Helicobacter pylori Genotypes May Determine Gastric Histopathology

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The outcome of *Helicobacter pylori* infection has been associated with specific virulence-associated bacterial genotypes. The present study aimed to investigate the gastric histopathology in Portuguese and Colombian patients infected with *H. pylori* and to assess its relationship with bacterial virulence-associated *vacA*, *cagA*, and *iceA* genotypes. A total of 370 patients from Portugal ($n = 192$) and Colombia ($n = 178$) were studied. Corpus and antrum biopsy specimens were collected from each individual. Histopathological features were recorded and graded according to the updated Sydney system. *H. pylori vacA*, *cagA*, and *iceA* genes were directly genotyped in the gastric biopsy specimens by polymerase chain reaction and reverse hybridization. Despite the significant differences between the Portuguese and Colombian patient groups, highly similar results were observed with respect to the relation between *H. pylori* genotypes and histopathology. *H. pylori vacA* s1, *vacA* m1, *cagA* + genotypes were significantly associated with a higher *H. pylori* density, higher degrees of lymphocytic and neutrophilic infiltrates, atrophy, the type of intestinal metaplasia, and presence of epithelial damage. The *iceA1* genotype was only associated with epithelial damage in Portuguese patients. These findings show that distinct *H. pylori* genotypes are strongly associated with histopathological findings in the stomach, confirming their relevance for the development of *H. pylori*-associ-

ated gastric pathology. (Am J Pathol 2001; 158:647–654)

Helicobacter pylori colonizes the human stomach and establishes a long-term infection of the gastric mucosa.¹ The persistent colonization induces gastritis and is associated with the development of peptic ulcer disease, atrophic gastritis, and gastric carcinoma.² In general, the prevalence of *H. pylori*-related diseases is not directly proportional to the prevalence of *H. pylori* colonization. This may be because of several factors, such as genetic diversity among humans,³ environmental factors, such as diet^{4–6} age at first infection, and the genetic variability of *H. pylori*. Despite the high degree of genetic heterogeneity among *H. pylori* isolates,⁷ there is evidence for the existence of distinct genetic lineages,⁸ that may play a role in its pathogenicity. The relevance of several specific *H. pylori* genes has been studied in the past. *vacA* encodes a vacuolating cytotoxin which is excreted by *H. pylori* and damages epithelial cells.^{9,10} The gene is present in all strains, and comprises two variable parts.¹¹ The s region (encoding the signal peptide) is located at the 5' end of the gene and exists as a s1 or s2 allele. Within type s1 several subtypes (s1a, s1b, and s1c) can be distinguished.¹² The m-region (middle) occurs as a m1 or m2 allele. The mosaic combination of s- and m-region allelic types determines the production of the cytotoxin and is associated with pathogenicity of the bacterium.¹¹ *vacA* m1 type strains have been associated with greater gastric epithelial damage than m2 strains.¹³ *cagA* (cytotoxin-associated gene) is considered as a marker for the presence of the pathogenicity (*cag*) island of ~35 kbp.¹⁴ Infection with *cagA* + strains increases the risk for the development of atrophic gastritis and gastric cancer.^{15,16} Recently, a novel gene has been discovered,

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designated *iceA* (induced by contact with epithelium). There are two main allelic variants of the gene, ie, *iceA1* and *iceA2*.¹⁷ The function of *iceA1* is not yet clear, but there is significant homology to a type II restriction endonuclease. The expression of *iceA1* is up-regulated on contact between *H. pylori* and human epithelial cells and is possibly associated with peptic ulcer disease,^{17,18} although other studies failed to confirm these initial results¹⁹ or found a reverse relationship.²⁰ The present study aimed to investigate the gastric histopathology in Portuguese and Colombian patients infected with *H. pylori* and to assess its relationship with bacterial virulence-associated *vacA*, *cagA*, and *iceA* genotypes.

Materials and Methods

Patients

A total of 370 *H. pylori*-infected patients from Portugal and Colombia were studied. Portuguese patients ($n = 192$, 173 males and 19 females; age 43.3 ± 6.9 years) were recruited from shipyard workers during a screening program for (pre)malignant lesions of the gastric mucosa. Patients provided informed consent and underwent standard gastroscopy in 1998 in Hospital de S. João (Porto, Portugal). None of the patients had gastric cancer, 21 (11.0%) had duodenal ulcer, and eight (4.2%) had a gastric ulcer. Biopsy specimens were taken from corpus and antrum.

Colombian patients ($n = 178$, 73 males and 105 females; age 55.9 ± 8.4 years) were from the Andean region of Nariño, a part of Colombia with an extremely high prevalence of gastric cancer. All patients were participants of a randomized 6-year chemoprevention trial of gastric dysplasia performed in the towns of Tuquerres and Pasto, Nariño. Only patients with atrophy were selected for this trial, which followed a factorial design comprising anti-*H. pylori* therapy, as well as vitamin C and β -carotene treatment. One of the patients had peptic ulcer disease and none had gastric cancer. Biopsy specimens were obtained in 1998 by gastroscopy from the corpus and antrum of the stomach.

Histopathology

Two gastric biopsy specimens from the antrum (one from the greater curvature and one from the incisura angularis) and one from the corpus were immersed in 10% formalin and embedded in paraffin. Sections were stained by hematoxylin and eosin, Alcian blue-periodic acid Schiff, and modified Giemsa. Only cases with adequately sized biopsy specimens of both antral and corpus mucosa were accepted for histological assessment by experienced pathologists, who were blinded with respect to the clinical information of each patient.

The following histopathological parameters were scored on an ordinal scale (0 to 3) using the criteria as described in the updated Sydney classification system²¹: *H. pylori* density, chronic inflammation, polymorphonuclear activity (neutrophil activity), and glandular atrophy.

Whenever present, intestinal metaplasia was typed as complete, mixed (complete and incomplete), or incomplete. Surface epithelial degeneration was scored as present or absent. Results from the two antrum biopsy specimens were combined and whenever differences were observed, the highest score was considered for statistical analyses.

DNA Isolation from Gastric Biopsies and *vacA*, *cagA*, and *iceA* Genotyping

Antral biopsies were obtained, immediately placed in liquid nitrogen, and then transferred in dry ice to -80°C freezers. Total DNA was extracted from gastric biopsy specimens using the method described by Boom and colleagues.²² Briefly, biopsies were homogenized in guanidinium isothiocyanate, using a sterile micropestle (Merck). DNA was captured onto silica particles, washed, and eluted in 100 μl of 10 mmol/L Tris-HCl, pH 8.3. Two μl was used for each polymerase chain reaction (PCR). Subsequently, *vacA* and *cagA* genotypes were determined by multiplex PCRs followed by reverse hybridization on a line probe assay (LiPA) as described earlier^{12,23} For amplification of the *iceA1* and *iceA2* genes, allele-specific PCR reactions were performed as described^{18,24}

Statistical Analyses

Data were analyzed in SPSS version 8.0. The Pearson chi-square test and Fisher's exact test were used to assess the relationships between individual genotypes and the presence of epithelial damage, atrophy, and intestinal metaplasia. Only cases containing single genotypes were included, and cases containing multiple genotypes were excluded. The *t*-test was used to analyze the age distributions between the different patient groups. Histopathological parameters, scored on ordinal scales (from 0 to 3), were analyzed by the Mann-Whitney test. Logistic regression analysis was used to relate the *vacA*, *cagA*, and *iceA* genotypes of *H. pylori*. Age (in 5-year classes) and gender were included as possible confounders. Selection of variables was based on a likelihood ratio test, using a significance level of 0.05 for inclusion or elimination.

Results

A total of 370 patients from Portugal and Colombia were endoscoped and gastric biopsy specimens were obtained from corpus and antrum. Comparison of the Portuguese and Colombian patients (Table 1) revealed several differences between the two groups. The mean age of the Colombian patients was significantly higher than that of the Portuguese patients ($P < 0.001$). Also, the gender distribution was different, because 90.1% of the Portuguese patients were male, versus only 40.4% of the Colombian patients ($P < 0.001$).

Histopathological parameters were scored according to the updated Sydney classification system in biopsy

Table 1. Characteristics of the Portuguese and Colombian Patients Participating in this Study

	Portugal n = 192	Colombia n = 178
Age (years)		
Mean	43.3 ± 6.9	55.9 ± 8.4
Range	24–62	37–74
Gender (M/F)	173/19	72/106
<i>vacA</i> genotype		
s1a/m1	3 (1.6%)	7 (3.9%)
s1a/m2a	1 (0.5%)	0 (0%)
s1b/m1	60 (31.3%)	145 (81.5%)
s1b/m2a	12 (6.2%)	5 (2.8%)
s2/m2a	57 (29.7%)	13 (7.3%)
Multiple*	59 (30.7%)	8 (4.5%)
<i>cagA</i> genotype		
Positive	116 (60.4%)	161 (90.4%)
Negative	76 (39.6%)	17 (9.6%)
<i>iceA</i> genotype		
A1	54 (28.1%)	55 (30.9%)
A2	68 (35.4%)	77 (43.3%)
Multiple†	63 (32.8%)	39 (21.9%)
Untyped‡	7 (3.7%)	7 (3.9%)

*More than one *vacA* genotype detected.
†More than one *iceA* genotype detected.
‡The *iceA* genotype could not be determined.

specimens from antrum and corpus and the grades of various parameters were determined using visual analogue scales.²¹ Genotypes were only determined in the antral biopsy specimens and not in the corpus biopsies.

The *vacA*, *cagA*, and *iceA* status was analyzed for all 370 *H. pylori*-infected patients (Table 1). The prevalence of *vacA* subtypes was different between Colombian and Portuguese patients. Subtype s1b was less prevalent in Portuguese (37.5%) as compared to Colombian patients (84.3%). Conversely, type s2 was more often found in Portuguese patients (29.7%) than in Colombian patients (7.3%). Type m1 seemed to be more prevalent in Colombian patients (85.4%) than in Portuguese cases (32.9%). Similarly, the *cagA*-positivity rate was higher among Colombian patients (90.4%) than among Portuguese patients (60.4%). The prevalence of the different *iceA* genotypes seemed to be similar among the two patient groups.

The prevalence of cases containing multiple *vacA* or *iceA* genotypes was much higher among the Portuguese patients than among Colombian cases ($P < 0.001$). Multiple *vacA* as well as *iceA* genotypes were found in 30 (15.6%) of the 192 Portuguese cases, whereas of the Colombian patients, only three (1.6%) of the 178 cases contained multiple genotypes for *vacA* as well as *iceA* ($P < 0.001$).

H. pylori Genotypes and the Density of *H. pylori* Colonization

The density of *H. pylori* was scored in antral and corpus biopsy specimens and the results are shown in Figure 1. In Portuguese patients, the average density scores in the antrum were significantly higher than in the corpus ($P < 0.001$). There also was a significant relationship between the density of *H. pylori* colonization in the corpus and *vacA*

Figure 1. Density of *H. pylori* colonization in Portuguese and in Colombian patients, according to the updated Sydney system. White = absent, light gray = rare, dark gray = moderate, black = abundant. Figures at the **left** show results for antral biopsy specimens, figures at the **right** show results for corpus biopsy specimens. A single **asterisk** above a pair of bars marks a significant difference at $P < 0.05$; **double asterisks** above a pair of bars marks a significant difference at $P < 0.01$ (Mann-Whitney test). Only cases containing a single genotype were included in the statistical analysis. Cases containing multiple genotypes were excluded.

s1 ($P = 0.003$), *cagA*+ ($P = 0.008$), and *iceA*1 ($P = 0.048$) genotypes, but not with the *vacA* m type. No significant relationships were observed between *vacA* s or m region, *cagA* status, or *iceA* genotypes and the density of *H. pylori* in the antrum biopsy specimens. In a total of seven (3.6%) antral biopsies no bacteria were observed (density = 0), whereas they were found positive by PCR.

Among Colombian patients the average *H. pylori* density scores in the antrum were lower than in the corpus ($P = 0.019$). The *H. pylori* density in the antrum was associated with the *vacA* s1 ($P = 0.006$) and m1 ($P = 0.001$) genotypes, but not with the *cagA* or *iceA* status. There were no significant relationships between genotypes and *H. pylori* density in the corpus. In 26 (14.6%) of the antral biopsies no bacteria were observed (density = 0), whereas they were found positive by PCR.

H. pylori Genotypes and Chronic Inflammation

Among the Portuguese patients (Figure 2A), there was a significant association between *vacA* s1, m1, and *cagA*+ genotypes and higher inflammation scores in both corpus and antrum biopsy specimens. The *iceA* status was only associated to the degree of inflammation in the corpus. Among Colombian patients (Figure 3A), *vacA* s1, m1, and *cagA*+ genotypes are associated to higher inflammation scores in the corpus, but not in the antrum. The *iceA* genotype was not associated to the degree of inflammation.

H. pylori Genotypes and Neutrophil Activity

Neutrophilic activity was investigated and results are shown in Figures 2B and 3B. In both Portuguese and Colombian patients, *vacA* s1, *vacA* m1, and *cagA*+ ge-

Portugal

Figure 2. Histological parameters determined in gastric biopsy specimens from Portuguese patients, according to the updated Sydney system. Patterns for lymphocytic infiltrates (A), neutrophil activity (B), and glandular atrophy (C) are as follows: white = absent, light gray = mild, dark gray = moderate, black = severe. Statistical analyses were performed using the Mann-Whitney test. **Asterisks** are identical to those in Figure 1.

notypes were strongly associated with higher activity in the corpus and in the antrum. The *iceA* status was not significantly associated with the neutrophil infiltration scores.

H. pylori Genotypes and Glandular Atrophy

Overall, the prevalence of atrophy among the Portuguese patients was lower than among Colombian cases. It should be taken into account that the Colombian patients were selected for presence of atrophy at baseline. Therefore, the association between presence of atrophy and *H. pylori* genotypes could not be assessed in Colombian patients. Nevertheless, the association between the degree of atrophy and *H. pylori* genotypes could still be analyzed.

Among the Portuguese patients, the ages were not normally distributed, and patients with atrophic gastritis (45.8 ± 6.8 years) were significantly older than patients with nonatrophic gastritis (42.0 ± 6.6 years) ($P < 0.001$). There was a strong association between the presence of glandular atrophy in the antrum and the individual *vacA* s1, m1, and *cagA* + genotypes, as compared to the *vacA* s2, m2, and *cagA* - genotypes, respectively, but not with the *iceA* genotype. The odds ratio of *vacA* s1 versus s2, *vacA* m1 versus m2, and *cagA* + versus *cagA* - strains for glandular atrophy were 32.21 (95% CI, 7.3 to 141.7),

Colombia

Figure 3. Histological parameters determined in gastric biopsy specimens from Colombian patients, according to the updated Sydney system. Patterns are identical to those in Figure 2 and **asterisks** are identical to those in Figure 1.

15.18 (95% CI, 5.7 to 40.3), and 13.12 (95% CI, 4.7 to 36.6), respectively.

For both patient groups, the associations between the *H. pylori* genotypes and the degree of atrophy were further analyzed, and results are shown in Figures 2C and 3C. In both Portuguese and Colombian patients, *vacA* s1, *vacA* m1, *cagA* + (but not the *iceA*) genotypes were each strongly correlated with the degree of glandular atrophy in the antrum. In the corpus of the Portuguese patients atrophy was found only in five cases, thus not permitting reliable analyses. In the corpus of Colombian patients, only an association between glandular atrophy and the *cagA* genotype was found.

H. pylori Genotypes and Intestinal Metaplasia

Firstly, a highly significant association was observed between the presence of intestinal metaplasia in the antrum and *H. pylori* genotypes. The prevalence of *vacA* s1, m1, or *cagA* + strains was much higher among patients with intestinal metaplasia than in cases without intestinal metaplasia. Among Portuguese patients, the odds ratios for intestinal metaplasia in the antrum of *vacA* s1 versus s2, *vacA* m1 versus m2, and *cagA* + versus *cagA* - strains were 16.2 (95% CI, 4.7 to 56.4), 9.3 (95% CI, 3.7 to 23.4), and 8.1 (95% CI, 3.1 to 21.2), respectively. In the corpus, intestinal metaplasia was observed in only a small number of patients. Among Colombian patients, the presence of metaplasia in the antrum was only significantly associated with the *vacA* s genotype with and odds ratio of 3.5

Figure 4. Type of intestinal metaplasia in Portuguese and in Colombian patients. White = absent, light gray = complete, dark gray = mixed, black = incomplete. Statistical analyses were performed using the Mann-Whitney test. Asterisks are identical to those in Figure 1.

(95% CI, 1.0 to 12.0). Presence of metaplasia in the corpus was not associated to any of the genotypes.

Secondly, the association between *H. pylori* genotypes and the type of intestinal metaplasia is shown in Figure 4. There was a strong association between the *vacA* s1, m1, and *cagA* + genotypes and the type of metaplasia in the antrum of the Portuguese patients. In Colombian patients, a similar trend was observed in the antrum, but this failed to reach statistical significance. In the corpus biopsy specimens, there were no significant associations observed, except for the *vacA* m type in Portuguese patients, although it should be noted that there were only five Portuguese patients with intestinal metaplasia in the corpus.

H. pylori Genotypes and Epithelial Damage

The presence or absence of epithelial damage was assessed and results are shown in Figure 5. Presence of *vacA* s1, *vacA* m1, and *cagA* + genotypes are strongly

Figure 5. Epithelial damage in Portuguese and in Colombian patients. White = absent, light gray = present. Statistical analyses were performed using the chi-square test. Asterisks are identical to those in Figure 1.

associated with the presence of epithelial damage in the corpus of both Portuguese and Colombian patients and in the antrum of the Portuguese patients. The *iceA* status was significantly associated with the presence of epithelial damage in the antrum and corpus of the Portuguese patients.

The association between the presence or absence of atrophic gastritis or intestinal metaplasia and the *H. pylori* *vacA* (s and m regions), *cagA*, or *iceA* genotypes, was analyzed by means of logistic regression in Portuguese patients. Because the *cagA*, *vacA*, and *iceA* genotypes were strongly associated, each was analyzed individually in separate regression models, correcting for age and gender as possible confounders. Presence of atrophy was associated with *vacA* s1 (OR, 46.9; 95% CI, 8.6 to 256.4), *vacA* m1 (OR, 10.1; 95% CI, 4.3 to 23.7), and *cagA* (OR, 11.2; 95% CI, 4.5 to 27.8), but not with the *iceA* genotype. Similarly, presence of intestinal metaplasia was associated with *vacA* s1 (OR, 14.9; 95% CI, 4.2 to 53.2), *vacA* m1 (OR, 6.8; 95% CI, 2.9 to 15.9), and *cagA* (OR, 4.8; 95% CI, 2.1 to 10.6), but not with the *iceA* genotype.

Separate analysis of a subgroup of 77 *cagA*-negative Portuguese cases revealed that in these patients, *vacA* s1 genotypes are significantly associated with the presence of atrophy ($P = 0.008$), the degree of atrophy ($P < 0.001$), presence of intestinal metaplasia ($P = 0.016$), the type of intestinal metaplasia ($P < 0.001$), and epithelial damage ($P = 0.024$) in the antrum. Similarly, the *vacA* m1 strains are associated with presence of atrophy ($P = 0.003$), the degree of atrophy ($P < 0.001$), the presence of intestinal metaplasia ($P = 0.006$), and the type of intestinal metaplasia ($P = 0.001$) in the antrum, but not with the presence of epithelial damage.

Discussion

H. pylori infection causes chronic gastritis and is associated with the development of peptic ulcer disease, gastric carcinoma, and MALT lymphoma.^{2,25,26} Although *H. pylori* infection causes chronic gastritis in virtually all patients, not all individuals develop more severe gastric pathology. Thus, there is a marked discrepancy between the number of individuals colonized and those with clinical symptoms. Although host and environmental factors are considered important,³ there is also evidence for a role of specific *H. pylori* virulence-associated genotypes. Patients infected with less virulent genotypes are more likely to have mild gastritis throughout their entire life, whereas patients infected with more virulent genotypes have a higher probability of developing peptic ulcer disease, atrophic gastritis, and eventually gastric carcinoma. Gastric carcinogenesis is a complex, multifactorial process, in which persistent *H. pylori* infection can play an important role.²⁶ Prolonged infection may lead to the loss of glands, resulting in multifocal atrophic gastritis, which can be followed by the development of intestinal metaplasia and dysplasia.⁵ As compared to the general population, patients with duodenal ulcer disease may have a reduced risk for the development of gastric cancer.²⁷

This finding, as well as other epidemiological observations,²⁸ suggest the existence of at least two distinct pathways, one resulting in duodenal ulcer disease, and the other associated with gastric ulcer disease, atrophic gastritis, and possibly gastric carcinoma. Atrophy is the first recognizable step in the multifocal atrophic gastritis complex that in more advanced stages may lead to intestinal metaplasia and dysplasia. *vacA* s- and m-region mosaicism can play an important role in such a chain of events. Several studies have consistently shown that the *vacA* s genotype is associated with disease in patients from various countries^{11,30–33} although others did not confirm these findings.¹⁹ The *vacA* m region genotype was not significantly associated with peptic ulcer disease.¹³ However, the present study revealed the strong significance of the *vacA* m1 genotype with respect to epithelial damage, neutrophilic and lymphocytic infiltrates, atrophic gastritis, and intestinal metaplasia in patients from two geographically distinct regions. Atherton and colleagues³⁴ also presented evidence that m1 strains were associated with greater gastric epithelial damage than m2 strains in North American patients. Increased epithelial damage may be explained by a higher level of cytotoxin production by *vacA* s1/m1 strains as compared to *vacA* s1/m2 strains.¹¹ Earlier studies³⁵ also revealed a significant relationship between the level of cytotoxin production and the severity of atrophy, confirming that the cytotoxin is a disease-associated factor. Because the production of cytotoxin primarily depends on the mosaic structure of the *vacA* gene, especially of the s and m regions,¹¹ the association between *vacA* genotypes and atrophy as presented here, is consistent with these earlier observations. Moreover, the present study showed that *vacA* and *cagA* genotypes not only are related to the presence, but also with the degree of atrophy and the type of intestinal metaplasia, suggesting that these genotypes may play an important role in the development of lesions considered as precursors of gastric carcinoma. Our findings that severe damage to the gastric epithelium is associated with *vacA* s1/m1 mosaicism suggests that such damage represents the primary effect of the cytotoxin¹⁰ and that leukocyte infiltrates and atrophy may be sequential in the cascade that characterizes the precancerous process. Furthermore, the m1 and m2 forms of the *VacA* cytotoxin may recognize different receptors on human gastric epithelial cells.³⁶

The *iceA* genotype, which has been associated with peptic ulcer disease in some populations^{17,18} seems to be of limited use in the patient populations studied here. *iceA1* was significantly associated to the degree of lymphocyte infiltrates and epithelial damage in Portuguese patients, but not with neutrophil activity, atrophy, and intestinal metaplasia. Therefore, the clinical relevance of *iceA* remains to be studied in more detail in patients from different geographic origins.

Several genes of the *cag* pathogenicity island encode proteins that enhance the virulence of the strain, eg, by increasing the production of interleukin-8 by gastric epithelial cells,³⁷ causing infiltration of polymorphonuclear neutrophils, resulting in more severe inflammatory activity of the gastric epithelium. The present study showed that

the great majority of patients have lymphocytic infiltrates in the corpus and antrum but neutrophil activity is found less frequently, and is strongly associated with *vacA*-s1, *vacA*-m1, and *cagA*+ genotypes. Several European and North American studies have shown that infection with *cagA*-positive *H. pylori* strains also increases the risk for atrophic gastritis and gastric cancer.^{15,38} However, several studies in Asian populations did not confirm these relationships,³⁹ indicating that there are important geographic differences. In general, the incidence of *H. pylori*-associated diseases, such as gastric cancer is not uniformly distributed over the globe and does not parallel the prevalence of *H. pylori* infection. The highest incidence of gastric cancer is found in East Asia (Japan and Korea), and in Central America and South America (especially the Andean countries).⁴⁰ Within Europe, the highest incidence is found in Portugal, Austria, Italy, and Spain.⁴¹ In contrast, high prevalence of infection is reported from such areas with low gastric cancer risk, such as Africa,⁴² India, and the coastal regions of Latin America.⁴³

We and others have recently shown that the *vacA* and *cagA* genotypes of *H. pylori* are not equally distributed over the world.^{19,20,44–46} There are significant differences between patient populations from Asia, different parts of Europe, and North and South America. Given the geographic distribution of specific *H. pylori* genotypes, it could be speculated that there might be an epidemiological relationship with the incidence of gastric cancer. The present study confirmed earlier findings in Portuguese patients showing that a high proportion are infected with multiple *H. pylori* strains.^{44,47}

With respect to age, gender, distribution of genotypes, as well as the prevalence of atrophy, there were significant differences between the Portuguese and Colombian patients, reflecting the different strategy used for selection of these cases. However, despite these differences, the highly similar relationships between bacterial genotypes and histopathological findings in both populations indicate that our observations are accurate and reliable.

Our study has several limitations. The biopsy specimens from each patient group were analyzed by two different pathologists, although they used the same scoring system.²¹ Also, only biopsies from the antrum of the stomach were used for genotyping analysis in each patient, and the influence of sampling errors cannot be excluded. Therefore, there may not be an absolute concordance between the genotype of the colonizing strain and underlying pathological observations in the corpus, because patients may carry different strains in the antrum and corpus. However, the antrum is the predominant site of *H. pylori* colonization⁴⁸ and the chance of false-negative results is lower than in corpus biopsies.⁴⁹

Furthermore, other possibly relevant factors, such as smoking, acid output, HLA-status, and other bacterial virulence factors were not studied. Despite the limitations of the present study, analysis of the *vacA*, *cagA*, and *iceA* virulence-associated genes directly in gastric biopsies permitted clinically relevant discrimination between *H. pylori* strains.

In conclusion, distinct *H. pylori* genotypes seem to be associated to the outcome of the infection and may have important clinical and epidemiological implications.

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