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Original Article

Relationship of *interleukin-1B* gene promoter region polymorphism with *Helicobacter pylori* infection and gastritis

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Abstract

Introduction: *Helicobacter pylori* infection is associated with gastritis, peptic ulcer disease and gastric carcinoma. The severity of damage is determined by the interplay between environmental/behavioral factors, bacterial pathogenicity genes and host genetic polymorphisms that can influence the secretion levels of inflammatory cytokines. Accordingly, this study aimed to identify polymorphisms in the *IL-1B* and *IL-1RN* genes and their associations with *H. pylori* infection, *cagA* gene of *H. pylori,* and gastroduodenal diseases.

Methodology: Gastric biopsy samples from 151 patients infected with *H. pylori* and 76 uninfected individuals were analyzed. *H. pylori* infection was diagnosed by histology and PCR. Polymorphisms at positions -511, -31 and +3954 of the *IL-1B* gene were detected by PCR-RFLP, and an analysis of the VNTR polymorphism of the *IL-1RN* gene was performed by PCR.

Results: It was observed that the presence of the T/T genotype at position -511 and the C/C genotype at position -31 were associated with *H. pylori* infection and with an increased risk of gastritis in *H. pylori*-positive patients. Additionally, strains from patients *H. pylori*-positive carrying the *cagA* gene was significantly related with the T/T genotype at position -511 of *IL-1B*. No association of polymorphisms at position +3954 of *IL-1B* and in the *IL-1RN* with *H. pylori* infection and with risk of severe gastric diseases was found.

Conclusions: We demonstrated that polymorphisms in the promoter region of the *IL-1B* gene (at positions -511 and -31) are associated with an enhanced risk of *H. pylori* infection as well as gastritis in *H. pylori*-positive patients.

Key words: *Helicobacter pylori*; genetic polymorphism; *Interleukin-1* gene; *cagA* gene; gastric diseases.

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Introduction

Helicobacter pylori is a bacterium that infects the gastric mucosa of more than half of the world's human population [1]. *H. pylori* infection has been associated with a variety of diseases, such as chronic gastritis, peptic ulcer and gastric carcinoma. However, the majority of people infected with *H. pylori* are asymptomatic, and only 15%–20% of them develop such gastroduodenal diseases [2]. It has been hypothesized that a combination of environmental/behavioral variables, host genetic factors and bacterial pathogenicity genes determine the clinical outcome of *H. pylori* infection and the severity of gastric damage [3].

H. pylori is genetically a highly diverse microorganism, and one of its major pathogenicity factors is the immunogenic protein CagA (cytotoxin-associated gene A), encoded by the *cagA* gene [4,5]. This gene is located at one end of a 40-kb DNA insertion called *cag* pathogenicity island (*cag*PAI), which encodes the components of the type IV secretion system (T4SS) that forms a pilus for the injection of pathogenicity factors into host target cells, including the CagA oncoprotein [6,7]. Patients infected with *cagA*-positive strains show a greater degree of inflammation of the gastric mucosa, severe atrophic gastritis and a higher risk of gastric carcinoma [8,9].

In addition to bacterial factors, the host immune system also appears to play an important role in the pathogenesis of gastroduodenal diseases by regulating the nature and intensity of the inflammatory response to *H. pylori* infection [10]. The inflammatory cells recruited to the gastric mucosa during infection

produce several pro-and anti-inflammatory cytokines, and the secretion levels of these cytokines are influenced by genetic polymorphisms [11,12]. Among these cytokines, it should be noted that interleukin (IL)-1 plays a central role in the regulation of immune and inflammatory responses. IL-1 consists of two pro-inflammatory cytokines, IL-1α and IL-1β, and a naturally occurring anti-inflammatory agent, IL-1 receptor antagonist (IL-1Ra) [13,14]; the genes encoding IL-1α (*IL-1A*), IL-1β (*IL-1B*), and IL-1Ra (*IL-1RN*) comprise a cluster on human chromosome 2q. The balance between IL-1β and IL-1Ra is the deciding factor of the degree of inflammation in local tissues, which has an important role in many diseases [15].

IL-1β, a powerful inhibitor of gastric acid secretion, is up-regulated in the presence of *H. pylori* and plays a major role in initiating and amplifying the inflammatory response to this infection [3,16]. Three single nucleotide polymorphisms (SNPs) associated with high levels of IL-1β secretion have been reported in the *IL-1B* gene: a T-C base transition at position -31 and C-T base transitions at positions -511 and +3954 from the transcriptional start site [17-19]. Increased production of IL-1β in the gastric mucosa might result in the enhanced suppression of gastric acid secretion, as well as enhanced inflammation, allowing the expansion of *H. pylori* colonization from the gastric antrum to the corpus. This event may lead to the development of hypochlorhydria, severe gastric atrophy, intestinal metaplasia, dysplasia and, ultimately, gastric carcinoma [17,20].

IL-1RN competitively binds to IL-1 receptors and counterbalances the potentially injurious pro-inflammatory effects of *IL-1B* [21,22]. *IL-1RN* shows an 86-bp variable number of tandem repeat (VNTR) polymorphism in intron 2, which leads to the presence of 5 different alleles: allele 1 (4 repeats), allele 2 (2 repeats), allele 3 (5 repeats), allele 4 (3 repeats), and allele 5 (6 repeats). The 4-repeat (*IL-1RN**1) and 2-repeat (*IL-1RN**2) alleles are the most common, whereas the other alleles occur at a combined frequency of < 5% [15]. Allele 2 (*IL-1RN**2) is associated with enhanced IL-1β production [23]. Polymorphisms of the *IL-1RN* gene have been associated with hypochlorhydria and with severe gastroduodenal lesions in the presence of *H. pylori* infection [24,25].

Based on this background, the genotyping of bacterial strains and the detection of host gene polymorphisms could be important in the early identification of individuals at a high risk of developing severe gastric disorders. Therefore, this study was performed to determine the frequency of polymorphisms in the *IL-1B* and *IL-1RN* genes in patients from South Brazil, to evaluate the relationship of these polymorphisms with *H. pylori* infection, and to investigate the association of these polymorphisms with gastritis, peptic ulcer disease and gastric carcinoma. Moreover, the relation of these polymorphisms with *cagA* gene of *H. pylori* was analyzed.

Methodology

Subjects and gastric biopsy specimens

The present study included a total of 227 patients (125 women and 102 men, with an average age of 53.4 years): 151 *H. pylori*-positive (23 with normal gastric mucosa, 86 with gastritis, 37 with peptic ulcer, 5 with gastric cancer) and 76 *H. pylori*-negative (15 with normal gastric mucosa, 45 with gastritis, 12 with peptic ulcer and 4 with gastric cancer) patients. Eight biopsy specimens were obtained from each patient. Of these, four were destined for histology (two from the gastric antrum and two from the gastric body), whereas the other four samples were intended for use in polymerase chain reaction (PCR) (two from the gastric antrum and two from the gastric body). *H. pylori* infection was diagnosed by histological examination and detection of the *ureA* and *glmM* genes by PCR, as described below. Patients were considered infected with the bacterium when positive results were obtained by at least two of the three methods and uninfected when the results of all diagnostic tests were negative. The diagnosis of gastroduodenal disease was based on endoscopic and histopathological examinations and established in accordance to the Sydney System Classification.

This work was approved by the Research Ethics Committee of Area Health (FURG - process number 23116.001044/2011-16) and carried out in accordance with the ethical standards outlined in the Helsinki Declaration. Written informed consent was obtained from all patients.

Histological examination, DNA extraction and PCR

The gastric biopsy specimens destined for histology were fixed in 10% formalin after collection and were then stained with Hematoxylin-Eosin (H&E) and Giemsa. Microscopic examination defined the degree of involvement of the gastric mucosa and the presence of *H. pylori*.

The specimens intended for PCR were kept in brain heart infusion Broth with 20% glycerol after

collection and stored at -70°C for further extraction. DNA was extracted using DNAzol Reagent and 10 µg/µL of Proteinase K, as described previously by Fonseca *et al.* [26]. The integrity of the DNA extracted was assessed by amplification of a 110-bases pairs (bp) fragment specific for human β-globin using initiator oligonucleotides and methodology reported in other study [27]. *H. pylori* infection was diagnosed by PCR using two sets of initiator oligonucleotides: UREA1–UREA2, which amplifies a 394-bp fragment corresponding to the *ureA* gene [28]; and GLM/MF-GLM/MR, which amplifies a 140-bp fragment corresponding to the *glmM* gene [29].

Detection of the cagA gene

The *cagA* gene was investigated in the gastric biopsy specimens from *H. pylori* positive patients and was present in the samples from 60 patients. The primers used were cagA1 (5′-ACCCTAGTCGGTAATGGGTTA-3′) and cagA2 (5′-GTAATTGTCTAGTTTCGC-3′) [30]. The PCR was performed as described by Batista *et al*. [31].

Identification of IL-1 gene polymorphisms

Polymorphisms at positions -511, -31 and +3954 of the *IL-1B* gene were detected by restriction fragment length polymorphism (PCR-RFLP).

To investigate polymorphism at position -511, we used the initiator oligonucleotides 5′-CTGCATACCGTATGTTCTCTGCC-3′ (forward) and 5′- GGAATCTTCCCACTTACAGATGG-3′ (reverse) [32]. PCR was performed under the conditions described by Erzin *et al*., with minor modifications [32]. The PCR products were digested with the restriction enzyme *Dde*I and then analyzed by electrophoresis on a 2.5% agarose gel with ethidium bromide staining. The genotypes were designated as follows: C/C - two bands of 140 and 49 bp; C/T - four bands of 140, 109, 49 and 31 bp and T/T - three bands of 109, 49 and 31 bp [32].

The initiator oligonucleotides used to analyze polymorphism at position -31 were 5′-AGAAGCTTCCACCAATACTC-3′ (forward) and 5′-AGCACCTAGTTGTAAGGAAG-3′ (reverse) [33]. The PCR was made as previously reported [34]. The PCR products were digested with the restriction enzyme *Alu*I and then visualized by electrophoresis on a 2% agarose gel with ethidium bromide staining. The genotypes were coded as follows: T/T - two bands of 137 and 102 bp; T/C - three bands of 239, 137 and 102 bp and C/C - a single band of 239 bp [33].

To determine polymorphism at position +3954, the initiator oligonucleotides 5′-GTTGTCATCAGACTTTGACC-3′ (forward) and 5′-TTCAGTTCATATGGACCAGA-3′ (reverse) were used [33]. The PCR was performed under the conditions described by Chiurillo *et al*. [34]. The PCR products were digested with the restriction enzyme *Taq*I and then analyzed by electrophoresis on a 2.5% agarose gel with ethidium bromide staining. The genotypes were designated as follows: C/C - two bands of 135 and 114 bp; C/T - three bands of 249, 135 and 114 bp and T/T - a single band of 249 bp [33].

The analysis of the variable numbers of tandem repeat polymorphism in intron 2 of the *IL-1RN* gene was performed by PCR, followed by electrophoresis on a 2% agarose gel. The initiator oligonucleotides used were 5′-CTCAGCAACACTCCTAT-3′ (forward) and 5′-TCCTGGTCTGCAGGTAA-3′ (reverse) [35]; and the PCR was performed as previously reported [5]. Polymorphism was based on the number of repeats of an 86-bp sequence. The alleles were coded conventionally, as follows: allele 1 = four repeats; allele 2 = two repeats; allele 3 = five repeats; allele 4 = three repeats; and allele 5 = six repeats [35]. For statistical analysis purpose and because of the low frequency of alleles 3, 4 and 5, the alleles were categorized into IL-1*RN**2, which contains at least one allele 2 and non-IL-1*RN**2, which does not contain allele 2 [36].

To verify the results obtained by PCR-RFLP for the *IL-1B* gene, 10% of the samples evaluated for each polymorphism were randomly selected and confirmed by sequencing.

Statistical Analysis

Data typing was carried out on a bank in the Microsoft Excel 2010 program. A consistency analysis, based on the creation and categorization of variables and verification of frequencies, was performed using SPSS version 18.0. To compare between proportions, the Chi-Squared Test or Fisher's Exact Test was used. P-values less than 0.05 were considered to be statistically significant. The prevalence ratio (PR) with 95% confidence interval (CI) was calculated to evaluate the relationship of cytokine gene polymorphisms with gastroduodenal diseases and with *H. pylori* infection.

Results

Genetic polymorphisms in *IL-1B* and *IL-1RN* were investigated in all 227 patients. For polymorphism at position -511 of the *IL-1B* gene, 41.0% (93/227) of the

subjects showed C/C homozygosity, 48.9% (111/227) showed C/T heterozygosity, and 10.1% (23/227) showed T/T homozygosity. With regard to polymorphism at position -31 of *IL-1B*, 39.2% (89/227) of the patients carried the T/T genotype, 47.6% (108/227) the T/C genotype, and 13.2% (30/227) the C/C genotype. Regarding polymorphism at position +3954 of *IL-1B*, C/C homozygosity was found in 63.5% (144/227) of the subjects, C/T heterozygosity in 33.0% (75/227), and T/T homozygosity in 3.5% (8/227). Polymorphism in the *IL-1RN* gene revealed that 53.3% (121/227) of the patients did not contain allele 2 (non-IL-1*RN**2), whereas 46.7% (106/227) contained at least one allele 2 (IL-1*RN**2).

Table 1 shows the frequencies of *IL-1* gene polymorphisms in the presence and absence of *H. pylori* infection. The presence of the T allele (PR = 1.16; $_{95\%}$CI = 1.02-1.32) and the T/T genotype (PR = 1.40; $_{95\%}$CI = 1.09-1.80) at position -511 of the *IL-1B* gene was associated with *H. pylori* infection, as did the presence of the C allele (PR = 1.20; $_{95\%}$CI = 1.06-1.37) and the C/C genotype (PR = 1.48; $_{95\%}$CI = 1.19-1.86) at position -31 of *IL-1B*. With respect to the polymorphism at position +3954 of *IL-1B* and in *IL-1RN*, we did not observe statistically significant relationship of these polymorphisms with *H. pylori* infection.

The distributions of *IL-1* gene polymorphisms in *H. pylori* positive and negative patients with normal gastric mucosa, gastritis, peptic ulcer disease and gastric carcinoma are presented in Table 2. *H. pylori* positive patients and carriers of the T/T genotype at position -511 and the C/C genotype at position -31 showed an increased risk of gastritis (PR = 1.52 and $_{95\%}$CI = 1.11-2.08, PR = 1.60 and $_{95\%}$CI = 1.21-2.13; respectively). No other association was found between *IL-1* polymorphisms and the risk of gastric disease. Despite this, when we analyzed the patients with peptic ulcer disease, the distribution of the T/T genotype at position -511 was higher in the *H. pylori* positive patients (21.6%) compared with the *H. pylori* negative patients (8.4%), as was the distribution of the C/C genotype at position -31 (29.7% *versus* 8.4%) and the distribution of carriers of the *IL-1RN**2 allele (59.5% *versus* 50.0%). Additionally, when we investigated the patients with gastric carcinoma, the frequencies of the T allele at position -511 of *IL-1B*, the C allele at position -31 of *IL-1B* and *IL-1RN**2 allele carriers were higher in patients infected with *H. pylori* compared with non-infected subjects.

Table 1. Frequencies of the polymorphisms of *IL-1* gene in patients *H. pylori* positive and negative

	***H. pylori* positive patients N (%)**	***H. pylori* negative patients N (%)**	**PR ($_{95\%}$CI)**
Alleles ➡ *IL-1B* (-511)			***p* = 0.017[a]**
C	187 (61.9%)	110 (72.4%)	1.0
T	115 (38.1%)	42 (27.6%)	1.16 (1.02-1.32)
Genotypes ➡ *IL-1B* (-511)			***p* = 0.069[b]**
C/C	55 (36.4%)	38 (50.0%)	1.0
C/T	77 (51.0%)	34 (44.7%)	1.17 (0.95-1.45)
T/T	19 (12.6%)	4 (5.3%)	1.40 (1.09-1.80)
Alleles ➡ *IL-1B* (-31)			***p* = 0.004[a]**
T	177 (58.6%)	109 (71.7%)	1.0
C	125 (41.4%)	43 (28.3%)	1.20 (1.06-1.37)
Genotypes ➡ *IL-1B* (-31)			***p* = 0.017[b]**
T/T	52 (34.5%)	37 (48.7%)	1.0
T/C	73 (48.3%)	35 (46.0%)	1.16 (0.93-1.44)
C/C	26 (17.2%)	4 (5.3%)	1.48 (1.19-1.86)
Alleles ➡ *IL-1B* (+3954)			***p* = 0.106 [a]**
C	247 (81.8%)	116 (76.3%)	1.0
T	55 (18.2%)	36 (23.7%)	0.89 (0.74-1.06)
Genotypes ➡ *IL-1B* (+3954)			***p* = 0.311[b]**
C/C	101 (66.9%)	43 (56.6%)	1.0
C/T	45 (29.8%)	30 (39.5%)	0.86 (0.69-1.06)
T/T	5 (3.3%)	3 (3.9%)	0.89 (0.52-1.54)
IL-1RN			***p* = 0.498[a]**
non-IL-1*RN**2	81(53.6%)	40 (52.6%)	1.0
IL-1*RN**2	70 (46.4%)	36 (47.4%)	0.99 (0.82-1.19)

PR: Prevalence ratio; $_{95\%}$CI: 95% confidence interval; [a] *P*-values were determined by Fisher´s Exact Test; [b] *P*-values were determined by Chi-Squared Test.

Table 2. Frequencies of the polymorphisms of *IL-1* gene in patients *H. pylori* positive and negative with normal gastric mucosa and with different gastric disorders

	Normal Gastric Mucosa			Gastritis			Peptic Ulcer Disease			Gastric Carcinoma		
	H. pylori positive patients N (%)	*H. pylori* negative patients N (%)	PR ($_{95\%}$CI)	*H. pylori* positive patients N (%)	*H. pylori* negative patients N (%)	PR ($_{95\%}$CI)	*H. pylori* positive patients N (%)	*H. pylori* negative patients N (%)	PR ($_{95\%}$CI)	*H. pylori* positive patients N (%)	*H. pylori* negative patients N (%)	PR ($_{95\%}$CI)
Alleles *IL-1B* (-511)			***p* = 0.747**[b]			***p* = 0.083**[b]			***p* = 0.468**[b]			***p* = 0.195**[b]
C	29 (63.0%)	20 (66.7%)	1.0	112 (65.1%)	68 (75.6%)	1.0	40 (54.1%)	15 (62.5%)	1.0	6 (60.0%)	7 (87.5%)	1.0
T	17 (37.0%)	10 (33.3%)	1.06 (0.73-1.54)	60 (34.9%)	22 (24.4%)	1.18 (0.99-1.40)	34 (45.9%)	9 (37.5%)	1.09 (0.87-1.36)	4 (40.0%)	1 (12.5%)	1.73 (0.83-3.61)
Genotypes *IL-1B* (-511)			***p* = 0.605**[b]			***p* = 0.157**[b]			***p* = 0.584**[b]			***p* = 0.500**[b]
C/C	8 (34.8%)	7 (46.7%)	1.0	34 (39.5%)	24 (53.3%)	1.0	11 (29.7%)	4 (33.3%)	1.0	2 (40.0%)	3 (75.0%)	1.0
C/T	13 (56.5%)	6 (40.0%)	1.28 (0.73-2.25)	44 (51.2%)	20 (44.5%)	1.17 (0.89-1.54)	18 (48.7%)	7 (58.3%)	0.98 (0.66-1.45)	2 (40.0%)	1 (25.0%)	1.67 (0.44-6.36)
T/T	2 (8.7%)	2 (13.3%)	0.94 (0.32-2.78)	8 (9.3%)	1 (2.2%)	1.52 (1.11-2.08)	8 (21.6%)	1 (8.4%)	1.21 (0.83-1.78)	1 (20.0%)	-	-
Alleles *IL-1B* (-31)			***p* = 0.828**[b]			***p* = 0.029**[b]			***p* = 0.286**[b]			***p* = 0.119**[a]
T	28 (60.9%)	19 (63.3%)	1.0	107 (62.2%)	68 (75.6%)	1.0	37 (50.0%)	15 (62.5%)	1.0	5 (50.0%)	7 (87.5%)	1.0
C	18 (39.1%)	11 (36.7%)	1.04 (0.72-1.51)	65 (37.8%)	22 (24.4%)	1.22 (1.03-1.45)	37 (50.0%)	9 (37.5%)	1.13 (0.90-1.41)	5 (50.0%)	1 (12.5%)	2.0 (0.94-4.27)
Genotypes *IL-1B* (-31)			***p* = 0.940**[b]			***p* = 0.061**[b]			***p* = 0.306**[b]			***p* = 0.242**[b]
T/T	8 (34.8%)	6 (40.0%)	1.0	32 (37.2%)	24 (53.3%)	1.0	11 (29.7%)	4 (33.3%)	1.0	1 (20.0%)	3 (75.0%)	1.0
T/C	12 (52.2%)	7 (46.7%)	1.11 (0.63-1.95)	43 (50.0%)	20 (44.5%)	1.19 (0.90-1.58)	15 (40.6%)	7 (58.3%)	0.93 (0.61-1.41)	3 (60.0%)	1 (25.0%)	3.0 (0.50-17.95)
C/C	3 (13.0%)	2 (13.3%)	1.05 (0.45-2.45)	11 (12.8%)	1 (2.2%)	1.60 (1.21-2.13)	11 (29.7%)	1 (8.4%)	1.25 (0.88-1.73)	1 (20.0%)	-	-
Alleles *IL-1B* (+3954)			***p* = 0.467**[b]			***p* = 0.365**[b]			***p* = 0.491**[b]			***p* = 0.617**[a]
C	37 (80.4%)	22 (73.3%)	1.0	140 (81.4%)	69 (76.7%)	1.0	63 (85.1%)	19 (79.2%)	1.0	7 (70.0%)	6 (75.0%)	1.0
T	9 (19.6%)	8 (26.7%)	0.84 (0.52-1.38)	32 (18.6%)	21 (23.3%)	0.90 (0.71-1.14)	11 (14.9%)	5 (20.8%)	0.89 (0.63-1.27)	3 (30.0%)	2 (25.0%)	1.11 (0.46-2.67)
Genotypes *IL-1B* (+3954)			***p* = 0.761**[b]			***p* = 0.380**[b]			***p* = 0.334**[a]			***p* = 0.785**[a]
C/C	15 (65.2%)	8 (53.3%)	1.0	57 (66.3%)	25 (55.6%)	1.0	26 (70.3%)	7 (58.3%)	1.0	3 (60.0%)	3 (75.0%)	1.0
C/T	7 (30.4%)	6 (40.0%)	0.83 (0.46-1.48)	26 (30.2%)	19 (42.2%)	0.83 (0.62-1.11)	11 (29.7%)	5 (41.7%)	0.87 (0.60-1.27)	1 (20.0%)	-	-
T/T	1 (4.4%)	1 (6.7%)	0.77 (0.18-3.16)	3 (3.5%)	1 (2.2%)	1.08 (0.60-1.93)	-	-	-	1 (20.0%)	1 (25.0%)	1 (0.20-4.95)
IL-1RN			***p* = 0.376**[b]			***p* = 0.668**[b]			***p* = 0.565**[b]			***p* = 0.357**[a]
non-IL-1*RN**2	11 (47.8%)	5 (33.3%)	1.0	53 (61.6%)	26 (57.8%)	1.0	15 (40.5%)	6 (50.0%)	1.0	2 (40.0%)	3 (75.0%)	1.0
IL-1*RN**2	12 (52.2%)	10 (66.7%)	0.79 (0.48-1.31)	33 (38.4%)	19 (42.2%)	0.95 (0.73-1.22)	22 (59.5%)	6 (50.0%)	1.10 (0.79-1.53)	3 (60.0%)	1 (25.0%)	1.88 (0.56-6.31)

PR: Prevalence ratio; $_{95\%}$CI: 95% confidence interval; [a] *P*-values were determined by Fisher´s Exact Test; [b] *P*-values were determined by Chi-Squared Test.

The relationship between the *cagA* gene of *H. pylori* and polymorphisms in *IL-1* was also evaluated (Table 3). Strains from patients *H. pylori* positive carrying the *cagA* gene was significantly associated with the T/T genotype at position -511 of *IL-1B* (p = 0.046). No other genotype of *IL-1* was related with the *cagA* gene.

Discussion

The frequencies of polymorphisms in the *IL-1B* and *IL-1RN* genes found in this study were similar to those described in European populations [37,38]. Genetic polymorphisms involved in the regulation of gastric acid secretion may to be associated with host susceptibility to the acquisition of *H. pylori* infection and to the development of gastric pathologies [18,36].

In this study, we observed that polymorphisms in the coding region of *IL-1B* (at position +3954) and in *IL-1RN* were not related to *H. pylori* infection. These finding are in agreement with those reported previously [10,39]. However, we did find that the presence of the T allele and the T/T genotype at position -511 as well as the C allele and the C/C genotype at position -31 were associated with *H. pylori* infection.

The presence of the T allele at position -511 and C allele at position -31 is associated with high levels of IL-1β [18]. IL-1 is a powerful inhibitor of gastric acid secretion; on a molar basis, IL-1 is estimated to be 100-fold more potent than proton pump inhibitors and 6000-fold more potent than H_2 receptor antagonists [16,40,41]. The decrease of the acid secretion causes hypochlorhydria, which favors *H. pylori* infection because the secretion of acid is an important host defense mechanism against bacterial colonization in the stomach. In addition, a reduction in the flow of gastric secretion may heighten mucosal damage by allowing the accumulation of bacterial toxins and the products of inflammation that would normally be diluted and flushed out [12,36,42].

The present work showed that *H. pylori* positive patients and carriers of genotypes T/T at position -511 and C/C at position -31 have an increased risk of gastritis. The presence of these genotypes appears to increase the levels of IL-1β and consequently inhibit gastric acid secretion and amplify the inflammatory response to *H. pylori* infection, which results in severe inflammation. This inflammation may lead to

Table 3. Frequencies of the polymorphisms of *IL-1* gene in *cagA*-positive and *cagA*-negative patients.

	***cagA*-positive patients N (%)**	***cagA*-negative patients N (%)**	**PR ($_{95\%}$CI)**
Alleles ➡ *IL-1B* (-511)			***p* = 0.248[a]**
C	71 (59.2%)	116 (63.7%)	1.0
T	49 (40.8%)	66 (36.3%)	1.12 (0.85-1.49)
Genotypes ➡ *IL-1B* (-511)			***p* = 0.046[b]**
C/C	23 (38.3%)	32 (35.2%)	1.0
C/T	25 (41.7%)	52 (57.1%)	0.78 (0.50-1.22)
T/T	12 (20.0%)	7 (7.7%)	1.51 (0.95-2.40)
Alleles ➡ *IL-1B* (-31)			***p* = 0.249[a]**
T	67 (55.8%)	110 (60.4%)	1.0
C	53 (44.2%)	72 (39.6%)	1.12 (0.85-1.48)
Genotypes ➡ *IL-1B* (-31)			***p* = 0.213[b]**
T/T	21 (35.0%)	31 (34.1%)	1.0
T/C	25 (41.7%)	48 (52.7%)	0.85 (0.54-1.34)
C/C	14 (23.3%)	12 (13.2%)	1.33 (0.82-2.17)
Alleles ➡ *IL-1B* (+3954)			***p* = 0.342[a]**
C	100 (83.3%)	147 (80.8%)	1.0
T	20 (16.7%)	35 (19.2%)	0.90 (0.61-1.31)
Genotypes ➡ *IL-1B* (+3954)			***p* = 0.655[b]**
C/C	41 (68.3%)	60 (65.9%)	1.0
C/T	18 (30.0%)	27 (29.7%)	0.99 (0.64-1.51)
T/T	1 (1.7%)	4 (4.4%)	0.49 (0.08-2.89)
IL-1RN			***p* = 0.075[a]**
non-IL-1*RN**2	37 (61.7%)	44 (48.4%)	1.0
IL-1*RN**2	23 (38.3%)	47 (51.6%)	0.72 (0.48-1.08)

PR: Prevalence ratio; $_{95\%}$CI: 95% confidence interval; [a] *P*-values were determined by Fisher´s Exact Test; [b] *P*-values were determined by Chi-Squared Test.

continuous injury to the tissue structure and function and to the development of gastroduodenal diseases of higher severity [16,18,43,44].

Interestingly, we did not observe significant association between IL-1 polymorphisms and increased risk of peptic ulcer disease and gastric carcinoma in the presence of *H. pylori* infection, possibly because of a limitation of this study, which had a low number of patients diagnosed with these disorders. Despite this fact, when we analyzed patients with peptic ulcer disease and gastric carcinoma, the frequency of the T/T genotype at position -511 of *IL-1B* was higher in *H. pylori* positive patients compared with *H. pylori* negative patients, as was the frequency of the C/C genotype at position -31 of *IL-1B* and the frequency of carriers of the 2 allele of *IL-1RN*. Previous studies have already demonstrated a positive relationship between *IL-1B* and *IL-1RN* gene polymorphisms and the development of peptic ulcer disease and gastric carcinoma in *H. pylori*-positive patients [22,45,46].

In this research, the relationship between *cagA* gene and *IL-1* polymorphisms was also analyzed because *cagA* positive strains induce higher levels of IL-1 expression than do *cagA* negative strains [47]. We found that strains from *H. pylori* positive patients that carried the *cagA* gene were significantly associated with the T/T genotype at position -511, suggesting a synergic interaction between the bacterium and host polymorphism, with more severe damage of the gastric mucosa [48].

Conclusions

We demonstrated that polymorphisms in the promoter region of the *IL-1B* gene (at positions -511 and -31) are significantly associated with *H. pylori* infection. Additionally, *H. pylori* positive patients and carriers of the T/T genotype at position -511 and the C/C genotype at position -31 have an increased risk of gastritis but not of peptic ulcer disease and gastric carcinoma, possibly because of the low number of patients diagnosed with ulcer and carcinoma, a limitation of this study. Nonetheless, it is worth noting that in the disorders that precede the development of gastric carcinoma, there is a marked decrease in the colonization of the gastric mucosa by *H. pylori*, which may lead to false negative results in infected patients who develop gastric carcinoma.

More extensive studies investigating polymorphisms in other cytokines with individuals from different ethnicities are required to understand the complex interplay between host and microorganism in the development of gastric disease. The identification of host biomarkers related to protection against or induction of gastric disorder will be able to allow a better prognosis for *H. pylori* infection.

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Authors' contributions

All authors contributed equally to this study.

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