

Helicobacter pylori and Interleukin 1 Genotyping: An Opportunity to Identify High-Risk Individuals for Gastric Carcinoma

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Background: Both *Helicobacter pylori* genotype and host genetic polymorphisms play a role in determining the clinical consequences of *H. pylori* infection. We investigated whether there are any combinations of bacterial and host genotypes that are particularly associated with the occurrence of gastric carcinoma. **Methods:** Genotypic variations in virulence-associated genes of *H. pylori* *vacA* (s and m regions) and *cagA* were determined in 221 subjects with chronic gastritis and 222 patients with gastric carcinoma by polymerase chain reaction (PCR)-line probe assay. Polymorphisms in the human interleukin 1 beta (IL-1B) gene (IL-1B-511*C or IL-1B-511*T) and in the IL-1 receptor antagonist gene (IL-1RN intron 2 variable number of tandem repeats) were evaluated by PCR and single-strand conformation polymorphism analysis. All statistical tests were two-sided. **Results:** Infection with *vacAs1*-, *vacAm1*-, and *cagA*-positive strains of *H. pylori* was associated with an increased risk for gastric carcinoma, with odds ratios (ORs) of 17 (95% confidence interval [CI] = 7.8 to 38), 6.7 (95% CI = 3.6 to 12), and 15 (95% CI = 7.4 to 29), respectively. IL-1B-511*T carriers (IL-1B-511*T/*T or IL-1B-511*T/*C) homozygous for the short allele of IL-1RN (IL-1RN*2/*2) had an increased gastric carcinoma risk (OR = 3.3, 95% CI = 1.3 to 8.2). For each combination of bacterial/host genotype, the odds of having gastric carcinoma were greatest in those with both bacterial and host high-risk genotypes: *vacAs1*/IL-1B-511*T carrier (OR = 87, 95% CI = 11 to 679), *vacAm1*/IL-1B-511*T carrier (OR = 7.4, 95% CI = 3.2 to 17), *cagA*-positive/IL-1B-511*T carrier (OR = 25, 95% CI = 8.2 to 77), *vacAs1*/IL-1RN*2/*2 (OR = 32, 95% CI = 7.8 to 134), *vacAm1*/IL-1RN*2/*2 (OR = 8.8, 95% CI = 2.2 to 35), and *cagA*-positive/IL-1RN*2/*2 (OR = 23, 95% CI = 7.0 to 72). **Conclusion:** Combined bacterial/host genotyping may provide an important tool in defining disease risk and targeting *H. pylori* eradication to high-risk individuals. [J Natl Cancer Inst 2002;94:1680-7]

Helicobacter pylori colonizes the human stomach and establishes a long-term infection of the gastric mucosa (1). This infection first induces chronic superficial (non-atrophic) gastritis, which can progress through chronic atrophic gastritis, intestinal metaplasia, and dysplasia toward gastric carcinoma (2). However, progression occurs in only some patients and seems to depend on a number of factors, including both bacterial and host genetic factors (3,4).

H. pylori are genetically highly diverse bacteria, and several genotypes have been associated with virulence and gastric disease risk (5,6). The *vacA* gene, which encodes a vacuolating

cytotoxin, is present in all *H. pylori* strains. This gene comprises two variable regions (7): the s region, which exists as an s1a, s1b, s1c, or s2 allele, and the m region, which occurs as an m1, m2a, or m2b allele (8). *H. pylori vacA* type s1 strains appear to be more virulent than type s2 strains and are associated with higher risks for peptic ulcer disease, gastric atrophy, and gastric carcinoma (9,10). The *vacAs1* and *vacAm1* strains are also strongly associated with a higher degree of inflammation and epithelial damage in the gastric mucosa (11,12).

The *H. pylori cagA* gene is a marker for the presence of the *cag* pathogenicity island (PAI) (13). The CagA protein is translocated by a type IV secretion system (encoded by the *cag* PAI) into gastric epithelial cells, where it induces changes in the tyrosine phosphorylation states of distinct cellular proteins (14). Several genes of the *cag* PAI encode proteins that increase the production of the pro-inflammatory interleukin 8 (IL-8) by the gastric epithelium (15). There is an association between infection with *cagA*-positive strains and risk for peptic ulcer disease (7,9), and for development of atrophic gastritis and carcinoma of the stomach (10,11,16,17).

Human genetic polymorphisms also appear to play a role in the disease susceptibility of the host. Recently, polymorphisms of the interleukin 1 beta (IL-1B) gene and the IL-1 receptor antagonist gene (IL-1RN) have been associated with an increased risk of both hypochlorhydria and gastric carcinoma (18,19). IL-1B encodes IL-1 β , a potent pro-inflammatory cytokine and powerful inhibitor of gastric acid secretion that plays a major role in initiating and amplifying the inflammatory response to *H. pylori* infection (20,21). A polymorphic allele with a T instead of a C at position -511 of the regulatory region of the IL-1B gene (IL-1B-511*T) is associated with increased IL-1 β production (18). IL-1RN encodes the IL-1 receptor antagonist (IL-1ra), an anti-inflammatory cytokine that competitively binds to IL-1 receptors, and thereby modulates the potentially damaging effects of IL-1 (22). The IL-1RN gene has a variable number of tandem repeats in intron 2, resulting in a short allele

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(IL-1RN*2, with two repeats) or long alleles (IL-1RN*L, with three to six repeats). The IL-1RN*2 allele is associated with increased IL-1b production (23,24). *H. pylori* infection in individuals with these alleles may therefore result in increased production of gastric IL-1b, leading to severe and sustained inflammation, gastric atrophy, and hypochlorhydria, and ultimately to the development of gastric carcinoma (18,25).

In this study we investigated whether there are combinations of bacterial and host genotypes that are highly associated with the occurrence of gastric carcinoma. Our aim was to relate *H. pylori* *vacA* and *cagA* virulence-associated genes and human IL-1B and IL-1RN susceptibility polymorphisms with the histopathologic features of gastritis and the risk for development of gastric carcinoma.

MATERIALS AND METHODS

Patients With Gastritis and Gastric Carcinoma

In total, 443 subjects were analyzed: 221 subjects with chronic gastritis and 222 with gastric carcinoma. Subjects with chronic gastritis (mean age = 43 years, median age = 43 years, range = 24–62 years, and male/female ratio of 13:1) were recruited from a group of shipyard workers and underwent standard gastroscopy in 1998 at the Hospital de S. João (Porto, Portugal) during a screening program for premalignant lesions of the gastric mucosa. Only individuals without evidence of past or present peptic ulcer disease were included. Patients with gastric carcinoma (mean age = 62 years, median age = 63 years, range = 26–90 years, and male/female ratio of 1.7:1) were diagnosed and underwent resection of cancer at the Hospital S. João/Medical Faculty and Instituto de Patologia e Imunologia Molecular da Universidade do Porto (IPATIMUP), Porto, Portugal. The cancers included 119 (53.6%) antral, 50 (22.5%) corpus, 4 (1.8%) fundus, and 39 (17.6%) cardia carcinomas. In 10 (4.5%) case patients, the tumors involved more than one anatomic region of the stomach. All procedures followed in the study were in accordance with the institutional ethical standards. All samples were delinked and unidentified from their donors. All individuals provided written informed consent.

Histopathology

Biopsy specimens from the antrum and corpus mucosa of chronic gastritis subjects and gastric carcinoma surgical specimens were formalin fixed, paraffin embedded, and stained with hematoxylin–eosin, Alcian blue–periodic acid–Schiff, and modified Giemsa. All 221 subjects with chronic gastritis had antral biopsy specimens but only 219 had corpus biopsy specimens available. All the available samples were accepted for histologic assessment. *H. pylori* density, chronic inflammation, polymorphonuclear activity, epithelial damage, glandular atrophy, and intestinal metaplasia were scored according to the updated Sydney system (26). Inflammation and activity scores were added, as previously described (27), for a combined (acute and chronic) inflammatory score with a maximum possible value of 6. Gastric carcinoma case patients were classified according to Laurén's classification (28) as intestinal ($n = 116$), diffuse ($n = 52$), or atypical ($n = 54$) carcinomas. Histologic slides were examined by two experienced pathologists (F. Carneiro and M. Sobrinho-Simões), blinded to the clinical information of the patients.

DNA Isolation

DNA was extracted from gastric antral biopsy specimens using the method described by Boom (29). Briefly, biopsy speci-

mens were homogenized in guanidinium isothiocyanate with a sterile micropestle. DNA was captured onto silica particles, washed, and then eluted in 100 μ L of 10 mM Tris–HCl (pH 8.3). DNA from non-neoplastic gastric mucosa of gastric carcinoma surgical specimens was isolated using a standard phenol/chloroform extraction procedure.

H. pylori vacA and *cagA* Genotyping

H. pylori DNA that was extracted from either gastric antral biopsies or surgical specimens was used for *vacA* and *cagA* genotyping. Two hundred eighteen (98.6%) of the 221 chronic gastritis subjects and 130 (58.6%) of the 222 carcinoma case subjects had material available for *H. pylori* genotyping. Genotyping was performed by multiplex polymerase chain reaction (PCR) followed by reverse hybridization on a line probe assay (LiPA), as described (8,30). Genotypes were obtained for all case subjects for the *vacA* s region and for 339 (97.4%) of the case subjects for the m region.

IL-1B-511 and IL-1RN Variable Number Tandem Repeats Genotyping

Two hundred seventeen (98.2%) chronic gastritis subjects and 221 (99.5%) carcinoma case subjects were genotyped for the IL-1B-511 polymorphism, and all subjects were genotyped for IL-1RN variable number tandem repeats (VNTR) in intron 2. The IL-1B-511*C/IL-1B-511*T bi-allelic polymorphism was genotyped by PCR–SSCP (polymerase chain reaction–single-strand conformation polymorphism), and the IL-1RN pentallelic VNTR was genotyped by PCR–standard agarose gel electrophoresis, as previously described (19). The IL-1RN alleles were coded as follows: allele 1 = four repeats, allele 2 = two repeats, allele 3 = five repeats, allele 4 = three repeats, and allele 5 = six repeats. For the purpose of statistical analysis and because of the rarity of alleles 3, 4, and 5, this polymorphism was treated as bi-allelic by dividing alleles into short and long (L) categories; those in the short allele category are those with two repeats (allele 2), and those in the long allele category are those with three or more repeats (alleles 1, 3, 4, and 5) (19). Genotype notation is as follows: C homozygote = IL-1B-511*C/*C, T carrier = IL-1B-511*T/*T or IL-1B-511*T/*C, L carrier = IL-1RN*L/*L or IL-1RN*L/*2, and 2 homozygote = IL-1RN*2/*2.

Statistical Analysis

Differences in combined inflammatory score and gastric bacterial colonization density among different genotypes were evaluated with the Mann–Whitney test. Associations between genotypes and the presence of epithelial damage, glandular atrophy, and intestinal metaplasia were assessed by the χ^2 test. Only subjects containing single *H. pylori* genotypes were included in the analyses of the histopathologic features of gastritis. Associations between bacterial genotypes and host genotypes and comparison of genotype frequencies between carcinoma patients and gastritis control subjects were assessed by the χ^2 test. Comparison of genotype frequencies between groups defined by age, sex, anatomic site, and histologic type was performed by the χ^2 test. The control group consisted of 136 individuals with no evidence of glandular atrophy or intestinal metaplasia. These 136 individuals were a subset of the 221 chronic gastritis subjects. Odds ratios (ORs) with 95% confidence intervals (CIs) and

unconditional logistic regression models were computed with SPSS software (version 9; SPSS Science, Chicago, IL). Differences were considered statistically significant when $P < .05$. All statistical tests were two-sided.

RESULTS

H. pylori and IL-1B/IL-1RN Genotypes and Histopathologic Features of Gastritis

In patients with gastritis, *H. pylori* vacAs1-, vacAm1-, and cagA-positive genotypes were each associated with higher combined inflammatory scores in corpus and antrum (Table 1). Statistically significant associations were also shown, both in corpus and in antrum, between the same *H. pylori* genotypes and active and chronic inflammatory scores when both parameters were examined independently.

The vacAs1-, vacAm1-, and cagA-positive genotypes each also showed an association with the presence of epithelial damage both in corpus and antrum (Fig. 1, A). These genotypes were also associated with *H. pylori* colonization density in the corpus but not in the antrum (Table 1).

Glandular atrophy and intestinal metaplasia were statistically significantly associated with vacAs1-, vacAm1-, and cagA-positive genotypes of *H. pylori* (Fig. 2, A and B). However, this association did not take into account the distribution within the stomach, because few cases of glandular atrophy and intestinal

metaplasia were detected in the corpus (seven and seven, respectively).

Neither the IL-1B-511 nor the IL-1RN VNTR genotype was individually associated with combined inflammatory score or with *H. pylori* colonization density in the corpus or the antrum (Table 1). The IL-1B-511*T carrier genotype was associated with epithelial degeneration in the corpus but not in the antrum, whereas the IL-1RN*2/*2 genotype was associated with this parameter in both areas of the stomach (Fig. 1, B).

Individuals who were IL-1B-511*T carriers and homozygous for IL-1RN*2 had a more marked corpus gastritis and a higher corpus *H. pylori* density than IL-1B-511*C homozygotes/IL-1RN*L carriers (Table 1). IL-1B-511*T carriers/IL-1RN*2 homozygotes also had a high risk of epithelial damage in both corpus and antrum (Fig. 1, B). These individuals also had a higher risk of glandular atrophy and intestinal metaplasia (Fig. 2, A and B).

H. pylori and IL-1B/IL-1RN Genotypes in Gastric Carcinomas Compared With Gastritis

To explore the effect of *H. pylori* strains on gastric disease phenotype and to assess the nature of any interaction with IL-1B/IL-1RN genotypes, we compared the strain and genotype data in the patients with gastric carcinoma with those data in the 136 individuals who had gastritis without evidence of glandular atrophy or metaplasia. Information regarding *H. pylori* strain

Table 1. Histopathologic features of corpus and antral biopsy specimens from subjects with gastritis carrying *Helicobacter pylori* strains with different vacA and cagA genotypes, and human interleukin 1B (IL-1B) and IL-1 receptor antagonist gene (IL-1RN) genotypes*

Biopsy specimen and histopathologic features	<i>H. pylori</i> genotypes†						IL-1B/IL-1RN genotypes					
	vacA s region		vacA m region		cagA		IL-1B-511		IL-1RN		IL-1B-511/IL-1RN	
	s1	s2	m1	m2	Positive	Negative	C homozygote	T carrier	L carrier	2 homozygote	C homozygote/ L carrier	T carrier/ 2 homozygote
Corpus (combined inflammatory score)												
Count	92	71	72	102	119	97	86	129	194	25	81	19
Range	0-6	0-4	0-6	0-4	0-6	0-4	0-5	0-6	0-6	0-5	0-5	0-5
Median	3	2	3	2	3	2	2	2	2	3	2	3
P value‡	<.001		<.001		<.001		.15		.13		.03	
Antrum (combined inflammatory score)												
Count	94	71	74	102	121	97	86	131	196	25	81	19
Range	1-5	2-6	1-5	1-6	1-6	1-6	1-5	1-6	1-6	1-6	1-5	1-5
Median	4	3	4	3	4	3	3.5	4	3.5	4	4	4
P value‡	<.001		<.001		<.001		.46		.26		.09	
Corpus (<i>H. pylori</i> density)												
Count	92	69	72	100	119	94	86	126	191	25	81	19
Range	0-3	0-3	0-3	0-3	0-3	0-3	0-3	0-3	0-3	0-3	0-3	1-3
Median	2	1	2	1	2	1	1	2	1	2	1	2
P value‡	<.001		.003		<.001		.15		.22		.012	
Antrum (<i>H. pylori</i> density)												
Count	94	71	74	102	121	97	86	131	196	25	81	19
Range	0-3	1-3	0-3	0-3	0-3	0-3	0-3	0-3	0-3	0-3	0-3	1-3
Median	2.5	2	2	2	2	2	2	2	2	2	2	3
P value‡	.81		.86		.75		.45		.46		.25	

*Results for totals may not agree because two case patients did not have a corpus biopsy specimen available and three case patients had a deficient inclusion of the corpus fragment, thus not allowing the proper *H. pylori* density evaluation. C homozygote = IL-1B-511*C/*C; T carrier = IL-1B-511*T/*T or IL-1B-511*T/*C; L carrier = IL-1RN*L/*L or IL-1RN*L/*2; 2 homozygote = IL-1RN*2/*2.

†*H. pylori* genotypes shown here do not include individuals infected with multiple vacA strains.

‡Mann-Whitney test.

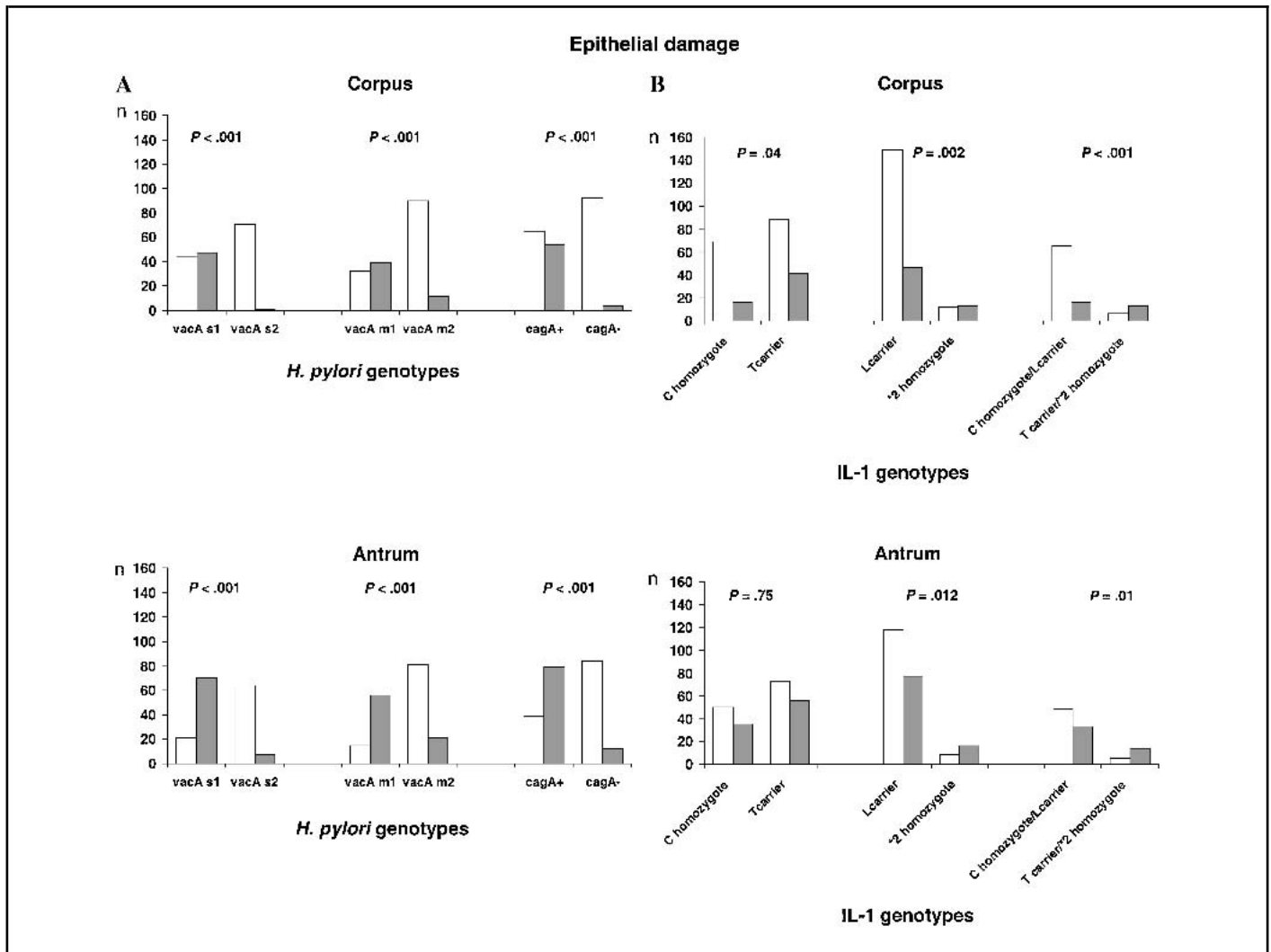


Fig. 1. Relationship between epithelial damage and *Helicobacter pylori* *vacA* and *cagA* genotypes (A) and interleukin 1B (IL-1B) and IL-1 receptor antagonist (IL-1RN) genotypes (B) in corpus and antrum of individuals with gastritis. **Open bars** = absence of epithelial damage; **solid bars** = presence of epithelial damage; n = number of subjects; C homozygote = IL-1B-511*C*/C; T carrier = IL-1B-511*T*/T or IL-1B-511*T*/C; L carrier = IL-1RN*L*/L or IL-1RN*L*/*2; *2 homozygote = IL-1RN*2*/2. P values were obtained from χ^2 tests.

was not available for the disease-free control group, and individuals with atrophic gastritis or intestinal metaplasia were excluded because of the well-documented association of these histologic features with gastric carcinoma.

Among individuals infected with single *H. pylori* *vacA*s and *vacA*m strains, *vacA*s1 and *vacA*m1 strains were statistically significantly more prevalent in patients with gastric carcinoma (92% [98/107] and 69% [68/98], respectively) than in subjects with non-atrophic gastritis (39% [39/101] and 25% [27/107], respectively), with ORs and 95% CIs for developing gastric carcinoma of 17 (7.8 to 38) and 6.7 (3.6 to 12), respectively (Table 2). *cagA*-Positive strains were also statistically significantly more prevalent in gastric carcinoma patients (91% [118/130]) than in non-atrophic gastritis subjects (40% [54/135]), with an OR (95% CI) for gastric carcinoma of 15 (7.4 to 29) (Table 2).

Infection with multiple *vacA*s or *vacA*m strains was more prevalent in patients with gastric carcinoma (72% [23/32] and 42% [22/52], respectively) than in non-atrophic gastritis subjects (35% [34/96] and 26% [28/108], respectively), with ORs (95% CIs) of 4.7 (1.9 to 11) and 2.1 (1.0 to 4.2), respectively.

In the gastric carcinoma patients, IL-1B-511*T carriers represented 69% (152/221) of the case subjects, which is statistically significantly higher than the proportion of this genotype in the non-atrophic gastritis group (55%) with an OR of 1.8 (95% CI = 1.2 to 2.8) (Table 3). The observed association between IL-1RN VNTR genotype and the risk of gastric carcinoma was not statistically significant. Genotype frequencies did not vary by sex or age in either control subjects or gastric carcinoma case patients.

There was some evidence that the risks associated with the IL-1B-511*T and IL-1RN*2 alleles act independently (Table 3), with the risk in combined *T carrier/*2 homozygotes being greater than the risk associated with each allele separately (OR = 3.3, 95% CI = 1.3 to 8.2). However, this observation should be treated with some caution because the individual ORs were not statistically significantly different. The estimated effects of the different bacterial and host genotypes were similar in subgroups of gastric carcinoma case patients defined by age, sex, histologic type, and anatomic site (Table 4).

To determine whether patients of a particular IL-1B or IL-1RN genotype were preferentially infected by specific *H. pylori*

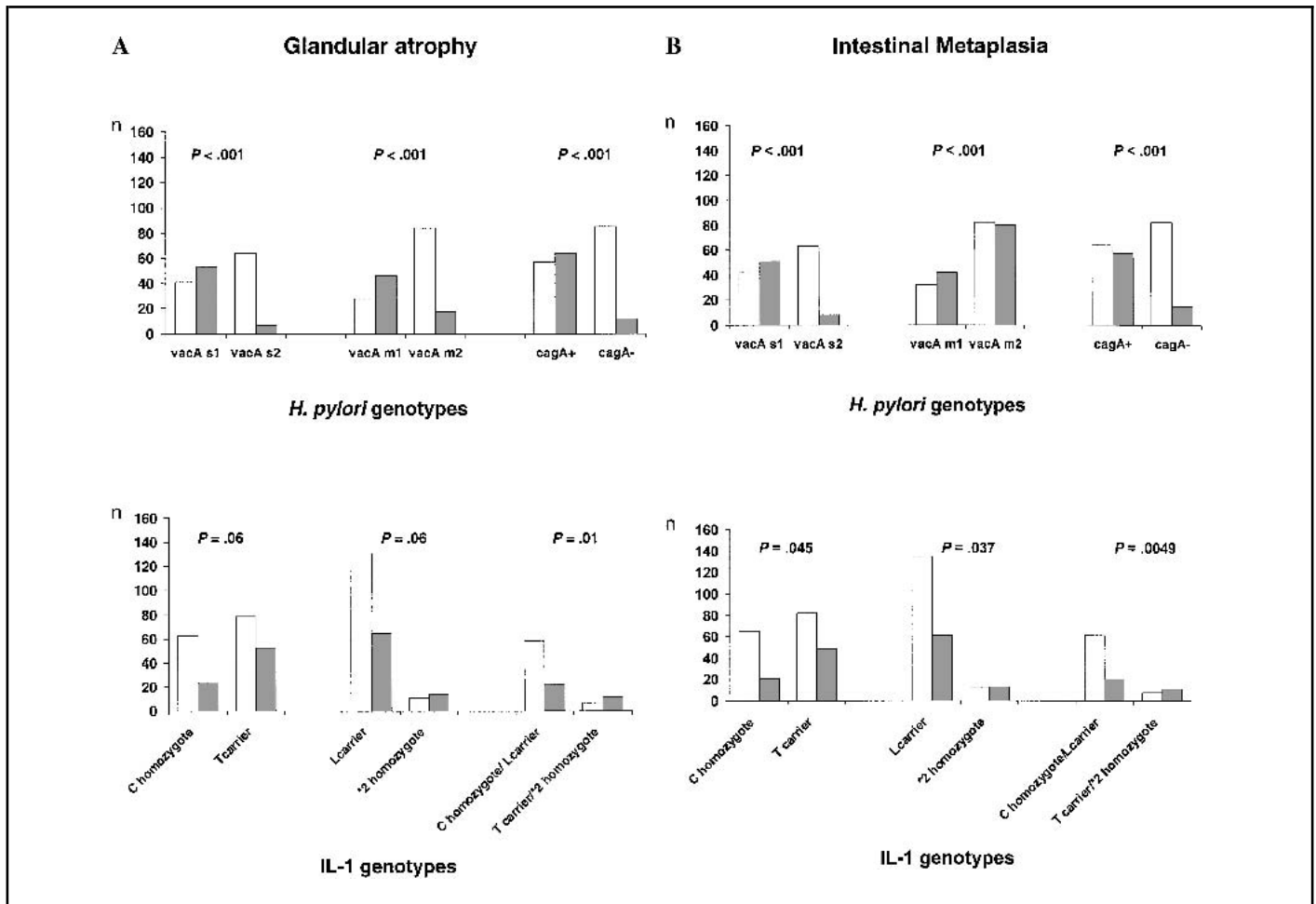


Fig. 2. Relationship between glandular atrophy (**A**) and intestinal metaplasia (**B**) and *Helicobacter pylori* vacA and cagA genotypes and interleukin 1B (IL-1B) and IL-1 receptor antagonist (IL-1RN) genotypes among individuals with gastritis. **Open bars** = absence of glandular atrophy or intestinal metaplasia; **solid bars** = presence of glandular atrophy or intestinal metaplasia; n = number of subjects; C homozygote = IL-1B-511*C/*C; T carrier = IL-1B-511*T/*T or IL-1B-511*T/*C; L carrier = IL-1RN*L/*L or IL-1RN*L/*2; 2 homozygote = IL-1RN*2/*2. *P values were obtained from χ^2 tests.

strains, we compared host and bacterial genotypes in the groups of individuals with gastritis and patients with gastric carcinoma. No association between infection with any of the bacterial vacAs, vacAm, or cagA genotypes and the human IL-1B or IL-1RN genotypes was observed (data not shown).

To assess the nature of the interaction between the high-risk bacterial and host genotypes, we compared the combined bacterial/host genotype frequencies in gastritis control subjects with those in case patients with gastric carcinoma (Tables 5 and 6). For each combination, the odds of having gastric carcinoma were greatest in those with high-risk genotypes in both the bacteria and the host. The highest risk was seen in IL-1B-511*T carriers infected with *H. pylori* of the vacAs1 genotype (Table 5). Such individuals represented 66% of the gastric carcinoma patients (71/107), although they made up only 22% of individuals with non-atrophic gastritis (22/101). Deviation from a multiplicative interaction model, which assumes independent effects for each risk factor, was assessed by including an interaction term in an unconditional logistic regression model. No interaction term was statistically significant, suggesting that the effects of the bacterial and host genotypes are independent. Subject numbers were too small to assess possible three-way interactions.

Table 2. *Helicobacter pylori* vacA and cagA genotypes in individuals with gastritis and gastric carcinoma

<i>H. pylori</i> genotypes	All Ga (n)*	NAGa (n)*	GC (n)*	Odds ratio (95% confidence interval)†	P value‡
vacA s region					
s2	71	62	9	1 (referent)	
Multiple	53	34	23	4.7 (1.9 to 11)	<.001
s1	94	39	98	17 (7.8 to 38)	<.001
vacA m region					
m2	102	80	30	1 (referent)	
Multiple	42	28	22	2.1 (1.0 to 4.2)	.038
m1	74	27	68	6.7 (3.6 to 12)	<.001
cagA status					
Negative	97	81	12	1 (referent)	
Positive	121	54	118	15 (7.4 to 29)	<.001

*All Ga = all subjects with gastritis; NAGa = subjects with non-atrophic gastritis; GC = patients with gastric carcinoma. Material was available for *H. pylori* genotyping for 218 of the 221 Ga subjects, 135 of the 136 NAGa subjects, and 130 of the 222 GC subjects. Ten samples from GC patients could not be genotyped for the vacAm region because of degraded DNA.

†Odds ratios and P values (χ^2 test) for developing GC using NAGa subjects as controls.

Table 3. Interleukin 1B (IL-1B) and IL-1 receptor antagonist (IL-1RN) genotypes in individuals with gastritis and gastric carcinoma

Human IL-1 genotypes	All Ga (n)*	NAGa (n)*	GC (n)*	Odds ratio (95% confidence interval)†	P value‡
IL-1B-511					
C homozygote	86	61	69	1 (referent)	
T carrier	131	75	152	1.8 (1.2 to 2.8)	.01
IL-1RN					
L carrier	196	125	190	1 (referent)	
2 homozygote	25	11	32	1.9 (0.9 to 3.9)	.078
IL-1B-511/IL-1RN					
C homozygote/L carrier	81	57	62	1 (referent)	
C homozygote/2 homozygote	5	4	7	1.6 (0.5 to 5.8)	.47
T carrier/L carrier	112	68	127	1.7 (1.1 to 2.7)	.023
T carrier/2 homozygote	19	7	25	3.3 (1.3 to 8.2)	.011

*All Ga = all subjects with gastritis; NAGa = subjects with non-atrophic gastritis; GC = patients with gastric carcinoma; C homozygote = IL-1B-511*C/*C; T carrier = IL-1B-511*T/*T or IL-1B-511*T/*C; L carrier = IL-1RN*L/*L or IL-1RN*L/*2; 2 homozygote = IL-1RN*2/*2. Material was available for IL-1B genotyping from 217 Ga subjects, 136 NAGa subjects, and 221 GC patients. All subjects were genotyped for IL-1RN.

†Odds ratios and P values (χ^2 test) for developing GC using NAGa subjects as controls.

Table 4. Interleukin 1B (IL-1B) and IL-1 receptor antagonist (IL-1RN) genotypes, histologic types, and anatomic sites of gastric carcinomas

Gastric carcinoma characteristic	IL-1B/IL-1RN genotypes			
	IL-1B-511		IL-1RN	
	C homozygote	T carrier	L carrier	2 homozygote
Anatomic site*				
Antrum	34	84	104	15
Corpus	20	30	41	9
Cardia	13	26	3	1
Fundus	1	3	33	6
P value†	0.55		0.75	
Histologic type				
Intestinal	28	87	94	22
Diffuse	20	32	48	4
Atypical	21	33	48	6
P value†	0.07		0.11	

*In 10 case patients, the tumors involved more than one anatomic region of the stomach; C homozygote = IL-1B-511*C/*C; T carrier = IL-1B-511*T/*T or IL-1B-511*T/*C; L carrier = IL-1RN*L/*L or IL-1RN*L/*2; 2 homozygote = IL-1RN*2/*2.

†Obtained from χ^2 test.

DISCUSSION

In this study, we have shown that infection with vacAs1-, vacAm1-, and cagA-positive *H. pylori* strains is associated with statistically significantly higher combined inflammatory score, epithelial damage, glandular atrophy, and intestinal metaplasia than infection with vacAs2-, vacAm2-, and cagA-negative strains. Furthermore, vacAs1-, vacAm1-, and cagA-positive strains were found more frequently in gastric carcinoma patients than in individuals with only gastritis. Our results show that vacAs1-, vacAm1-, and cagA-positive strains are associated with an increased risk for gastric carcinoma, with ORs of 17, 6.7, and 15, respectively. These results are in agreement with previously reported data (10–12,16,17) regarding the associations with both histopathologic features of gastritis and gastric carcinoma risk. In addition, we observed that infection with multiple vacAs or vacAm strains was also associated with increased risk for gastric carcinoma, with ORs of 4.7 and 2.1, respectively. This association can be interpreted as a strain dose effect, as-

Table 5. Combination of *Helicobacter pylori* and interleukin 1B (IL-1B) genotypes in non-atrophic gastritis subjects and gastric carcinoma patients

<i>H. pylori</i> and IL-1B-511 genotypes	NAGa*	GC*	Odds ratio (95% confidence interval)	P value†
vacA s region				
vacAs2/C homozygote	27	1	1 (referent)	
vacAs2/T carrier	35	8	6.2 (0.7 to 52)	.1
vacAs1/C homozygote	17	27	43 (5.3 to 345)	<.001
vacAs1/T carrier	22	71	87 (11 to 679)	<.001
vacA m region				
vacAm2/C homozygote	38	13	1 (referent)	
vacAm2/T carrier	42	17	1.2 (0.5 to 2.8)	.7
vacAm1/C homozygote	8	20	7.3 (2.6 to 21)	<.001
vacAm1/T carrier	19	48	7.4 (3.2 to 17)	<.001
cagA status				
cagA ⁺ /C homozygote	35	4	1 (referent)	
cagA ⁺ /T carrier	46	8	1.5 (0.4 to 5.5)	.52
cagA ⁺ /C homozygote	26	38	13 (4.1 to 40)	<.001
cagA ⁺ /T carrier	28	80	25 (8.2 to 77)	<.001

*NAGa = subjects with non-atrophic gastritis; GC = patients with gastric carcinoma; C homozygote = IL-1B-511*C/*C; T carrier = IL-1B-511*T/*T or IL-1B-511*T/*C. Material was available for *H. pylori* genotyping for 135 of the 136 NAGa subjects and 130 of the 222 GC subjects. Ten samples from GC patients could not be genotyped for the vacAm region because of degraded DNA. NAGa and GC subjects infected with multiple vacAs strains (n = 34 and n = 23, respectively) or multiple vacAm strains (n = 28 and n = 22, respectively) were not included.

†Obtained from χ^2 test.

suming that an individual infected with a vacAs1 strain has a greater dose of the more virulent bacteria than someone infected simultaneously with vacAs1 and vacAs2 strains. The *H. pylori* colonization density in the corpus of subjects infected with vacAs1 strains was also statistically significantly higher ($P = .035$) than that in subjects infected with multiple vacAs strains (data not shown). Further studies are necessary to assess the impact of infection with multiple *H. pylori* strains on disease outcome.

It has been shown recently that specific genotypes of the IL-1B and IL-1RN genes increase the likelihood of hypochlorhydria and the development of gastric carcinoma (18,19). We confirmed that these pro-inflammatory genotypes (IL-1B-511*T carriers/IL-1RN*2 homozygotes) are statistically significantly

Table 6. Combination of *Helicobacter pylori* and interleukin 1 receptor antagonist (IL-1RN) variable number tandem repeat genotypes in non-atrophic gastritis subjects and gastric carcinoma patients

<i>H. pylori</i> and IL-1RN genotypes	NAGa*	GC*	Odds ratio (95% confidence interval)	P value†
vacA s region				
vacAs2/L carrier	58	9	1 (referent)	
vacAs2/2 homozygote	4	0	—	.98
vacAs1/L carrier	36	83	15 (6.7 to 33)	<.001
vacAs1/2 homozygote	3	15	32 (7.8 to 134)	<.001
vacA m region				
vacAm2/L carrier	76	26	1 (referent)	
vacAm2/2 homozygote	4	4	2.9 (0.7 to 13)	.15
vacAm1/L carrier	24	59	7.2 (3.7 to 14)	<.001
vacAm1/2 homozygote	3	9	8.8 (2.2 to 35)	.002
cagA status				
cagA ⁻ /L carrier	75	12	1 (referent)	
cagA ⁻ /2 homozygote	6	0	—	.98
cagA ⁺ /L carrier	49	100	13 (6.3 to 26)	<.001
cagA ⁺ /2 homozygote	5	18	23 (7.0 to 72)	<.001

NAGa = subjects with non-atrophic gastritis; GC = patients with gastric carcinoma; L carrier = IL-1RN^L/^L or IL-1RN*^L/²; 2 homozygote = IL-1RN*²/². Material was available for *H. pylori* genotyping for 135 of the 136 NAGa subjects and 130 of the 222 GC subjects. Ten samples from GC patients could not be genotyped for the vacAm region because of degraded DNA. NAGa and GC subjects infected with multiple vacAs strains (n = 34 and n = 23, respectively) or multiple vacAm strains (n = 28 and n = 22, respectively) were not included.

†Obtained from χ^2 test.

more prevalent among patients with gastric carcinoma than among subjects in a control group with gastritis only. We observed an increased risk for gastric carcinoma in IL-1B-511*^T allele carriers who are homozygous for the IL-1RN*² allele, with an OR of 3.3. We have also shown that IL-1B-511*^T carriers/IL-1RN*² homozygotes have more severe gastritis, i.e., higher combined inflammatory score in the corpus, epithelial damage in both corpus and antrum, and presence of glandular atrophy. These results provide indirect evidence of the increased pro-inflammatory effect associated with the IL-1B-511*^T and IL-1RN*² alleles. Moreover, the association between higher inflammatory scores in the corpus and pro-inflammatory IL-1 genotypes fits in with the pattern of gastritis typically associated with *H. pylori*-induced gastric carcinogenesis, i.e., gastritis involving the acid-secreting corpus leading to hypochlorhydria, gastric atrophy, and increased risk of gastric carcinoma (31). In contrast, gastritis confined to the antral region is associated with excessive acid production and higher risk of duodenal ulcer disease (31).

Analysis of the combined bacterial and host genotypes showed that, for each combination, the odds of having gastric carcinoma were greatest in those individuals with both the bacterial and the host high-risk genotypes. IL-1B-511*^T carriers infected with vacAs1-, vacAm1-, and cagA-positive strains were found to have increased risk for developing gastric carcinoma, with ORs of 87, 7.4, and 25, respectively. IL-1RN*² homozygotes infected with vacAs1-, vacAm1-, and cagA-positive strains also had increased risk for developing gastric carcinoma, with ORs of 32, 8.8, and 23, respectively. Statistical analysis did not reveal any significant interaction between these two groups of factors, suggesting that the risks for developing gastric carcinoma conferred by the *H. pylori* and IL-1 genotypes are inde-

pendent. However, given our sample size, our power to detect a small interaction was low. The analysis of the distribution of *H. pylori* vacA and cagA genotypes and human IL-1 polymorphisms in subjects with gastritis and/or in patients with gastric carcinoma showed no statistically significant associations, which indicates that there is no preferential colonization of specific hosts (as defined by these IL-1 polymorphisms) by specific bacterial strains.

Our results support the hypothesis that the extent of gastric mucosal injury may be related to *H. pylori* strain differences, inflammatory responses governed by host genetics, and interactions between host and bacterial determinants (4). The combination of these factors, favoring a set of responses with higher magnitude, can eventually result in hypochlorhydria, corpus atrophy, and an increased risk of gastric carcinoma. Nevertheless, we observed several individuals with high-risk IL-1 genotypes who were infected with virulent *H. pylori* strains but had only gastritis. Conversely, several gastric carcinoma patients had low-risk IL-1 genotypes and were infected with vacAs2-, vacAm2-, or cagA-negative strains. Gastric carcinogenesis is a complex and multifactorial cascade of events in which additional factors probably play a crucial role (2). Bacterial virulence factors such as babA (related to binding to blood-group antigens) and other outer membrane proteins have been shown to influence *H. pylori* virulence (32). Other host genetic factors associated with the inflammatory response, such as human leukocyte antigen and tumor necrosis factor- α polymorphisms (25,33), may also be associated with the outcome of infection. Finally, it is likely that the genetic background of gastric acid production plays a role in determining the topography of *H. pylori* stomach colonization and in the development of gastric carcinoma-associated corpus gastritis (31).

The extent to which *H. pylori* eradication decreases the risk of gastric carcinoma is unknown and controversial, raising the question of whether population-based *H. pylori* screening and treatment should be undertaken (34–36). Insufficient evidence of costs and benefits of gastric carcinoma prevention, the increase in antibiotic resistance, and the controversial hypothesis of potential negative effects of eradication in certain clinical entities have been hampering this practice (34). Our findings indicate that *H. pylori* and host genotyping can be important in better defining disease risk and preferentially targeting *H. pylori* eradication to high-risk individuals.

These findings address a major health issue, especially in countries where the prevalence of *H. pylori* is very high. As put forward by Peek and Blaser (4), individuals with genetic polymorphisms associated with high levels of IL-1b expression who are colonized by, for example, vacAs1- or cagA-positive strains are most likely to benefit from *H. pylori* eradication because such treatment could result in substantially reduced gastric carcinoma risk. According to our results, an intervention aimed at the 22% of gastritis individuals with the very high risk host/bacterial genotype (i.e., IL-1B-511*^T carrier infected with vacAs1 genotype) has the potential to reduce gastric cancer substantially because this group accounts for 66% of the carcinoma patients.

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NOTES

Supported by Fundação para a Ciência e Tecnologia (PRAXIS/P/SAU/14293/1998 and POCTI/CBO/41550/2001), Ministério da Saúde (Projecto 152/01), and by Programa Operacional Ciência, Tecnologia e Inovação (POCTI) do Quadro Comunitário de Apoio (QCA) II.

Manuscript received February 20, 2002; revised August 20, 2002; accepted September 11, 2002.