**homB Status of Helicobacter pylori as a Novel Marker To Distinguish Gastric Cancer from Duodenal Ulcer**

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The hom family of Helicobacter pylori outer-membrane proteins, especially the homB gene, has been suggested as a novel virulence factor; however, the clinical association and function of this gene are still unclear. We evaluated the presence of the homA, homB, and cagA genes in 286 strains isolated from patients in the U.S. and Colombian populations (126 with gastritis, 96 with duodenal ulcer, and 64 with gastric cancer) by PCR. The results were compared with the clinical presentation and gastric injury. The prevalence of the homB gene was significantly higher in strains isolated from gastric-cancer patients (71.9%) than in those from duodenal ulcer patients (52.1%) (P = 0.012). In a multivariate analysis, the presence of the cagA gene significantly increased the risk for developing gastric cancer and duodenal ulcer, with the presence of the homB gene acting as a factor that could distinguish gastric cancer from duodenal ulcer (adjusted odds ratio, 3.033; 95% confidence interval, 1.37 to 6.73). cagA status was correlated with homB status (r = 0.323; P < 0.01). A histological analysis showed that cagA status was associated with inflammation and atrophy both in the antrum and in the corpus, while homB status was associated with inflammation and atrophy in the corpus. homB gene status might be susceptible to gastric-cancer development such that the homB gene is used as a factor for discriminating the risk of gastric cancer from that of duodenal ulcer.

**Helicobacter pylori** infection is one of the most common infections of mankind and is etiologically associated with gastritis, peptic ulcer disease (PUD), gastric cancer (GC), and gastric mucosa-associated lymphoid tissue lymphoma (19). Most infected people remain asymptomatic. Factors thought to be associated with clinical gastroduodenal diseases include *H. pylori* virulence, host genetics, and environmental factors, such as diet (11). Putative *H. pylori* virulence factors associated with an increased risk of a clinical outcome include the cag pathogenicity island, CagA, VacA, BabA, and OipA (5, 9, 22). However, none have been exclusively linked to a specific *H. pylori*-related disease (e.g., GC).

The hom family is a small paralogous family of proteins that contain the C-terminal alternating hydrophobic motif and signal sequences typical of outer-membrane proteins. The homB and homA genes are 90% identical; the differences are confined to the central domain (1). Recent studies suggested that there was a close association between the presence of the homB gene and interleukin-8 secretion from human gastric epithelial cells and that the number of *H. pylori* isolates binding to gastric cells was related to the number of homB copies present (15). Moreover, the authors proposed that the presence of the homB gene was significantly associated with PUD in Portuguese children and adults less than 40 years of age and that it may be a new *H. pylori* virulence factor (15, 16). However, there is no study for the association between the homB gene and *H. pylori*-related diseases in other countries. This study investigated whether there was an association between the homA and homB genes and clinical gastroduodenal diseases and the severity of gastric inflammation in the U.S. and Colombian populations.

**MATERIALS AND METHODS**

**Patients and H. pylori.** *H. pylori* strains were obtained from the gastric mucosa of *H. pylori*-infected patients who underwent endoscopy at Universidad Nacional de Colombia Bogotá (Bogotá, Colombia) and Michael E. DeBakey Veterans Affairs Medical Center (Houston, TX). Presentations included gastritis, duodenal ulcer (DU), and advanced noncardiac gastric adenocarcinoma. DU and GC were identified by endoscopy and histopathology. Gastritis was defined as *H. pylori* gastritis in the absence of peptic ulcers or gastric malignancy. Specimens were obtained under informed consent, which was obtained from all patients under protocols approved by the local ethics committees.

**Gastric histology.** Gastric biopsy specimens were obtained from the antrum (pyloric-gland area) and the corpus (fundic-gland area). Each biopsy specimen was placed in a separate bottle of formalin and routinely processed. Serial sections were stained with hematoxylin and eosin and Genta stains and examined by a pathologist blinded to the patient’s clinical diagnosis or the characteristics of the *H. pylori* strain isolated. Each specimen was scored for *H. pylori* density, neutrophil infiltration, and atrophy. All the variables were scored using a visual analogue scale graded from 0 (absent/normal) to 5 (maximal intensity), as described previously (7).

**H. pylori genotyping.** Antral biopsy specimens were obtained for the isolation of *H. pylori* using standard culture methods as previously described (23, 25). Chromosomal DNA was extracted from confluent plate cultures expanded from a single colony using a commercially available kit (Qiagen, Inc., Valencia, CA). PCR amplification was performed by using the synthetic oligonucleotide primers 5'-AGA GGG TGT TTG AAA CCA GCG TCA ATA-3' and 5'-GAT GAA TCT TCC TTC TGC GGT TG-3', described by Olearco et al. (16). The PCR conditions were 95°C for 5 min and then 35 cycles of 95°C for 30 s, 60°C for 30 s, 72°C for 17 s, and finally 72°C for 7 min. According to those authors, the set of F1-jhp870/jhp6049 and R1-jhp870/jhp6049 primers was used and hybridized both homA and homB, generating PCR products of 128 bp and 161 bp, respectively. The cagA status was determined by PCR methods using primer pair 5'-GAT AAC AGG CAA GCT TTT GAG G-3' and 5'-CTG CAA AGG ATT GTT TGG CAG A-3' as described previously (24).

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Data analysis. Variables such as gender (male or female), mean age, and status of homA, homB, and cagA (negative or positive) were evaluated. Statistical differences in demographic characteristics among the different disease groups were determined by one-way analysis of variance or the chi-square test. The univariate association between each genotype status and the clinical outcomes was quantified by the chi-square test and Student’s t test. A multivariate logistic regression model was used to calculate the odds ratios (OR) of the clinical outcomes by including age, sex, and the H. pylori genotypes. All determinants with a P value of <0.25 were entered together in the full model of logistic regression, and the model was reduced by excluding variables with P values of <0.05. OR and 95% confidence intervals were used to estimate the risk. Spearman rank coefficients (r) were also determined to evaluate the association between the different genotypes of the strains. Statistically significant differences in the histological scoring among genotype groups were determined using the Mann-Whitney U test. A P value of less than 0.05 was accepted as statistically significant. We used the SPSS statistical software package version 15.0 (SPSS, Inc., Chicago, IL) for all statistical analyses.

RESULTS

A total of 286 patients were studied, 173 from the United States (91 with gastritis, 60 with DU, and 22 with GC) and 113 from Colombia (35 with gastritis, 36 with DU, and 42 with GC). The characteristics of the patients and the distribution of homA, homB, and cagA status among the disease groups are shown in Table 1. There were significant differences in age, homB status, and cagA status among the disease groups of gastritis, DU, and GC (P < 0.01, 0.04, and 0.04, respectively).

Prevalence of homA, homB, and cagA genes. PCR analyses showed that 272 of the strains had at least one gene corresponding to homA (128 bp) or homB (161 bp). Intermediate-length PCR products were observed in the remaining four strains (1.4%). A sequence analysis showed that the PCR products with intermediate lengths had sequences that differed from both the homA and homB genes related to deletions of different sizes and sites (Fig. 1). These four cases were regarded as negative results for both the homA and homB genes. As there were no disease-related significant differences in the prevalences of the homA and homB genes between the U.S. and Colombian strains (Table 1), the data from the two countries were combined and analyzed as Western strains.

The prevalences of the homA, homB, and cagA genes were 61.9% (177 strains), 61.2% (175 isolates), and 82.2% (235 isolates), respectively (Table 2). Overall, the prevalence of the homA gene was not significantly different among the disease groups. However, that of the homB gene was significantly higher in strains from GC patients and the lowest among strains from DU patients (Table 2).

A univariate analysis showed that the prevalence of cagA in
The majority of the strains with the homB homA contrast, the presence of the 172) also possessed the VOL. 47, 2009 with explanatory variables (age, sex, and genotypes); CI, confidence interval.

There was a positive correlation between the presence of the homB gene was significantly lower in strains from GC patients than those from gastritis patients (51.6% versus 66.7%; P = 0.043), and the presence of the homB gene was significantly higher in strains from GC patients than those from DU patients (71.9% versus 52.1%; P = 0.012).

Table 3 shows the association of the presence of the homA, homB, and cagA genes and clinical outcomes. In the multivariate analysis, only the presence of the cagA gene was an independent discriminating factor for both DU and GC compared with gastritis (adjusted OR, 2.08 and 4.12, respectively). The presence of the homB gene was an independent discriminating factor for GC compared with that for DU (adjusted OR, 3.03) (Table 3).

Correlations between cagA and homA or homB. There was a weak, but significant, negative correlation between the presence of the homA and cagA genes (r = −0.140; P = 0.02). In contrast, there was a positive correlation between the presence of the homB and cagA genes (r = 0.323; P < 0.01) (Table 4). The majority of the strains with the homB gene (92.0% [161 of 172]) also possessed the cagA gene. However, 66.7% of homB-negative strains were also cagA positive. Therefore, we investigated the distribution of genotypes for the homB gene according to the diseases in relation to cagA status. Among the cagA-positive cases, the prevalence of the homB gene was significantly higher in strains from GC patients (74.1%) and gastritis patients (75%) than those from DU patients (56.8%) (P = 0.01 and P = 0.036, respectively). Among the cagA-negative cases, homB-positive strains were more prevalent from GC patients (50%) than DU patients (26.7%) and gastritis patients (23.3%), but the differences were not statistically significant (P = 0.35 and P = 0.18, respectively) (Fig. 2).

Relationship between homA, homB, or cagA status and gastric mucosal histology. The gastric mucosa of the GC patient is generally atrophic, whereas that of the DU patient is typically associated with enhanced antral inflammation with corpus sparing. Thus, the inclusion of those patients can potentially bias histological analyses, and we limited the evaluation of the histological analyses to gastritis cases alone. The histological analysis showed that there was no association between the presence of the homA gene and histology (data not shown). In contrast, neutrophil infiltration and mucosal atrophy scores were significantly higher in gastric mucosae infected with homB-positive strains than homB-negative strains in the corpus (median [25 to 75% range], 2.0 [1.3 to 3.0] versus 1.5 [0.5 to 2.0] for neutrophil infiltration and 0 [0 to 1.0] versus 0 [0 to 1.8] for atrophy) but not in the antrum (Fig. 3A). Neutrophil infiltration scores were significantly greater in gastric mucosae infected with cagA-positive strains than those with cagA-negative strains both in the antrum (median [25 to 75% range], 2.5 [2.0 to 3.0] versus 2.0 [1.0 to 2.5]) and in the corpus (median [25 to 75% range], 2.0 [1.0 to 3.0] versus 1.0 [0 to 2.0]) (Fig.

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a P values among diseases (gastritis, DU, and GC) were 0.206, <0.01, 0.127, 0.038, and 0.038 for the number of male/female patients, age, and percentages of patients with homA+, homB+, and cagA+, respectively.

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<th>TABLE 3. H. pylori factors associated with clinical presentation</th>
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a P values of the univariate analysis were determined by χ² test.

b OR, adjusted odds ratio based on multivariate logistic regression analysis with explanatory variables (age, sex, and genotypes); CI, confidence interval.

FIG. 2. Distribution of genotypes for cagA and homB according to Helicobacter pylori-related gastritis, DU, and GC. Among the cagA-positive strains, homB was more prevalent in strains from GC and gastritis patients than those from DU patients.
and 90th percentiles.

and 90th percentiles, and circles indicate all data outside the 10th
inside each box marks the median. Capped bars indicate the 10th
extents indicate the 25th and 75th percentiles in the column; a line
in the antrum and in the corpus. Data are graphed as boxes whose
positive strains, neutrophil infiltration was significantly greater both
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cagA
3B). Atrophy scores were also higher in gastric mucosae in-
ected with cagA-positive strains than cagA-negative strains both in
the antrum and in the corpus; however, the differences were not statistically significant. There was no relationship
between the density of H. pylori and the presence of the homA, homB, or cagA genes (data not shown).

**DISCUSSION**

This study suggests that the homB gene may be an independent discriminating factor for distinguishing GC from DU. Previous associations with putative H. pylori virulence genes have shown an association with an increased risk of both PUD and GC (6, 14). The exception is the dupA gene, which was originally reported to be associated with an increased risk for DU and reduced risk for GC (12); however, this association is still controversial (2, 3, 10). homB status is a putative new marker for the risk of development of GC. This conclusion differs from the first study reporting that the homB gene was less prevalent in adenocarcinoma patients than PUD patients (16). In that study, there were only 15 GC strains (n = 15). Another study reported that homB was significantly associated with PUD in children and in adults less than 40 years of age (15). However, in both previous studies, PUD included both gastric ulcer (GU), which is associated with pangastritis, and an increased risk of GC as well as DU, which is not (8, 13), and the association of the homB gene with the pathological finding of gastric mucosa was not described. It is well known that DU and GC are clinically divergent gastroduodenal diseases such that it is rare for GC to develop among DU patients (17, 20). Therefore, our study excluded strains from GU and, moreover, clearly showed an association of the homB gene with corpus inflammation which is more indicative of GU and GC than of DU (4, 18). The prevalence of the homB gene in strains from gastritis patients was between the prevalences of the homB gene in strains from DU and GC patients. This is not unexpected since the gastritis group contains a mixture of patients, some of whom would be destined to develop clinical outcomes. What is really needed are longitudinal studies where the outcome can be assessed against the genotypes of the strains responsible for the chronic infections.

We observed a positive correlation between homB status and cagA status. Interestingly, the prevalence of the homB gene in cagA-positive strains was higher in GC patients than in DU patients. Even among cagA-negative strains, homB-positive strains in GC patients seemed to be more prevalent than in DU patients. Moreover, pathological analyses showed that homB status was related to inflammation and atrophy only in the corpus, consistent with it having a role in atrophic gastritis, the precursor lesion of GC, while cagA was associated with increasing the scores of neutrophil infiltration and atrophy both in the antrum and corpus. These findings further suggest that the homB status of H. pylori affects the risk for GC independently of cagA status. Therefore, although cagA-negative strains are less likely to be associated with GC, homB status might be considered in eradicating H. pylori for high-risk patients infected with cagA-negative strains.

Although our study showed the association between the homB gene and GC as well as corpus inflammation, a causal relationship between these is still not clear. Since outer-membrane protein can contribute to improving the adaptation of a strain to a specific host environment, strains containing the homB gene are more likely to survive in circumstances such as corpus atrophic gastritis. H. pylori infection is typically acquired in childhood and persists throughout life; thus, the gastric mucosa gradually develops chronic atrophic gastritis and GC. From this point of view, the presence of the homB gene is thought to be more prevalent in strains from elderly patients. However, in this study, there was no significant difference in mean age between homB-positive and homB-negative groups (50.3 and 53.3, respectively; P = 0.12). Therefore, the homB gene may be involved in the development of GC and corpus gastritis.

Finally, this study showed no geographic differences in the prevalence of the homB gene from the two Western countries. However, the number of GC patients in the United States was very small, precluding a reliable analysis of disease-specific associations in each country separately. It is possible that the
difference relates to variations among strains isolated from different countries which are well recognized (6, 14, 21, 23, 25–27). Further studies with a large number of GC patients in the United States will be necessary to test the hypothesis of the association. Studies in Asian countries are also necessary to test the hypothesis that the associations are universal.

In conclusion, the presence of the homB gene is proposed as an independent discrimination factor for GC and is associated with corpus inflammation and atrophy. However, the biological analysis of homA and homB, including whether the deletion interrupts a protein product or results in the change in the amino acid sequence, is unclear. Therefore, additional in vitro and in vivo studies are necessary to elucidate the causal relationship between the homB gene and gastric pathology and to investigate the mechanisms of how the homB gene product correlates corpus inflammation and GC. Moreover, larger population groups and samples from other countries will be required to increase evaluations of these hypotheses.

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REFERENCES


