2009

Interleukin-1 beta single-nucleotide polymorphism's C allele is associated with elevated risk of gastric cancer in helicobacter pylori-infected Peruvians

Sebastian Gehmert
University of Texas M.D. Anderson Cancer Center

Billie Velapatiño
Universidad Peruana Cayetano Heredia

Phabiola Herrera
AB PRISMA

Jaqueline Balqui
AB PRISMA

Livia Santivañez
AB PRISMA

See next page for additional authors

Am. J. Trop. Med. Hyg., 81(5), 2009, pp. 804–810
doi:10.4269/ajtmh.2009.08-0494

Interleukin-1 Beta Single-Nucleotide Polymorphism's C Allele is Associated with Elevated Risk of Gastric Cancer in *Helicobacter pylori*-Infected Peruvians

Sebastian Gehmert,* Billie Velapatiño, Phabiola Herrera, Jaqueline Balqui, Livia Santivañez, Jaime Cok, Gloria Vargas, Juan Combe, Douglas J. Passaro,† Sijin Wen, Frank Meyer, Douglas E. Berg, and Robert H. Gilman

Department of Molecular Pathology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas; Department of Surgery, Otto-von-Guericke University, Magdeburg, Germany; Laboratorios de Investigación y Desarrollo, Facultad de Ciencias, Universidad Peruana Cayetano Heredia, Lima, Peru; Asociacion Benefica PRISMA, Department of Bioresearch, Lima, Peru; Department of Pathology, Hospital Nacional Cayetano Heredia, Lima, Peru; Department of Gastroenterology, National Major San Marcos University, Lima, Peru; Department of Gastroenterology, National Institute of Neoplastic Diseases, Lima, Peru; Division of Epidemiology and Biostatistics, University of Illinois School of Public Health, Chicago, Illinois; Department of Biostatistics, The University of Texas M. D. Anderson Cancer Center, Houston, Texas; Departments of Molecular Microbiology, Genetics, and Medicine, Washington University Medical School, St. Louis, Missouri; Department of International Health, The Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland

Abstract. Particular alleles of the *interleukin-1B (IL-1B)* gene have been correlated with increased risk of atrophic gastritis and gastric cancer in the populations of East Asia and Europe. No such data exist from Peru, a developing country with a population genotypically different from others studied and with a high prevalence of *Helicobacter pylori* infection and gastric cancer. We conducted a case-control study comparing 334 hospitalized patients with atrophic gastritis or gastric cancer with 158 nonatrophic gastritis patients (controls). Conditional logistic regression analysis revealed that an increased risk of atrophic gastritis (odds ratio, 5.60) and gastric cancer (odds ratio, 2.36) was associated with the *IL-1B*-511 C allele. Our study is the first to establish this allele as a risk for these conditions. Given the high prevalence of *H. pylori* and recurrence rate after treatment, *IL-1B*-511 single-nucleotide polymorphism analysis may identify those individuals who would benefit most from robust *H. pylori* eradication efforts in Peru.

INTRODUCTION

Chronic infection by the gastric pathogen *Helicobacter pylori* is an early risk factor for gastric cancer, one of the most frequently lethal malignancies in Latin America, East Asia, and Eastern Europe.[1,2] However, only a small fraction of the people infected with *H. pylori* develop this disease.[3]

The development of gastric cancer (GC) is considered to be multifactorial and probably conditioned by particular aspects of the human genotype and other factors, including nutrition, environment, and virulence of infecting *H. pylori* strains.[4,5] It has recently been suggested that interaction between factors related to bacterial virulence and immune response of the hosts affects the outcome of *H. pylori* infection, which may be influenced by polymorphisms in both bacteria and host.[6,7] In particular, polymorphisms in *IL-1B* and *IL-1RN* genes were found to be associated with increased risk for developing GC owing to an increased *IL-1* beta level in the gastric mucosa in response to infection with *H. pylori*.[7,8]

However, reports from different parts of the world concerning association of gene polymorphisms with GC are not consistent, and the primarily Amerindian populations of Latin America differ significantly from most other well-studied human populations, not only in environment and lifestyle but also in human and *H. pylori* genotype.[9,10] In Peru, gastric cancer is still the leading cause of death for both sexes; however, no data exist on whether particular *IL-1B*-511 and *IL-1RN* alleles are associated with development of chronic atrophic gastritis (ChAG) or GC in Peruvians. Most Peruvians are of low socioeconomic status, have had a chronic *H. pylori* infection since infancy, and are at high risk of GC.[11,12] The purpose of this prospective study was to determine whether alleles of the cytokine *IL-1B* gene (site -511) and of the IL1 receptor antagonist (*IL-1RN*) associated with an increased risk of cancer in some populations are also associated with an increased risk for ChAG or GC in a Peruvian Amerindian population. We conducted a case-control study of 658 hospitalized patients diagnosed for gastric cancer, atrophic gastritis, or nonatrophic gastritis (controls).

MATERIALS AND METHODS

Patients. We conducted a hospital-based case-control study among 658 hospitalized patients recruited sequentially in two municipal hospitals in Lima, Peru, from January 2005 to December 2006. Patients with a history of cancer, a pregnancy, or a biopsy specimen that was inadequate for histopathologic study were excluded from further analysis (N = 324). Gastric biopsies without gastritis, though typical of modern industrialized societies, are a rarity in Peru because of high rates of chronic *H. pylori* infection (over 90% in adults from a low socioeconomic group). Thus, from the 334 subjects that were included in our study, we used 158 with nonatrophic gastritis (NAG) as our reference control group. For subjects with noncardia gastric cancer (intestinal type, n = 92; diffuse type, n = 41), controls were matched 1:1, and for cases with chronic atrophic gastritis (n = 43), controls were matched 2:1. Controls were frequency matched to subjects in age (±10 years), gender, and environmental risk factors (house material, water preparation, waste removal), because significant differences have been found between the groups.

All participants gave written consent for participation in this research study and were interviewed using a 130-item questionnaire that included personal characteristics, environmental risk factors, and personal and family medical history. Ethylenediamine-tetraacetic acid (EDTA) blood samples (4 mL) were obtained and stored at −20°C for later DNA extraction.

*Address correspondence to Sebastian Gehmert, Department of Molecular Pathology, The University of Texas M. D. Anderson Cancer Center, 7435 Fannin Street, Houston, TX 77054. E-mail: s.gehmert@gmail.com
† Deceased.

The protocol and consent were approved by the human study–ethics committees of The Johns Hopkins School of Public Health (Baltimore, MD) and AB PRISMA in Lima, Peru.

Endoscopy. All endoscopic examinations were routinely performed as described by Soto and others.[13] Two gastric biopsies were obtained from the midantrum, two gastric biopsies from the midcorpus (lesser and greater curvature), and one gastric biopsy from the incisura angularis; gastric biopsies were also obtained from each visible lesion. One biopsy from each gastric site was subjected to histologic preparation, and one biopsy from midcorpus and midantrum was used for culture and polymerase chain reaction (PCR). All samples were coded for anonymity and accurate recordkeeping.

***Helicobacter pylori* detection.** *Helicobacter pylori* infection was detected in gastric biopsies by culture, silver stain, and PCR for urease *(ureB)*. Biopsies were considered positive if any of the three tests was positive and were considered negative only when all three tests were negative.

Culture and staining. One gastric corpus biopsy was homogenized, and the tissue suspension was spread on BHI blood Agar and on Columbia CNA Agar plates (Becton, Dickinson and Co., Sparks, MD) and incubated under microaerobic conditions (O_2, 5%; CO_2, 10%; N_2, 85%) at 37°C for 4 to 7 days, as previously described.[13] Visible *H. pylori* colonies were identified by characteristic morphology, a positive urease test, and bacterial morphology after Gram staining (Sigma, St. Louis, MO). In addition, pathohistologic analysis was performed on biopsies from antrum, corpus, and incisura using Warthin-Starry silver stain, as described previously.[14]

Polymerase chain reaction. One biopsy sample from the gastric antrum was used for DNA preparation using the QIAmp DNA Mini Kit (QIAGEN AG, Basel, Switzerland) according to the manufacturer's instructions to detect *H. pylori* with PCR. Briefly, PCR was carried out in a 25 µL volume containing 10–20 ng of extracted DNA, 0.4 mM of each primer specific for a 460 bp segment of the *ureB* gene (UreB-sense 5′-CGT CCG GCA ATA GCT GCC ATA GT-3′ and UreB-antisense 5′-GTA GGT CCT GCT ACT GAA GCC TTA-3′), standard PCR buffer, 0.25 mM of dNTP mix, 2.5 mM of $MgCl_2$, 0.02 U of recombinant *Taq* DNA Polymerase (Invitrogen, Carlsbad, CA), and 0.1 µg/µL of bovine serum albumin, with the following cycling parameters: denaturation at 94°C for 5 minutes, 35 cycles of 94°C for 1 minute, annealing at 67°C for 1 minute, and elongation at 72°C for 1 minute; a final step of 5 minutes was used to complete DNA extensions at 72°C. The PCR products were electrophoresed in a 2% UltraPure (Invitrogen) agarose gel in 1X TAE buffer containing 0.5 mg/mL ethidium bromide (Fisher Scientific, Fair Lawn, NJ) and were visualized using Kodak digital science 1D software (Eastman Kodak Company, Rochester, NY) on a Dell workstation (Dell Computer Corporation, Round Rock, TX).

Pathohistology. One biopsy sample each from the antrum, corpus, and incisura were fixed in buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin (Sigma). An experienced pathologist (JC) examined the coded slides without knowledge of the area of origin of the microsection, clinical data, or endoscopic characteristics. Grades of gastritis were classified according to the updated Sydney Classification System[15] and are presented in Table 1.

Gastric carcinoma patients were subdivided according to Lauren's classification as intestinal type and diffuse type. Each subject was diagnosed based on the most severe or pronounced finding from the pathohistologic study of the gastric biopsies.

TABLE 1
Characterization of gastritis in the study population

Characteristic	NAG* n (%)	ChAG† n (%)	*P* value
Subjects (n)	158	43	
Chronicity of gastritis			
L1	58 (36.7)	16 (37.2)	0.17
L2	76 (48.1)	25 (58.1)	
L3	24 (15.2)	2 (4.7)	
Activity of gastritis			
G0	51 (32.3)	24 (55.8)	0.036
G1	40 (25.3)	9 (20.9)	
G2	47 (29.7)	7 (16.3)	
G3	20 (12.7)	3 (7.0)	
Atrophy			
No	158 (100.0)	0	< 0.001
Light	0	18 (41.9)	
Moderate	0	8 (18.6)	
Severe	0	17 (39.5)	
Topography of atrophy			
Focal		34 (79.1)	NA‡
Diffuse		9 (20.9)	
Intestinal metaplasia			
None	117 (91.4)	13 (30.2)	< 0.001
Type I	10 (7.8)	28 (65.1)	
Type II–III	1 (0.8)	2 (4.7)	
Location of gastritis			
Predominant antrum	47 (29.7)	32 (74.4)	< 0.001
Predominant corpus	48 (30.4)	7 (16.3)	
Antrum and corpus	63 (39.9)	4 (9.3)	

* Nonatrophic gastritis.
† Chronic atrophic gastritis.
‡ Not applicable.

***IL-1B* and *IL-1RN* genotyping.** The DNA was extracted from gastric biopsy or blood specimens using the QIAmp DNA Mini Kit (QIAGEN AG) according to the manufacturer's instructions. The PCRs were performed in a 25 µL reaction mixture filled to full volume with PCR water (Sigma).

For genotyping the position -511 cytokine *IL-1B* gene single-nucleotide polymorphism (SNP), a 305 bp fragment was amplified with 0.5 µM of each primer, 5′-TGG CAT TGA TCT GGT TCA TC -3′ (sense) and 5′-GTT TAG GAA TCT TCC CAC TT -3′ (antisense), using standard PCR protocol (see above) and optimized with 1.5 µM of $MgCl_2$ and 0.06 U of recombinant *Taq* DNA Polymerase. Cycling parameters were denaturation at 95°C for 5 minutes followed by 35 cycles of 95°C for 30 seconds, 50°C for 30 seconds, 72°C for 30 seconds, and a final extension step for 5 minutes at 72°C. Thereafter, the PCR products were digested for 18 hours at 37°C with 10 U of *Ava*I enzyme (New England BioLabs, Beverly, MA) to distinguish allele T (*Ava*I-resistant; one fragment of 305 bp) versus allele C (*Ava*I-cut: fragments of 115 and 190 bp). The digested DNAs were electrophoresed on a 2% agarose gel, and data were recorded as detailed previously.

Either an intact fragment of 305 bp (allele T) or two fragments of 190 bp and 115 bp (allele C) were obtained using electrophoresis. Human genotypes could be homozygous C/C, T/T, or heterozygous C/T.

The diagnostic variable number of tandem repeat (VNTR) marker for *IL-1RN* alleles was genotyped by PCR as follows: 0.5 µM of each primer, 5′-CTC AGC AAC ACT CCT AT- 3′ (sense) and 5′-TCC TGG TCT GCA GGT AA- 3′ (antisense), were used with the cycling parameters used for *IL-1B*-511 genotyping, but with an annealing temperature of 55°C and 3 mM of $MgCl_2$ in the buffer. The *IL-1RN* alleles were coded

as follows: Allele 1 (four repeats of the 86 bp region, 412 bp PCR product), allele 2 (two repeats, 240 bp), and allele 3 (three repeats, 326 bp). For statistical analysis, this polymorphism was treated as bi-allelic using categories of short-allele (S allele) with two repeats (allele 2) and long-allele (L allele) with three or more repeats (alleles 1 and 3).

Samples assayed by PCR product sequencing were also used as positive controls for internal validation of enzyme digestion. In addition, one-fifth of the analyzed samples were tested again using the same assay, with completely concordant results.

Statistics. Data were collected in a computerized database using double data entry to ensure accurate recording. Descriptive statistical analysis was calculated, including proportions, percentage, means, and standard deviations. Chi-square (χ^2) and *t* test were used to assess differences of frequency distributions among demographic characteristics of the study population. To calculate odds ratios (ORs) and 95% confidence intervals (CIs) for *IL-1B*-511 and *IL-1RN* genotypes, a conditional logistic regression model adjusted for *H. pylori* infection was applied. Stratification in an unconditional logistic regression analysis was used to evaluate whether the association between the cytokine polymorphisms and gastric cancer risk differed by histology type or location where it was diagnosed. This study's dependent variable was the histologic diagnosis.

Differences were considered statistically significant if $P < 0.05$. Statistical analysis was performed with SPSS software (version 11.5, SPSS Science, Chicago, IL) and SPLUS (version 2000, Insightful Corporation, Seattle, WA). Hardy-Weinberg equilibrium of alleles at individual loci was assessed using the program Linkage Disequilibrium Analyzer 1.0 (available at http://www.chgb.org.cn/lda/lda.htm).

RESULTS

In total, 334 patients were successfully genotyped for the two polymorphisms in the *IL-1* gene cluster. The demographic comparison of matched cases and controls is summarized in Table 2. Although 80.1% of patients were infected with *H. pylori*, the genotype frequencies in cases and controls did not deviate from the Hardy-Weinberg equilibrium (Table 3). Distribution of gastritis location and intestinal metaplasia (IM) were different between patients diagnosed for NAG and ChAG ($P < 0.001$). ChAG occurred more frequently in the antrum region and were more often associated with IM. No significant differences were revealed between both groups (NAG, ChAG) with regard to the chronicity of gastritis ($P = 0.17$) (Table 1).

Association between polymorphisms of *IL-1* gene cluster and the risk of chronic atrophic gastritis. We found that subjects with the C allele of the *IL-1B*-511 SNP had a higher frequency of atrophic gastritis (OR, 5.60; 95% CI, 2.02–15.51) than did subjects with nonatrophic gastritis homozygous for the T allele. Also subjects with the C allele homozygote had this premalignant lesion more frequently (OR, 11.22; 95% CI, 2.27–55.37) than did subjects with C/T heterozygotes (OR, 4.79; 95% CI, 1.65–13.83) (Table 4). We also found a higher frequency of chronic atrophic gastritis in the antrum region in C/C homozygous subjects (OR, 12.72; 95% CI, 2.12–76.43) than in subjects with T/T homozygotes (Table 6). This association has not been revealed for corpus region ChAG. Furthermore, none of the *IL-1RN* VNTR states were associated with increased frequency of chronic atrophic gastritis (Table 4).

Association between polymorphisms of *IL-1* gene cluster and the risk of gastric cancer. Carriage of the C allele of the *IL-1B*-511 SNP either in homozygous or heterozygous condition was more frequent in subjects with GC than in controls (OR, 4.15; 95% CI, 1.33–12.93 and OR, 2.17; 95% CI, 1.23–3.84, respectively) (Table 5), mirroring the association seen between premalignant chronic atrophic gastritis and IL-1B SNP. Stratification by location and histologic type showed that the C allele was associated with intestinal but not with diffuse types of GC. Homozygosity for the C allele in particular was associated with a 6-fold increased frequency of intestinal GC in the gastric corpus compared with nonatrophic gastritis controls (OR, 6.29; 95% CI, 1.17–33.76) (Table 6).

None of the *IL-1RN* alleles showed particular associations with GC, nor was a higher frequency of GC or chronic-atrophic gastritis seen with any combination of *IL-1B*-511 and *IL-1RN* alleles (data not shown).

DISCUSSION

Given a high rate of infection by virulent (CagA positive, VacA toxigenic) *H. pylori* strains in the Amerindian Peruvian population,[9] it has seemed likely that host genetic factors affect the risk of emergence and progression of premalignant lesions (chronic atrophic gastritis) in *H. pylori*-infected Peruvians, as in other populations. Here, we found that homozygosity for the C allele of the *IL-1B*-511 SNP was strongly associated with chronic atrophic gastritis (OR, 11.22) and GC (OR, 4.15), even

TABLE 2
Demographic variables of matched cases and controls with different diagnoses

Variable	Patients for ChAG risk analysis			Patients for GC risk analysis		
	NAG*†	ChAG*‡	*P* value	NAG**	GC**§	*P* value
Subjects (n)	86	43		133	133	
Sex [male/female (%/%)]	52/34 (60.5/39.5)	26/17 (60.5/39.5)	1.0	61/72 (45.9/54.1)	61/72 (45.9/54.1)	1.0
Mean age ± SD (y)	64.0 (14.43)	67.2 (12.73)	0.85	61.4 (14.84)	64.0 (14.76)	0.2
H. pylori infection (%)	88.4	76.7	0.12	88.0	74.4	0.006
Hospital						
Loyaza	63 (73.3)	28 (65.1)		96 (72.2)	80 (60.2)	
INEN	23 (26.7)	15 (34.9)	0.41	37 (27.8)	53 (39.8)	0.052
Location						
Antrum	61 (70.9)	36 (83.7)		95 (71.4)	70 (52.6)	
Corpus	25 (29.1)	7 (16.3)	0.09	38 (28.6)	63 (47.4)	0.002

* Controls are matched 2:1 (**1:1) to cases according age (± < 10 years), sex, and environmental risk factors.
† Nonatrophic gastritis.
‡ Chronic atrophic gastritis.
§ Gastric cancer.

TABLE 3
Hardy Weinberg Equilibrium (HWE) for matched cases and controls

	Hardy Weinberg equilibrium											
	Patients for ChAG risk analysis						Patients for GC risk analysis					
	NAG*†			ChAG*‡			NAG**			GC**§		
Genotype	Obs. no.#	(exp.)¶	*P* value⊥	Obs.	(exp.)	*P* value	Obs.	(exp.)	*P* value	Obs.	(exp.)	*P* value
IL-1B-511												
C/C	3	(3.36)		7	(7.12)		7	(6.54)		15	(15.91)	
C/T	28	(27.28)		21	(20.76)		45	(45.91)		62	(60.18)	
T/T	55	(55.36)	1.0	15	(15.12)	1.0	81	(80.54)	0.8	56	(56.91)	0.85
IL-1RN												
*2/*2	10	(12.66)		8	(8.15)		16	(18.80)		16	(19.94)	
*2/L	46	(40.67)		21	(20.70)		68	(62.41)		71	(63.12)	
L/L	30	(32.66)	0.26	13	(13.15)	1.0	49	(51.80)	0.36	46	(49.94)	0.2

* Controls and cases are matched 2:1 for age, sex, and environmental factors.
** Controls and cases are matched 1:1 for age, sex, and environmental factors.
† Nonatrophic gastritis.
‡ Chronic atrophic gastritis.
§ Gastric cancer.
⊥ * Fisher's test.
Observed.
¶ Expected.

when heterozygosity was associated with these pathologies (OR, 4.79 and 2.17, respectively).

Our results agree with reports from Asia[16–18] that also showed higher GC risk for subjects with the *IL-1B*-511 C allele. In addition, Ikehara and others[19] reported that the C allele was associated with progression of gastric cancer, and Matsukura and others[20] found that the *IL-1B*-511 C/C genotype was more prevalent in Japanese subjects with severe mucosal atrophy. In contrast, previous studies have reported that the *IL-1B*-511 T allele and the *IL-1RN**2 allele are associated with ChAG or GC in Caucasians.[21–24] The results of the *IL-1B*-511 C allele risk association are very interesting, because four meta-analyses[25–28] did not reveal concurrent evidence for the *IL-1B*-511 T allele, and significant associations between *IL-1B* SNP and GC were only found for Caucasians.

Differences in GC prevalence, *H. pylori* infection, allele frequency, and ethnicity might account for the varied results found in Caucasian and Asian populations. This theory is supported by a study from China that showed a significant risk associated with *IL-1B*-511 only for the population where gastric cancer was less prevalent.[29] Rates of GC and *H. pylori* infection in Peru are among the highest in the world.[13,30] Moreover, the T allele frequency for control patients in our study population (T allele frequency = 0.8) is higher than that reported in Caucasians (T allele frequency ~0.3) and Asians (T allele frequency ~0.46). Our data agrees with previous data that have shown that variation of several factors might affect gene polymorphism associations.[20,29]

Previous studies from Peru have shown that ChAG is found predominantly in the antrum region and is more severe than ChAG that is found in the body of the stomach.[31–33] In the present study, we also found ChAG to occur more frequently in the antrum region, and only in this gastric site was an association between ChAG and the C allele statistically significant. However, conflicting results regarding mucosal *IL-1β* levels and the *IL-1B*-511 SNP indicate that this association is dependent on the study population.[8,34,35]

Our stratification analysis for the effects of the *IL-1B* allele type on the subtype and location of GC reflected an increased risk for intestinal types of GC in the corpus (OR, 6.29) and for *IL-1B*-511 C allele homozygosity. In line with these findings, Uemura and others[3] reported that patients with *H. pylori* infection, predominantly in the corpus, were at a significantly higher risk for intestinal gastric cancer. This finding emphasizes the importance of chronic atrophic gastritis and how its site of occurrence is affected by previous *H. pylori* infection.

Taken together, the C allele has been associated with higher *IL-1B* promoter activity and thus with *IL-1β* synthesis.[36] In

TABLE 4
Conditional logistic regression analysis for the risk of chronic atrophic gastritis using nonatrophic gastritis subjects as control

NAG*		ChAG†		
Genotype	*n* (%)	*n* (%)	OR (95% CI)‡	*P* value‡
IL-1B-511				
T/T	55 (64.0)	15 (34.9)	1.0 (Reference)	
C/T	28 (32.6)	21 (48.8)	4.8 (1.65–13.83)	0.004
C/C	3 (3.5)	7 (16.3)	11.2 (2.27–55.37)	0.003
C carrier§	31 (36.0)	28 (65.1)	5.6 (2.02–15.51)	0.001
IL-1RN				
L/L	30 (34.9)	13 (31.0)	1.0 (Reference)	
*2/L	46 (53.5)	21 (50.0)	1.0 (0.44–2.34)	0.98
*2/*2	10 (11.6)	8 (19.0)	1.6 (0.48–5.39)	0.43
*2 carrier¶	56 (65.1)	29 (69.0)	1.1 (0.50–2.48)	0.79

Controls and cases are matched 2:1 for age, sex, and environmental factors.
* Nonatrophic gastritis (controls).
† Chronic atrophic gastritis (cases).
‡ Conditional logistic regression model adjusted for *H. pylori* infection.
§ Subjects with genotype C/T or C/C.
¶ Subjects with genotype *2/L or *2/*2.

TABLE 5
Conditional logistic regression analysis for the risk of gastric cancer using nonatrophic gastritis subjects as control

NAG*		GC†		
Genotype	*n* (%)	*n* (%)	OR (95% CI)‡	*P* value‡
IL-1B-511				
T/T	81 (60.9)	56 (42.1)	1.0 (Reference)	
C/T	45 (33.8)	62 (46.6)	2.17 (1.23–3.84)	0.007
C/C	7 (5.3)	15 (11.3)	4.15 (1.33–12.93)	0.014
C carrier§	52 (39.1)	77 (57.9)	2.36 (1.36–4.11)	0.002
IL-1RN				
L/L	49 (36.8)	46 (34.6)	1.0 (Reference)	
*2/L	68 (51.1)	71 (53.4)	1.03 (0.59–1.80)	0.91
*2/*2	16 (12.0)	16 (12.0)	0.86 (0.34–2.14)	0.75
*2 carrier¶	84 (63.2)	87 (65.4)	0.99 (0.57–1.71)	0.99

Controls and cases are matched 1:1 for age, sex, and environmental factors.
* Nonatrophic gastritis (controls).
† Gastric cancer (cases).
‡ Conditional logistic regression model adjusted for *H. pylori* infection.
§ Subjects with genotype C/T or C/C.
¶ Subjects with genotype *2/L or *2/*2.

TABLE 6
Risk of getting ChAG or GC in subjects that are homozygous carriers of the IL-1B-511C allele by anatomic location

Location	Matched pairs		IL-1B-511 genotype C/C	C/T	C carrier	T/T	C/C vs. T/T OR (95% CI)†	*P* value†	C carrier* vs. T/T OR (95% CI)†	*P* value†
	Control	NAG‡	12 (13.3)§	21 (34.4)	23 (37.7)	38 (62.3)				
	Cases	ChAG¶	16 (16.7)	19 (52.8)	25 (69.4)	11 (30.6)	12.72 (2.1–76.4)	0.005	4.00 (1.6–9.8)	0.002
Antrum	Control	NAG	15 (15.3)	32 (33.7)	37 (38.9)	58 (61.1)				
	Cases	GC	13 (14.3)	36 (51.4)	39 (55.7)	31 (44.3)	11.07 (0.2–4.8)	0.93	1.96 (1.0–3.7)	0.038
		IT-GC⊥	12 (14.0)	25 (50.0)	27 (54.0)	23 (46.0)	11.03 (0.2–5.8)	0.97	1.85 (0.9–3.8)	0.087
		DT-GC**	11 (15.0)	11 (55.0)	12 (60.0)	18 (40.0)	11.30 (0.1–13.2)	0.82	2.36 (0.9–6.3)	0.089
	Control	NAG	11 (14.0)	17 (28.0)	18 (32.0)	17 (68.0)				
	Cases	ChAG	11 (14.3)	12 (28.6)	13 (42.9)	14 (57.1)	4.84 (0.1–360.6)	0.47	1.23 (0.1–12.6)	0.86
Corpus	Control	NAG	12 (15.3)	13 (34.2)	15 (39.5)	23 (60.5)				
	Cases	GC	12 (19.0)	26 (41.3)	38 (60.3)	25 (39.7)	5.56 (1.1–29.0)	0.034	2.46 (1.1–5.7)	0.037
		IT-GC	19 (21.4)	17 (40.5)	26 (61.9)	16 (38.1)	6.29 (1.2–33.8)	0.032	2.60 (1.0–6.5)	0.042
		DT-GC	13 (14.3)	19 (42.9)	12 (57.1)	19 (42.9)	5.00 (0.7–37.3)	0.116	2.27 (0.7–7.0)	0.155

* Subjects with genotype C/C and C/T.
† Unconditional logistic regression model adjusted for *H. pylori* infection.
‡ Nonatrophic gastritis.
§ n (%).
¶ Chronic atrophic gastritis.
⊥ Intestinal-type gastric cancer.
** Diffuse-type gastric cancer.

terms of possible mechanisms for GC risk in Peruvians, the increased *IL-1β* mucosal levels might cause profound inhibition of gastric acid secretion, which makes the gastric corpus (where most parietal cells are located) more hospitable for *H. pylori* colonization after initial infection in the antrum.[37,38] Local infection-induced inflammation in the corpus can exacerbate the elevated *IL-1β* levels and thereby increase gastric mucosal pH, which paves the way for a progression of premalignant mucosal changes.[39] We propose that these bacterial-host synergisms explain the strong 511 C allele SNP gastric cancer association reported here in our results. The fact that the *IL-1B*-511 C allele was not associated with an increased risk for the diffuse-subtype GC suggests a different pattern for the emergence of or at least a lower correlation to atrophy in gastric mucosa.

Further study is needed on the polymorphic *IL-10* gene's affect regarding the occurrence of premalignant lesions and GC in Peruvians. *IL-10* serves as a counter balance in the inflammatory orchestra and antagonizes proinflammatory cytokines, especially *IL-1β*.[40] The low *IL-10* haplotype (*IL-10-1082A, IL-10-819T,* and *IL-10-592A*) has been reported to significantly increase the risk of noncardia gastric cancer, most likely by uncontrolled *IL-1β* activity causing a hyperinflammatory milieu.[21] However, similar to *IL-1B,* contradictory data exist for the reported polymorphism in the *IL-10* gene.[41]

Our *IL-1RN* polymorphism analyses did not show any significant association between *2 allele and gastric disease. This contrasts with several studies in Caucasian populations in which the risk of GC was associated with the presence of the *IL-1RN**2 allele.[21–23,42,43] The *IL-1RN**2 allele has been reported with elevated *IL-1ra* levels and thereby with higher *IL-1β* levels in *in-vitro* cell cultures.[44] *Helicobacter pylori* virulence, other environmental factors, or human genetic factors may diminish the importance of these *IL-1ra* variants as regulators of *IL-1B* receptor binding in the Peruvian population.

In conclusion, our study is the first to establish the C allele of the *IL-1B*-511 SNP as a risk factor for intestinal-type GC and its premalignant lesion, gastric atrophy, in a largely Amerindian population. These findings are consistent with the progression of histologic changes from chronic atrophic gastritis to intestinal types of GC and furthermore suggest differences in the processes leading to diffuse types of GC.

Our findings support the theory that inter-individual genetically determined differences of cytokine production affect outcomes in *H. pylori* infection and GC.[45,46]

In view of a high *H. pylori* prevalence and a high recurrence rate of treated infections in Peruvians,[13] *IL-1B*-511 SNP might be an indicator of which patients would benefit from rigorous anti-*H. pylori* therapy and vigilant surveillance of their gastric mucosal changes by intermittent gastroscopies.

Received September 24, 2008. Accepted for publication July 14, 2009.

Acknowledgments: We thank Paula Maguiña for technical support. We are grateful for the assistance of the gastroenterologists at the Hospital Arzobispo Loayaza and the Instituto Especializado de Enfermedades Neoplasticas and for the generous cooperation of patients from those hospitals. We thank Kristi M. Speights from the Department of Scientific Publications at The University of Texas M. D. Anderson Cancer Center for critically reviewing and editing the manuscript. Our condolences to the family of Dr. Passaro, a brilliant epidemiologist, whose ideas initiated this study.

Financial support: This study was supported by an NIH grant (R01 DK63041) to R. Gilman.

Authors' addresses: Sebastian Gehmert, Department of Molecular Pathology, The University of Texas M. D. Anderson Cancer Center, 7435 Fannin Street, Houston, TX, 77054, Tel: 1-713-834-6115, Fax: 1-713-834-6105, E-mail: s.gehmert@gmail.com. Billie Velapatiño, Universidad Peruana Cayetano Heredia, Department of Microbiology, Av. Honorio Delgado, No. 430, Urb. Ingeniería, San Martin de Porres, Lima, Peru, Tel: 011-511-3190025, Fax: 011-511-3284038, E-mail: bvelap@yahoo.com. Phabiola Herrera, AB PRISMA, Bioresearch, Carlos Gonzáles No. 251, Urb. Maranga - San Miguel, Lima, Peru, Tel: 011-511-464 0221, Fax: 011-511-4640781, E-mail: phabiola@gmail.com. Jaqueline Balqui and Livia Santivañez, AB Prisma, Bioresearch, Carlos Gonzáles No. 251, Urb. Maranga, San Miguel, Lima, Peru, Tel: 011-511-464 0221, Fax: 011-511-4640781, E-mails: jbalqui@yahoo.es and lisantivanez@yahoo.es. Jaime Cok, Department of Pathology, Hospital Nacional Cayetano Heredia, Av. Honorio Delgado 262, Urb. Ingeniería, San Martin de Porres, Lima, Peru, Tel: 011-511-482-0402, Fax: 011-511-482-0402 ext. 229, E-mail: jaimecok20@yahoo.com. Gloria Vargas, Department of Gastroenterology, National Major San Marcos University, Av. Germán Amezaga s/n, Lima, Peru, Tel: 011-619-7000, Fax: 011-619-7000, E-mail: gvargas@inen.sld.pe. Juan Combe, Department of Gastroenterology, Hospital Arzobispo Loayza, Av. Alfonso Ugarte Nro. 848, Lima, Peru, Tel: 011-511-4313710, Fax: 011-511-4313710, E-mail: jcombe@inen.sld.pe. Sijin Wen, The University of Texas M. D. Anderson Cancer Center, Department of Biostatistics, 1515 Holcombe Boulevard, Houston, TX 77030-4009, Tel: 1-713-794-4168, Fax: 1-713-563-4242, E-mail:

sijinwen@mdanderson.org. Frank Meyer, Otto-von-Guericke University Magdeburg, Department of Surgery, University Hospital, Magdeburg Leipziger Str. 44, 39112 Magdeburg, Tel: 0049-391-6715500, Fax: 0049-391-6715570, E-mail: Frank.Meyer@med.ovgu.de. Douglas E. Berg, Washington University School of Medicine, Department of Molecular Microbiology, 660 South Euclid Avenue, St. Louis, MO 63110-1093, Tel: 1-314-362-2772, Fax: 1-314-362-7325, E-mail: berg@borcim.wustl.edu. Robert H. Gilman, Department of International Health, The Johns Hopkins Bloomberg School of Public Health, 615 North Wolfe Street, Baltimore, MA, 21205, Tel: 1-410-614-3959, Fax: 1-410-614-6060, E-mail: RGilman@jhsph.edu.

Reprint requests: Sebastian Gehmert, Department of Molecular Pathology, The University of Texas M. D. Anderson Cancer Center, 7435 Fannin Street, Houston, TX 77054, E-mail: s.gehmert@gmail.com. Robert H. Gilman, Department of International Health, The Johns Hopkins Bloomberg School of Public Health, 615 North Wolfe Street, Baltimore, MD 21205, E-mail: RGilman@jhsph.edu.

REFERENCES

1. Parkin DM, Pisani P, Ferlay J, 1999. Global cancer statistics. *CA Cancer J Clin 49:* 33–64.
2. Stewart BW, Kleihues P, 2003. *World Cancer Report*. Stewart BW, Kleihues P, eds. Lyon: IARC Press, 12–19.
3. Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ, 2001. *Helicobacter pylori* infection and the development of gastric cancer. *N Engl J Med 345:* 784–789.
4. Correa P, 1992. Human gastric carcinogenesis: a multistep and multifactorial process–First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res 52:* 6735–6740.
5. Covacci A, Telford JL, Del Giudice G, Parsonnet J, Rappuoli R, 1999. *Helicobacter pylori* virulence and genetic geography. *Science 284:* 1328–1333.
6. Leung WK, Chan MC, To KF, Man EP, Ng EK, Chu ES, Lau JY, Lin SR, Sung JJ, 2006. *H. pylori* genotypes and cytokine gene polymorphisms influence the development of gastric intestinal metaplasia in a Chinese population. *Am J Gastroenterol 101:* 714–720.
7. Rad R, Prinz C, Neu B, Neuhofer M, Zeitner M, Voland P, Becker I, Schepp W, Gerhard M, 2003. Synergistic effect of *Helicobacter pylori* virulence factors and interleukin-1 polymorphisms for the development of severe histological changes in the gastric mucosa. *J Infect Dis 188:* 272–281.
8. Rad R, Dossumbekova A, Neu B, Lang R, Bauer S, Saur D, Gerhard M, Prinz C, 2004. Cytokine gene polymorphisms influence mucosal cytokine expression, gastric inflammation, and host specific colonisation during *Helicobacter pylori* infection. *Gut 53:* 1082–1089.
9. Kersulyte D, Mukhopadhyay AK, Velapatino B, Su W, Pan Z, Garcia C, Hernandez V, Valdez Y, Mistry RS, Gilman RH, Yuan Y, Gao H, Alarcon T, Lopez-Brea M, Balakrish NG, Chowdhury A, Datta S, Shirai M, Nakazawa T, Ally R, Segal I, Wong BC, Lam SK, Olfat FO, Boren T, Engstrand L, Torres O, Schneider R, Thomas JE, Czinn S, Berg DE, 2000. Differences in genotypes of *Helicobacter pylori* from different human populations. *J Bacteriol 182:* 3210–3218.
10. Santos FR, Gerelsaikhan T, Munkhtuja B, Oyunsuren T, Epplen JT, Pena SD, 1996. Geographic differences in the allele frequencies of the human Y-linked tetranucleotide polymorphism DYS19. *Hum Genet 97:* 309–313.
11. The Gastrointestinal Physiology Working Group of the Cayetano Heredia and the Johns Hopkins University, 1990. *Helicobacter pylori* and gastritis in Peruvian patients: relationship to socioeconomic level, age, and sex. *Am J Gastroenterol 85:* 819–823.
12. The Gastrointestinal Physiology Working Group of the Cayetano Heredia and the Johns Hopkins University, 1992. Ecology of *Helicobacter pylori* in Peru: infection rates in coastal, high altitude, and jungle communities. *Gut 33:* 604–605.
13. Soto G, Bautista CT, Roth DE, Gilman RH, Velapatino B, Ogura M, Dailide G, Razuri M, Meza R, Katz U, Monath TP, Berg DE, Taylor DN, The Gastrointestinal Physiology Working Group in Peru, 2003. *Helicobacter pylori* reinfection is common in Peruvian adults after antibiotic eradication therapy. *J Infect Dis 188:* 1263–1275.
14. Doglioni C, Turrin M, Macri E, Chiarelli C, Germana B, Barbareschi M, 1997. HpSS: a new silver staining method for *Helicobacter pylori*. *J Clin Pathol 50:* 461–464.
15. Dixon MF, Genta RM, Yardley JH, Correa P, 1996. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol 20:* 1161–1181.
16. Chang YW, Jang JY, Kim NH, Lee JW, Lee HJ, Jung WW, Dong SH, Kim HJ, Kim BH, Lee JI, Chang R, 2005. Interleukin-1B (IL-1B) polymorphisms and gastric mucosal levels of IL-1beta cytokine in Korean patients with gastric cancer. *Int J Cancer 114:* 465–471.
17. Yamada S, Matsuhisa T, Makonkawkeyoon L, Chaidatch S, Kato S, Matsukura N, 2006. *Helicobacter pylori* infection in combination with the serum pepsinogen I/II ratio and interleukin-1beta-511 polymorphisms are independent risk factors for gastric cancer in Thais. *J Gastroenterol 41:* 1169–1177.
18. Yang J, Hu Z, Xu Y, Shen J, Niu J, Hu X, Guo J, Wei Q, Wang X, Shen H, 2004. Interleukin-1B gene promoter variants are associated with an increased risk of gastric cancer in a Chinese population. *Cancer Lett 215:* 191–198.
19. Ikehara SK, Ikehara Y, Matsuo K, Hirose K, Niwa T, Ito H, Ito S, Kodera Y, Yamamura Y, Nakanishi H, Tatematsu M, Tajima K, 2006. A polymorphism of C-to-T substitution at -31 IL1B is associated with the risk of advanced gastric adenocarcinoma in a Japanese population. *J Hum Genet 51:* 927–933.
20. Matsukura N, Yamada S, Kato S, Tomtitchong P, Tajiri T, Miki M, Matsuhisa T, Yamada N, 2003. Genetic differences in interleukin-1 betapolymorphisms among four Asian populations: an analysis of the Asian paradox between *H. pylori* infection and gastric cancer incidence. *J Exp Clin Cancer Res 22:* 47–55.
21. El Omar EM, Rabkin CS, Gammon MD, Vaughan TL, Risch HA, Schoenberg JB, Stanford JL, Mayne ST, Goedert J, Blot WJ, Fraumeni JF Jr, Chow WH, 2003. Increased risk of noncardia gastric cancer associated with proinflammatory cytokine gene polymorphisms. *Gastroenterology 124:* 1193–1201.
22. Figueiredo C, Machado JC, Pharoah P, Seruca R, Sousa S, Carvalho R, Capelinha AF, Quint W, Caldas C, van Doorn LJ, Carneiro F, Sobrinho-Simoes M, 2002. *Helicobacter pylori* and interleukin 1 genotyping: an opportunity to identify high-risk individuals for gastric carcinoma. *J Natl Cancer Inst 94:* 1680–1687.
23. Machado JC, Pharoah P, Sousa S, Carvalho R, Oliveira C, Figueiredo C, Amorim A, Seruca R, Caldas C, Carneiro F, Sobrinho-Simoes M, 2001. Interleukin 1B and interleukin 1RN polymorphisms are associated with increased risk of gastric carcinoma. *Gastroenterology 121:* 823–829.
24. Ruzzo A, Graziano F, Pizzagalli F, Santini D, Battistelli V, Panunzi S, Canestrari E, Catalano V, Humar B, Ficarelli R, Bearzi I, Cascinu S, Naldi N, Testa E, Magnani M, 2005. Interleukin 1B gene (IL-1B) and interleukin 1 receptor antagonist gene (IL-1RN) polymorphisms in *Helicobacter pylori*-negative gastric cancer of intestinal and diffuse histotype. *Ann Oncol 16:* 887–892.
25. Camargo MC, Mera R, Correa P, Peek RM Jr, Fontham ET, Goodman KJ, Piazuelo MB, Sicinschi L, Zabaleta J, Schneider BG, 2006. Interleukin-1beta and interleukin-1 receptor antagonist gene polymorphisms and gastric cancer: a meta-analysis. *Cancer Epidemiol Biomarkers Prev 15:* 1674–1687.
26. Kamangar F, Cheng C, Abnet CC, Rabkin CS, 2006. Interleukin-1B polymorphisms and gastric cancer risk-a meta-analysis. *Cancer Epidemiol Biomarkers Prev 15:* 1920–1928.
27. Vincenzi B, Patti G, Galluzzo S, Pantano F, Venditti O, Santini D, Ruzzo A, Schiavon G, Caraglia M, Marra M, Graziano F, Tonini G, 2008. Interleukin 1beta-511T gene (IL1beta) polymorphism is correlated with gastric cancer in the Caucasian population: results from a meta-analysis. *Oncol Rep 20:* 1213–1220.
28. Wang P, Xia HH, Zhang JY, Dai LP, Xu XQ, Wang KJ, 2007. Association of interleukin-1 gene polymorphisms with gastric cancer: a meta-analysis. *Int J Cancer 120:* 552–562.
29. Zeng ZR, Hu PJ, Hu S, Pang RP, Chen MH, Ng M, Sung JJ, 2003. Association of interleukin 1B gene polymorphism and gastric cancers in high and low prevalence regions in China. *Gut 52:* 1684–1689.

30. Bardhan PK, 1997. Epidemiological features of *Helicobacter pylori* infection in developing countries. *Clin Infect Dis 25:* 973–978.
31. The Gastrointestinal Physiology Working Group of the Cayetano Heredia and the Johns Hopkins University, 1986. Rapid identification of *pyloric Campylobacter* in Peruvians with gastritis. *Dig Dis Sci 31:* 1089–1094.
32. Recavarren-Arce S, Leon-Barua R, Rodriguez C, Cok J, Berendson R, Gilman RH, 1995. *Helicobacter pylori*-associated chronic gastritis in Peruvian adolescents is very common and severe. *J Clin Gastroenterol 20:* 335–337.
33. Recavarren-Arce S, Ramirez-Ramos A, Gilman RH, Chinga-Alayo E, Watanabe-Yamamoto J, Rodriguez-Ulloa C, Miyagui J, Passaro DJ, Eza D, 2005. Severe gastritis in the Peruvian Andes. *Histopathology 46:* 374–379.
34. Hwang IR, Kodama T, Kikuchi S, Sakai K, Peterson LE, Graham DY, Yamaoka Y, 2002. Effect of interleukin 1 polymorphisms on gastric mucosal interleukin 1beta production in *Helicobacter pylori* infection. *Gastroenterology 123:* 1793–1803.
35. Xuan J, Deguchi R, Watanabe S, Ozawa H, Urano T, Ogawa Y, Fukuda R, Kijima H, Koga Y, Takagi A, 2005. Relationship between IL-1beta gene polymorphism and gastric mucosal IL-1beta levels in patients with *Helicobacter pylori* infection. *J Gastroenterol 40:* 796–801.
36. Chakravorty M, Ghosh A, Choudhury A, Santra A, Hembrum J, Roychoudhury S, 2006. Interaction between IL1B gene promoter polymorphisms in determining susceptibility to *Helicobacter pylori* associated duodenal ulcer. *Hum Mutat 27:* 411–419.
37. Danon SJ, O'Rourke JL, Moss ND, Lee A, 1995. The importance of local acid production in the distribution of *Helicobacter felis* in the mouse stomach. *Gastroenterology 108:* 1386–1395.
38. Fukui H, Franceschi F, Penland RL, Sakai T, Sepulveda AR, Fujimori T, Terano A, Chiba T, Genta RM, 2003. Effects of *Helicobacter pylori* infection on the link between regenerating gene expression and serum gastrin levels in Mongolian gerbils. *Lab Invest 83:* 1777–1786.
39. Bayerdorffer E, Lehn N, Hatz R, Mannes GA, Oertel H, Sauerbruch T, Stolte M, 1992. Difference in expression of *Helicobacter pylori* gastritis in antrum and body. *Gastroenterology 102:* 1575–1582.
40. de Waal MR, Abrams J, Bennett B, Figdor CG, de Vries JE, 1991. Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J Exp Med 174:* 1209–1220.
41. Garza-Gonzalez E, Hold G, Perez-Perez GI, Bosques-Padilla FJ, Tijerina-Menchaca R, Maldonado-Garza HJ, el Omar E, 2003. Role of polymorphism of certain cytokines in gastric cancer in Mexico. Preliminary results. *Rev Gastroenterol Mex 68:* 107–112.
42. Garcia-Gonzalez MA, Lanas A, Santolaria S, Crusius JB, Serrano MT, Pena AS, 2001. The polymorphic IL-1B and IL-1RN genes in the aetiopathogenesis of peptic ulcer. *Clin Exp Immunol 125:* 368–375.
43. Garcia-Gonzalez MA, Lanas A, Savelkoul PH, Santolaria S, Benito R, Crusius JB, Pena AS, 2003. Association of interleukin 1 gene family polymorphisms with duodenal ulcer disease. *Clin Exp Immunol 134:* 525–531.
44. Santtila S, Savinainen K, Hurme M, 1998. Presence of the IL-1RA allele 2 (IL1RN*2) is associated with enhanced IL-1beta production *in vitro. Scand J Immunol 47:* 195–198.
45. El Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, Herrera J, Lissowska J, Yuan CC, Rothman N, Lanyon G, Martin M, Fraumeni JF Jr, Rabkin CS, 2000. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature 404:* 398–402.
46. Perez-Perez GI, Garza-Gonzalez E, Portal C, Olivares AZ, 2005. Role of cytokine polymorphisms in the risk of distal gastric cancer development. *Cancer Epidemiol Biomarkers Prev 14:* 1869–1873.