Lewis Antigen Expression and Other Pathogenic Factors in the Presence of Atrophic Chronic Gastritis in a European Population

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To study the relationship between Helicobacter pylori cagA and vacA status and the expression of Lewis (Le) antigens and between these characteristics and atrophic chronic gastritis (ACG), H. pylori infection was assessed by culture and by histologic and serologic tests, cagA and vacA were assessed by a polymerase chain reaction–based reverse hybridization assay, and bacterial Le expression was assessed by immunoblotting. ACG was any form of antral or fundic atrophy with or without intestinal metaplasia. Of the 215 isolates, 64% were cagA+ and 100% were vacA+ (s1m1, 42%; s1m2, 29%; s2m2, 29%; and s2m1, 0). Le typing of 155 isolates showed that 6 (4%) were Le+, 31 (20%) were Le-, 87 (56%) were Le+, and 31 (20%) were neither Le+ nor Le-. Two main clusters of isolates were identified by multiple correspondence analysis: s1a/m1/cagA+/Le+ or s1a/m1/cagA+/Le+ (n = 44; 29.7%) and s2/m2a/cagA+/Le+ or Le-/+ (n = 29; 19.7%). Among patients with ACG, 54% of their isolates were from cluster s1a/m1/cagA+/Le+ or s1a/m1/cagA+/Le+, which was associated with the presence of ACG (odds ratio, 7.4; 95% confidence interval, 1.5–37.0).

Helicobacter pylori colonizes the stomachs of most people worldwide [1], causing chronic active gastritis [2]. Although most persons remain asymptomatic, H. pylori is implicated in gastric carcinogenesis [3], as well as peptic ulcer disease, more specifically with the development of precancerous lesions (i.e., atrophic chronic gastritis [ACG]) [4].

The development of ACG may depend on the presence of specific virulence factors of the bacterial genotype [5]. The 2 most studied of these factors are the cytotoxin-associated gene (cagA) [6] and the vacuolating cytotoxin–associated gene (vacA) [7]. Their respective roles in pathogenesis have been explored in detail elsewhere [8–13]. The cagA gene is present in all H. pylori isolates [14] and is a marker of the presence of a pathogenicity island [15], which is itself associated with clinical outcomes such as ACG [16, 17]. Conversely, the vacA gene always is present in H. pylori and contains 2 variable parts [7, 18]: the s (signal peptide) region, with s1 or s2 alleles (within s1, thus far, 3 subtypes have been identified: s1a, s1b, and s1c [19]), and the m (middle) region, with m1 or m2 alleles. The different combinations of alleles determine the pathogenicity of the isolate, by means of cytotoxin production [20].

H. pylori has developed survival strategies to colonize its ecologic niche [21, 22]. Some studies have focused on the mode of bacterial adaptation to the environment on the basis of an adaptive relationship to host immune response. In this respect, a mechanism commonly studied relates to how the bacterium mimics host gastric structures [23–29]. The chemical structure of the O-specific chain of lipopolysaccharides (LPSs) of the H. pylori type isolate has been established and was found to mimic Lewis (Le) blood group antigens, Le+ or Le− in particular [30–32]. In other bacterial infections, mimicry of host structures has been proven to contribute to pathogenesis, for example, by inducing the formation of antibodies cross-reacting with host structures and contributing to disease by an autoimmune process [32–34]. In addition, in H. pylori infections, microbial expression of host Le antigens has been suggested to be a mechanism of camouflage to escape elimination by the host immune response [26] or to facilitate adherence of the bacteria to the gastric mucosa [35, 36].

Although it is still difficult to determine the exact role of the presence of Le determinants, this is another factor possibly involved in pathogenicity [29, 32]. Indeed, some researchers have found a relationship between Le antigen expression, cagA status, and alleles of the vacA gene of H. pylori [25], whereas others have not [37]. Therefore, additional information, obtained by an epidemiologic approach, is needed on how the different characteristics of H. pylori isolates aggregate and whether they play a role in the development of ACG.

The aims of this study, based on the analysis of H. pylori isolates from patients at inclusion in the Eurohepygast cohort, were to determine the genotype and expression of Le determinants of...
these isolates, to assess whether this expression was related to cagA and vacA genotypes, to evaluate how these characteristics aggregated among the different isolates, and to examine the relationship of these bacterial characteristics with the presence of ACG.

### Population and Methods

#### Study Sample

This cross-sectional study was based on data collected at the inclusion of patients into the Eurohepygast cohort [38]. This cohort was set up to follow the evolution of chronic gastritis for 3 years in 451 patients recruited in 14 different European countries. Inclusion requirements for patients were as follows: consultation for dyspepsia between 1995 and 1997 at one of the 19 participating European teaching hospitals, no ulcer detected on endoscopy, chronic gastritis diagnosed from histologic samples, and age between 18 and 75 years. A questionnaire was filled out by the clinician, and a blood sample was taken from nonfasting patients. The serum was kept at 4°C until transfer to a −80°C freezer.

#### Endoscopy

For each patient, 4 biopsy samples were taken from the midantrum (2 for histologic testing and 2 for culture) [39]. Three biopsy samples were taken from the midcorpus on the greater curve (2 for histologic testing and 1 for culture). Biopsy samples for histologic testing were placed in 10% formalin, and the remainder were frozen immediately at −80°C before examination.

**H. pylori Culture and Extraction of DNA**

Antral and corpus biopsy samples were cultured locally according to a common protocol [39] agreed upon by the participating bacteriologists. In brief, biopsy samples were ground for 2–3 s with an electric tissue homogenizer (Ultraturax; LaboModerne) before inoculation onto a selective in-house medium made of Wilkins Chalgren agar (Oxoid) enriched with 10% human blood and rendered selective by the addition of antibiotics (vancomycin, 10 mg/L; cefsulodin, 5 mg/L; trimethoprim, 5 mg/L; and cycloheximide, 100 mg/L). The plates were incubated under microaerobic conditions at 37°C for up to 12 days. The organisms were identified as *H. pylori* by Gram staining, as well as by urease, oxidase, and catalase activities. Isolates were transported frozen in dry ice by a courier and were kept frozen at −80°C until processing.

For DNA extraction, the isolates were subcultured on the same medium for 48 h, were harvested in 1 mL of Brucella broth (BBL Microbiology Systems), and subsequently were centrifuged at 3000 g for 15 min, and the bacterial pellet was resuspended in 1 mL of extraction buffer (20 mM Tris-HCl [pH 8] and 0.5% Tween 20) and was treated with 10% SDS and proteinase K (100 µg/mL). After at least 1 h at 56°C, the proteins were eliminated by solvent extraction by means of a standard protocol [40]. Nucleic acids were precipitated in the presence of 70% ethanol and 0.3 M sodium acetate (pH 5.2) at −80°C for 30 min. After centrifugation and washing with 70% ethanol, the DNA was dissolved in an appropriate volume of sterile water and was stored at −20°C. The DNA concentration was determined as an optical density at 260 nm.

#### cagA and vacA Detection

**Multiplex polymerase chain reaction (PCR).** Multiplex PCR was used without cloning, to amplify the cagA gene and the s and m regions of the vacA gene. All 11 biotinylated primers used in this study have been described elsewhere by van Doorn et al. [19]. The PCR mixture (50 µL) consisted of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 200 µM each dNTP, 25 pmol of each primer, 1.5 U of Ampli Taq Gold DNA polymerase (Perkin-Elmer), and 2 µL of DNA. The PCR conditions were as follows: after a step of 9 min at 94°C, 40 cycles were applied, each consisting of 30 s at 94°C, 45 s at 50°C, and 45 s at 72°C. A final elongation step of 7 min at 72°C was added. Amplifications were done in a 480 Perkin-Elmer apparatus.

**Reverse-hybridization line probe assay (LiPA).** Amplicons were analyzed by a reverse-hybridization LiPA (Inno-LiPA; Innogentics) [19]. In brief, 10 µL of PCR products was denatured and added to a LiPA strip on which oligonucleotidic probes corresponding to the different genotypes (n = 15) were immobilized. After hybridization at 50°C (±0.5°C) for 1 h, the strips were washed, and streptavidin–alkaline phosphatase and substrate were successively added. Hybrids are visible as purple lines, and hybridization patterns were interpreted visually.

**Amplification of the cagA gene.** In 19 cases, anti-CagA antibodies were present, yet the isolate was negative for the cagA genotype by Inno-LiPA. Conventional PCR amplifications then were done with 2 sets of primers: the first allowed for the amplification of a 394-bp fragment (nt 910–1626). The second amplification was done with 2 sets of primers: the first allowed for the amplification of a 394-bp fragment (nt 157–550), and the second amplified a 80-bp fragment (nt 105–184). The first amplification was done only when the first yielded negative results. The PCR was done in a volume of 50 µL containing 5 µL of DNA, 67 mM Tris-HCl (pH 8.8), 16 mM [NHEt]2SO4, 0.1% Tween 20, 1.5 mM MgCl2, 1 mM each primer, 200 µM each dNTP, and 1 U of Eurobio Taq polymerase (Eurobio). The cycling program, preceded by a 5-min denaturing step, consisted of 40 cycles each of 1 min at 94°C, 1 min at 58°C, and 1 min at 72°C, followed by a final extension step of 7 min at 72°C. The PCR products were visualized in agarose gels. A cagA-positive status was defined when the cagA gene was detected by at least 1 of the 2 primer pairs.

#### Analysis of Le' and Le'' Expression in H. pylori Isolates

**Preparation of proteinase K–treated whole cell samples.** *H. pylori* was cloned, and biomass was harvested from blood agar plates in 0.1 M PBS (pH 7.2) and was washed twice in PBS by centrifugation (5000 g for 30 min). Subsequently, proteinase K–treated whole cell extracts, LPS minipreparations, were prepared as described elsewhere [41]. In brief, *H. pylori* cells were suspended in PBS to an optical density of 0.3 at 600 nm, were heated to 100°C for 10 min for dissolution, were treated with proteinase K (0.1 mg/mL) at 60°C for 1 h, and then were heated to 100°C for 5 min to denature the enzyme. Samples were stored at −20°C before use.
Serologic analysis of Le\textsuperscript{a} and Le\textsuperscript{y}. Le\textsuperscript{a} and Le\textsuperscript{y} antigen expression was analyzed by the serodot method described by Hynes and Moran [42], with minor modifications. The proteinase K–treated whole cell extracts (1 \( \mu \)L) were placed directly onto a nitrocellulose membrane (Amersham) in a large serodot assay in which 60 samples were applied by use of a template and were allowed to air dry before probing with antibodies. Nonspecific binding of antibodies was prevented by blocking the nitrocellulose membrane with 1% skim milk in 10 mM Tris-buffered saline (TBS) for 30 min at 20°C. After 3 washes (10 min each) in TBS containing 0.1% Tween 20, the proteinase K–treated whole cell extracts were probed with anti-Le\textsuperscript{a} and anti-Le\textsuperscript{y} murine monoclonal antibodies (Signet Laboratories), diluted 1:1000 in 0.5% skim milk, in TBS at 37°C for 1 h. Subsequently, the membranes were washed twice in TBS–0.1% Tween for 10 min. The membranes were incubated with horseradish peroxidase–conjugated goat anti–mouse IgM (Sigma) as the secondary antibody, diluted 1:1000, at 37°C for 1 h, and immunoreactants were precipitated by use of a commercial color development kit (Bio-Rad). The specificity of the monoclonal antibodies was validated by use of purified \textit{H. pylori} LPS (2 \( \mu \)g) of known Le expression [27, 42].

Histopathologic Diagnosis

Biopsy samples were routinely embedded in paraffin blocks and then were sectioned and stained in each local center. Hematoxylin–eosin staining was essentially used. Silver staining was used for \textit{H. pylori} detection. Special stains for intestinal metaplasia were indicated in the study protocol, such as Alcian blue (pH 2.5) or periodic acid–Schiff, with a preference for Gomori–aldehyde fuchsin or high-iron diamine stain. The stained slides subsequently were centralized and examined by a single expert gastrointestinal pathologist (P. Sipponen, Espoo, Finland), who used the updated Sydney classification [43, 44]. Two histopathologic entities were delineated, non-ACG and ACG, without further grading of ACG into subgroups. The non-ACG patients were those who presented with any grade of inflammation with no atrophy in either the corpus or the antrum. ACG was defined as antral atrophy with or without intestinal metaplasia, or corpus atrophy with or without corpus intestinal metaplasia. Patients with missing or inadequate biopsy samples from either of the 2 sites, antrum or fundus, were excluded from the analysis.

Statistical Analysis

Data were summarized in 2-way tables, and the distribution of characteristics of isolates was compared by \( \chi^2 \) test. Analysis was done with STATA statistical software (version 5.0; StataCorp). Sample size depended on data available for each variable \((n = 215)\) when genotypes were compared and \(n = 148\) when the comparison included Le expression (figure 1).

To create a variable describing how the different characteristics of the isolates, \textit{cagA} gene, alleles of \textit{vacA} gene (s1a, s1b, s2, m1, and m2a), and Le antigen determinants (Le\textsuperscript{a}, Le\textsuperscript{y}, and Le\textsuperscript{xy}) were associated, a multiple correspondence analysis (MCA) was done; that is, a multivariate descriptive analysis was done of qualitative variables only. To confirm the results, a cluster analysis was used to draw a dendrograms based on the same variables, by use of the minimum aggregation method and Euclidean distance. Both analyses were done with the Statbox 2.5 program (Grimmer Logiciels). These analyses were done for 148 patients for whom information on genotype and Le expression was available.

The odds for the presence of ACG were compared within groups of isolates with the different genotypes and Le determinants, as well as between the different strata of the summary variable. The unadjusted odds ratio (OR) and its 95% confidence interval (CI) were calculated. An OR adjusted for age also was calculated for the variable issued from the MCA analysis. This analysis was done for 108 patients for whom histologic diagnosis was available, in addition to genotype and Le expression data.

Results

Sample. Of the 451 patients included in the Eurohepygast cohort, 108 patients with or without ACG also had data available regarding \textit{cagA} and \textit{vacA} genotype and Le determinants. The description of the 451 patients is detailed elsewhere [38]. The Inno-LiPA was done on 221 \textit{H. pylori} isolates from the Eurohepygast patients, to define the \textit{cagA} status and \textit{vacA} genotype of each isolate. Four of these isolates had indeterminate results for \textit{cagA} status after the second control PCR but not for \textit{vacA} genotype, and 2 had indeterminate results for the \textit{vacA} mid-region genotype but not for \textit{cagA} status. Therefore, data were available regarding \textit{cagA} for 217 isolates, regarding \textit{vacA} for 219 isolates (table 1), and regarding both \textit{cagA} and \textit{vacA} for 215 isolates (figure 1). Mixed infections were present in some cases, and the decision was made to consider the more virulent profile for the analysis. It occurred in 7 cases for the m allele and in 8 cases for the s allele of \textit{vacA}.

451 patients included in Eurohepygast

- 2 strains with \textit{cagA} or \textit{vacA} status not available

215 patients with \textit{cagA} and \textit{vacA} status

- Strains not recovered after transportation \((n = 67)\)

148 patients with Lewis determination\(*\)

- Slides for histology of insufficient quality \((n = 40)\)

108 patients with histological diagnosis

Figure 1. Flow chart of population under study, according to data available for patients or \textit{Helicobacter pylori} isolates in the Eurohepygast study. *A total of 155 patients had Lewis determination done, but, for 7 of them, \textit{cagA} or \textit{vacA} status was not known.
Le typing was done for *H. pylori* isolates from 155 patients. A total of 148 patients had complete data for Le, *cagA*, and *vacA* status, but only 108 patients had complete data when histologic diagnosis was added (figure 1). The available characteristics of the 236 patients lacking *cagA* and *vacA* genotype were compared with those of the 215 analyzed, and no differences were found between these groups. The variables compared were age; sex; body mass index; profession; education level; country of birth; use of coffee, alcohol, cigarettes, and tranquilizers; anti-CagA and anti-VacA antibodies; and histopathologic diagnosis. These variables are described in detail elsewhere [38].

The description of the characteristics of the *H. pylori* isolates in the global sample is presented in table 1. In the global sample, 138 (64%) of the 217 isolates examined for *cagA* were *cagA*+; of the 219 isolates typed for *vacA* genotype, the alleles were distributed as follows: s1m1, 91 (42%); s1m2, 64 (29%); and s2m2, 64 (29%). Of the 155 isolates with Le results available, 31 (20%) did not express any Le determinant, and 124 (80%) expressed at least 1 Le determinant: 87 (56%) expressed both Lex and Ley, only 6 (4%) isolates expressed Le x alone, and 31 (20%) expressed Ley alone.

**Table 1.** Genotype and distribution of expression of Lewis determinants in isolates of dyspeptic patients included in the Eurohepygast study.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td></td>
</tr>
<tr>
<td><em>cagA</em></td>
<td>217</td>
</tr>
<tr>
<td><em>cagA</em>+</td>
<td>79 (36)</td>
</tr>
<tr>
<td><em>cagA</em>−</td>
<td>138 (64)</td>
</tr>
<tr>
<td><em>vacA</em></td>
<td>219</td>
</tr>
<tr>
<td>s1m1</td>
<td>72 (33)</td>
</tr>
<tr>
<td>s1m2</td>
<td>19 (9)</td>
</tr>
<tr>
<td>s2m1</td>
<td>5 (2)</td>
</tr>
<tr>
<td>s2m2</td>
<td>0</td>
</tr>
<tr>
<td>Lewis expression</td>
<td></td>
</tr>
<tr>
<td>Lex−/Ley−</td>
<td>31 (20)</td>
</tr>
<tr>
<td>Lex+/Ley−</td>
<td>6 (4)</td>
</tr>
<tr>
<td>Lex−/Ley+</td>
<td>31 (20)</td>
</tr>
<tr>
<td>Lex+/Ley+</td>
<td>87 (56)</td>
</tr>
</tbody>
</table>

Le typing was done for *H. pylori* isolates from 155 patients. A total of 148 patients had complete data for Le, *cagA*, and *vacA* status, but only 108 patients had complete data when histologic diagnosis was added (figure 1).

The available characteristics of the 236 patients lacking *cagA* and *vacA* genotype were compared with those of the 215 analyzed, and no differences were found between these 2 groups. The characteristics of the 67 patients lacking Le determination were compared with the 148 patients for whom these data were available: more patients had s1m1 or s2m2 and fewer patients had s1m2 alleles of the *vacA* gene among the 148 patients with Le data, compared with the 67 patients without Le data available (44% vs. 34%, 32% vs. 25%, and 24% vs. 41%, respectively; \( P = .034 \)). The available characteristics of the 40 patients lacking histologic diagnosis were compared with those of the 108 analyzed: more patients harbored anti-CagA antibodies among the 108 patients with histologic diagnosis than among the 40 patients with missing data (71% vs. 48%; \( P = .016 \)). This difference also was observed regarding the presence of the gene *cagA*, but the difference did not reach significance (66% vs. 50%; \( P = .081 \)). No other differences were found between these groups. The variables compared were age; sex; body mass index; profession; education level; country of birth; use of coffee, alcohol, cigarettes, and tranquilizers; anti-CagA and anti-VacA antibodies; and histopathologic diagnosis. These variables are described in detail elsewhere [38].

The correlation between *cagA* and *vacA* alleles. There was a strong correlation between the presence of *cagA* and the s1m1 allele of *vacA* and between the absence of *cagA* and the s2m2 allele of *vacA* (figure 2). Among the 138 *cagA*+ isolates, the proportions of alleles were as follows: s1m1, 91 (42%); s1m2, 64 (29%); and s2m2, 64 (29%). Of the 155 isolates with Le results available, 31 (20%) did not express any Le determinant, and 124 (80%) expressed at least 1 Le determinant: 87 (56%) expressed both Le x and Ley, only 6 (4%) isolates expressed Le x alone, and 31 (20%) expressed Ley alone.

**Figure 2.** Distribution of alleles of *vacA* gene, according to *cagA* status of *Helicobacter pylori* isolates from dyspeptic patients included in the Eurohepygast study (\( n = 215 \)).
genotype s1a (81%), whereas, for cagA− isolates, the s2 allele was predominant (72%).

**Correlation of Le* or Le† expression with cagA and vacA alleles of *H. pylori* isolates.** The expression of Le* and Le† differed markedly between cagA+ and cagA− isolates and between vacA alleles (figures 3 and 4). Of the 91 cagA+ isolates for which Le expression was available, 80 (87.9%) expressed at least 1 Le determinant, compared with 38 (66.7%) of 57 cagA− isolates ($\chi^2 = 8.59; P = .0034$). Of the 91 cagA+ isolates, 64 (70.3%) were positive for both Le* and Le†, compared with 20 (35.1%) of the 57 cagA− isolates ($\chi^2 = 17.74; P < .0001$; figure 3). When Le* and Le† determinants were considered separately, the association between Le* and cagA status did not reach statistical significance: of the 91 cagA+ isolates, 3 (3%) expressed Le†, versus 3 (5%) of the 57 cagA− isolates ($\chi^2 = 0.87; P = .87$). On the other hand, for Le† the difference was borderline: of the 91 cagA+ isolates, 13 (14%) expressed Le†, versus 15 (26%) of the 57 cagA− isolates ($\chi^2 = 3.31; P = .069$).

Concerning the relationship between Le determinants and the vacA alleles, at least 1 Le determinant was detected in 94 (90.4%) of the 104 isolates with the s1 allele, versus 26 (55.3%) of the 47 isolates with the s2 allele. A linear decreasing trend was observed in the proportion of Le* and Le† determinants expressed in isolates grouped according to s1m1, s1m2, and s2m2 alleles (72%, 64%, and 28%, respectively; $\chi^2$ for trend, 23.4; $P < .0001$). Moreover, as shown in figure 4, compared with s2m2-bearing isolates, the distribution of Le determinants was similar in isolates possessing s1m1 and s1m2 alleles. Furthermore, in isolates with the s2m2 allele, those not expressing Le determinants predominated over those expressing Le groups. These results indicate that expression of Le determinants correlates with the presence of cagA and vacA genotypes.

**Results of the MCA for construction of the summary variable.** The MCA was done for 148 patients for whom complete data were available for cagA status, vacA alleles, and Le determinants (figure 5), yielding the F1/F2 plane, which accounted for 71.4% of the total information (60.4% for the horizontal axis [F1] and 11.0% for the vertical axis [F2]). Two main clusters were iden-
tified on each side of the horizontal axis: m1, s1a, cagA+, Le+, and Le- isolates on one side, and isolates with s2, m2a, cagA-, Le+, or no Le determinants on the other. The dendrogram (figure 6) drawn from the results of the cluster analysis confirmed the presence of the 2 clusters. Of interest, isolates expressing only Le+ determinants belonged to both main groups. Therefore, they were removed from the principal classification, and these 6 patients were included in a third group (i.e., patients harboring isolates with a different combination of the defined characteristics). According to these results, the 148 H. pylori isolates were divided into 3 groups: cagA+/s2/m2a/Le+ or no Le determinants (n = 29; 19.6%; group A); cagA+/s1a (or s1b)/m1/Le+ and Le- (n = 44; 29.7%; group C); and isolates having none of these characteristics (n = 75; 50.7%; group B). The same MCA analysis was done on the 75 patients in the latter group whose isolates were not classified. The analysis did not show any important aggregation between characteristics, except for those expressing Le+ and Le- determinants and the m2 allele (36 of 75 isolates had these 3 characteristics). Therefore, a subclassification was created for these remaining 75 cases: m2/Le+ and Le- (n = 36; 24.3%; group B1), and other types of isolates (n = 39; 26.3%; group B2).

Description of the summary variable. Globally, the study of the distribution of the summary variables classified into 3 strata showed interesting differences among countries (figure 7). The highest prevalence of group C isolates was found in Bulgaria and Greece, 9 (47.4%) of 19 and 7 (53.8%) of 13 isolates, respectively. The lowest prevalence of this type of isolate was found in Ireland and Portugal, with 1 (7.1%) of 14 and 0 of 11 isolates, respectively. A heterogeneous distribution also was noted between age groups, with the highest rate of the same isolates among patients 41–50 years old or 51–60 years old: 17 (32.7%) of 52 and 15 (45.5%) of 33 isolates, respectively. In the other age groups, the distribution of this type of isolate was as follows: 19–30 years old, 5 (23.8%) of 21; 31–40 years old, 4 (16.0%) of 25; and ≥61 years old, 3 (17.6%) of 17. No differences were observed according to sex, blood group or Rhesus group, and family history of digestive disease.

Relationship between characteristics of the isolates and the presence of ACG. The possible relationship between the presence of ACG and the characteristics of isolates cultured from these patients was analyzed. As shown in table 2, the proportion of ACG was higher in patients harboring isolates with the cagA gene, or the s1m1 allele of vacA, or expressing Le+ determinants,

Figure 5. Multiple correspondence analysis plot of cagA and vacA alleles and Lewis expression characteristics of 148 Helicobacter pylori isolates from dyspeptic patients included in the Eurohepygast study.

Figure 6. Dendrogram showing how Helicobacter pylori isolates aggregate according to their cagA status, alleles of vacA gene, and expression of Lewis determinants in dyspeptic patients included in the Eurohepygast study (n = 148).
or expressing Le⁺ and Le⁻ determinants (30.4%, 38.1%, 29.4%, and 29.7%, respectively) than in patients harboring isolates without the cagA gene, or with the s2m2 allele of vacA, or of the corresponding Le baseline group (11.3%, 15.6%, 15.6%, and 16.3%, respectively). The difference between these proportions was statistically significant between cagA, as well as the s1m1 allele of vacA and their respective baseline groups, but not for any of the Le determinant combinations. The calculated ORs were in line with these results.

When analyzing the summary variables classified into 3 or 4 strata according to the MCA results (table 2), it was remarkable that the odds of having ACG among patients harboring group C isolates (OR, 7.4; 95% CI, 1.5–37.0) was >7 times higher than that for patients harboring group A isolates (baseline risk). Patients harboring isolates classified in group B of the summary variables or in 1 of the 2 subgroups, B1 or B2, were not at risk for ACG, compared with patients harboring group A isolates. These results were adjusted for age.

Discussion

The interesting findings in this study were the clustering among established pathogenic factors and Le determinants and the relationship between the cluster and the presence of atrophy. The results of the MCA and the cluster analysis (2 independent methods) were identical. Therefore, this argues in favor of the existence of the 2 main clusters, A and C, identified in the analysis and the deterministic clustering of isolates. Explanations for the aggregation of Le determinants with the established virulence characteristics of H. pylori isolates may include mechanisms to facilitate bacterial adherence to host cells [35, 36], which leads bacteria to escape elimination by the host immune response and persist [26]. The majority of the isolates expressing Le⁺ and Le⁻ antigens were also cagA⁺ and possessed the s1m1 vacA allele. What the presence of Le⁺ and Le⁻ on isolates adds to the presence of cagA and s1m1, in terms of pathogenicity, remains a complex issue. Camouflage may be a plausible hypothesis. The aggregation of pathogenic characteristics may be the result of a selective advantage. First, cagA⁺ s1m1 isolates being more aggressive [11–13, 45] are more exposed to the immune system, and camouflage by an efficient mimicry process therefore could help them to persist in the stomach of their host. Thus, the ability to express Le⁺ and Le⁻ would be an advantage for the isolate [46]. Second, alternatively, bacterial Le antigen expression could contribute to adherence to the gastric mucosa and aid delivery of secreted products [32]. These secreted products may include those associated with the cag pathogenicity island, including CagA, which, although contributing to the development of inflammation and pathology, may liberate nutrients of benefit to the bacterium.
The results of the present analysis are in agreement with the results of others [26, 27, 47] in that the development of ACG is related to the presence of antigenic mimicry by *H. pylori*. However, when considering only Le expression, isolates expressing Le⁺ and Le⁻ were not linked to ACG (OR, 2.2; 95% CI, 0.85–5.5; table 2) and did not thereby express any pathogenic features. Thus, the lack of association, even if borderline to significance, of the expression of Le⁺ or Le⁻ antigen does not support the hypothesis that isolates expressing Le⁺ and Le⁻ without the presence of other pathogenic factors are virulent. Nevertheless, these factors aggregated together, and, when Le antigens were added to isolates of the cagA+ and s1m1 genotype (group C isolates), the point estimates of the risk for ACG increased 2-fold. Of note, among the 6 patients who harbored isolates that were cagA+, who had the s1m1 allele of the vacA gene, and who did not express Le⁺ or Le⁻, 5 were non-ACG and only 1 had ACG.

Furthermore, one should bear in mind that, when the MCA variable was taken into account, there was no difference in risk for the occurrence of atrophy in patients between group A and group B of *H. pylori* isolates. Only group C isolates were more virulent and strongly associated with ACG. In contrast to findings of Covacci et al. [48], this study did not indicate any gradient of pathogenicity among the isolates, and there was no progression in the risk of occurrence of ACG (i.e., increasing OR from type A to type C isolates). However, the question remains whether colonization by a type C isolate precedes the occurrence of ACG (and, therefore, could be considered as causal) or whether its presence is the consequence of a selective process following the progression of the gastric mucosa from non-ACG to ACG. It is, therefore, of interest that there was no difference in the distribution of more virulent isolates between persons <50 old and those >50 years old, which would favor the first alternative. The cutoff of 50 years was chosen because this age has been proven to be an important step in the pathologic development from gastritis toward ACG [49] and is in agreement with the results of a previous analysis of this sample [38].

Even though only 108 of the 451 patients included in the Eurohepygast study had complete data, this group is still large and was strictly comparable to the 155 patients who had Le typing done. The study would be strengthened if Le typing had been done on all strains, but the maximum data available were used to remain as close as possible to the original sample. It is difficult to evaluate the bias induced by missing data; however, the different comparisons between the subsamples show few differences, and it may be possible that only the difference in the distribution of the vacA alleles may have affected the results. On the other hand, both s1m1 and s2m2 were more prevalent in the subgroup with Le determination, which counterbalances their effect. Another point is the possibility that strains with pathogenic features recover more easily than strains without, but information is

Table 2. Relationship between genotype of *Helicobacter pylori* isolates, expression of Lewis determinants, and presence of atrophic chronic gastritis (ACG) in patients included in Eurohepygast study.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total no. of patients</th>
<th>No. of patients with ACG</th>
<th>Percentage of patients with ACG (95% CI)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cagA</td>
<td>155</td>
<td>37</td>
<td>23.9 (17–31)</td>
<td></td>
</tr>
<tr>
<td>cagA⁺</td>
<td>53</td>
<td>6</td>
<td>11.3 (14–23)</td>
<td>Baseline</td>
</tr>
<tr>
<td>cagA⁻</td>
<td>102</td>
<td>31</td>
<td>30.4 (22–40)</td>
<td>3.4 (1.3–8.8)</td>
</tr>
<tr>
<td>vacA</td>
<td>158</td>
<td>38</td>
<td>24.0 (18–31)</td>
<td></td>
</tr>
<tr>
<td>s2m2</td>
<td>45</td>
<td>7</td>
<td>15.6 (6–29)</td>
<td>Baseline</td>
</tr>
<tr>
<td>s2m1</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>s1m2</td>
<td>50</td>
<td>7</td>
<td>14.0 (6–27)</td>
<td>0.9 (0.3–2.7)</td>
</tr>
<tr>
<td>s1m1</td>
<td>63</td>
<td>24</td>
<td>38.1 (26–51)</td>
<td>3.3 (1.3–8.7)</td>
</tr>
<tr>
<td>Lewis expression</td>
<td>113</td>
<td>27</td>
<td>23.9 (16–33)</td>
<td></td>
</tr>
<tr>
<td>Le⁺/Le⁻⁻ or Le⁺⁻/Le⁺⁻</td>
<td>45</td>
<td>7</td>
<td>15.6 (6–29)</td>
<td>Baseline</td>
</tr>
<tr>
<td>Le⁺⁺/Le⁺⁻ or Le⁺⁺/Le⁺⁻</td>
<td>68</td>
<td>20</td>
<td>29.4 (19–42)</td>
<td>2.3 (0.9–5.9)</td>
</tr>
<tr>
<td>Le⁺⁻/Le⁺⁺ or Le⁺⁻/Le⁺⁻</td>
<td>49</td>
<td>8</td>
<td>16.3 (7–30)</td>
<td>Baseline</td>
</tr>
<tr>
<td>Le⁺⁺/Le⁺⁺</td>
<td>64</td>
<td>19</td>
<td>29.7 (19–42)</td>
<td>2.2 (0.85–5.5)</td>
</tr>
<tr>
<td>MCA summary variable</td>
<td>108</td>
<td>26</td>
<td>24.1 (16–33)</td>
<td></td>
</tr>
<tr>
<td>1st MCA variable</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A</td>
<td>21</td>
<td>2</td>
<td>9.5 (1–30)</td>
<td>Baseline</td>
</tr>
<tr>
<td>Group B</td>
<td>55</td>
<td>10</td>
<td>18.2 (9–31)</td>
<td>2.1 (0.4–10.6)</td>
</tr>
<tr>
<td>Group C</td>
<td>32</td>
<td>14</td>
<td>43.8 (26–62)</td>
<td>7.4 (1.5–37)</td>
</tr>
<tr>
<td>2nd MCA variable</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A</td>
<td>21</td>
<td>2</td>
<td>9.5 (1–30)</td>
<td>Baseline</td>
</tr>
<tr>
<td>Group B1</td>
<td>28</td>
<td>4</td>
<td>14.3 (4–33)</td>
<td>1.6 (0.3–9.6)</td>
</tr>
<tr>
<td>Group B2</td>
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<td>6</td>
<td>22.2 (9–42)</td>
<td>2.7 (0.5–15.1)</td>
</tr>
<tr>
<td>Group C</td>
<td>32</td>
<td>14</td>
<td>43.8 (26–62)</td>
<td>7.4 (1.5–37)</td>
</tr>
</tbody>
</table>

NOTE. Odds ratios (ORs) are presented as univariate results, except for multiple correspondence analysis (MCA) summary variable; ORs are adjusted for age. CI, exact binomial 95% confidence interval. Group A: s2m2, cagA*, and Le⁺⁻/Le⁺⁻ or Le⁺⁺/Le⁺⁺; group B1: m2 and Le⁺/Ley⁺; group B or B2: isolates with other combinations; group C: s1m1, cagA⁺, and Le⁺⁺/Le⁺⁺.
lacking on this subject. Nonetheless, these missing data affect the generalizability of the results. Furthermore, the fact that cagA+ isolates were more prevalent than cagA− isolates in this European population may be the consequence of a selection bias: only symptomatic dyspeptic patients consulting gastroenterologists were included in the present study, and thus an increased proportion of pathogenic isolates were examined, which possibly do not represent the isolates circulating in the general population. Although the distribution of the various types of isolates appears to be different according to country, no concrete conclusion can be drawn from this diversity, given the small sample sizes. Nevertheless, the results of the present study help explain why previous studies carried out in the Irish population did not find any association between the expression of Le determinants and cagA+ isolates [37].

The MCA combined different characteristics of the isolates and permitted a better description of the way isolates aggregate spatially. Future work, with use of the same methodology, should explore other putative genes implicated in H. pylori pathogenesis. Such studies have methodologic limitations because of a need for large sample size (depending on the number of genes under study) and the cost of analysis. The emergence of new technology for gene analysis will certainly facilitate such an approach. However, in no way does this type of study demonstrate why these characteristics aggregate or whether this aggregation is the cause or consequence of the disease. Nevertheless, it allows for an exact description of a combination of various factors and a measurement of the strength of the association with the studied disease. Causality is a more complex issue that requires long-term follow-up of patients starting before the onset of the disease.

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References