

CLINICAL–ALIMENTARY TRACT

Increased Risk of Noncardia Gastric Cancer Associated With Proinflammatory Cytokine Gene Polymorphisms

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Background & Aims: Genetic variations in proinflammatory and anti-inflammatory cytokine genes influence individual response to carcinogenic exposures. Polymorphisms in interleukin (IL)-1 β and its endogenous receptor antagonist are associated with risk of *Helicobacter pylori*-related gastric cancer. The aim of this study was to evaluate the role of proinflammatory cytokine gene polymorphisms in gastric and esophageal cancers defined by anatomic subsite. **Methods:** We assessed polymorphisms of the *IL-1* gene cluster and 4 other cytokine genes in a population-based case-control study of upper gastrointestinal cancers, including gastric cardia (n = 126) and noncardia adenocarcinoma (n = 188), esophageal squamous cell carcinoma (n = 53), and adenocarcinoma (n = 108), and frequency-matched controls (n = 212). ORs for the different cancers were computed from logistic regression models adjusted for potential confounding factors. **Results:** Proinflammatory genotypes of tumor necrosis factor α and *IL-10* were each associated with more than doubling of the risk of noncardia gastric cancer. Carriage of multiple proinflammatory polymorphisms of *IL-1B*, *IL-1* receptor antagonist, tumor necrosis factor A, and *IL-10* conferred greater risk, with ORs (and 95% confidence intervals) of 2.8 (1.6–5.1) for one, 5.4 (2.7–10.6) for 2, and 27.3 (7.4–99.8) for 3 or 4 high-risk genotypes. In contrast, these polymorphisms were not consistently related to the risks of esophageal or gastric cardia cancers. Polymorphisms in *IL-4* and *IL-6* were not associated with any of the cancers studied. **Conclusions:** A proinflammatory cytokine genetic profile increases the risk of noncardia gastric adenocarcinoma but not other upper gastrointestinal cancers, possibly by inducing a hypochlorhydric and atrophic response to gastric *H. pylori* infection.

cell carcinoma of the esophagus, adenocarcinoma of the esophagus, adenocarcinoma of the gastric cardia, and adenocarcinoma of the distal (noncardia) stomach. The available evidence for these tumors suggests that different pathogenic mechanisms are involved. For example, noncardia gastric adenocarcinoma occurs excessively in patients with gastric atrophy and hypochlorhydria,² which seem to protect against the more proximal cardia and esophageal adenocarcinomas.³ Time trends also differ; the incidence rates for adenocarcinomas of the esophagus and gastric cardia have increased steeply in industrialized countries since the mid-1970s, while rates for esophageal squamous cell carcinoma and noncardia gastric adenocarcinoma have remained stable or decreased.^{4,5} The decline in incidence of noncardia gastric adenocarcinoma has paralleled the decreasing prevalence of infection with *Helicobacter pylori*, which plays an essential role in initiating this cancer⁶ but could possibly protect against the more proximal cancers.^{7,8}

Host genetic factors are emerging as key determinants of disease risk for many cancers.^{9,10} We recently reported that functional polymorphisms in the genes for interleukin 1 β (*IL-1B*) and its endogenous receptor antagonist (*IL-1RN*) were associated with increased risk of gastric cancer and its precursors.^{11,12} These polymorphisms have been shown to significantly affect gastric mucosal IL-1 β production in response to *H. pylori* infection.¹³ The effect of these polymorphisms is therefore most likely mediated

Cancers of the upper gastrointestinal tract are collectively the second most common type of cancer worldwide¹ and comprise 4 distinct entities: squamous

Abbreviations used in this paper: CI, confidence interval; IL, interleukin; *IL-1RN*, interleukin-1 receptor antagonist; OR, odds ratio; TNF, tumor necrosis factor.

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Table 1. Sequences of Primers and Probes Used in the 5' Nuclease (TaqMan) Assays

Gene	Primers	Probes
<i>IL-1B</i> -511 C/T	F: 5'-TCCTCAGAGGCTCCTGCAAT-3' R: 5'-TGTGGGTCTCTACCTGGGTG-3'	FAM:TGTTCTCTGCCTCGGGAGCTCTCTG VIC:CTGTTCTCTGCCTCAGGAGCTCTCTGTC
<i>IL-4</i> -590 C/T	F: 5'-CCTGTCCTTCTCAAACACTAACTTG-3' R: 5'-GCAGAATAACAGGCAGACTCTCCTA-3'	FAM:AACATTGTCCCCAGTCTGGG VIC:AGAACATTGTCCCCAGTCTGGG
<i>IL-6</i> -174 G/C	F: 5'-CAATGACGACCTAAGCTGCACT-3' R: 5'-GCTGATTGGAAACCTTATTAAGATTGT-3'	FAM:AGTTGTGTCTTGGCATGCTAAAGGACG VIC:AGTTGTGTCTTGGCATGCTAAAGGACG
<i>IL-10</i> -1082 G/C	F: 5'-CACACACACAAAATCCAAGACAA-3' R: 5'-GCTGGATAGGAGGTCCCTTACTTT-3'	FAM:AAGGCTTCTTTGGGAGGGGGAAGTAG VIC:AAGGCTTCTTTGGGAGGGGGAAGTAGG
<i>IL-10</i> -819 C/T	F: 5'-GGGTGAGGAAACCAAATTCTCA-3' R: 5'-CATGACCCTACCGTCTCTATTTTA-3'	FAM:TACAGGTGATGAACATCTCTGTGCCTCAG VIC:TGTACAGGTGATGAATATCTCTGTGCCTCAGT
<i>IL-10</i> -592 C/A	F: 5'-GGTAAAGGAGCCTGGAACACATC-3' R: 5'-CCAAGCAGCCCTTCCATT-3'	FAM:ACCCCGCCTGTCTGTAGGAAGC VIC:ACCCCGCCTGTACTGTAGGAAGC
<i>TNF-A</i> -308 G/A	F: 5'-CCCCAAAAGAAATGGAGGC-3' R: 5'-TCTTCTGGGCCACTGACTGAT-3'	FAM:AGGGGCATGGGGACGGG VIC:TGAGGGGCATGAGGACGGG

F, forward primer; R, reverse primer; FAM, wild-type allele; VIC, mutant allele.

through the higher production of IL-1 β , which is an important proinflammatory cytokine and a powerful inhibitor of acid secretion.¹⁴ However, our initial study only addressed gastric cancer, which consisted predominantly of the noncardia gastric tumors.

The genetic control of inflammation may affect other upper gastrointestinal cancers as well. Both proinflammatory and anti-inflammatory cytokine genes have recognized polymorphisms that alter gene transcription and/or expression.¹⁵ In addition to the *IL-1* gene cluster, candidate genes include those encoding the proinflammatory cytokines tumor necrosis factor α (TNF- α) and IL-6, as well as the anti-inflammatory IL-4 and IL-10. We therefore undertook a study to further investigate the relationship between inflammatory cytokine polymorphisms and the risk of upper gastrointestinal tract cancers.

Materials and Methods

Study Population

The subjects in this study came from a multicenter, case-control study of esophageal and gastric cancer conducted in 3 geographic areas of the United States with population-based tumor registries.¹⁶ Population-based controls were frequency matched by 5-year age group and sex, and were selected by random-digit dialing and Health Care Financing Administration roster sampling as previously described.¹⁶ Genomic DNA was obtained from peripheral blood samples that had been collected at some of the participating centers¹⁷ or from paraffin-embedded tissue sections. DNA samples were available for 161 subjects with esophageal cancer (53 with squamous cell carcinoma and 108 with adenocarcinoma), 314 subjects with gastric adenocarcinoma (126 with cardia and 188 with noncardia adenocarcinomas), and 212 controls. *H. pylori* serologic status was available on 214 subjects (46%) with cancer and all controls as previously reported.¹⁷

Genotyping

We assessed a total of 7 single nucleotide polymorphisms in *IL-1B* (-511 C/T), *IL-4* (-590 C/T), *IL-6* (-174 G/C), *IL-10* (-1082 G/A, -819 C/T, -592 C/A), and *TNF-A* (-308 G/A), as well as the variable number of tandem repeat polymorphism of *IL-1RN*. Single nucleotide polymorphisms were discriminated by 5' nuclease polymerase chain reaction assays (TaqMan) using methods as previously described.¹¹ The sequences of the primers and probes used in the TaqMan assays are provided in Table 1 and were all developed in our laboratory except for *IL-4*-590 (C/T), which was previously reported by Holloway et al.¹⁸

For the *IL-1RN* variable number of tandem repeat polymorphism, genomic DNA was polymerase chain reaction amplified and resultant products sized by gel electrophoresis as previously described¹¹; the rarer alleles 3 and 4 were grouped in the statistical analysis.

Genotype data were complete for the *IL-1B*, *IL1-RN*, *IL-10*, and *TNF-A* markers in all of the cases and in 210 controls; 2 control samples with degraded DNA could not be typed completely and were excluded from analysis. The *IL-4* and *IL-6* polymorphisms were only genotyped for subjects with peripheral blood DNA because tissue DNA samples were limited. For 27 subjects with DNA from both blood and tissue samples, genotype results for the other 6 polymorphisms were more than 99% identical in quality control analyses.

Statistical Analysis

Hardy-Weinberg equilibrium of alleles at individual loci was assessed by χ^2 statistics. Odds ratios (OR) with Cornfield 95% confidence intervals (CIs) were computed by logistic regression using STATA version 7.0 software (STATA Press, College Station, TX). ORs for the different cancers were adjusted for age (categorized as younger than 50, 50-59, 60-69, and 70 years or older), sex, and race (categorized as white and all other); alternative analyses stratified by race provided similar estimates. Additional models were adjusted for the effects of the genetic polymorphisms on one another,

Table 2. Selected Characteristics of Patients With Different Types of Upper Gastrointestinal Cancer and Controls

Characteristic	Esophageal cancer		Gastric cancer		Controls (n = 210)
	Squamous cell carcinoma (n = 53)	Adenocarcinoma (n = 108)	Cardia (n = 126)	Noncardia (n = 188)	
Age (median years)	66	65	66	70	66
Sex (% male)	89	86	87	77	85
Race (% white)	75	97	97	85	94
Body mass index (median kg/m ²)	24.3	26.6	25.8	25.1	25.3
Cigarette Users (%)					
Current	47	32	33	27	24
Former	43	47	47	48	45
Alcohol (% regular users)	98	76	80	70	85
History of reflux symptoms (%)	21	61	40	38	30
History of peptic ulcer (%)					
Gastric	13	11	10	19	7
Duodenal	4	10	9	12	6
Family history of cancer (%)					
Esophageal	0	2	2	2	2
Gastric	8	8	8	14	4
Lauren classification (%)					
Diffuse (%)	—	14	24	47	—
Intestinal (%)	—	73	59	34	—
<i>H. pylori</i> immunoglobulin G antibody (% positive)	58	32	26	48	40
DNA source (% peripheral blood)	45	61	46	35	100

histologic subtype (Lauren classification), cigarette smoking (categorized as current, former, and never), alcohol consumption (categorized as greater or less than one drink per month), history of gastroesophageal reflux disease,¹⁹ body mass index (in quartiles), history of gastric or duodenal ulcer disease, *H. pylori* serologic status,¹⁷ and family history of esophageal or gastric cancer. Population-attributable fractions of the various cancers were estimated from logistic regression models comparing subjects with one or more polymorphisms to subjects with none.

The study was approved by the institutional review boards of the participating centers, and written informed consent was obtained from all subjects.

Results

Demographic and risk-factor characteristics of the subjects with cancer and the controls (Table 2) were similar to those reported in the previous study.¹⁶

Among controls, the alleles at all of the individual loci studied were in Hardy–Weinberg equilibrium, with nominally nonsignificant χ^2 values (Table 3). Nevertheless, only 9 (4%) had the homozygous variant genotype of *IL-1B-511*, whereas 16 (7%) were expected based on allele frequencies (Hardy–Weinberg $\chi^2 = 5.6$; $P = 0.07$, 2 *df*). Based on genotype frequencies reported from other white populations, the paucity of *IL-1B-511T/T* among the controls was an anomaly that had the effect of exaggerating the case-control comparisons for this genotype. In all subjects, there was 100% linkage disequilibrium

between the *IL-10-819* and *-592* alleles as previously reported.^{20,21}

Associations With Polymorphisms in the *IL-1* Gene Cluster

Carriers of the proinflammatory *IL-1B-511T* allele had a significantly increased risk of noncardia gastric cancer; the association seemed to be greater in homozygotes than in heterozygotes due to the low frequency of homozygous controls (Table 4). In a logistic regression model that included the other genetic markers, the adjusted OR for *IL-1B-511T+* carriers (homozygotes and heterozygotes) was 2.3 (95% CI, 1.4–3.8). Although homozygotes also had statistically significant excess risks of the other 3 cancers, these increases may have been artifactual because there were no increases in risk for heterozygotes or for *IL-1B-511T+* carriers overall (Table 5).

Additionally, *IL-1RN*2* was associated with an increased risk of both noncardia gastric cancer and esophageal squamous cell cancer but only in homozygotes, with adjusted ORs of 3.6 (95% CI, 1.7–7.6) and 4.2 (95% CI, 1.3–13.9), respectively (Table 5). Carriers of the rarer *IL-1RN* alleles 3 or 4 had an increased risk of esophageal adenocarcinoma of marginal statistical significance ($P = 0.03$).

Table 3. Cytokine Genotype Frequencies in Patients With Different Types of Upper Gastrointestinal Cancer and Controls

Genotype	Esophageal cancer (%)		Gastric cancer (%)		Controls (%) (n = 210)
	Squamous cell carcinoma (n = 53)	Adenocarcinoma (n = 108)	Cardia (n = 126)	Noncardia (n = 188)	
<i>IL-1B</i> -511					
C/C	21 (40)	50 (46)	57 (45)	47 (25)	104 (50)
C/T	21 (40)	46 (43)	56 (44)	98 (52)	97 (46)
T/T	11 (20)	12 (11)	13 (11)	43 (23)	9 (4)
<i>IL-1RN</i>					
1/1	30 (57)	49 (46)	69 (55)	81 (43)	114 (55)
1/2	12 (23)	40 (37)	42 (33)	59 (31)	76 (36)
1/3,4	2 (3)	10 (9)	3 (2)	2 (1)	7 (3)
2/2	8 (15)	8 (7)	12 (10)	45 (24)	13 (6)
2/3	1 (2)	1 (1)	0 (0)	1 (1)	0 (0)
<i>IL-4</i> -590					
G/G	11 (46)	44 (67)	41 (71)	37 (58)	153 (73)
G/T	11 (46)	17 (26)	16 (27)	21 (33)	46 (22)
T/T	2 (8)	5 (7)	1 (2)	6 (9)	10 (5)
<i>IL-6</i> -174					
G/G	13 (54)	20 (30)	22 (38)	33 (51)	83 (40)
G/C	6 (25)	33 (50)	31 (53)	21 (32)	98 (47)
C/C	5 (21)	13 (20)	5 (9)	11 (17)	28 (13)
<i>IL-10</i> -1082					
G/G	9 (17)	23 (21)	30 (24)	31 (16)	48 (23)
G/A	28 (53)	59 (55)	62 (49)	71 (38)	103 (49)
A/A	16 (30)	26 (24)	34 (27)	86 (46)	59 (28)
<i>IL-10</i> -592					
C/C	35 (66)	72 (67)	80 (64)	98 (52)	127 (61)
C/A	15 (28)	29 (27)	38 (30)	63 (34)	70 (33)
A/A	3 (6)	7 (6)	8 (6)	27 (14)	13 (6)
<i>IL-10</i>					
GCC/ GCC	8 (15)	23 (21)	30 (24)	31 (16)	48 (23)
Other ^a	43 (81)	79 (73)	88 (70)	131 (70)	150 (71)
ATA/ATA	2 (4)	6 (6)	8 (6)	26 (14)	12 (6)
<i>TNF-A</i> -308					
G/G	41 (77)	81 (75)	91 (72)	110 (59)	152 (72)
G/A	10 (19)	24 (22)	30 (24)	57 (30)	52 (25)
A/A	2 (4)	3 (3)	5 (4)	21 (11)	6 (3)

^a*IL-10* genotypes other than GCC/GCC or ATA/ATA.

Associations With Polymorphisms in Other Proinflammatory Cytokine Genes

Carriers of the proinflammatory *TNF-A*-308A allele had a significantly increased risk of noncardia gastric cancer that was more pronounced for homozygotes than heterozygotes. Adjusted for the effects of the other genetic markers, the OR for *TNF-A*-308A+ was 2.2 (95% CI, 1.4–3.7). *TNF-A*-308A+ carriers were not at increased risk for the other gastrointestinal cancers (Table 5). No statistically significant associations of *IL-6*-174C were seen for any of the 4 cancers.

Associations With Polymorphisms in Anti-inflammatory Cytokine Genes

Subjects homozygous for the hypoactive *IL-10* alleles *IL-10*-1082A and *IL-10*-592A, as well as for the

“low *IL-10*” haplotype ATA (*IL-10*-1082A, *IL-10*-819T, and *IL-10*-592A)²¹ had significantly increased risks of noncardia gastric cancer. Compared with homozygotes for the “high *IL-10*” haplotype GCC, the adjusted OR for low *IL-10* homozygotes was 2.5 (95% CI, 1.1–5.7). The low *IL-10* haplotype was not associated with risk for the other cancers (Table 5). Homozygotes for the *IL-4*-590T allele were not at altered risk for any of the cancers, although heterozygotes had an excess of esophageal squamous cell cancer of marginal statistical significance ($P = 0.02$; Table 4).

Association With Composite of Cytokine Polymorphisms

To analyze the combined association with increases in proinflammatory cytokines and decreases in

Table 4. Age-, Sex-, and Race-Adjusted ORs (and Cornfield 95% CIs) for the Association of Cytokine Gene Polymorphisms With Different Types of Upper Gastrointestinal Cancer

Genotype	Esophageal cancer		Gastric cancer	
	Squamous cell carcinoma (n = 53)	Adenocarcinoma (n = 108)	Cardia (n = 126)	Noncardia (n = 188)
<i>IL-1B-511</i>				
C/C	1.0	1.0	1.0	1.0
C/T	0.9 (0.5–1.9)	1.0 (0.6–1.6)	1.1 (0.7–1.8)	2.4 (1.5–3.8)
T/T	6.6 (2.2–19.8)	2.93 (1.1–7.7)	3.1 (1.2–8.0)	9.5 (4.0–22.7)
<i>IL-1RN</i>				
1/1	1.0	1.0	1.0	1.0
1/2	0.7 (0.3–1.6)	1.2 (0.7–2.0)	0.9 (0.5–1.4)	1.2 (0.7–1.9)
1/3,4	2.1 (0.4–12.0)	3.3 (1.1–9.5)	0.7 (0.2–2.9)	0.8 (0.2–4.0)
2/2	3.3 (1.2–9.2)	1.3 (0.5–3.5)	1.6 (0.7–3.7)	5.4 (2.6–11.1)
<i>IL-4-590</i>				
G/G	1.0	1.0	1.0	1.0
G/T	3.2 (1.2–8.3)	1.4 (0.7–2.7)	1.4 (0.7–2.9)	1.8 (0.9–3.5)
T/T	1.7 (0.2–14.2)	2.0 (0.5–7.6)	0.6 (0.1–4.9)	1.4 (0.3–5.9)
<i>IL-6-174</i>				
G/G	1.0	1.0	1.0	1.0
G/C	0.5 (0.2–1.5)	1.4 (0.8–2.8)	1.1 (0.6–2.1)	0.7 (0.3–1.3)
C/C	1.2 (0.4–4.1)	1.9 (0.8–4.4)	0.6 (0.2–1.8)	1.3 (0.5–3.1)
<i>IL-10-1082</i>				
G/G	1.0	1.0	1.0	1.0
G/A	1.3 (0.5–3.0)	1.2 (0.7–2.2)	1.0 (0.6–1.8)	1.0 (0.6–1.8)
A/A	1.1 (0.4–2.9)	0.8 (0.4–1.7)	1.1 (0.6–2.1)	1.9 (1.1–3.5)
<i>IL-10-592</i>				
C/C	1.0	1.0	1.0	1.0
C/A	0.6 (0.3–1.2)	0.8 (0.5–1.3)	0.9 (0.6–1.5)	1.2 (0.8–1.9)
A/A	0.3 (0.1–1.4)	1.3 (0.5–3.8)	1.4 (0.5–3.9)	2.4 (1.1–5.1)
<i>IL-10</i>				
GCC/GCC	1.0	1.0	1.0	1.0
Other ^a	1.5 (0.7–3.6)	1.1 (0.6–1.9)	1.0 (0.6–1.7)	1.2 (0.7–2.1)
ATA/ATA	1.0 (0.1–6.0)	1.2 (0.3–4.3)	1.7 (0.5–6.1)	3.4 (1.3–8.8)
<i>TNF-A-308</i>				
G/G	1.0	1.0	1.0	1.0
G/A	0.8 (0.4–1.7)	0.9 (0.5–1.6)	1.0 (0.6–1.6)	1.5 (0.9–2.4)
A/A	1.3 (0.2–6.9)	1.2 (0.3–5.3)	1.5 (0.4–5.0)	4.8 (1.8–12.8)

^a*IL-10* genotypes other than GCC/GCC or ATA/ATA.

anti-inflammatory cytokines, subjects were classified according to their number of inflammation-promoting cytokine polymorphisms, defined as follows: (1) carriage of *IL-1B-511T*, (2) homozygosity for *IL-1RN*2*, (3) carriage of *TNF-A-308A*, and (4) homozygosity for the low *IL-10* haplotype ATA. The OR for noncardia gastric

cancer increased progressively with increasing number of proinflammatory genotypes to 27.3 (95% CI, 7.4–99.8; Table 6) for 3 or 4 polymorphisms. In contrast, the number of proinflammatory cytokine polymorphisms did not significantly affect the risk of esophageal squamous cell carcinoma, adenocarcinoma, or gastric cardia cancer,

Table 5. Multivariate Model–Estimated ORs (and Cornfield 95% CIs) for the Association of Proinflammatory Genotypes With Different Types of Upper Gastrointestinal Cancer

Genotype	Esophageal cancer		Gastric cancer	
	Squamous cell carcinoma (n = 53)	Adenocarcinoma (n = 108)	Cardia (n = 126)	Noncardia (n = 188)
<i>IL-1B-511T+</i>	0.9 (0.4–2.0)	1.0 (0.6–1.7)	1.2 (0.7–1.9)	2.3 (1.4–3.8)
<i>IL-1RN*2/*2</i>	4.2 (1.3–13.9)	1.0 (0.4–2.8)	1.4 (0.6–3.3)	3.6 (1.7–7.6)
<i>IL-10</i> ATA/ATA	0.3 (0.1–2.1)	1.5 (0.5–4.4)	1.3 (0.5–3.6)	2.5 (1.1–5.7)
<i>TNF-A-308A+</i>	0.8 (0.4–1.7)	1.0 (0.6–1.8)	1.1 (0.6–1.8)	2.2 (1.4–3.7)

Table 6. Frequencies and Age-, Sex-, and Race-Adjusted ORs (and Cornfield 95% CIs) for the Association of 1–4 Proinflammatory Polymorphisms in *IL-1B*, *IL-1RN*, *IL-10*, and *TNFA* With Noncardia Gastric Cancer

Polymorphisms	Cases (n = 188)	Controls (n = 210)	OR (95% CI)
0	22	75	(Referent)
1	74	85	2.8 (1.6–5.1)
2	62	46	5.4 (2.7–10.6)
3	28	4	26.3 (7.1–97.1)
4	2	0	∞ (Undefined)

with ORs for carriage of 3 or 4 proinflammatory genotypes of 0 (95% CI, 0–10.5), 1.3 (95% CI, 0.2–8.0), and 3.6 (95% CI, 0.8–16.7), respectively.

Sixteen percent of the subjects with noncardia gastric cancer but only 2% of the controls had 3 or 4 high-risk genotypes, whereas 37% of the controls compared with 12% of the subjects with noncardia gastric cancer had no high-risk genotypes. Assuming these associations are causal, the combined population-attributable fraction of noncardia gastric cancer due to the effect of these alleles is estimated to be 67%.

Similar results were obtained after exclusion of subjects with DNA from tumor specimens and in separate analyses for each histologic subtype defined by Lauren classification. The estimated effects of the proinflammatory genotypes were not altered substantially after adjustment for the effects of possible confounders of the risk of noncardia gastric cancer (data not shown).

Effects of Cytokine Polymorphisms in *H. pylori*-Seropositive Subjects

Less than half of the subjects with cancer had blood available for *H. pylori* serologic analysis. Among those with *H. pylori* seropositivity, the OR estimates for noncardia gastric cancer were 4.2 for *IL-1B*-511*T, 1.6 for *IL-1RN**2*2, 2.9 for *IL-10* ATA/ATA, and 2.9 for *TNF-A*-308*2. For those with CagA-positive serology, the OR estimates were higher (9.9, 2.9, 5.2, and 6.2 for the above markers, respectively). For the composite estimates, the *H. pylori*-seropositive and CagA-seropositive subjects had OR estimates of 5.4 and 6.6 for one marker, 8.3 and 19.5 for 2 markers, and 42.0 and undefined for 3 markers, respectively. *H. pylori* serologic markers did not apparently affect the OR estimates for the other upper gastrointestinal cancers.

Discussion

We have identified a proinflammatory profile of genetic polymorphisms in *IL-1B*, *IL-1RN*, *IL-10*, and

TNF-A associated with an increased risk of gastric cancer that was limited to noncardia tumors. No associations were seen with cancers of the gastric cardia or esophagus. The *IL-1* gene cluster polymorphisms were associated with gastric cancer in 2 previous reports.^{11,22} The ORs are similar in all 3 studies (all in predominantly white populations), underscoring the central role of *IL-1β* in the pathogenesis of *H. pylori*-associated gastric cancer. A novel finding in our study is the identification of the proinflammatory genotypes of *TNF-A* and *IL-10* as additional risk factors.

TNF-α is up-regulated early in *H. pylori* colonization and induces the transcription of a wide range of other proinflammatory cytokines and chemokines, amplifying the inflammatory cascade against the infection.²³ Although not as potent as *IL-1β*, *TNF-α* also inhibits gastric acid secretion,²⁴ which paradoxically promotes spread of the organism. The association with the low *IL-10* haplotype may be related to the role of *IL-10* as an anti-inflammatory cytokine that down-regulates *IL-1β*, *TNF-α*, interferon gamma, and other proinflammatory cytokines. Relative deficiency of *IL-10* may result in a Th-1-driven hyperinflammatory response to *H. pylori* with greater damage to the gastric mucosa.^{25–28} Imbalances among proinflammatory and anti-inflammatory mediators and acid production may favor the development of gastric atrophy and its progression to cancer.

The association of these polymorphisms with gastric cancer requires the presence of *H. pylori* and may be most important early in the disease process. When *H. pylori* infects gastric mucosa, it induces a vigorous inflammatory response with high levels of *IL-1β* and *TNF-α*. The direct effects of these cytokines are advantageous to eradication of *H. pylori* organisms, but the concomitant inhibition of acid secretion may extend the area of colonization affecting the corpus mucosa, which is otherwise protected by an acidic environment. The critical role of acid in determining the pattern of gastritis is well shown by the changes resulting from its pharmacologic inhibition. *H. pylori*-infected patients on long-term proton pump inhibitor therapy may experience an exacerbation of gastritis, with a shift to corpus predominance and an increased risk of gastric atrophy.²⁹

In addition, a decreased flow of gastric secretions may promote accumulation of the genotoxic by-products of inflammation, causing more mucosal damage and an increased rate of mutation. As the inflammatory process extends across the corpus, the acid secretion is further inhibited in a continuing cycle that accelerates glandular loss and onset of gastric atrophy. The hypochlorhydric

milieu also promotes growth of non-*H. pylori* bacteria, which may contribute to mucosal damage and/or production of carcinogenic *N*-nitrosocompounds.³⁰ With progression from mild gastritis through severe gastritis, atrophy, and intestinal metaplasia, it becomes more difficult for *H. pylori* to colonize and be detected.³¹

While *H. pylori* infection and host genetics interact to initiate a hypochlorhydric and atrophic phenotype, other factors may contribute to subsequent neoplastic transformation, which does not depend on continued presence of the infection. Diet may be particularly relevant, because greater consumption of fresh fruits and vegetables has been shown to protect against the risk of gastric cancer. In particular, dietary vitamin C reduces the formation of *N*-nitrosocompounds and scavenges mutagenic reactive oxygen metabolites generated by gastric inflammation,³² and supplemental vitamin C was associated with a significantly lower risk of noncardia gastric cancer in this study population³³; furthermore, vitamin C concentrations and bioavailability are reduced in the presence of *H. pylori* infection.^{34,35} Another factor for transformation is cigarette smoking, which was found to nearly double the risk of transition from atrophic gastritis to dysplasia in a high-risk population.³⁶ Thus, cytokine gene polymorphisms represent one component of a complex interplay among the host, pathogen, and environmental factors involved in gastric carcinogenesis.

In the present study, we did not have an opportunity to fully assess the interaction between host genetic factors and *H. pylori* virulence factors, because gastric biopsy material for isolation of *H. pylori* strains was not available. Our limited analysis using the subgroup of subjects with serologic data showed a strong effect of the proinflammatory cytokine gene polymorphisms in the presence of CagA seropositivity. Our data thus concur with the recent report by Figueiredo et al.³⁷ that the risk of gastric cancer is greatest in subjects with both bacterial and host high-risk genotypes.

Notably, the proinflammatory cytokine profile did not affect the risk of the other upper gastrointestinal tract cancers. Esophageal adenocarcinoma is believed to arise from Barrett's esophagus, a precancerous metaplasia induced by chronic gastroesophageal reflux of acidic gastric contents.³⁸⁻⁴⁰ Theoretically, *H. pylori*-induced gastritis and secondary hypochlorhydria reduce the amount of acid production and refluxate. Accordingly, it has been argued that *H. pylori* colonization,⁴¹ particularly with the CagA-positive strains, may be protective against gastroesophageal reflux disease and its complications, such as Barrett's esophagus, dysplasia, and adenocarcinoma.^{7,17,42,43} It might be expected that proinflamma-

tory genotypes enhancing gastric acid inhibition would be protective against esophageal and possibly gastric cardia adenocarcinoma, but our data do not provide support for this hypothesis.

Our study also showed that homozygous carriage of the proinflammatory allele of *IL-1RN* was associated with risk of esophageal squamous cell carcinoma. However, risk was not related to the other proinflammatory markers associated with response to *H. pylori*, suggesting differences in the cytokine repertoire predisposing to the distinctive etiologic factors for esophageal squamous cell cancer, including alcohol, smoking, and nutritional deficiency. The associations we observed with *IL-1RN* and *IL-4* polymorphisms warrant further investigation in larger studies of esophageal cancer.

Although required for inclusion in this study, DNA collection was unlikely to have been influenced by cytokine genotype and therefore should not have biased our results. The absence of selection or survival bias was further supported by the similarity in genotyping findings based on subjects with DNA from blood or from tumor tissue. In addition, there was no evidence that DNA availability for the present study was associated with demographic or risk-factor characteristics previously reported for these populations.¹⁶

In conclusion, our findings indicate that a proinflammatory host genotype favors the development of a hypochlorhydric, atrophic response to gastric infection with *H. pylori*, which in turn predisposes to noncardia gastric adenocarcinoma but not to cardia or esophageal cancers. Pathophysiologic considerations indicate that cytokine dysregulation is important in initiating this disease process, but the multistep progression from atrophy to neoplastic transformation depends on other factors, including diet and tobacco. Better understanding of the risk factors involved in the early and late stages of gastric carcinogenesis should enhance the prospects for preventive and therapeutic interventions.

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