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Lack of Association of *IL-12 p40* Gene Polymorphism With Peptic Ulcer Disease

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ABSTRACT: Interleukin 12 (IL-12) is a proinflammatory cytokine composed by two chains, p40 and p35, that plays a key role in the promotion of a Th1 immune response in the gastrointestinal mucosa. An enhanced expression of IL-12 mRNA in gastric mucosa has been reported in individuals infected by *Helicobacter pylori*. The aim of our study was to assess whether a functional polymorphism located at position 1188 (A→C) of the *IL-12 p40* (*IL12B*) gene is associated with the susceptibility and clinical features of peptic ulcer disease. Genotyping of 184 unrelated white Spanish patients with peptic ulcer and 107 healthy controls was performed by polymerase chain reaction and restriction fragment length polymorphism. *Helicobacter pylori* status and nonsteroidal antiinflammatory drugs use were studied in patients and controls. There were no significant differences in carriage, genotype, and allele frequencies of the *IL-12 p40* gene polymorphism between patients with peptic ulcer and controls. Moreover, no differences were found with respect to the localization of the ulcer, *Helicobacter pylori* status, nonsteroidal antiinflammatory drug use, age, sex, bleeding episodes, and family history of peptic ulcer. Our data reveal that the *IL12B* 1188 (A→C) gene polymorphism is not involved in defining the genetic basis of the susceptibility to and final outcome of peptic ulcer disease. *Human Immunology 66, 72-76 (2005).* © American Society for Histocompatibility and Immunogenetics, 2005. Published by Elsevier Inc.

KEYWORDS: polymorphism; peptic ulcer; cytokine; *Helicobacter pylori*; IL-12

ABBREVIATIONS

CI	confidence interval
IL	interleukin
NSAID	nonsteroidal antiinflammatory drug
OR	odds ratio
PCR	polymerase chain reaction
Th	helper T cell

INTRODUCTION

Helicobacter pylori (H. pylori) infection is considered the most important causative agent in the genesis of peptic ulcer disease [1, 2]. A key element in the response to *H. pylori* infection is the prompt production of proinflammatory cytokines in gastric mucosa. Several bacterial infection models have demonstrated that the host response is initiated by interleukin (IL)-12, a cytokine produced mostly by phagocytic cells, B cells, and other antigen-presenting cells that play a critical role in the regulation of the inflammatory cascade network [3–5]. IL-12 induces interferon-γ synthesis by T cells and natural killer cells and promotes a Th1-type cytokine profile to enhance the inflammatory response to infection [6, 7]. IL-12 is composed of two disulfide-linked subunits, p40 and p35, which are encoded by different genes (*IL12B* and *IL12A,* respectively) located on different chromosomes (5q31–33 and 3p12–3q13.2) [8]. The coexpression of both subunits is required to generate a heterodimer (p70) biologically active [9], which appears to be predominantly regulated at the level of *IL12B* transcription.

Over the last few years, IL-12 is emerging as a key cytokine in determining the outcome of the effector Th

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cell response in the gastrointestinal mucosa. Several studies have reported high levels of IL-12 secretion and enhanced expression of IL-12 mRNA in gastric mucosa of individuals infected by *H. pylori* [10–12]. In addition, D'Elios *et al.* have described a higher expression of IL-12 mRNA and other Th1 cytokines in gastric mucosa of patients with peptic ulcer infected with *Helicobacter pylori* compared with those *H. pylori*–positive individuals with chronic gastritis only [13]. Interindividual variations in the production levels of cytokines have been reported to be genetically determined. Recently, a TaqI polymorphism (A→C) located at position +1188 in the 3′-untranslated region of the *IL-12 p40* gene (*IL12B*) [14] has been found to be biologically relevant. Seegers *et al.* demonstrated that the IL12B 1188C allele was related to an increased IL-12 p70 secretion by stimulated monocytes [15]. Furthermore, this polymorphism has been associated with disease susceptibility to several chronic inflammatory, autoimmune, and infectious diseases such as insulin-dependent diabetes mellitus [16], multiple sclerosis [17], psoriasis [18], and cerebral malaria [19]. On the basis of these associations, the aim of our study was to investigate whether the 1188 (A→C) polymorphism in the *IL-12 p40* gene is involved in the susceptibility to and clinical outcome of peptic ulcer disease.

PATIENTS AND METHODS

Subjects

A total of 184 unrelated white Spanish patients with peptic ulcer disease (124 with duodenal ulcer, 50 with gastric ulcer, and ten with both duodenal and gastric ulcer) attending the Hospital Clínico in Zaragoza, Spain, were included in the study. One hundred seven ethnically matched healthy volunteers without active or peptic ulcer history served as controls. The healthy control group comprised blood donors and healthy volunteers who did not have any symptoms of gastrointestinal disease. The diagnosis of gastric or duodenal ulcer and the presence of complications were made on the basis of conventional clinical and endoscopic findings. *H. pylori* status and nonsteroidal antiinflammatory drug (NSAID) use were studied in patients and controls either at the time of the diagnosis or at recruitment. All patients and controls provided written informed consent to the study, which was conducted in accordance with the Ethical Committee of the Hospital.

Methods

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 histologic examination from biopsy samples 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 found to be negative by invasive methods. In controls, the presence of *H. pylori* was diagnosed by ^{13}C-urea breath test and serology that used a commercial immunoglobulin G enzyme-linked immunosorbent assay kit (Plate Helicobacter IgG, Roche; Cortesec Diagnostics Ltd., Clwyd, UK). Both tests have been validated in our area [20, 21], and controls were considered positive for the *H. pylori* infection if either of the two tests was positive for the infection.

NSAID use. The use of NSAIDs at the time of the diagnosis of duodenal and gastric ulcers was determined by structured data collection. A patient was considered positive if the drug had been taken within the week before the hospital admission of the endoscopic diagnosis of peptic ulcer [22].

IL-12 p40 1188 (A→C) genotyping. Genomic DNA was extracted from EDTA-preserved whole blood by a standard proteinase-K digestion and phenol/chloroform procedure [23].

A biallelic TaqI restriction fragment length polymorphism (RFLP) (A→C) at position +1188 of the *IL-12 p40* gene was analyzed by polymerase chain reaction (PCR) according to previously described methods [24]. Briefly, PCR fragments were generated using the oligonucleotides 5′-TTC TAT CTG ATT TGC TTTA-3′ and 5′-TGA AAC ATT CCA TAC ATCC-3′ as primers. Reaction mixtures (25μl) contained 50 ng of genomic DNA in 1× Taq buffer, 200 μM of each dNTP, 0.2 μM of each primer, and 0.2 U of Taq DNA polymerase. Polymerase chain reaction amplifications were carried out according to the following parameters: 30 cycles of 95°C for 30 seconds, 43°C for 30 seconds, and 72°C for 60 seconds, followed by a final elongation at 72°C for 7 minutes. Ten microliters of the PCR products were digested with 2 units of the restriction enzyme *Taq*I (Invitrogen) for 16 hours at 65°C and resulted in an intact fragment of 233 bp (allele IL12B 1188A) or in two fragments of 165 and 68 bp (allele IL12B 1188C).

Statistical Analysis

Comparison of the genotype and allele frequencies of patients and healthy controls were performed using the χ^2 test or Fisher's exact test, if needed. The magnitude of the association of the *IL12B* 1188 (A→C) polymorphism in each group was estimated by odds ratio (OR) and 95% confidence intervals (CI). A two-sided p value <0.05 was considered statistically significant. In addition, unconditional logistic regression analysis was carried out using the BMDP Dynamic 7.0 program (BMDP Statistical Software Inc.) to quantify the influence of both genetic and environmental factors for duodenal and gas-

tric ulcers as dependent variables. Starting with age and sex, the model was constructed with a stepwise forward conditional method.

RESULTS

Clinical and demographic characteristics of patients with peptic ulcer and controls are summarized in Table 1. The group of patients with combined gastric ulcer and duodenal ulcer (ten patients) was too small to perform statistical analysis for assessing its significance. Genotypes, carriage rate, and distribution of allele frequencies in patients and controls for the *IL12B* 1188 (A→C) polymorphism are listed in Table 2. In both patients and controls, genotype and allele frequencies did not deviate significantly from those expected from Hardy-Weinberg's equilibrium. No significant differences in carriage rate, genotype, and allele frequencies of the *IL12B* 1188 (A→C) gene polymorphism were found between patients and controls, either when patients were analyzed as a whole group (patients with peptic ulcer) or when they were classified according to the localization of the ulcer (duodenal or gastric).

In addition, available clinical characteristics of patients with peptic ulcer were analyzed for possible associations with the different genotypes or alleles of *IL12B* 1188 (A→C) gene polymorphism. No significant differences in carriage rate, genotype, and allele frequencies were found when patients were categorized according to *H. pylori* status, NSAID use, sex, age at onset, smoking habit, ulcer history, type of complication (stenosis, perforation, and gastrointestinal bleeding), number of bleeding episodes, recurrence of the ulcer, and need for surgical treatment (data not shown). Finally, of the environmental and genetic factors evaluated in this study, and after controlling for confounding factors, logistic regression analysis identified *H. pylori* infection (OR 6.09; 95%CI 2.63–14.12) and NSAID use (OR 23.13; 95%CI 8.67–61.72) as independent risk factors for the development of peptic ulcer disease.

DISCUSSION

Current studies have indicated that the immune response to *H. pylori* infection in the gastric mucosa of patients with peptic ulcer is more polarized toward a predominantly Th1 profile [13]. IL-12 is a proinflammatory cytokine that modulates the immune response by favoring the differentiation of native Th cells into the Th1 phenotype. Sequencing of the *IL-12 p40* gene has revealed several intronic polymorphisms and a TaqI restriction fragment length polymorphism in the 3′ untranslated region at position 1188 [14] that is associated with interindividual variations in the production levels of IL-12 [15]. These variations in IL-12 production may influence T-cell responses, which are crucial for either mediating or protecting against infectious or immune diseases. In the current study, we analyzed the role of the *IL12B* 1188 (A→C) gene polymorphism in the susceptibility to and final outcome of peptic ulcer disease. Genotype and allele frequencies of this polymorphism in our control population were similar to those reported by several European studies [15, 17, 24, 25]. No differences in carriage, genotype, and allele frequencies of the *IL12B* 1188 (A→C) gene polymorphism were found between patients and controls, either when patients were analyzed as a whole group (patients with peptic ulcer) or when they were classified according to the localization of the ulcer (duodenal or gastric). The lack of association with other several clinical characteristics such as sex, age at onset, smoking habit, bleeding episodes, *H. pylori* status, and NSAID use suggests that the *IL12B* 1188 (A→C) gene polymorphism does not constitute a genetic risk factor for the predisposition to and final outcome of peptic ulcer disease. However, this finding does not

TABLE 1 Demographics and clinical characteristics of healthy controls and patients with peptic ulcer

Characteristic	Controls (n = 107)	Duodenal ulcer (n = 124)	Gastric ulcer (n = 50)	Gastric + duodenal ulcer (n = 10)
Age, years, mean ± SD (range)	44.8 ± 19.1	46.7 ± 13.1	56.7 ± 13.6	63.7 ± 9.3
Sex, *n* (%)	(20–83)	(17–75)	(16–83)	(47–77)
Female	43 (40.2%)	30 (24.2%)	18 (36%)	2 (20%)
Male	64 (59.8%)	94 (75.8%)	32 (64%)	8 (80%)
H. pylori positive, *n* (%)	73 (68.2%)	118 (95.2%)[a]	41 (82%)	10 (100%)
NSAID use, *n* (%)	7 (6.5%)	44 (35.5%)[a]	41 (82%)[a]	6 (60%)
Cigarette smoking, *n* (%)	32 (29.9%)	46 (37.1%)	19 (38%)	3 (30%)
Family history of ulcer, *n* (%)	13 (12.1%)	40 (32.2%)	13 (26%)	1 (10%)
GI bleeding history, *n* (%)	—	75 (60.5%)	42 (84%)	7 (70%)

Abbreviations: GI = gastrointestinal; NSAID = nonsteroidal antiinflammatory drug.

[a] $p < 0.001$ versus healthy controls.

TABLE 2 Genotype and allele frequencies of the *1188* (A → C) polymorphism in the *IL-12 p40* gene in healthy controls and patients with peptic ulcer according to the localization of the ulcer

	Genotype *n* (%)			Allele frequency (%)	
IL-12 p40	A/A	A/C	C/C	A	C
Healthy controls ($n = 107$)	75 (70.1)	27 (25.2)	5 (4.7)	82.7	17.3
Peptic ulcer ($n = 184$)	117 (63.6)	58 (31.5)	9 (4.9)	79.3	20.7
Duodenal ulcer ($n = 124$)	77 (62.2)	39 (31.4)	8 (6.4)	77.8	22.2
Gastric ulcer ($n = 50$)	33 (66)	16 (32)	1 (2)	82	18

exclude a key role for this cytokine in the regulation of inflammatory events in these diseases.

As we previously reported, different pathogenic mechanisms are involved in the development of gastric and duodenal ulcers. Although *H. pylori* infection plays a key role especially in duodenal ulcers (OR 10.63; 95% CI 3.81–29.64), the use of NSAIDs is considered the major risk factor in gastric ulcers (OR 69.03; 95% CI 20.81–229). In this regard, Hida *et al.* reported an increased antral IL-12 mRNA expression in the majority of *H. pylori*–infected patients with duodenal ulcers, but not in those with gastric ulcers or without ulcers. The authors suggest that in patients with duodenal ulcer, mucosal Th1 responses may predominate and be a factor in the pathogenesis of duodenal ulceration [12]. In relation to these findings, we have previously reported a strong association of *IL-1B* and *IL-1RN* gene polymorphisms in patients with duodenal ulcers [26]. Thus, the simultaneous carriage of IL-1B^{+3954} allele T and IL-1RN allele 2 was associated with reduced risk for duodenal ulcer disease. Interestingly, no association of these polymorphic genes was found in patients with gastric ulcer and in controls. That possible different genetic background existing between patients with duodenal and gastric ulcers was not corroborated in the current study, where no differences in genotype, carriage, and allele frequencies of the *IL12B* 1188 (A→C) gene polymorphism between gastric and duodenal ulcer patients were found. We conclude that this polymorphism does not participate in defining the susceptibility to and the nature of the clinical course of peptic ulcer. In spite of this lack of association, we believe that further studies are needed in order to evaluate whether other polymorphisms within this gene or its receptors may denote susceptibility to peptic ulcer.

To our knowledge, this is the first study analyzing the possible link between the *IL12B* 1188 (A→C) gene polymorphism and peptic ulcer disease. When we take into account the fact that cytokines form a complex interacting network, we think that other proinflammatory and antiinflammatory cytokines genes involved in the inflammatory response to *H. pylori* infection merit further study. The study of cytokine gene polymorphisms might help define patient subgroups and may lead to the development of a different approach to the treatment of peptic ulcer.

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