos | Neg | Neg | 1.25 (1) | 1.25 (1) |
| Pos | Neg | Pos | 0 (0) | 2.50 (2) |
| Neg | Neg | Neg | 21.25 (17) | 32.50 (26) |

Pos, positive; Neg, negative.

**Table 2**
Distribution of *cag* genotype profiles in the gastroesophageal mucosa of the same patient by PCR

| % of *H. pylori*-positive patients ($n = 80$) | PCR results of *cag* genotypes in mucosa | | | | | |
|---|---|---|---|---|---|---|
| | Gastric | | | Esophageal | | |
| | *cag*A | *cag*E | *vir*B11 | *cag*A | *cag*E | *vir*B11 |
| Single *cag* ($n = 40$) | | | | | | |
| 60.0 (24) | Pos | Pos | Pos | Pos | Pos | Pos |
| 7.5 (3) | Neg | Pos | Pos | Neg | Pos | Pos |
| 32.5 (13) | Neg | Neg | Neg | Neg | Neg | Neg |
| Mixed *cag* ($n = 40$) | | | | | | |
| 42.5 (17) | Pos | Pos | Pos | Neg | Pos | Pos |
| 7.5 (3) | Pos | Pos | Pos | Neg | Neg | Neg |
| 5.0 (2) | Pos | Pos | Pos | Pos | Neg | Pos |
| 17.5 (7) | Neg | Pos | Pos | Neg | Neg | Neg |
| 5.0 (2) | Neg | Pos | Pos | Pos | Pos | Pos |
| 2.5 (1) | Neg | Pos | Pos | Pos | Neg | Neg |
| 2.5 (1) | Neg | Pos | Pos | Neg | Neg | Pos |
| 5.0 (2) | Neg | Neg | Neg | Neg | Pos | Pos |
| 2.5 (1) | Neg | Neg | Neg | Neg | Neg | Pos |
| 2.5 (1) | Neg | Neg | Neg | Pos | Pos | Pos |
| 5.0 (2) | Neg | Neg | Pos | Neg | Neg | Neg |
| 2.5 (1) | Pos | Neg | Neg | Neg | Neg | Neg |

Pos, positive; Neg, negative.

stomach were detected in 82.5% and 87.5%, respectively, of the 40 patients with mixed genotypes, showing a significant correlation ($r = 0.821$, $p < 0.05$); these genes in the esophagus were found in 55.0% and 65.0% of the 40 patients, also showing a significant correlation ($r = 0.811$, $p < 0.05$) (Table 2).

### 3.2. Histopathology of the gastric and esophageal mucosa

Of the 80 *H. pylori*-positive patients, it was possible to perform a histopathological study of gastric biopsies for only 74 and of esophageal biopsies for only 57. All 74 patients (100%) had histopathological alterations in the antrum. Of these subjects, 14 (18.9%) showed acute inflammation characterized by neutrophil and monocyte infiltration and bacteria, and 60 (81.0%) had varying degrees of superficial and chronic atrophic gastritis (Table 3). Seventeen out of these 60 patients (28.3%) had superficial gastritis with or without dysplasia, with monocyte infiltration and bacteria being the most frequent findings. On the other hand, 43 patients (71.7%) had chronic atrophic gastritis with or without dysplasia, neutrophil infiltration, malgun (clear) cells, and bacteria (Table 3). Other histopathological alterations, such as the presence of scarce mucosa-associated lymphoid tissue (MALT)-like follicles, were associated with gastritis and observed in 16 patients (22.0%) (Table 3).

We found dysplasia, neutrophil infiltration, bacteria, microabscesses, hyperplasia, and plasmocytes in all 57 (71.3%) esophageal mucosa samples. Infiltration, microabscesses, and hyperplasia were the most frequent alterations. The presence of chronic inflammation in these 57 patients evidenced a marked cellular immune response.

### 3.3. Relationship between different cag genotypes and histopathological outcomes

We found significant correlations between single or mixed *cag* genotype profiles and different histopathological variables in each mucosa on a per-patient basis. The correlation analysis of the intact and deleted single *cag* genotypes with histopathological variables in the gastric and esophageal mucosa is shown in Table 4.

The correlation analysis of the mixed *cag* genotypes on a per-patient basis, i.e., intact *cag* genotype in the stomach and partial *cag* genotype in the esophagus with the histopathological variables, showed significance between MALT-like follicles and infiltration 2 ($r = 0.577$, $p < 0.05$) (stomach) and between pseudogoblet cells and metaplasia ($r = 0.674$, $p < 0.05$) (esophagus). The partial *cag* genotype in the stomach, but deleted in the esophagus, showed significance between superficial gastritis and reflux ($r = 1$, $p < 0.01$) and chronic atrophic gastritis and reflux ($r = 1$, $p < 0.05$), while no correlations were found in the esophagus.

## 4. Discussion

The present study of 150 symptomatic patients demonstrated that the overall prevalence of *H. pylori* infection in the gastroesophageal mucosa was 64.7% (97 patients). Of these patients, we found *H. pylori* infection in the esophagus of 82.5% (80 patients), indicating that its colonization is linked to infection in the stomach. Esophageal infection was correlated with different signs of inflammation (pseudogoblet cells and metaplasia, microabscesses and bacteria), as we have reported previously.[14] The

**Table 3**
Histopathological results and frequencies in gastroesophageal mucosal biopsies from dyspeptic patients

| Histopathological alterations | Gastric ($n = 74$) | | | | Esophageal ($n = 57$) $n$ (($n$/57) × 100)[b] |
|---|---|---|---|---|---|
| | Acute inflammation[a] $n$ (($n$/14) × 100) | Superficial gastritis[a] $n$ (($n$/17) × 100) | Chronic atrophic gastritis[a] $n$ (($n$/43) × 100) | Total $n$ (($n$/74) × 100) | |
| Dysplasia | 0 (0) | 10 (59%) | 28 (65%) | 38 (51%) | 39 (68%) |
| Metaplasia | 0 (0) | 3 (18%) | 2 (5%) | 5 (7%) | 9 (17%) |
| Infiltration 0 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | - |
| Infiltration 1 | 13 (93%) | 3 (18%) | 5 (12%) | 8 (11%) | - |
| Infiltration 2 | 1 (7%) | 7 (41%) | 16 (37%) | 24 (32%) | - |
| Infiltration 3 | 0 (0) | 7 (41%) | 23 (53%) | 30 (41%) | - |
| Neutrophil infiltration | 7 (50%) | 9 (53%) | 43 (100%) | 59 (80%) | 47 (82%) |
| Monocyte infiltration | 8 (57%) | 16 (94%) | 28 (65%) | 52 (70%) | - |
| Malgun cells | 2 (14%) | 3 (18%) | 22 (51%) | 72 (42%) | - |
| MALT-like follicles | 1 (7%) | 7 (41%) | 8 (19%) | 16 (22%) | - |
| Bacteria | 14 (100%) | 17 (100%) | 40 (93%) | 71 (96%) | 45 (79%) |
| Microabscesses | - | - | - | - | 51 (89%) |
| Pseudogoblet cells | - | - | - | - | 5 (9%) |
| Hyperplasia | - | - | - | - | 49 (86%) |
| Plasmocytes | - | - | - | - | 38 (67%) |

MALT, mucosa-associated lymphoid tissue.
[a] $n$ = numbers of patients with a given histopathological alteration found in 14, 17, and 43 out of 74 patients according to the updated Sydney classification for the stomach (antrum).
[b] $n$ = numbers of patients with a given histopathological alteration found in 57 esophageal biopsies.

**Table 4**
Analysis of significant correlations between different histopathological variables in intact and deleted single *cag* genotype profiles from the same patient

| Comparison among the different variables in the gastroesophageal mucosa | | Correlation significance analysis by Spearman's rank | |
|---|---|---|---|
| | | *r* | *p*-Value |
| Intact single *cag* genotype | *Stomach* | | |
| | Dysplasia and presence of chronic atrophic gastritis | 0.6 | 0.018[a] |
| | Neutrophil infiltration and chronic atrophic gastritis | 0.577 | 0.024[a] |
| | Malgun cells and chronic atrophic gastritis | 0.577 | 0.024[a] |
| | *Esophagus* | | |
| | Neutrophil infiltration and dysplasia | 0.681 | 0.005[b] |
| | Neutrophil infiltration and microabscesses | 0.681 | 0.005[b] |
| | Pseudogoblet cells and metaplasia | 0.65 | 0.009[b] |
| | Microabscesses and bacteria | 0.65 | 0.009[b] |
| Deleted single *cag* genotype | *Stomach* | | |
| | Malgun cells and infiltration 2 | 0.745 | 0.034[a] |
| | *Esophagus* | | |
| | Plasmocytes and microabscesses | 1 | 0.0001[b] |
| | Neutrophil infiltration and bacteria | 1 | 0.0001[b] |

[a] $p < 0.05$.
[b] $p < 0.01$.

histopathological changes suggest that the occurrence of *H. pylori* in the esophageal mucosa might be linked to gastric acidity and/or reflux.[10,19] The relationship between *H. pylori* and reflux-related disorders is controversial. Some studies have shown that reflux diseases in *cag*A-positive *H. pylori* patients tend to be less severe than in *H. pylori*-negative ones;[20] however, others have reported that *H. pylori*-positive patients with non-atrophic and atrophic gastritis have an increased risk of esophageal adenocarcinoma and esophageal squamous cell carcinoma.[20] Our results demonstrate an association between *H. pylori* and histopathological damage in the esophagus.

*H. pylori* pathogenicity could be related not only to the presence of the *cag*A gene, but also to other *cag*-PAI genes, which may have pathogenic potential due to their critical functions. Indeed, *vir*B11 participates in the assembling of the *cag*-PAI as a complex and constitutes the minimum core structure necessary for type IV transporter biogenesis.[5] *cag*E is involved in the expression of interleukin-8 in the gastric epithelial cells.[21] Studies in a single gastric biopsy and in a single-colony *H. pylori* isolated from the same patient have shown different *cag* genotypes.[6,7]

We found frequencies in the stomach for an intact *cag*-PAI of 57.5%, partial of 21.3%, and deleted of 21.3%, whereas in the esophagus these values were 33.8%, 33.8%, and 32.5%, respectively. These results indicate that the intact *cag*-PAI is highly prevalent in the stomach, while in the esophagus, intact or partial *cag*-PAI had the same frequency of *cag*A-positive and negative strains. This suggests a heterogeneous distribution in the esophagus, where *H. pylori* strains may coexist in similar proportions with different *cag* genotypes.

There is a remarkable genetic diversity among single- or pooled-colony isolates and gastric biopsies, demonstrating genotypic variation among strains and variations in *H. pylori* populations within a single human host.[6,7,22,23] This is in accordance with the hypothesis that an intact *cag*-PAI in the stomach confers a selective advantage to these *H. pylori* strains to colonize the highly acidic gastric environment (pH 2.0). However, in the less acid environment of the esophagus (pH 6.0), *cag*A-positive may not have a selective advantage over the other strains, allowing the colonization of strains with partial or deleted *cag*-PAI.[6]

When we assessed the distribution of *cag* genotype profiles in the gastric and esophageal mucosae on a per-patient basis, it was found that half of the individuals carried a single *cag* genotype, whereas the other half carried a mixed *cag* genotype in both mucosae (Table 2). In the single *cag* genotype profile, the intact and the deleted *cag*-PAI were the most prevalent (60.0% and 32.5%, respectively). The presence of *H. pylori* in both mucosae with the same strain type suggests that the esophageal epithelium may have been modified by changing its microenvironmental conditions, making it similar to the stomach and allowing its colonization by this bacterium.

In the mixed *cag* genotype profile, *cag*A varied from 57.5% in the stomach to 15.0% in the esophagus, while the *cag*E and *vir*B11 genes showed similar frequencies between them by mucosa, with significant positive correlations. These correlations were higher in the stomach than in the esophagus. These results suggest that both genes are highly conserved within the *cag*-PAI, which is in accordance with the fact that we showed an intact *cag*-PAI in 57.5% (46 of 80 patients) with virulent genotypes in the stomach and 33.8% (27 of 80 patients) in the esophagus. The prevalence of *cag*E and *vir*B11 in the gastric mucosa is in accordance with previous reports in dyspeptic patients with duodenal ulcer, and non-atrophic or atrophic gastritis as being important factors for the *H. pylori* pathogenesis.[6,7,24,25] These authors found that *cag*E was present in 73.7–96.6%[6,7,24,25] of the cases, while *vir*B11 was present in 90.0–94.7%.[6,7] In this study we show the occurrence of *cag*E and *vir*B11 in the esophageal mucosa for the first time, indicating that these genes can also be used as markers of *cag*-PAI in the esophagus, as proposed by some authors for the *cag* island in the stomach.[6,7,25] The composition of the *cag* island in the mixed genotype profile, within the same individuals, in the stomach was 55.0%, 35.0%, and 10.0% for intact, partial, and deleted respectively, while in the esophagus the prevalences were 7.5%, 60%, and 32.5%. The variation in *cag*-PAI composition in the esophagus may be due to adaptations of *H. pylori* to different microenvironmental conditions than in the stomach (i.e., bacterial growth, host immune response, and pH susceptibility), allowing growth without selective advantage among strains, which could explain its heterogeneity.

The frequency of the *cag*A gene was higher in the stomach (47/80 patients; 58.8%) than in the esophagus (30/80 patients; 37.5%), regardless of the single and mixed *cag* genotype profile found (Table 2). This difference in the prevalences indicates that *cag* genotypes possess a higher virulence in the stomach in relation to less virulent genotypes in the esophagus. In Venezuela, as in occidental countries, *H. pylori* strains carrying Western type CagA with EPIYA-C variants (ABC, ABCC, or ABCCC) have been described.[26–29] The number of these variants significantly increases the risk of developing severe gastritis and gastric carcinoma. We found a stronger inflammation pattern in the stomach of patients with chronic atrophic gastritis accompanied

by dysplasia and/or metaplasia, malgun cells, and bacteria, as reported previously.[14] The different histopathological correlations were higher in intact and partial *cag*-PAI genotypes compared with a deleted *cag* island in the stomach. This demonstrates that the presence of virulence-associated genotypes is necessary for the development of histopathological damage in the stomach. Our results are in accordance with those of other studies associating the presence of an intact *cag*-PAI with the development of severe gastrointestinal damage, including gastric diseases and cancer.[4,6,23,30]

To understand whether a relationship exists between single and mixed *cag* genotypes and histopathological outcomes in both mucosae, we assessed their correlations and found that the presence of an intact single *cag*-PAI genotype was significantly associated with more severe damage in the gastric mucosa, as has been observed in *vac*A s1m1/*cag*A genotypes in severe atrophic gastritis.[9] Dysplasia, microabscesses with neutrophil infiltration, pseudogoblet cells and metaplasia, and microabscesses and bacteria were found in the esophagus (Table 4). These results indicate that a single *cag*-PAI genotype in both mucosae confers an increased risk of developing histological damage.

In conclusion, our PCR-based study showed heterogeneity of *cag* genotypes of *H. pylori* detected in the esophagus from the population and on a per-patient basis. *H. pylori* may coexist in similar proportions without dominance of one *cag* genotype. The *cag*E and *vir*B11 genes can be used as markers of *cag*-PAI in the esophagus. A detailed genetic analysis of different colonies from a single patient is required to establish the colonization by single or multiple *H. pylori* strains in the esophagus.

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## References

1. Atherton JC, Blaser MJ. Coadaptation of *Helicobacter pylori* and humans: ancient history, modern implications. *J Clin Invest* 2009;**119**:2475–87.
2. Blaser MJ, Atherton JC. *Helicobacter pylori* persistence: biology and disease. *J Clin Invest* 2004;**113**:321–3.
3. Frenck Jr RW, Clemens J. Helicobacter in the developing world. *Microbes Infect* 2003;**5**:705–13.
4. Lima VP, de Lima MA, Ferreira MV, Barros MA, Rabenhorst SH. The relationship between *Helicobacter pylori* genes *cag*E and *vir*B11 and gastric cancer. *Int J Infect Dis* 2010;**14**:e613–7.
5. Covacci A, Telford JL, Del Giudice G, Parsonnet J, Rappuoli R. *Helicobacter pylori* virulence and genetic geography. *Science* 1999;**284**:1328–33.
6. Sozzi M, Tomasini ML, Vindigni C, Zanussi S, Tedeschi R, Basaglia G, et al. Heterogeneity of *cag* genotypes and clinical outcome of *Helicobacter pylori* infection. *J Lab Clin Med* 2005;**146**:262–70.
7. Tomasini ML, Zanussi S, Sozzi M, Tedeschi R, Basaglia G, De Paoli P. Heterogeneity of *cag* genotypes in *Helicobacter pylori* isolates from human biopsy specimens. *J Clin Microbiol* 2003;**41**:976–80.
8. Ghose C, Perez-Perez GI, van Doorn LJ, Domínguez-Bello MG, Blaser MJ. High frequency of gastric colonization with multiple *Helicobacter pylori* strains in Venezuelan subjects. *J Clin Microbiol* 2005;**43**:2635–41.
9. Chiurillo MA, Moran Y, Cañas M, Valderrama E, Granda N, Sayegh M, et al. Genotyping of *Helicobacter pylori* virulence-associated genes shows high diversity of strains infecting patients in western Venezuela. *Int J Infect Dis* 2013;**17**:e750–6.
10. Pei Z, Bini EJ, Yang L, Zhou M, Francois F, Blaser MJ. Bacterial biota in the human distal esophagus. *Proc Natl Acad Sci U S A* 2004;**101**:4250–5.
11. Gutierrez O, Akamatsu T, Cardona H, Graham DY, El-Zimaity HM. *Helicobacter pylori* and heterotopic gastric mucosa in the upper esophagus (the inlet patch). *Am J Gastroenterol* 2003;**98**:1266–70.
12. Weiss J, Tsang TK, Meng X, Zhang H, Kilner E, Wang E, et al. Detection of *Helicobacter pylori* gastritis by PCR: correlation with inflammation scores and immunohistochemical and CLOtest findings. *Am J Clin Pathol* 2008;**129**:89–96.
13. Pei Z, Yang L, Peek RM, Levine Jr SM, Pride DT, Blaser MJ. Bacterial biota in reflux esophagitis and Barrett's esophagus. *World J Gastroenterol* 2005;**11**:7277–83.
14. Contreras M, Salazar V, García-Amado MA, Reyes N, Aparcero M, Silva O, et al. High frequency of *Helicobacter pylori* strains in the esophageal mucosa of dyspeptic patients and its possible association with histopathological alterations. *Int J Infect Dis* 2012;**16**:e364–70.
15. Germani Y, Dauga C, Duval P, Huerre M, Levy M, Pialoux G, et al. Strategy for the detection of *Helicobacter* species by amplification of 16S rRNA genes and identification of *H. felis* in a human gastric biopsy. *Res Microbiol* 1997;**148**:315–26.
16. Kansau I, Raymond J, Bingen E, Courcoux P, Kalach N, Bergeret M, et al. Genotyping of *Helicobacter pylori* isolates by sequencing of PCR products and comparison with the RAPD technique. *Res Microbiol* 1996;**147**:661–9.
17. Peek Jr RM, Miller GG, Tham KT, Pérez-Pérez GI, Cover TL, Atherton JC, et al. Detection of *Helicobacter pylori* gene expression in human gastric mucosa. *J Clin Microbiol* 1995;**33**:28–32.
18. Rugge M, Busatto G, Cassaro M, Shiao YH, Russo V, Leandro G, et al. Patients younger than 40 years with gastric carcinoma: *Helicobacter pylori* genotype and associated gastritis phenotype. *Cancer* 1999;**85**:2506–11.
19. Herbella FA, Patti MG. Gastroesophageal reflux disease: from pathophysiology to treatment. *World J Gastroenterol* 2010;**16**:3745–9.
20. McColl KE. *Helicobacter pylori* and oesophageal cancer—not always protective. *Gut* 2007;**56**:457–9.
21. Audibert C, Burucoa C, Janvier B, Fauchère JL. Implication of the structure of the *Helicobacter pylori cag* pathogenicity island in induction of interleukin-8 secretion. *Infect Immun* 2001;**69**:1625–9.
22. Israel DA, Salama N, Krishna U, Rieger UM, Atherton JC, Falkow S, et al. *Helicobacter pylori* genetic diversity within the gastric niche of a single human host. *Proc Natl Acad Sci U S A* 2001;**98**:14625–30.
23. Blaser MJ. Heterogeneity of *Helicobacter pylori*. *Eur J Gastroenterol Hepatol* 2012;**9**:S3–7.
24. Chiurillo MA, Moran YH, Cañas M, Valderrama EJ, Armanie E. Infection with specific *Helicobacter pylori-cag* pathogenicity island strains is associated with interleukin-1B gene polymorphisms in Venezuelan chronic gastritis patients. *Dig Dis Sci* 2011;**56**:449–56.
25. Ikenoue T, Maeda S, Ogura K, Akanuma M, Mitsuno Y, Imai Y, et al. Determination of *Helicobacter pylori* virulence by simple gene analysis of the *cag* pathogenicity island. *Clin Diagn Lab Immunol* 2001;**8**:181–6.
26. Xia Y, Yamaoka Y, Zhu Q, Matha I, Gao X. A comprehensive sequence and disease correlation analysis for the C-terminal region of CagA protein of *Helicobacter pylori*. *PLoS One* 2009;**4**:e7736.
27. Higashi H, Tsutsumi R, Fujita A, Yamazaki S, Asaka M, Azuma T, et al. Biological activity of the *Helicobacter pylori* virulence factor CagA is determined by variation in the tyrosine phosphorylation sites. *Proc Natl Acad Sci U S A* 2002;**99**:14428–33.
28. Argent RH, Hale JL, El-Omar EM, Atherton JC. Differences in *Helicobacter pylori* CagA tyrosine phosphorylation motif patterns between Western and East Asian strains, and influences on interleukin-8 secretion. *J Med Microbiol* 2008;**57**:1062–7.
29. Torres Izarra KE, Moran Borges YH, Valderrama Rios EJ, Chiurillo Siervo MA. Variants of the EPIYA motif of *Helicobacter pylori* CagA protein in gastric biopsies from patients with chronic gastritis from the center-occidental region of Venezuela. *Rev Soc Ven Microbiol* 2013;**33**:18–23.
30. Nilsson C, Sillén A, Eriksson L, Strand ML, Enroth H, Normark S, et al. Correlation between *cag* pathogenicity island composition and *Helicobacter pylori*-associated gastroduodenal disease. *Infect Immun* 2003;**71**:6573–81.