Clin Exp Immunol 2003; **134**:525–531 doi:10.1046/j.1365-2249.2003.02325.x

Association of interleukin 1 gene family polymorphisms with duodenal ulcer disease

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(Accepted for publication 7 October 2003)

SUMMARY

Cytokine genes taking part in the immunological response to *Helicobacter pylori* infection are good candidates to study for genetic predisposition to duodenal ulcer disease (DU). Among cytokines, interleukin (IL)-1β and its natural specific inhibitor, the interleukin-1 receptor antagonist, are cytokines that play a key role in regulating gastric acid secretion and modulating the immune response in the gastrointestinal mucosa. We aimed to investigate whether polymorphisms in the *IL-1B* and *IL-1RN* genes are involved in the susceptibility to duodenal ulcer. DNA from 131 unrelated Spanish Caucasian patients with DU and 105 ethnically matched healthy controls was typed for the *IL-1B*-511, *IL-1B*-31, and *IL-1B* + 3954 gene polymorphisms, and the VNTR polymorphism in intron 2 of the *IL-1RN* gene by polymerase chain reaction (PCR)-based methods and *Taq*Man assays. *H. pylori* status and non-steroidal anti-inflammatory drugs (NSAIDs) use was determined in all patients and controls. Logistic regression analysis identified *H. pylori* infection (OR: 9·74; 95%CI = 3·53–26·89) and NSAIDs use (OR: 8·82; 95%CI = 3·51–22·17) as independent risk factors for DU. In addition, the simultaneous carriage of *IL-1RN*2*, *IL-1B*-511**C*, *IL-1B*-31**T* and *IL-1B* + 3954**C* alleles was a genetic risk factor for DU in patients with *H. pylori* infection (OR: 3·22; 95%CI = 1·09–9·47). No significant differences in *IL-1RN* and *IL-1B* genotypes were found when patients were categorized according to gender, age of onset, smoking habit, NSAIDs use, type of complication and positive family history. Our results provide further evidence that host genetic factors play a key role in the pathogenesis of duodenal ulcer.

Keywords cytokine duodenal ulcer *Helicobacter pylori* polymorphism NSAIDs

INTRODUCTION

Helicobacter pylori infection is the major environmental factor involved in the pathogenesis of duodenal ulcer (DU). It is estimated that this organism colonizes more than half the world population. However, it is still unknown why only a small proportion of infected individuals develop the disease. A large number of studies have focused on the role of *H. pylori* as the key factor in initiating the inflammatory response in the gastric mucosa. Infection with $CagA^+$ and $VacA^+$ strains is associated with enhanced epithelial cell injury and a higher degree of gastric inflammation in peptic ulceration [1–5]. However, none of the currently identified bacterial virulence factors seem to be disease specific. In fact, both $CagA^+$ and $VacA^+$ strains have been equally associated with such divergent diseases as gastric cancer and duodenal ulcer disease [6–9]. In these cases, it seems reasonable to consider the influence of host factors as key determinants to explain the varying clinical presentations of *H. pylori* infection.

Among host factors, the immune system might play an important role in the pathogenesis of duodenal ulcer by controlling the nature and intensity of the inflammatory response to *H. pylori* infection. The inflammatory reaction is characterized by an infiltration of polymorphonuclear neutrophils, lymphocytes, macrophages and plasma cells into the gastric mucosa [10,11]. Persistent inflammation with the subsequent release of toxic metabolites and lysosomal enzymes might lead to local tissue injury, but weak responses might favour uncontrolled organism growth [12]. The main biological mediators in this immune-inflammatory response are cytokines, low-molecular-weight peptide molecules produced by a large variety of cells that possess a

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broad range of physiological functions. Among cytokines, interleukin (IL)-1 is a proinflammatory cytokine that plays a key role in initiating and amplifying the immune-inflammatory response to *H. pylori* infection. The interleukin-1 family involves two agonist polypeptides (IL-1α and IL-1β) and the interleukin-1 receptor antagonist (IL-1ra). IL-1α and IL-1β bind to the IL-1 receptor type I, eliciting signal transduction. IL-1ra blocks the binding of IL-1 to receptors acting as a competitive inhibitor of IL-1/IL–1R interactions [13]. IL-1β is up-regulated in the presence of *H. pylori*. Several studies have reported high levels of IL-1β in the gastric mucosa of *H. pylori*-infected patients with gastritis and duodenal ulcer disease [14,15]. In the stomach, IL-1β is known to be a cytoprotective agent, and is involved in reducing gastric damage induced by a wide variety of ulcerogenic agents such as ethanol and non-steroidal anti-inflammatory drugs (NSAIDs) [16,17]. In addition, IL-1β is the most powerful inhibitor of gastric acid and pepsinogen secretion [18,19]. Takashima *et al*., using the Mongolian gerbil model of *H. pylori* infection, have shown that decreased acid secretion produced by *H. pylori* infection is associated with enhanced expression of IL-1β mRNA levels in the gastric mucosa, and that this effect is significantly restored by the administration of IL-1ra [20]. Moreover, some studies suggest a direct inhibitory effect of IL-*1*β on parietal cells as well as an indirect effect by stimulating the synthesis of prostaglandins E_2 [19,21,22].

The significant role of IL-1β in the regulation of acid secretion and cytoprotection of the gastrointestinal mucosa has brought substantial interest in studying the genes that encode the IL-1 family proteins. *IL-1 A*, *IL-1B* and *IL-1RN* genes are clustered located on the long arm of human chromosome 2 (2q13–14) [23]. Several polymorphisms, such as a variable number of an 86-base pair (bp) tandem repeat polymorphism (VNTR) in intron 2 of the *IL-1RN* gene [24] and three bi-allelic restriction fragment length polymorphisms (RFLP) at positions –511, –31, and + 3954 of the *IL-1B* gene [25,26], have been reported to be related to variations in the production levels of IL-1β and IL-1ra [26–29]. Furthermore, these polymorphic genes have been related to a wide number of chronic inflammatory and autoimmune diseases such as multiple sclerosis [30,31], inflammatory bowel diseases [32,33] and rheumatoid arthritis [34].

In recent years, some fascinating studies have reported the association of specific variants of the *IL-1* gene cluster with *H. pylori*-related diseases. El-Omar and coworkers described the association of the proinflammatory genotypes *IL-1B*-31*T and *IL-1RN**2/*2 with a chronic hypochlorhydric response to *H. pylori* infection and increased risk of developing gastric cancer [35]. Machado *et al*. provided further support to this association in patients with intestinal-type gastric carcinoma. However, no significant relationship was observed regarding patients with diffuse-type and atypical carcinomas [36]. According to these findings we could then speculate that those genotypes, which predispose to gastric cancer through producing hypochlorhydria, would be protective against duodenal ulcer disease, regarded traditionally as disease induced by high acid production. We have reported previously, in a study carried out in a population of peptic ulcer patients from the North of Spain, a strong association of *IL-1B* + 3954 and *IL-1RN* gene polymorphisms in a subgroup of patients with duodenal ulcers [37]. Interestingly, no association of these polymorphic genes was found in gastric ulcer patients and in controls, suggesting a difference in the immunogenetic background for suffering from gastric or duodenal ulcer.

Because the state of knowledge about the involvement of cytokine genes in the pathogenesis of duodenal ulcer is still at an early stage, the aim of our study was to determine the genotype and allele frequencies of the *IL-1B*-511, *IL-1B*-31, *IL-1B* + 3954 and *IL-1RN* gene polymorphisms in a Spanish Caucasian population of duodenal ulcer patients and healthy controls, and to assess whether these genes are involved in the susceptibility to and final outcome of duodenal ulcer disease.

MATERIALS AND METHODS

Subjects

The subjects in this study included 131 unrelated Spanish Caucasian patients (100 males, 31 females; mean age 47 years) with duodenal ulcer disease (DU) attending the Hospital Clínico Universitario Lozano Blesa in Zaragoza, Spain, and 105 ethnically matched healthy volunteers (72 males, 33 females; mean age 47 years) without active or past peptic ulcer history (HC). The healthy control group comprised blood donors and healthy volunteers who did not have any symptoms of gastrointestinal disease. The diagnosis of DU and the presence of complications were established on the basis of conventional clinical and endoscopic findings. Endoscopy was not performed in the group of healthy individuals because, apart from ethical reasons, the probability of finding an active ulcer in patients without symptoms is very low. All subjects gave informed consent to the study, which was conducted in accordance with the Ethical Committee of the hospital. *H. pylori* status and NSAID use were studied in all patients and controls either at the time of the diagnosis or recruitment. Duodenal ulcer patients with gastrointestinal bleeding taking either aspirin or NSAIDs suffered from osteoarthritis, cardiovascular disease or had taken the drug over-the counter as pain killer medication.

H. pylori diagnosis

The presence of *H. pylori* infection was determined in patients by both, urease test (CLO-test; Delta West Ltd, Canning Vale, Bentley, Australia) and histological examination from biopsies taken at the antrum and corpus of the stomach during the endoscopic procedure. In addition, a ^{13}C-urea breath test (Isomed, Madrid, Spain) was performed in all patients who were negative by invasive methods. In controls, the presence of *H. pylori* was diagnosed by ^{13}C-urea breath test, and serology using a commercial IgG ELISA kit (Plate Helicobacter IgG, Roche; Cortesec Diagnostics Ltd, Clwyd, UK). Both tests have been validated in our area [38,39] and controls were considered positive for the *H. pylori* infection if any one of the two tests was positive for the infection.

In order to determine whether patients of a particular *IL-1B* or *IL-1RN* genotype were infected preferentially by specific *H. pylori* strains, a subset of individuals with *H. pylori* infection selected at random (49 of 78 in controls, and 48 of 125 in DU patients) were analysed further to determine in serum the presence of antibodies to CagA and/or to VacA by Western blot analysis (Bioblot Helicobacter, Biokit SA, Barcelona, Spain). All serum samples were aliquoted and stored at –70°C until analysis.

NSAIDs use

The use of NSAIDs at the time of the diagnosis of duodenal ulcer was determined by structured data collection. A patient was

considered positive if the drug had been taken within the week prior to the hospital admission of the endoscopic diagnosis of duodenal ulcer [40].

IL-1RN and IL-1B genotyping

Genomic DNA was extracted from peripheral blood leucocytes according to a conventional proteinase-K digestion and phenol/chloroform procedure [41].

The region containing the polymorphism at position −31 in the TATA sequence of the *IL-1B* promoter was analysed by 5′ nuclease assay (*Taq*Man) [35]. Polymerase chain reaction (PCR) fragments were generated using the oligonucleotides 5′-CCCTTTCCTTTAACTTGATTGTGA-3′ and 5′-GGTTTG GTATCTGCCAGTTTCTC-3′ as primers. Probes (PE Applied Biosystems) for the T or the C allele were 5′ labelled with either FAM (6-carboxyfluoresceine) or VIC fluorogenic dyes and 3′- labelled with TAMRA (6-carboxytetramethylrhodamine) quencher dye (PE Applied Biosystems, Foster City, CA, USA). The PCR reaction mixture (25 μl) contained 50 ng of genomic DNA in 1 × *Taq*Man Universal Master Mix (PE Applied Biosystems), 200 nM of each probe and 900 nM of each primer. PCR amplification was carried out in a thermal cycler Perkin-Elmer 9700 (PE Applied Biosystems) according to the following parameters: 50°C for 2 min, 95°C for 10 min and 40 cycles of 95°C for 15 s and 62°C for 1 min. In each amplification, a sample containing the PCR cocktail with sterile high performance liquid chromatography (HPLC) water instead of genomic DNA was used as a negative PCR control. PCR end-point analysis was performed in an ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems).

The fragment containing the *Ava* I polymorphic site at position −511 in the *IL-1B* gene [25] was amplified using the oligonucleotides 5′-TGGCATTGATCTGGTTCATC-3′ and 5′-GTTTAGGAATCTTCCCACTT-3′ as primers. PCR conditions were as follows: 94°C for 1 min followed by 30 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min, and a final elongation at 72°C for 10 min. PCR products were digested with 3 units of *Ava* I for 5 h at 37°C and fragments were analysed in 2% agarose gels stained with ethidium bromide. The digestion resulted in fragments that either remained intact (allele T) or were cut into two fragments of 190 and 115 base pairs (bp) (allele C).

The fragment containing the *Taq* I polymorphic site within exon 5, at position + 3954 of the *IL-1B* gene [26] was amplified by PCR using the following oligonucleotides: 5′- GTTGTCATCA GACTTTGACC-3′ and 5′-TTCAGTTCATATGGACCAGA-3′. PCR conditions were as follows: 94°C for 60 s, 55°C for 90 s and 72°C for 60 s for three cycles followed by 32 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 30 s, and a final elongation at 72°C for 10 min. PCR products were digested with 2 units of *Taq* I for 5 h at 65°C and resulted in an intact fragment of 249 bp (allele T) or in two fragments of 135 and 114 bb (allele C).

The penta-allelic variable number of an 86-bp VNTR in intron 2 of the *IL-1RN* gene was analysed by PCR according to methods described previously [33]. Briefly, the oligonucleotides 5′-CTCAGCAACACTCCTAT-3′ and 5′-TCCTGGTCTGCAG GTAA-3′ were used as primers. The PCR program was: an initial denaturation of 94°C for 1 min followed by 35 cycles of 94°C for 1 min, 60°C for 1 min and 72°C for 1 min, and a final elongation of 72°C for 5 min. The PCR products of 410 bp (allele1 = four repeats of the 86-bp region), 240 bp (allele 2 = two repeats), 500 bp (allele 3 = five repeats), 325 bp (allele 4 = three repeats) and 595 bp (allele 5 = six repeats) were analysed by electrophoresis on 2% agarose gels stained with ethidium bromide.

Statistical analysis

Bivariate analysis was performed by χ^2 test with Yates's correction for the comparison of phenotype frequencies between different groups of patients and healthy controls. Hardy–Weinberg equilibrium was tested in the control samples by a χ^2 test with 1 degree of freedom (d.f.). The strength of the association between *IL-1* gene polymorphisms in each group was estimated by the odds ratio (OR), and the 95% confidence intervals (CI) after performing Fisher's exact test (2 × 2 contingency tables). A two-sided *P*-value < 0·05 was considered statistically significant. Estimated haplotype frequencies and linkage disequilibrium coefficients for each pair of *IL-1* loci in patients and controls were calculated using the estimating haplotype frequencies (EH) software program (available from ftp://linkage.rockefeller.edu/software/eh).

Unconditional logistic regression analysis was carried out using the BMDP Dynamic version 7·0 program (BMDP Statistical Software Inc., Cork, Ireland) to quantify the influence of both genetic and environmental factors on duodenal ulcer disease. Starting with age and sex, the model was constructed with a stepwise forward conditional method. A variable was entered into the model if the significance level of its coefficient was less than 0·05 and was removed if it was greater than 0·10. Sex and age variables were present in all the models. Categorical variables included in the models were codified as dummy variables. Interaction terms were also tested in the different models.

Based on a previous study performed in a Spanish population of patients with peptic ulcer [37], we estimated that it was necessary to study 102 subjects in each group in order to have 0·80 power and an α value of 0·05.

RESULTS

Clinical and demographic characteristics of patients and controls

Clinical and demographic characteristics of duodenal ulcer patients and controls are given in Table 1. *H. pylori* infection was significantly more frequent in DU (95.%) patients than in controls (74%) (OR: 7·21, 95% CI = 2·85–18·25) and it was distributed equally among males and females in all study groups. NSAID use was also more frequent in DU patients than in controls (33·6% *versus* 7·7%) (OR: 6·13, 95% CI = 2·74–13·74). Seventy-eight patients with DU (61% of the total DU group) had experienced an episode of gastrointestinal bleeding, which was associated with NSAID use in 51% of cases. However, the frequency of *H. pylori* in this population was very high and similar to that observed in the whole group (96·1% *versus* 95%).

Serum antibodies against CagA and VacA *H. pylori* antigens were determined in a subset of infected patients and controls selected at random (49 of 78 *H. pylori* positive controls, and 48 of 125 *H. pylori* positive DU patients). CagA positive *H. pylori* strains were distributed similarly in infected patients, regardless of sex and age, and a similar distribution was found in infected controls. CagA positive strains were significantly more frequent in DU patients than in controls (70·8% *versus* 36·7%) (OR: 4·18, 95% CI = 1·78–9·80). VacA positive strains were also more frequent in DU patients than in controls (47·9% *versus* 28·6%), although the difference did not reach statistical significance (*P* = 0·08).

IL-1RN and IL-1B gene polymorphisms

Genotypes and allele frequencies of the *IL-1RN* and the *IL-1B*-511, *IL-1B*-31 and *IL-1B* + 3954 gene polymorphisms in patients and controls according to their *H. pylori* status are shown in Table 2. Genotype frequencies of these polymorphisms did not deviate significantly from Hardy–Weinberg expectation in the control group. The *IL-1B*-31 locus was in complete linkage disequilibrium with the *IL-1B*-511 locus (–511T/–31C and –511C/–31T) in both control and DU groups. Therefore, we have restricted the analysis to the *IL-1B*-511 locus. There was also strong linkage disequilibrium between *IL-1B*-511 and *IL-1RN* in both study groups (D′ = 0·39 in HC and D′ = 0·21 in DU) and between *IL-1B*-511 and *IL-1B* + 3954 (D′ = – 0·47 in HC and D′ = – 0·48 in DU). However, the two groups differed in relation to LD between *IL-1B* + 3954 and *IL-1RN*. While no significant LD was found in the control population, LD in DU patients was strong (D′ = –0·80). There were no significant differences in carriage rate, genotype and allele frequencies of the *IL-1RN* and the *IL-1B* gene polymorphisms between DU patients and controls when they were singly analysed. When considering the *H. pylori* status, no significant differences were found between *H. pylori*-infected and non-infected individuals. Moreover, no differences in genotype with respect to NSAIDs use were found between DU patients. However, when we analysed possible associations between specific alleles of the *IL-1B* and *IL-1RN* genes we found that the simultaneous carriage of *IL-1RN*2*, *IL-1B*-511**C*, *IL-1B*⁻ 31**T* and *IL-1B* + 3954**C* alleles was increased significantly in *H. pylori*-positive DU patients who did not take NSAIDs when compared to *H. pylori* infected healthy controls (OR: 3·70, 95% CI = 1·16–11·78) (Table 3). The CagA/VacA status within the DU and HC groups was not linked to the genotype distribution or allele frequency of *IL-1RN* and *IL-1B* gene polymorphisms (data not shown).

Of the environmental and genetic factors evaluated in this study, and after controlling for confounding factors, logistic regression analysis identified *H. pylori* infection and NSAIDs use as independent risk factors for duodenal ulcer disease. The simultaneous carriage *IL-1RN*2*, *IL-1B*-511**C*, *IL-1B*-31**T* and *IL-1B* + 3954**C*, was also an independent risk factor (regardless of *H. pylori* status), associated with increased risk of suffering from duodenal ulcer disease (OR: 3·46, 95% CI = 1·25–9·56) (Table 4). Because the vast majority of the duodenal ulcer patients included in our study were infected by *H. pylori* (125 of 131 patients), we performed additional logistic regression analysis restricted to those *H. pylori* positive patients. This analysis showed that the carriage of *IL-1RN*2*, *IL-1B-31*T*, *IL-1B-511*C* and *IL-1 + 3954*C* was also an independent risk factor (OR: 3·22; 95% CI = 1·1–9·5) in this population, which was in addition to that intrinsic risk associated with the infection (OR: 9·74; 95% CI = 3·5–26·9).

Table 1. Demographics and clinical characteristics of healthy controls and duodenal ulcer patients

	Healthy controls *n* = 105 (%)	Duodenal ulcer *n* = 131 (%)
Age year ± s.d. (range)	47·6 ± 18·9 (20–83)	47·5 ± 13·2 (17–75)
Sex	72 M (68·6%)	100 M (76·3%)
	33 F (31·4%)	31 F (23·7%)
H pylori +	78 (74·3%)	125 (95·4%)*
CagA +	18/49 (36·7%)	34/48 (70·8%)**
VacA +	14/49 (28·6%)	23/48 (47·9%)
CagA+/VacA +	11/49 (22·4%)	19/48 (39·6%)
NSAID-use	8 (7·7%)	44 (33·6%)*
Cigarette smoking	36 (34·3%)	48 (36·7%)
Family history-ulcer	12 (11·4%)	40 (30·5%)**
Past ulcer history	–	59 (45%)
GI bleeding history	–	78 (61·2%)
H pylori⁺		75/78 (96·1%)
NSAID⁺		40/78 (51·3%)

n = Number of patients; M = male; F = female. **P* < 0·001, ***P* < 0·05 *versus* healthy controls.

Table 2. Genotype and allele frequencies of *IL-1RN* and *IL-1B* gene polymorphisms in HC and DU patients according to their *H. pylori* status

		HC Hp^{neg} *n* = 27		HC Hp^{pos} *n* = 78		DU Hp^{pos} *n* = 125	
Polymorphism	Genotype	*n* (%)	A.F.	*n* (%)	A. F.	*n* (%)	A.F.
IL-1B–31	T/T	14 (51·8)	0·70	38 (48·7)	0·67	62 (49·6)	0·71
	C/T	10 (37·1)		28 (35·9)		53 (42·4)	
	C/C	3 (11·1)	0·30	12 (15·4)	0·33	10 (8)	0·29
IL-1B–511	C/C	14 (51·8)	0·70	38 (48·7)	0·67	62 (49·6)	0·71
	T/C	10 (37·1)		28 (35·9)		53 (42·4)	
	T/T	3 (11·1)	0·30	12 (15·4)	0·33	10 (8)	0·29
IL-1B + 3954	C/C	17 (63)	0·81	49 (62·8)	0·79	77 (61·6)	0·78
	C/T	10 (37)		25 (32)		41 (32·8)	
	T/T	–	0·19	4 (5·2)	0·21	7 (5·6)	0·22
IL-1RN	1/1	14 (51·8)	0·70	42 (53·8)	0·71	61 (48·8)	0·70
	1/2	10 (37)		26 (33·4)		47 (37·6)	
	2/2	3 (11·2)	0·30	8 (10·2)	0·28	9 (7·2)	0·27
	2/3	–		1 (1·3)		2 (1·6)	
	1/3, 4, 5	–		1 (1·3)	0·1	6 (4·8)	0·3

n = Number of individuals. A.F = Allele frequency. Six DU patients were not infected with *H. pylori*. Their genotype results are not shown in the table.

Table 3. Frequencies of simultaneous carriage of alleles *IL-1RN**2, IL-1B* + 3954*T and *IL-1B*-511*T in controls and duodenal ulcer patients after stratification for NSAIDs intake in *H. pylori* positive patients (*Hp* pos)

IL-1RN*2 Carriership	IL-1B + 3954*T Carriership	IL-1B-511*T Carriership	Controls *Hp* pos $n = 78$ n (%)	Duodenal ulcer *Hp* pos nsaids pos $n = 41$ n (%)	Duodenal ulcer *Hp* pos nsaids neg $n = 84$ n (%)
–	–	+	11 (14·1)	6 (16·3)	8 (9·5)
–	–	–	15 (19·2)	9 (21·9)	9 (10·7)
–	+	+	5 (6·4)	6 (14·6)	9 (10·7)
–	+	–	13 (16·6)	5 (12·2)	16 (19)
+	–	+	18 (23·1)	9 (21·9)	20 (23·8)
+	–	–	4 (5·1)	2 (4·9)	14 (16·7)
+	+	+	6 (7·7)	3 (7·3)	2 (2·4)
+	+	–	6 (7·7)	1 (2·4)	6 (7·1)

+ = Carrier; – = non-carrier; n = number of individuals.

Table 4. Adjusted odds ratios and 95% confidence intervals of duodenal ulcer associated with different environmental and genetic factors by logistic regression

Factor	OR	95% (CI)	*P*-value
H. pylori infection	9·74	3·53–26·89	<0·001
NSAIDs use	8·82	3·51–22·17	<0·001
Carriage of *IL-1RN**2, $IL\text{-}1B^{-31}$*T, $IL\text{-}1B^{-511}$*C and $IL\text{-}1B^{+3954}$*C	3·46	1·25–9·56	0·017

Age and sex were included in the model.

Finally, no significant differences in carriage rate, genotype and allele frequencies of the *IL-1RN* and the *IL-1B* gene polymorphisms were found when patients were categorized according to gender, age of onset, smoking habit, past ulcer history, positive family history, type of complication (stenosis, perforation and namely gastrointestinal bleeding), number of bleeding episodes, recurrence of the ulcer and need for surgical treatment (data not shown).

DISCUSSION

It is now well accepted that the clinical outcome of *H. pylori* infection is most probably the result of complex interactions among host, bacteria and environmental factors. In spite of the strong clinical association of *H. pylori* infection and duodenal ulcer [42,43], no disease specificity has been shown to date by any of the currently identified virulence factors. This highlighted the need to study the importance of host genetic factors in the development and final outcome of the disease.

In this study we investigated the role of *IL-1RN*, *IL-1B*-511, *IL-1B*-31 and *IL-1B* + 3954 gene polymorphisms in the susceptibility to and final outcome of duodenal ulcer. Genotype and allele frequencies of the *IL-1RN* and the *IL-1B* gene polymorphisms in our control population did not differ from those reported by several European studies [32,33,35,36]. Because these polymorphic genes are clustered located in a 360-kb region on the long arm of human chromosome 2 (2q14), allelic associations are very probable. We found a significant linkage disequilibrium (LD) between the 4-*IL-1RN/IL-1B* loci. As El-Omar *et al*. [35] reported previously, the *IL-1B*-31 locus was in complete LD with the *IL-1B*-511 locus (– 511T/–31C and –511C/–31T). Also, *IL-1B*-511 and *IL-1B* + 3954 were linked tightly. However, no significant linkage disequilibrium between *IL-1B* + 3954 and *IL-1RN* was found in the control population, which is in accordance with other studies reported previously in Western populations [33,35].

Recently, Furuta *et al*. showed in a study performed in a Japanese population that allele 2 of the *IL-1RN* gene polymorphism had a significant protective effect against duodenal ulcer disease [44]. Moreover, the *IL-1B*-511 T/T genotype significantly protected against DU recurrence in patients older than 60 years. Although the T/T genotype had a trend towards protection, the authors did not find a significant association between the *IL-1B*-511 polymorphism and risk of duodenal ulcer. In our study, the *IL-1B*-511 T/T genotype was also found to be less frequent in duodenal ulcer patients than in *H. pylori* infected controls (8% *versus* 15·4%), and showed the same non-significant trend reported by Furuta *et al*. [44]. However, we did not find any difference when patients were categorized according to the age of onset of the disease. Concerning the *IL-1RN* polymorphism, our study shows no differences in carriage rate, genotype and allele frequencies between DU patients and controls. The *IL-1RN**2/*2 genotype, reported by El Omar *et al*. to be associated with an increased risk of developing gastric cancer [35], was slightly less frequent in duodenal ulcer patients than in controls (7·2% *versus* 10·2%), but the value did not reach statistical significance. Furuta *et al*. showed an association between allele 2 of the *IL-1RN* gene and a significant protective effect against duodenal ulcer disease [44]. However, the frequency of *IL-1RN**2 in Japan (6%) is much lower than reported in other studies performed in white populations from the Netherlands (24%) [45], Scotland (34%) [35], Portugal (28%) [36] and our control population from the North of Spain (29%). Ethnic origin might explain these differences in the frequencies of allelic variants, and highlights the need to perform further studies in different populations in order to assess the importance of these polymorphisms as genetic markers of duodenal ulcer disease.

No significant differences in carriage rate, genotype and allele frequencies of the *IL-1RN* and the *IL-1B* gene polymorphisms between DU patients and controls were found when

they were analysed singly. However, multivariate analysis identified the simultaneous carriage of *IL-1B*-511**C*/*IL-1B*-31**T*/*IL-1B* + 3954**C*/*IL-1RN**2* as an independent risk factor for duodenal ulcer disease. The fact that data regarding the effects of *IL-1B* and *IL-1RN* gene polymorphisms on IL-1β and IL-1ra production are still controversial [26–29] makes it difficult to elaborate a hypothesis about the real functional significance of this association. This includes three alleles, theoretically low producers of IL-1β (*IL-1B*-511**C*, *IL-1B*-31**T* and *IL-1B* + 3954**C*), and one controversial allele concerning the production of IL-1ra (*IL-1RN**2). While some studies [46] reported the association of the presence of *IL-1RN**2 with slightly higher IL-1ra production, other studies [28] showed that IL-1ra concentrations were significantly lower in the colonic mucosa of patients carriers of the *IL-1RN* allele 2. Based on the former studies we could then speculate that the combination associated whit a higher risk for the development of duodenal ulcer (*IL-1RN**2, *IL-1B*-511**C*, *IL-1B*-31**T* and *IL-1B* + 3954**C*) would represent a phenotype low producer of Il-1ra and Il-1β. Thus, patients with duodenal ulcers would lack the protective inhibition of gastric acid and pepsinogen secretion mediated by IL-1β. However against this interpretation are the findings reported by Santtila *et al*. [47], showing that *IL-1RN* allele 2 strongly increased *in vitro* production of IL-1β independently of the associated *IL-1B* alleles. In addition, Hwang *et al*. [48] reported that the simultaneous carriers of both *IL-1B*-511T/T genotype and *IL-1RN**2 allele presented severe gastric inflammation and had the highest levels of IL-1β in gastric mucosa. Therefore, further studies are crucial in order to clarify the exact functional consequences of these polymorphisms.

In order to determine the potential relationship between both host and environmental factors we also determined the presence of *H. pylori* status in all patients and controls. Because endoscopy was performed only in patients and not in the group of healthy individuals, determination of serological *H. pylori*-specific antibodies was performed by Western blot analysis in patients and controls. Although this was not the primary objective of the study, we thought that the analysis of these aspects could be of interest, as it could provide some clues for more specific studies in the future. Most of the duodenal ulcer patients were infected by *H pylori* (95·4%), which is in accordance with previous results reported in the literature showing a strong association between duodenal ulcer and *H. pylori* infection [42,43]. Infection with CagA positive strains, which are related to a higher degree of inflammation and epithelial damage in the gastric mucosa [1,49], were also more frequent in duodenal ulcer patients than in controls. Finally, the CagA/VacA status within duodenal ulcer patients and controls was not linked to the genotype distribution or allele frequency of *IL-1RN* and *IL-1B* gene polymorphisms. Although the number of individuals *H. pylori*$^+$ tested for the presence in serum of CagA/VacA antibodies was restricted (49 controls, and 48 DU patients), our results are in agreement with previous studies showing no association between *IL-1RN*/*IL-1B* genotypes and infection with *H. pylori* cagA/vacA strains [50,51]. As suggested by Figueiredo *et al*. [50], this finding indicates that there is no preferential colonization of specific hosts (defined by IL-1 polymorphisms) by specific bacterial strains.

In previous studies we have reported that the major aetiological factor of ulcer diseases, *H. pylori*, plays a similar role in both complicated and uncomplicated ulcers [37,52], which suggest that both phenotypes have similar background for the underlying disease. In this study, 87 patients with duodenal ulcer (61% of the total duodenal ulcer group) presented with an episode of gastrointestinal bleeding, which was associated with NSAID use in 51% of cases. This suggests that NSAIDs use have probably played a major role in the complication event, but a minor role in the induction of the ulcer itself. In any case, no significant differences in carriage rate, genotype and allele frequencies of the *IL-1RN* and the *IL-1B* gene polymorphisms were found when patients were classified according to the presence of gastrointestinal bleeding, number of bleeding episodes, recurrence of the ulcer and need for surgical treatment.

In summary, our results provide further evidence that genetic factors are important in the pathogenesis of duodenal ulcer disease. We show in this paper that the carriage of the *IL-1B*-511**C*/*IL-1B*-31**T*/*IL-1B* + 3954**C*/*IL-1RN**2 allele combination in addition to bacterial and environmental factors play a key role in the development of duodenal ulcer. Additional research in larger studies and different ethnic groups is needed in order to confirm the importance of these polymorphisms as genetic markers of duodenal ulcer disease. Genotyping of the *IL-1RN*/*IL-1B* polymorphisms could help to identify potential high-risk individuals and may have direct implications for a novel therapeutic approach of the disease.

ACKNOWLEDGEMENTS

This study was supported by grants from the 'Fondo de Investigaciones Sanitarias' (Exp: 01/3141), Instituto de Salud Carlos III (C03/02), 'Diputación General de Aragón' (Exp: P020/2001), 'Sociedad Aragonesa de Patología Digestiva' and 'Club Español del *Helicobacter pylori*'.

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