A possible role of IL-1RN gene polymorphism in the outcome of gastrointestinal diseases associated with *H. pylori* infection

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**Objective:** To verify whether the variable number of tandem repeat (VNTR) polymorphism in the IL-1RN gene that encodes the interleukin (IL)-1 receptor antagonist (IL-1Ra) plays a role in the outcome of gastrointestinal diseases associated with *Helicobacter pylori* (*H. pylori*) infection.

**Methods:** Patients with normal endoscopy (*n* = 71), inflammation of the upper gastrointestinal tract only (*n* = 196), gastric ulcer (*n* = 28), duodenal ulcer (*n* = 76), and gastric cancer (*n* = 19) were studied. *H. pylori* infection was diagnosed by the urease test, histological examination, and polymerase chain reaction. The IL-1 receptor antagonist gene (*IL-1RN* intron 2 VNTR) was analyzed by polymerase chain reaction. Gastritis was scored according to the updated Sydney system of classification.

**Results:** *H. pylori* infection was an independent risk factor for mild (odds ratio [OR] = 5.53 [95% confidence interval [CI] = 2.63–11.64; *P* < 0.05]), moderate (OR = 83.93 [95% CI = 29.7–237.18; *P* < 0.05]) and marked (OR = 47.47 [95% CI = 5.39–418.05; *P* < 0.05]) gastritis. The carriage of IL-1RN*2/*2 had a significant protective effect of *H. pylori* infection (OR = 0.31 [95% CI = 0.17–0.57; *P* < 0.05]). *H. pylori* infection was identified as an independent risk of inflammation, duodenal ulcer, and gastric ulcer. The carriage of IL-1RN*2/*2 was an independent risk factor for gastric cancer (OR = 5.16 [95% CI = 1.06–31.98; *P* < 0.05]); nonetheless, the carriage of allele 2 (IL-1RN*2/*2 plus IL-1RN*L/*2) had an independent protective effect on duodenal ulcer (OR = 0.45 [95% CI = 0.22–0.91; *P* < 0.05]).

**Conclusions:** Allele 2 of the VNTR IL-1RN polymorphism had a protective effect against duodenal ulcer and *H. pylori* infection; however, it increased the risk of gastric cancer.

**Keywords:** *Helicobacter pylori*, IL-1RN polymorphism, gastric cancer, peptic ulcer

**Introduction**

*Helicobacter pylori* (*H. pylori*) infection is considered to be the etiological cause of chronic gastritis and peptic ulcer disease, and it has been associated with gastric cancer.¹ The prevalence of *H. pylori* infection varies from 7% (Czech Republic) to 87% (South Africa); the prevalence is lower in Europe (7%–33%) than in South America (48%–78%).² In Brazil, there are differences in the prevalence of *H. pylori* infection depending on the local sanitary conditions.³ The poor communities show higher prevalence (from 73.3%–87%) of *H. pylori* infection than those with a high standard of living, such as that in the city of São Paulo in Brazil (53%).³,⁴,⁵

The lifetime risk for peptic ulcer in *H. pylori*-positive subjects increases with age and among males, and it reached 33.4% of patients in a tertiary care hospital of São Paulo.⁶ Other factors have been reported to play a role in the outcome of gastrointestinal
diseases in *H. pylori*-positive subjects. Antral predominant gastritis with higher acid production is more common in the peptic ulcer and non-ulcer dyspepsia groups. Subjects with body-predominant and atrophic gastritis affecting the gastric body have low acid production, and they are at an increased risk for gastric cancer. *H. pylori* virulence factors encoded by cytotoxin-associated gene (cag) pathogenicity island (a cluster of genes) have been associated with peptic ulcer disease and gastric cancer. Another issue to be considered in this interplay is that of the host genetic polymorphisms that control the inflammatory response against the bacterium, by either accentuating or attenuating the inflammatory response and affecting the disease outcome.

Inflammatory cells in the gastric mucosa produce proinflammatory cytokines (interleukin [IL]-1β, IL-2, IL-6, IL-8, and tumor necrosis factor [TNF]-α) and anti-inflammatory cytokines (IL-4 and IL-10) in response to chronic *H. pylori* infection. Gastric mucosal cytokine levels may be affected by genetic polymorphisms, which result in different grades of inflammation, acid secretion, and outcome of the gastrointestinal disease.

Among the proinflammatory cytokines, genetic polymorphisms in the IL-1B and the IL-1RN genes that encode IL-1β and IL-1ra (endogenous receptor antagonist of IL-1β), respectively, have been widely studied in the presence of *H. pylori* infection, modulating the risk of hypochlorhydria, gastric atrophy, and gastric cancer. The IL-1RN gene has a penta-allelic 86-bp variable number of tandem repeat (VNTR) polymorphism in intron 2, resulting in a short allele with two repeats (IL-1RN*2*) or long alleles (IL-1RN*2L): allele 1 (four repeats), allele 3 (five repeats), allele 4 (three repeats), and allele 5 (six repeats). Allele 1 is more frequently found, followed by allele 2; the others may not be present.

The presence of a single *IL-1RN*2 allele was associated with higher levels of IL-1β and two alleles (*IL-1RN*2/*2) with gastric acid suppression. The increase of IL-1β associated with less gastric acid secretion predisposes to gastric atrophy and gastric cancer development; however, it may protect against duodenal ulcer because the gastric acid output is the principal cause. While the reports of studies conducted in Brazil and Portugal have shown association of *IL-1RN*2 allele with an increased risk of gastric cancer and chronic gastritis, other authors in Brazil failed to show this association.

In a study conducted in Bogota, an inverse association was detected. Additionally, an increased risk of gastric ulcer has been reported. The levels of IL-1β were higher when the *IL-1RN*2 allele was present; however, in other studies, low levels of IL-1β were associated with the *IL-1RN*2 allele. Thus, the results concerning *IL-1RN*2 allele have been contradictory.

The aim of this study was to verify whether the VNTR polymorphism in the *IL-1RN* gene that encodes IL-1 receptor antagonist (IL-1Ra) has a role in the outcome of gastrointestinal diseases associated with *H. pylori* infection.

**Materials and methods**

This study was approved by the local Ethics Committee (CAPPesq n 799/04), and the patients gave written informed consent. Consecutive dyspeptic patients who underwent upper gastrointestinal endoscopy at the Endoscopy Section of Sapopemba Hospital, a primary care health center, were invited to participate in the study. Patients were selected from those who were not taking nonsteroidal anti-inflammatory drugs and proton pump inhibitors for more than 15 days. From a total of 500 consecutive endoscopies, 379 patients were included in the study. Normal endoscopy was observed in 71 patients, inflammation of the upper gastrointestinal tract only in 196 patients (esophagitis, 31; gastritis, 138; duodenitis, 27), gastric ulcer in 28 patients, duodenal ulcer in 76 patients, and diffuse type gastric cancer in eight patients. Deoxyribonucleic acid (DNA) previously extracted from the leukocytes of eleven intestinal type gastric cancer patients that were identified as leu 1, leu 2, leu 5, leu 6, leu 8, leu 9, leu 11, leu 12, leu 13, leu 21, and leu 22 subtypes were included because of the low number of gastric cancer patients, although there was no information on the *H. pylori* status. Therefore, the total number of cases that were analyzed was 390 with a mean age of 44.18 ± 15.42 years (69.23% were women).

**DNA extraction and PCR**

DNA was extracted from gastric biopsies taken for the urease test by a salting out procedure described previously. The primers, *IL-1RN* sense 5′-CTCAGCAACACTCTTAT-3′ and *IL-1RN* antisense 5′-TCCTGGTCTGACGGTAA-3′, described by Bioque et al in 1995, were used to amplify the region within the second intron of the *IL-1RN* gene that encompasses the 86 bp VNTR polymorphism. Genomic DNA (1 µL) was used as a template in a reaction volume of 25 µL, containing 1.5 mM MgCl2, 20 mM Tris-HCl (pH 8), 50 mM KCl, 0.2 mM of each deoxyribonucleotide triphosphate, 10 pmol of each primer, and 2.0 U of *Taq* DNA polymerase (Life Technologies, Carlsbad, CA, USA). Amplification was performed as follows: at 95°C for 5 minutes followed by 35 cycles at 95°C for 30 seconds, annealing at 50°C for
30 seconds and at 72°C for 30 seconds. The final extension at 72°C was performed for 5 minutes. The PCR products were visualized on a 2% agarose gel stained by ethidium bromide. The samples showing a band of 410 bp (four repeats of the 86 bp region) were classified as allele 1, a band of 240 bp (two repeats of 86 bp region) as allele 2, a band of 500 bp (five repeats of 86 bp region) as allele 3, a band of 325 bp (three repeats of 86 bp region) as allele 4, and a band of 595 bp (six repeats of 86 bp region) as allele 5. Alleles were categorized as allele 2 (the short one) and alleles 1, 3, 4, and 5, the long alleles, as L. Genotype was reported as IL-1RN*L/*L, IL-1RN*L/*2 (allele L/allele2 heterozygote), and IL-1RN*2/*2 (allele 2 homozygote).

H. pylori infection status
Cases were considered to be H. pylori-positive when at least two of the following examinations were positive.

PCR
PCR for the diagnosis of H. pylori infection was performed according to the previously described technique, by using a set of primers (P1 5′-TGGCGTGTCTATTGACAGCGAGC-3′, and P2 5′-CCTGCTGGGCATATCTACCATG-3′) that amplifies a 26-kDa antigen gene (Accession: M55507) present in all the strains of H. pylori. The conditions for amplification were as follows: 94°C for 5 minutes, followed by 40 cycles at 93°C for 1 minute, 57°C for 2 minutes, and 70°C for 2 minutes, thereby resulting in a 298 bp product stained by ethidium bromide on a 1% agarose gel.

Urease test
The biopsy samples taken from the antrum and corpus of the stomach were inserted into the homemade urease test tubes according to the previously described technique. The urease test tube was examined over the next 24 hours.

Histology
Gastric biopsy samples from the antrum and corpus were fixed in 10% formalin and stained with hematoxylin and eosin, and also by Giemsa’s solution (Vetec Química Fina Ltda, Duque de Caxias, RJ, Brazil) for H. pylori identification, and they were scored according to the updated Sydney system of classification and the grading of gastritis.

Statistical analysis
Statistical analysis was performed by R version 2.12.1 (R Core Team, R Foundation for Statistical Computing Vienna, Austria, http://www.R-project.org). A P-value < 0.05 was considered significant. In the cases that were categorized according to the endoscopic findings, age was analyzed by analysis of variance and Tukey’s test, and age was included as a categorical variable, with 45 years as the cutoff value. Categorical variables in cases that were categorized according to the endoscopic findings, such as age, gender, H. pylori status, grading of chronic gastritis, and allele 2, were analyzed by Fisher’s exact test. Categorical variables were subjected to simple multinomial logistic regression and multiple multinomial logistic regressions. Factors associated with H. pylori infection were subjected to binary logistic regression.

Results
Patients with gastric ulcer, duodenal ulcer, and gastric cancer were older than those with normal endoscopy (P < 0.05). Differences in gender distribution were not significant, except in the gastric cancer group. H. pylori infection was significantly more frequent in the inflammation, gastric ulcer, and duodenal ulcer groups than in the normal endoscopy group (Table 1).

The genotypic frequencies of the VNTR IL-1RN polymorphism were the following: 219 individuals (56.2%) had *1/*1 genotype, 103 (26.4%) had *1/*2 genotype, 55 (14.1%) had *2/*2 genotype, four (1%) had *1/*3 genotype, four (1%) had *1/*4 genotype, three (0.7%) had *2/*3 genotype, one (0.3%) had *2/*4 genotype, and one (0.3%) had *4/*4 genotype (data not shown).

The analysis of frequencies of the genotypes, considering alleles 1, 3, and 4 as long allele (L), showed that the differences among the groups and between H. pylori-positive and negative cases were significant (Table 2). Analysis of the frequency of allele 2 according to the histologic type of gastric cancer showed that this value was higher in the

| Endoscopy (n) | Mean age ± 16.7* | Male gender (%) | Helicobacter pylori-positive (%) |
|--------------|-----------------|-----------------|---------------------------------|
| Normal (71)  | 39.0 ± 13.0     | 15 (21.1)       | 41 (57.7)                       |
| Inflammation (196) | 46.6 ± 14.9 | 59 (30.1)       | 144 (73.5)*                     |
| Gastric ulcer (28) | 53.3 ± 14.4*   | 8 (28.6)        | 28 (100)*                       |
| Duodenal ulcer (76) | 45.4 ± 14.8*  | 27 (35.5)       | 73 (96.1)*                      |
| Gastric cancer (19) | 62.4 ± 16.7*    | 11 (57.9)*     | 7 (87.5) of 8 cases             |
| Total         | 390             | 120 (30.8)      | 293 (77.3)                      |

Note: *P < 0.05. Abbreviation: n, number.
Table 2 VNTR IL-1RN polymorphism genotypes among the different groups and Helicobacter pylori infection status (%)

| Groups (total number) | *L/*L | *2/*2 | *L/*2 | All *2 |
|-----------------------|-------|-------|-------|--------|
| Normal (71)           | 36 (50.7) | 12 (16.9) | 23 (32.4) | 35 (49.3) |
| Inflammation (196)    | 108 (55.1) | 32 (16.3) | 56 (28.6) | 88 (44.9) |
| Gastric ulcer (28)    | 18 (64.3) | 1 (3.6) | 9 (32.1) | 10 (35.7) |
| Duodenal ulcer (76)   | 53 (69.7) | 5 (6.6) | 18 (23.7) | 23 (30.3) |
| Gastric cancer (19)   | 13 (68.4) | 5 (26.3) | 1 (5.3) | 6 (31.6) |
| Helicobacter pylori-positive (293) | 171 (58.4) | 30 (10.2) | 92 (31.4) | 122 (41.6) |
| Helicobacter pylori-negative (86) | 48 (55.8) | 23 (26.7) | 15 (17.4) | 38 (44.2) |

Notes: *L/*L, allele 1, 3, or 4; *2, allele 2. All *2, the sum of *L/*2 with *2/*2.

Abbreviations: VNTR, variable number of tandem repeat; IL, interleukin; L, long allele.

diffuse-type than in the intestinal-type, though the difference was not significant (data not shown).

All of the cases were of antral-predominant nonatrophic gastritis, except for one case of gastric ulcer that had atrophic gastritis, and therefore, the analysis was performed considering the histology of the antrum. Chronic gastritis in the disease groups was usually moderate compared to that in the normal endoscopy group (P < 0.05). The cases that were H. pylori negative frequently had no histological inflammation when compared to those with H. pylori infection, which usually presented with a moderate and with marked chronic gastritis (P < 0.05). The carriage of IL-1RN*2/*2 showed a protective effect for mild (OR = 0.43 [95% CI = 0.18–0.99; P < 0.05]) and moderate (OR = 0.41 [95% CI = 0.19–0.88; P < 0.05]) gastritis by simple logistic regression. By multiple multinomial logistic regression, this estimated protective effect was not observed, and H. pylori infection was an independent risk factor for gastric cancer (OR = 0.47 [95% CI = 0.16–1.39; P = 0.05]).

Analysis of the factors for the disease groups and for the H. pylori infection by simple logistic regression showed that H. pylori infection was a significant risk factor for inflammation, gastric ulcer, and duodenal ulcer (Table 3). The carriage of IL-1RN*2/*2 had a significant protective effect against gastric cancer (OR = 0.12 [95% CI = 0.01–0.93; P < 0.05]); however, this gene increased the risk of H. pylori infection (OR = 5.81 [95% CI = 1.06–31.98; P < 0.05]). In contrast, the carriage of IL-1RN*2/*2 had a significant protective effect against H. pylori infection (OR = 0.31 [95% CI = 0.17–0.57; P < 0.05]) and a tendency to protect against duodenal ulcer (OR = 0.35 [0.12–1.04; P = 0.059]). The carriage of allele 2 (the sum of *L/*2 with *2/*2) had a significant protective effect against duodenal ulcer (OR = 0.44 [95% CI = 0.23–0.87; P < 0.05]) (Table 3).

Factors that had a tendency to be significant, or were significant by simple logistic regression for the disease groups, were analyzed by multiple multinomial logistic regression (Tables 4–6). After controlling for confounding factors, H. pylori infection was identified as an independent risk factor for inflammation, duodenal ulcer, and gastric ulcer. The carriage of IL-1RN*2/*2 was an independent risk factor for gastric cancer (OR = 5.81 [95% CI = 1.06–31.98; P < 0.05]); nonetheless, the carriage of allele 2 (IL-1RN*2/*2 plus IL-1RN*L/*2) had an independent protective effect against duodenal ulcer (OR = 0.45 [95% CI = 0.22–0.91]).

Discussion

The acquisition of H. pylori infection occurs early in life, and persists lifelong; however, the infection may spontaneously be

Table 3 Adjusted odds ratio and 95% confidence intervals for the disease groups by simple logistic regression and for Helicobacter pylori infection by binary logistic regression

| Factor               | Duodenal ulcer | Gastric ulcer | Inflammation | Gastric cancer | Helicobacter pylori |
|----------------------|----------------|---------------|--------------|----------------|---------------------|
| Age                  | 2.01 (1.03–3.92)* | 8.17 (2.89–23.08)* | 1.73 (0.98–3.06) | 8.33 (2.47–28.08)* | 1.36 (0.83–2.22) |
| Gender               | 2.05 (0.98–4.33) | 1.49 (0.55–4.05) | 1.60 (0.84–3.06) | 5.16 (1.75–15.15) | 1.37 (0.79–2.38) |
| Helicobacter pylori  | 17.81 (5.08–62.45)* | 129604750.98* | 2.03 (1.15–3.59)* | 0.43 (0.15–1.21) | 2.17 (1.18–3.99)* |
| *L/*2                | 0.65 (0.31–1.34) | 0.99 (0.39–2.54) | 0.84 (0.46–1.5) | 0.12 (0.01–0.93)* | 0.31 (0.17–0.57)* |
| *2/*2               | 0.35 (0.12–1.04) | 0.18 (0.02–1.49) | 0.96 (0.47–1.98) | 1.75 (0.53–5.79) | 0.31 (0.17–0.57)* |
| All *2             | 0.44 (0.23–0.87)* | 0.57 (0.23–1.41) | 0.84 (0.48–1.45) | 0.47 (0.16–1.39) | 0.9 (0.56–1.46) |

Notes: *P < 0.05; 95% confidence interval impossible to calculate; *L, (allele 1, 3, or 4); *2, allele 2; All *2, the sum of *L/*2 and *2/*2.

Abbreviation: L, long allele.
eradicated in young children. Macrolides and penicillins that are used may be associated with the loss of *H. pylori* infection in very young children; nonetheless, other children with no previous usage of antibiotics showed loss of infection. In developing countries with high levels of exposure to *H. pylori* at a young age, some individuals never develop persistent *H. pylori* infection. It is not the high levels of exposure to *H. pylori* that may prevent or clear an established infection. High levels of exposure increases the risk to acquire the infection. Thus, host genetic factors seemed to be involved in *H. pylori* infection persistence.

*H. pylori* infection induces the synthesis of IL-1β that is important in initiating and amplifying the inflammatory response against the bacteria, however, IL-1β may also induce proliferation of gastric epithelial cells that could be involved in carcinogenesis and IL-1β inhibits gastric acid secretion. Low gastric acid secretion, although it may protect against duodenal ulcer, favors the corpus colonization, causing atrophic gastritis that may evolve to gastric carcinoma.

The IL-1 receptor antagonist (IL-1Ra) competitively binds to the IL-1 receptor without activating the target cell, modulating IL-1β effects. The intensity of mucosal damage by the inflammatory response is mediated by a balance between these cytokines. Polymorphism in the second intron of *IL-1RN* (VNTR of 86-bp repeat sequence), resulting in the short allele 2 (*IL-1RN*2), is associated with increased levels of IL-1β.

In this study, the carriage of *IL-1RN*2/*2 had a 31% lower risk of *H. pylori* infection with a higher allelic frequency in those with normal endoscopy, as in the controls of a study conducted in Bogota. To our knowledge, this is the first time that an association of homozygous allele 2 with a lower risk of *H. pylori* infection has been demonstrated, and this finding should be evaluated further. The association between *IL-1RN*2 and chronic inflammatory disorders suggested that the *IL-1RN*2 homozygote with increased proinflammatory immunity would combat microbial infection or colonization more efficiently. This finding was previously reported in the study of vaginal colonization by the mycoplasmas, *Ureaplasma urealyticum* and *Mycoplasma hominis*, and in HIV-infected Brazilian women who had significantly lower levels of HIV-1 RNA in their circulation than in the women with other IL-1Ra genotypes.

El Omar et al demonstrated an increased risk of hypochlorhydria when *IL-1RN*2/*2 was present in the homozygous state, and not in the heterozygous state. In the present study, we observed that the *IL-1RN*2 allele (the sum of *2/*2 plus *L*/2) was associated with a decreased risk of duodenal ulcer, and that the carriage of *IL-1RN*2/*2 was a risk factor for gastric cancer, as was previously described. In Portugal, this was a risk factor for developing intestinal-type gastric carcinoma; in this report, *IL-1RN*2/*2 was more frequently found in diffuse-type gastric carcinoma, although the number of cases was small and the difference between these subtypes was not significant. This finding should be further evaluated in a higher number of gastric cancer cases, which was one of the limitations of this study.

Despite this effect, and different from previous reports, allele 2 had no association with the grading of gastritis of the gastric antrum and with gastric ulcer. *H. pylori* infection was...
demonstrated as an independent risk factor for histological gastritis, inflammation, and peptic ulcer.\textsuperscript{3,4,33,35}

**Conclusions**
Allele 2 of the VNTR IL-1RN polymorphism had a protective effect against duodenal ulcer and *H. pylori* infection; however, this allele increased the risk of gastric cancer.

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**Disclosure**
The authors report no conflicts of interest in this work.

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