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Int J Cancer. Author manuscript; available in PMC 2012 February 1.

Published in final edited form as:
Int J Cancer. 2011 February 1; 128(3): 668–675. doi:10.1002/ijc.25385.

Association of haplotypes of inflammation-related genes with gastric preneoplastic lesions in African Americans and Caucasians

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Abstract

Identification of biomarkers is needed for development of screening programs to prevent gastric cancer. Because racial differences exist in cancer rates, we aimed to evaluate the association between polymorphisms in inflammation-related genes and gastric preneoplastic lesions in African Americans and Caucasians from Louisiana, United States. Gastric biopsies from 569 adults (361 African Americans and 208 Caucasians) undergoing diagnostic endoscopy were used for histological diagnosis and genomic DNA extraction. Polymorphisms within 8 genes (*IL1B*, *IL8*, *IL6*, *TNF*, *PTGS2*, *ARG1*, *IL10*, and *TGFB1*) were investigated by TaqMan. *cagA* status of *Helicobacter pylori* infection was assessed by PCR. Haplotype logistic regression models were used to identify variables associated with intestinal metaplasia or dysplasia. African Americans carrying the haplotype *IL1B−511T/−31C/+3954T*, which includes the three risk-associated alleles at the *IL1B* locus, were more likely to being diagnosed with intestinal metaplasia or dysplasia than those carrying the most common haplotype T-C-C (adjusted OR: 2.51, 95% CI: 1.1–5.5). None of the polymorphisms were associated with intestinal metaplasia and dysplasia in Caucasians. Age and *cagA*-positive status were independent factors associated with these lesions. Haplotypes at the *IL1B* locus may participate in mediating the susceptibility to gastric carcinogenesis and might be useful as markers of advanced pre-malignant lesions in African Americans. Interestingly, carriage of *IL1B+3954T* allele seems to be the key factor, even though the role played by other polymorphisms cannot be excluded.

Keywords

Single nucleotide polymorphisms; gastric inflammatory lesions; inflammation; *Helicobacter pylori*

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Introduction

Gastric cancer is one of the most common cancers worldwide and the second leading cause of death due to cancer (estimated 934,000 new cases and 700,000 deaths for 2002) (1). Populations at highest risk for gastric cancer are mainly located in East Asia, Eastern Europe, and the Andean regions of Latin America (1). In the United States, differences in incidence and mortality exist by race/ethnicity. African Americans, Asian American/Pacific Islanders, and Indian Americans/Alaska Natives are at higher risk of gastric cancer than Caucasians (2). In the State of Louisiana, the age-adjusted incidence rates of gastric cancer in males are 17.5 and 10.2 (per 100,000 individuals) for African Americans and Caucasians, respectively; while the rates in females are 9.1 in African Americans and 4.7 in Caucasians (3). Chronic inflammatory conditions, resulting from infectious or non-infectious agents, are known to promote and influence malignant transformation by several mechanisms, including epithelial hyperproliferation and DNA damage (4;5). Gastric adenocarcinoma of the intestinal type is the most common type of gastric cancer worldwide. It is believed to be the result of a multifactorial and multistep process, which starts with *Helicobacter pylori* (*H. pylori*) infection, a recognized risk factor for gastric cancer (6). The process involves the consecutive stages of progression of multifocal atrophic gastritis, intestinal metaplasia, and dysplasia (7).

Genetic variants of both the host and *H. pylori* may influence the type and intensity of the inflammatory response eventually leading to malignant transformation (8). *H. pylori* infection upregulates a wide variety of pro- and anti-inflammatory molecules. *H. pylori* strains possessing the cytotoxin-associated gene pathogenicity island (cag PAI) are associated with a more severe form of gastritis and increased risk of cancer (9–11). Single nucleotide polymorphisms (SNPs) in genes encoding the pro-inflammatory cytokines interleukin (IL)-1β (*IL1B−511, IL1B−31, IL1B+3954*) and tumor necrosis factor (TNF)-α (*TNF−308*) have been linked with increased production of the respective cytokines and with susceptibility to gastric precancerous lesions and gastric cancer (11–18). The production of IL-8 in the gastric mucosa is stimulated by *H. pylori* infection. A haplotype in the *IL8* gene (*IL8−251A/+396G/+781T*) was associated with gastric cardia adenocarcinoma in China (19). Interleukin 10 (IL-10) is an anti-inflammatory cytokine that downregulates the effects of proinflammatory mediators. A haplotype in the *IL10* gene (*IL10−1082A/−819T/−592A*) has been associated with increased risk of noncardia gastric cancer (20). Interleukin 6 (IL-6) is a multifunctional cytokine with proinflammatory properties. A polymorphism in the promoter of *IL6* (*IL6-174* G>C) has been associated with plasma levels of IL-6 (21). Studies of association between *IL6* variants (*−174* and *−597*) and risk of gastric cancer have been negative (20;22). Polymorphisms in the transforming growth factor-β (TGF-β) and its receptor (TGF-βRII) genes have been associated with reduced risk of both duodenal ulcer (*TGFB1+869*) (23) and gastric cancer (*TGFB1−509* and *TGFBR2−875*) (23;24). The expression of cyclooxygenase 2 (COX-2) in the gastric mucosa is associated with *H. pylori* infection (25) and the presence of gastric premalignant lesions (26). A polymorphism (*PTGS2-1195G>A*) has been associated with high expression of COX-2 in gastric mucosa and with risk of gastric cancer (26). Arginase I is an enzyme that hydrolyzes L-arginine to urea and L-ornithine, the latter involved in the production of polyamines required for cell proliferation. Cytokines regulate the expression of arginase I, which is associated with T cell anergy (27). Arginase I may play a role in the pathogenesis of gastric carcinogenesis through various mechanisms, including inhibition of nitric oxide generation, increased polyamine and proline synthesis, and suppression of the immune system. Polymorphisms in *ARG1* have not been previously investigated in gastric carcinogenesis.

The influence of genetic variants in inflammation-related genes on the development of gastric preneoplastic lesions has not been comprehensively investigated in African

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Americans, a population at increased risk of gastric cancer. The identification of host susceptibility markers is needed for the design of screening programs. This study is aimed at evaluating the association of polymorphisms in genes involved in pro-inflammatory (*IL1B, IL8, IL6, TNF*) and anti-inflammatory (*IL10, TGB1*) processes, immune-related molecules (*PTGS2, ARG1*) and *H. pylori* infection in relation to the presence of precancerous gastric lesions in African Americans and Caucasians from Louisiana, United States. Since the effect of single SNPs can be masked by the proximity of other SNPs (28–31), the importance of the evaluation of haplotypes rather than single SNPs is highlighted in this study.

Materials and methods

Patients

All patients attending the gastrointestinal services at the Medical Center of Louisiana and the Oschner Baptist Medical Center (formerly Memorial Medical Center), both in New Orleans, Louisiana between March 1995 and August 2005 were invited to participate in the study. All exclusion and inclusion criteria were reported previously (18), and included pregnancy, previous gastrectomy, and major diseases present at the time of the recruitment. All individuals provided informed consent. A total of 569 individuals were included (208 Caucasians and 361 African-Americans). Sixty-five subjects were excluded because of the following reasons: racial group other than African American or Caucasian (n=37), gastric adenocarcinoma (n=3), inadequate tissue samples for histologic diagnosis (n=14), duplicated cases (n=4), and missing demographic data (n=7).

Gastric mucosa biopsies were obtained from each patient and used for histological examination as follows: one from the antrum (greater curvature, within 5 cm of the pylorus), one from the lesser curvature (at the *incisura angularis*) and one from the corpus (mid anterior wall). In addition, a second biopsy from the lesser curvature was used for DNA extraction. Sociodemographic information was obtained from clinical records and interviews. The Institutional Review Board of Louisiana State University Health Sciences Center and the Institutional Review Committee of Memorial Medical Center approved the protocol of this study.

Histologic Diagnosis

All the protocols and guidelines for the staining of the gastric sections and for the histopathological classification have been previously described (18). Briefly, after fixation in buffered formalin, the biopsy samples were embedded in paraffin. Four-micron thick sections were stained with hematoxylin and eosin. Histopathological diagnoses were independently assessed by a single pathologist (M.B.P.) according to established guidelines (32;33) as follows: normal, non-atrophic gastritis (NAG), multifocal atrophic gastritis without intestinal metaplasia (MAG), intestinal metaplasia (IM), and dysplasia (DYS). All biopsies displaying MAG or DYS were additionally examined and classified by a second experienced gastrointestinal pathologist (P.C.) (34). Pathologists were unaware of any clinical information or the results of genotyping.

DNA extraction

DNA from biopsies were extracted with DNAzol (Invitrogen; Carlsbad, CA), as recommended by the manufacturer, after homogenization of the tissue. The DNA was resuspended in 8 mM NaOH, pH 8.0 (adjusted with 0.1 M HEPES), and stored at −20°C until use.

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Single nucleotide polymorphism examination

Twenty-two SNPs in 8 cytokine genes were analyzed in the study (see Table 1). Genotypes of *TGFB1* (rs1982073 and rs1800471), *IL6-174* (rs1800795), and *PTGS2-765* (rs20417) SNPs were determined using TaqMan assays according to the conditions given at the National Cancer Institute SNP web site (http://snp500cancer.nci.nih.gov). All the remaining genotypes were determined by TaqMan genotyping assays (Assays on Demand, Applied Biosystems, Foster City, CA) with reporter probes (either FAM or VIC). Genomic DNA (5 ng) was denatured at 95°C for 10 min and amplified for 40 cycles of 15 sec at 92°C and 1 min at 58°C, in the presence of 2X TaqMan Universal Master Mix (Applied Biosystems), water, and the respective primer and probe mix. The reaction was analyzed using a 7900 HT instrument (Applied Biosystems), for the presence of VIC or FAM fluorescence, or both, using the Sequence Detection System (Applied Biosystems) to determine the genotype. Controls included 12 individuals of known genotype and blanks without DNA. In addition, 15% of the samples which were run twice in separate assays and the allele classification compared. The individuals of known genotype and blanks without DNA were included for each SNP in every batch.

Evaluation of the cagA status

To determine the *cagA* status of *H. pylori*, we amplified the *cagA* gene using primers and conditions previously reported (35). All DNAs were also tested by PCR for the presence of *H. pylori* harboring an empty cagA site, as reported (36). As controls we used DNA extracted from *H. pylori* strains 43504 (*cagA*-positive) and 51932 (*cagA*-negative) (ATCC, Manassas, VA) using the QIAamp DNA Mini Kit (QIAGEN; Valencia, CA) as recommended by the manufacturer. Laboratory personnel performing host and bacteria genotyping were blinded to the histological diagnoses and race of the participants.

Statistical analysis

Chi-square, Fisher's exact, and student-t tests were used as appropriate to assess the statistical significance of the differences between the racial groups regarding sociodemographic variables, haplotype frequencies, histological diagnosis, and *cagA* status. Haplotype frequencies were inferred using log-linear modeling embedded within an expectation-maximization algorithm. Quality control procedures (including evaluation of genotyping efficiency and Hardy-Weinberg equilibrium in the reference groups), assessment of linkage disequilibrium between markers as well as haplotype association analysis were performed separately in Caucasians and African-Americans.

For the statistical analysis, we considered IM and DYS as advanced preneoplastic lesions and patients with normal histology, NAG and MAG, as the reference group. Subjects with MAG were included in the reference group because MAG is the last reversible stage in the precancerous cascade and because most subjects (77%) with MAG displayed mild atrophy. Odds ratios (OR) and corresponding 95% confidence intervals (CI) for inferred haplotypes were calculated for IM and DYS using logistic regression. Regression models were run for each racial group and each locus separately, comparing the most common haplotype with every other haplotype. Logistic regression models were adjusted for *cagA* status, sex, age, smoking, and education level. Rare haplotypes with a frequency of less than 5% were included in a single "others" group. Statistical analyses were performed with R (37), Haploview version 3.2 (Whitehead Institute for Biomedical Research, USA) (38), and Stata, version 9.2 (Stata Corporation, College Station, Texas, USA).

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Results

Table 1 shows the identity and genomic location of the evaluated SNPs as well as the results of Hardy-Weinberg equilibrium (HWE) tests of the different SNPs in the reference groups. All SNPs were in HWE in Caucasians, while some were out of HWE in African Americans. In both racial groups, the *IL1B−511T* allele was in very strong linkage disequilibrium with the *IL1B−31C* allele (r^2=0.8 in Caucasians, r^2=0.7 in African Americans). In both African Americans and Caucasians, there was also strong linkage disequilibrium (r^2>0.50) between SNPs in *IL10*, *IL6*, and *ARG1* (data not shown).

Characteristics of the study subjects and their associations with the histological diagnosis are presented in Table 2. Among the Caucasian individuals, 186 (89.4%) were classified as normal/NAG/MAG and 22 (10.6%) as IM/DYS. The latter represents cases of IM since no DYS cases were observed in the Caucasians. Among the African American individuals, 299 (82.8%) were classified as normal/NAG/MAG, while 62 (17.2%) were classified as IM/DYS (60 cases as IM and 2 as DYS). Normal histology was more frequent in Caucasians (53.4%), while precancerous lesions (MAG, IM or DYS) were more common in African American patients (42.1%). Age and education level were found to be associated with IM/DYS in both racial groups. Overall, *H. pylori* infection was significantly more prevalent in African Americans than in Caucasians (43% and 13%, respectively; p<0.001). Among *H. pylori* infected subjects, the proportion of infection with *cagA*-positive strains was significantly greater in African Americans (p<0.001).

Analysis restricted to subjects with normal histology showed significant differences in haplotype frequencies between the two racial groups in 6 of the 8 loci. For example, haplotypes in the *IL1B* gene including 2 and 3 risk-associated alleles were more frequent in African Americans than in Caucasians (50.8% and 33.6%, respectively; p=0.004). Similarly, haplotypes within the *IL10* gene including risk-associated alleles (e.g. ATA haplotype) were more prevalent among African Americans (p=0.044) (data not shown).

Table 3 shows the ORs of IM/DYS for *IL1B* haplotypes adjusted for sex, age, smoking, education level, and *cagA* status by race. African American subjects carrying the *−511T/−31C/+3954T* haplotype, including the three risk-associated alleles, had a 2.5 fold increased likelihood of a diagnosis of IM or DYS (95% CI: 1.1–5.5) in comparison to the common haplotype T-C-C including 2 risk-associated alleles. Analyses of the remaining genetic variants showed no significant associations in this racial group. In Caucasians, none of the examined gene polymorphisms analyzed was associated with IM/DYS.

Based on multivariable analyses, age and *H. pylori* infection were identified as independent risk factors for the presence of IM/DYS in both racial groups. In African Americans, using uninfected subjects as the reference group, subjects infected with *cagA*-positive strains were significantly more likely to had a diagnosis of IM or DYS (OR: 2.1; 95% CI: 1.1–4.1). A positive but non-significant association with IM/DYS was observed in African American subjects carrying *cagA*-negative strains (OR: 1.4; 95% CI 0.5–3.7). In Caucasians, considering also uninfected subjects as reference, ORs for IM/DYS for those infected with *cagA*-positive and *cagA*-negative strains were 11.8 (95% CI 2.5–56.2) and 4.6 (95% CI 1.0–22.0), respectively. Finally, smoking was inversely associated with the presence of IM/DYS in Caucasians, most likely indicating “reverse causality” (i.e. the outcome influencing the exposure) (OR: 0.2; 95% CI 0.10-0.9).

Discussion

In this study, we examined 22 SNPs in eight inflammation-related genes, some of them previously associated with gastric cancer risk, to determine whether any of these genetic

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factors were associated with the presence of advanced gastric premalignant lesions in two racial groups with contrasting gastric cancer risk. Since the study of the physiological effect of a single polymorphism may not reveal the overall functional effect in combination with other polymorphisms, our analysis was based on haplotypes rather than on single SNPs.

Our main finding is that African American subjects carrying the haplotype *−511T/−31C/+3954T* at the *IL1B* locus had an increased likelihood of a diagnosis of IM or DYS when compared with the most common haplotype T-C-C. Interestingly, the presence of allele T at *IL1B+3954* marks the only difference between the two compared haplotypes. *IL1B+3954T* has been previously associated with increased production of IL-1β (17) and more recently with the risk of IM (39) and gastric cancer in Costa Rica (40), a country with one of the highest incidence rates of gastric cancer in the world.

In the gastric mucosa, the colonization and survival of *H. pylori* is promoted by increased production of the proinflammatory cytokine IL-1β which is a potent inhibitor of gastric acid secretion (41;42). It has been hypothesized that prolonged hypochlorhydria may lead to gastric atrophy a step in the pre-cancerous process. Hypochlorhydria may also lead to increased production of gastrin, a potent cell growth factor implicated in several processes, including neoplastic transformation (43). Similar to the allele T at *IL1B+3954*, the alleles T and C of *IL1B−511* and *IL1B−31*, respectively, have been associated with IL-1β production and with development of gastric precancerous lesions and with gastric cancer (11;13;16–18). Race specific associations have been reported for certain *IL1B* gene polymorphisms in gastric cancer. *ILB−511T* and *+3954T* alleles were found to be significantly associated with the neoplasia in Caucasians and in Asians, respectively (44). The limited number of studies conducted in Hispanics, and the lack of evidence in African Americans restricted the evaluation and interpretation in those racial groups.

Chen *et al*. (28) demonstrated that the activity of the *IL1B* promoter depends on the haplotype context. However, due to the goals of their work, only SNPs in the promoter region of the gene were included. Since the SNP (*IL1B+3954T*) is located in exon 5 of the *IL1B* gene, it is possible that, instead of being involved in increasing the transcription of the *IL1B* gene, this SNP may confer gastric cancer susceptibility by increasing the stability of the IL1β mRNA. More IL1β mRNA available thus leads to accumulation of IL-1β with deleterious effects on the gastric mucosa. However, this hypothesis remains to be proven. Alternatively, *IL1B+3954T* may be linked to additional upstream regulatory sequences which may promote secretion of products that may induce parietal cell damage and changes in the phenotype of the cell, acquiring functions of intestinal cell types.

Using genotype-based analyses, and including 269 subjects (172 African Americans and 97 Caucasians) we reported previously that subjects carrying *IL1B+3954T* were more likely to have preneoplastic lesions (18). The association was particularly significant in African American subjects. The present study, an extended analysis of our previous report, provides further support to the importance of the host genetic factors showing that in African Americans the simultaneous carriage of *IL1B−511T, −31C,* and *+3954T* alleles increases the odds of having IM/DYS. Interestingly, this risk-associated haplotype was not observed in Caucasians. Therefore, it is likely that differences in haplotype structure between these two groups may play a role in gastric carcinogenesis.

We did not find any association between precancerous lesions and variants in *IL8*, *IL6*, *IL10*, *TNF*, *TGFB1*, *PTGS2*, and *ARG1* genes. Consistent with our previous work, differences in haplotype frequencies were found between African Americans and Caucasians with normal gastric histology.

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Screening of populations at high risk of gastric cancer seems to be more cost-effective than mass population screening in countries where gastric cancer is highly incident (45). Although histopathological examination of biopsy specimens obtained through endoscopy is the most accurate method to detect a gastric neoplasia and its precursors, screening by endoscopy is likely to be impractical due to high cost, invasiveness, and availability of endoscopy instruments (46). According to the Japanese experience, the combination of serum pepsinogen levels and anti-*H pylori* antibodies status provides a good predictive marker for the development of gastric cancer (47). Identification of alleles or haplotypes associated with advanced precancerous lesions, e.g. carrying of the *ILB−511T/−31C/+3954T* haplotype, in combination with *H. pylori* IgG antibodies (particularly against CagA) and serum pepsinogen status may help predict the presence of advanced gastric lesions. If subjects with atrophic gastritis can be identified by means of this strategy, it would be possible to offer them treatment for *H. pylori* infection, an endoscopic evaluation with biopsies and, if warranted by the histopathological findings, endoscopic surveillance.

The greater prevalence of advanced precancerous lesions we observed in African Americans may be explained by several factors: 1) an increased proportion of African American subjects infected with *H. pylori*, and among those, a greater proportion of infection with *cagA*-positive *H. pylori* strains; 2) a delay in the diagnosis after onset of the symptoms, assuming that, in general, African Americans may have less accessibility to health services; 3) a genetic background predisposing to differences in nature and severity of the inflammatory process.

To fully exploit high-density maps, it may be more useful to focus on the transmission of multilocus haplotypes, as opposed to alleles at individual loci. Because each new allele is associated with its own chromosomal history, haplotype-based analyses can detect unique chromosomal segments that harbor disease-predisposing alleles. Further, the use of multilocus analyses in the SNP setting can improve the information content of genomic regions (48;49). The identification and study of the transmission of haplotypes, however, requires knowledge of phase information about the individuals studied. Methods for determining phase and assigning haplotypes usually require either laborious chromosomal isolation or other laboratory-based strategies or genotypic information on relatives of the individuals studied. Thus, analysis of unrelated individuals, as in case-control studies where simple genotypic data is collected, is problematic.

Due to the exploratory nature of this work no correction for multiple comparisons was made. A limitation of this study is the small sample size, particularly in the Caucasian series and consequently, limited statistical power. However, our results in African Americans regarding inflammatory haplotypes and infection with more virulent *H. pylori* strains, constitutes one step forward in the understanding of the higher gastric cancer risk of this racial group. Further studies with larger sample size are needed to replicate our findings.

In conclusion, a haplotype at the *IL1B* locus (*−511T/−31C/+3954T*) may play a role in mediating the susceptibility to gastric carcinogenesis and might be useful as a genetic marker of advanced gastric pre-malignant lesions in African Americans. Interestingly, the presence of the allele T of *IL1B+3954* seems to be the key factor predicting the presence of these lesions, although it is possible that other SNPs in the *IL1B* gene may be also involved in modifying the risk of these lesions.

Acknowledgments

We thank Drs. Luis Balart and William Ferrante at the Oschner Baptist Medical Center (formerly Memorial Medical Center) and Medical Center of Louisiana at New Orleans, respectively, and the staff of the Gastroenterology program at LSUHSC for their invaluable contribution in the collection of the gastric mucosa

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biopsies. We wish to acknowledge our former staff members at LSUHSC: Helen McMillan for recruitment and interview of patients, and Violeta Sanchez and Raquel Nin for the processing of the tissues. This study was supported in part by a grant from National Cancer Institute (PO1CA028842) and by a grant from the Health Excellence Fund of the Board of Regents of the State of Louisiana (HEF 2000-05-03).

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Table 1

rs number, genomic location and HWE of the SNPs included in this study

rs number	SNP	Base change	SNP region	Chromosome	Caucasians		African Americans	
					HWE p-value	MAF	HWE p-value	MAF
rs16944	*IL1B−511*	C>T	−1070	2q14	0.72	0.36	0.86	0.50
rs1143627	*IL1B−31*	T>C	−580	2q14	0.84	0.38	0.80	0.42
rs1143634	*IL1B+3954*	C>T	EX5+14	2q14	0.35	0.21	1	0.15
rs1799724	*TNF−857*	C>T	−1037	6p21.3	0.49	0.13	1	0.04
rs361525	*TNF−238*	G>A	−414	6p21.3	1	0.07	1	0.05
rs1800629	*TNF−308*	G>A	EX5+14	6p21.3	0.33	0.15	0.23	0.13
rs1982073	*TGFB1 10*	T>C	Ex1−327	19q13.2	0.83	0.37	0.01	0.44
rs1800471	*TGFB1 25*	G>C	Ex1−282	19q13.2	0.09	0.05	0.01	0.08
rs1800896	*IL10−1082*	G>A	−1116	1q31–q32	0.19	0.43	0.34	0.32
rs1800871	*IL10−819*	C>T	−853	1q31–q32	1	0.27	1	0.42
rs1800872	*IL10−592*	C>A	−626	1q31–q32	0.79	0.28	0.97	0.43
rs2227307	*IL8+230*	G>T	IVS1+230	4q13–q21	0.49	0.49	0.03	0.49
rs1803205	*IL8−47*	C>T	Ex1−47	4q13–q21	1	0.01	0.10	0.02
rs1800795	*IL6−174*	C>G	−236	7p21	0.28	0.37	0.22	0.08
rs2069832	*IL6−180*	G>A	IVS2−180	7p21	0.32	0.39	0.18	0.10
rs1800797	*IL6−598*	G>A	−660	7p21	0.37	0.37	0.04	0.08
rs20417	*PTGS2−765*	G>C	−898	1q25.2–q25.3	1	0.18	0.39	0.31
rs689466	*PTGS2−1195*	A>G	−1328	1q25.2–q25.3	0.41	0.19	0.13	0.11
rs5275	*PTGS2+837*	T>C	Ex10+837	1q25.2–q25.3	1	0.36	0.16	0.46
rs2781659	*ARG1*	A>G		6q23	0.15	0.34	0.14	0.36
rs2781667	*ARG1*	C>T	IVS1+665	6q23	0.24	0.32	0.43	0.29
rs2246012	*ARG1*	T>C	IVS2+333	6q23	0.22	0.17	0.68	0.12

Abbreviations: HWE= Hardy-Weinberg equilibrium; SNP= single nucleotide polymorphism; MAF= minor allele frequency

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Table 2

Sociodemographic characteristics, *H. pylori* and cagA status according to histological diagnosis by race

Characteristics	Race (n=569)					
	Caucasians (n=208)			African Americans (n=361)		
	Normal/NAG/MAG (n=186)	IM/DYS (n=22)	Two-sided p-value [a]	Normal/NAG/MAG (n=299)	IM/DYS (n=62)	Two-sided p-value [a]
Age in years, mean (SD)	48(12)	56(12)	0.007	48(12)	54(11)	0.001
Sex, %						
Female	64.5	63.6	0.935	76.3	80.7	0.455
Male	35.5	36.4		23.7	19.3	
Body Mass Index [b,c], %						
Underweight (<18)	3.3	0.0	0.452	1.4	0.0	0.145
Normal (18.5–24.9)	36.8	22.7		20.8	34.5	
Overweight (25–29.9)	32.4	45.5		24.3	17.2	
Obese (≥30)	27.5	31.8		53.5	48.3	
Years of education [c], %						
<9	8.5	13.6	0.036	10.4	23.0	0.017
9–12	46.6	68.2		66.0	62.3	
≥13	44.9	18.2		23.6	14.7	
Current smoker [c], %						
No	58.8	81.8	0.039	68.2	65.6	0.695
Yes	41.2	18.2		31.8	34.4	
H. pylori status [c], %						
Uninfected	90.3	59.1	<0.001	59.2	46.5	0.188
Infected, cagA negative	6.5	18.2		10.7	12.1	
Infected, cagA positive	3.2	22.7		30.1	41.4	

Abbreviations: SD: Standard deviation, NAG: Non-atrophic gastritis, MAG: Multifocal atrophic gastritis; IM: Intestinal metaplasia.

[a] p-values for difference from chi-square, Fisher exact or *t* tests as appropriate.

[b] Classification according to Centers for Disease Control and Prevention

[c] Minimum number of missing values was present

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Table 3

Odds ratio and 95% confidence intervals of intestinal metaplasia/dysplasia for *IL1B* haplotypes by race

Haplotypes [a]	Race					
	Caucasians			African Americans		
	Normal/NAG/MAG	IM/DYS	OR [b](95 CI)	Normal/NAG/MAG	IM/DYS	OR [b] (95 CI)
IL1B (*−511/−31/+3954*), %						
C-T-C	44	50	1.0 (reference)	34	40	1.7 (1.0–2.9)
C-T-T	17	18	0.9 (0.3–2.7)	6	3	0.6 (0.1–2.7)
C-C-C	0	0	NA	8	8	1.0 (0.4–2.6)
T-C-C	30	32	0.8 (0.3–2.1)	41	30	1.0 (reference)
T-C-T	0	0	NA	10	16	2.5 (1.1–5.5)
Others	9	0	NA	1	3	5.9 (0.9–41.3)

Abbreviations: OR: odds ratio; CI: Confidence interval; NAG: Non-atrophic gastritis, MAG: Multifocal atrophic gastritis; IM: Intestinal metaplasia.

[a]The estimation maximization algorithm accounts for missing alleles when phasing ambiguous haplotypes.

[b]ORs adjusted for sex, age, smoking, education, and *cag*A status.