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ORIGINAL ARTICLE

Ethnic differences in cytokine gene polymorphisms: potential implications for cancer development

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Abstract Differences in incidence and outcome of cancer among ethnic groups may be explained by biological and/or socio-economic factors. Genetic variations that affect chronic inflammation, a potentially important risk factor for carcinogenesis, may differ across ethnic groups. Such differences may help explain cancer disparities among these groups. Single nucleotide polymorphisms (SNPs) within cytokine genes can affect cytokine levels and the degree of inflammation. Associations between cancer and some cytokine SNPs have been suggested. However, these have not been consistently replicated among populations, suggesting that SNP function may differ according to ethnicity, or that SNPs alone do not completely account for regulation of inflammation. We examined seven polymorphisms in African-American ($n = 294$) and Caucasian ($n = 299$) newborns in Louisiana: *IL1B-511C > T, IL1B-31T > C, IL1B + 3954C > T, IL1RN*2, IL10-1082G > A, IL10-592C > A*, and *TNF-308G > A*. African-American newborns had significantly higher frequencies of *IL1B-511T, IL1B-31C, IL10-1082A* and *IL10-592A* alleles and complete linkage equilibrium between *IL1B + 3954* and *IL1B-31*. In contrast, *IL1B + 3954T, IL1RN*2*, and *TNF-308A* were more frequent in Caucasian newborns and exhibited strong linkage disequilibrium between *IL1B + 3954* and *IL1B-31*. All allelic frequencies were significantly different between groups. We hypothesize that these dissimilarities may contribute to differences in the inflammatory response and cancer incidence and mortality between African-Americans and Caucasians in Louisiana.

Keywords Inflammation · Single nucleotide polymorphisms · Cytokines · Cancer

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Introduction

Chronic inflammation is a risk factor for many types of cancers [10, 39, 48, 50]; however, the mechanisms that affect the levels of inflammation and ultimately cancer risk are unclear. Incidence and mortality rates of cancer are higher in African-Americans than in Caucasians in the US [58]. Genetic variants, specifically single nucleotide polymorphisms (SNPs) in cytokine genes can affect cytokine production, and therefore, may in part modulate the inflammatory response. Differences in SNP allelic distributions

between cases and controls have been used to assess or predict susceptibility to cancer, but the data have not been consistent among different ethnic groups. Therefore, either the role of these SNPs differs among groups for some unknown reasons, or their distribution is such that significant associations cannot be assessed with equal power in the different populations studied.

Several cytokine SNPs have previously been associated with the expression of cytokines and/or cancer risk. For example, a C to T transition at position -511 in the gene encoding IL1β (*IL1B-511C > T*) is associated with increased secretion of IL1β [25], and with gastric [13, 35, 52], breast and pancreatic cancer [3, 32] in several populations. High levels of IL1β inhibit gastric acid secretion [5], and elevated gastric pH promotes colonization by *Helicobacter pylori (H. pylori)*, a recognized Class I carcinogen [26]. Other studies have also found *IL1B + 3954T* to be associated with gastric cancer [1, 64] and with disease-free survival in breast cancer patients [53]. Similarly, allele 2 of the IL1 receptor antagonist gene (*IL1RN*), containing 2 repeats of the 86 bp variable number tandem repeats (VNTR) polymorphism [60], has been associated with increased production of IL1β [24, 47] and increased risk of gastric, cervical and ovarian cancer [1, 13, 34]. Tumor necrosis factor alpha (TNF-α) also inhibits gastric acid secretion [5]. Specifically, a G to A transition at position -308 of the TNF-α gene (*TNF-308A*) was associated with increased protein expression in vitro [28], and increased risk of gastric cancer and non-small cell lung carcinoma [14, 34, 51]. Interleukin 10 is an important anti-inflammatory cytokine [36], and a haplotype containing three SNPs (*-1082A/-819T/-592A*) (ATA) in the *IL10* gene is associated with reduced levels of circulating IL-10 [11, 56]. Alleles of the *IL-10* gene are reported to be associated with increased risk of gastric, renal, lung and skin cancer [14, 20, 43, 49].

Although most of the studies cited above suggest that individual SNPs can be used to predict cytokine expression, recent work by Chen et al. [9] on *IL1B* demonstrated that haplotypes, and not individual SNPs, need to be studied in order to understand transcriptional regulation. Similar results, regarding the importance of haplotype in regulation, have been shown for interleukin 6 (*IL6*) [55]. We therefore decided to study the frequency of haplotypes as well as single polymorphisms in *IL1B* and other cytokine genes in a population-based sample of African-American and Caucasian newborns in Louisiana, as part of an ongoing study aimed at understanding the inflammatory response to *H. pylori* infection and ultimately cancer risk. We compared the distribution of alleles, genotypes and haplotypes of *IL1B-511C > T, IL1B-31T > C, IL1B + 3954C > T, IL10-1082G > A, IL10-592C > A, TNF-308G > A*, and the presence of allele 2 of the *IL1RN* VNTR in 593 newborns. Because these polymorphisms may be important in cancer risk, the patterns of genetic variation in these populations may help us to better understand disparities in cancer incidence between African-American and Caucasian populations.

Materials and methods

DNA samples

Dried blood spots (DBS) collected on specimen collection paper (Whatman Inc., Florham Park, NJ) were obtained from the Louisiana Public Health Laboratory Newborn Screening Program, which screens infants from participating centers, representing approximately 90% of the newborns in the State of Louisiana (United States). Briefly, after routine newborn screening was completed, the dried blood spots were selected by accession number to ensure that no sample would be tested twice. The samples were segregated by gender and categorized under the ethnic information entered with sample submission: African-American, Caucasian or other ethnic group. Only African-American and Caucasian samples were used for this study, since they constitute the majority of the inhabitants of Louisiana. Excluded from the study population were any infants with positive screens for phenylketonuria/hyperphenylalaninemia (incidence 1 in 10,000 live births), congenital hypothyroidism (1 in 3,600 to 5,000), biotinidase deficiency (1 in 70,000), galactosemia (1 in 60,000), sickle cell anemia or abnormal hemoglobins revealed by high performance liquid chromatography and isoelectric focusing (1 in 385 African-American live births). After the selection, all accession numbers were removed, preventing reverse access to the identifying information. The sample size of 593 was selected from 12,000 samples received for routine newborn screening over a 10-week period in 2004. The study group samples were randomly selected from an overall pool of samples available, with exclusions listed above, from non-consecutive days of submission.

DNA extraction

DNA was extracted from the specimen paper in one 1.5 ml tube using 1 ml of DNAzol (Invitrogen; Carlsbad, CA). The sample was mixed by vortex at a maximum speed for 30 min at room temperature (RT). The sample was then centrifuged at 10,000*g* for 10 min. at RT and the supernatant was transferred to a new tube. Absolute ethanol, 500 µl, was added, and the sample was mixed by inversion and incubated at RT for 3 min. After centrifugation at 10,000*g* for 10 min, 1 ml of 75% ethanol was added to the sample, which was centrifuged again. Finally, the DNA was dissolved in 100 µl of 8 mM NaOH, pH 8.0.

SNP detection

Polymorphisms studied in this work were *IL1B -511, IL1B -31, IL1B + 3954, IL1RN* VNTR, *IL10-1082, IL10-592* and *TNF -308*. The SNPs were analyzed by TaqMan assays (Assays-on-Demand; Applied Biosystems, Foster City, CA), containing probes for both alleles labeled with either FAM or VIC dyes. Briefly, 2–5 ng DNA were mixed in a total volume of 5 µl with TaqMan Universal Master Mix (Applied Biosystems) and water, and then subjected to 95°C for 10 min followed by 40 cycles of 15 sec at 92°C and 1 min at 60°C. The PCR product was analyzed on a 7,900 HT instrument (Applied Biosystems), and the allele determined according to the VIC or FAM fluorescence detected. Controls of known genotype for each polymorphic locus were always run in parallel with each experiment. In addition, 20% of the samples were randomly selected and analyzed by PCR-RFLP as previously described [11, 17, 31]. The concordance between techniques was greater than 98.5%. In cases where concordance was not achieved, the samples were checked by both techniques, and the genotype obtained by TaqMan was used. Alleles of the *IL1RN* VNTR were analyzed by PCR followed by electrophoresis in 2% agarose gels.

Statistical analyses

Statistical analysis was performed with Stata®, version 9 (Stata Corporation, College Station, Texas, USA), PowerMarker [33] and Haploview [4]. Allele frequencies were estimated and tested for fit to the expectations of Hardy-Weinberg equilibrium (HWE) using the χ^2 goodness of fit test, with 1 degree of freedom (each ethnic group was tested separately). For the *IL1RN* VNTR analysis, we grouped genotypes into those containing allele 2 (the short allele) and all others (long alleles 1, 3, 4 or 5). Genotype and allele frequencies of African-Americans and Caucasians were compared by Chi-square statistics or Fisher's exact tests. Linkage disequilibrium (LD) matrices were estimated for the *IL1RN* VNTR and the SNPs in *IL1B* (in each ethnic group separately), using the standardized summary statistics D' and r^2 calculated by the Haploview program, version 2.0.2 (Whitehead Institute for Biomedical Research, USA). The D' confidence interval algorithm in Haploview was used to assign haplotype blocks in our unphased genotype data [15].

Haplotype frequencies of *IL1B* (*IL1RN, IL1B-511, IL1B-31* and *IL1B + 3954*) and *IL10* (*IL10-1082* and *IL10-592*) were estimated using log-linear modeling embedded within an expectation-maximization algorithm using Stata® and PowerMarker. The haplotype frequency estimates did not differ significantly between the two programs. Differences in the haplotype frequency distributions between African-Americans and Caucasians were estimated with the program PowerMarker. Logistic regression was used to determine the probability, expressed as exposure odds ratios (OR) and confidence intervals (CI), of a specific haplotype being present in either African-Americans or Caucasians as compared with the reference haplotype.

Results

A total of 593 samples from newborns were studied for SNPs and VNTR polymorphism. The two ethnic groups did not differ in gender distribution (52.2% males for African-Americans and 49% for Caucasians, $P = 0.772$). A total of 537 (90.6%) samples were successfully genotyped for all seven loci. The remaining 9.4% of the subjects were not classified for one or more polymorphisms due to technical reasons, including DNA degradation, and inability to detect a specific SNP. All the evaluated SNPs were in Hardy–Weinberg equilibrium (HWE). The *IL1B-511T* allele was found in near complete linkage disequilibrium with the *IL1B-31C* allele in both ethnic groups ($D' = 0.93$ in African-Americans and $D' = 0.95$ in Caucasians, $P < 0.0001$ in both groups), as previously reported [13, 46].

Differences in genotype frequencies between the two ethnic groups were observed in six of the seven polymorphisms (Table 1), the exception being *TNF-308* that was almost significant ($P = 0.062$). All allele frequencies were significantly different between the groups. The *IL1B-511T/T* and *IL1B-31C/C* genotypes were more common in African-Americans (26.8 and 35.2%, respectively) than in Caucasians (12.1 and 13.8%, respectively). Similarly, the *IL10-1082A/A* and *IL10-592A/A* genotypes were more frequent in African-American newborns (41.2 and 16.3%, respectively) than in Caucasians (26.0 and 3.5%, respectively). In contrast, Caucasian newborns had a higher frequency of genotype *IL1RN2/2* (8.9 versus 1.4% in African-Americans) as well as higher frequency of *IL1B + 3954C/T* heterozygotes (32.2 versus 19.7% in African-Americans). Similarly, Caucasian newborns also had a higher frequency of genotypes that have at least one of the following alleles, *IL1RN2, IL1B + 3954T* and *TNF -308A* (49.5, 36.0, 32.9%, respectively) compared to African-American newborns (17.0, 22.5, 24.9%, respectively).

Differences between the two ethnic groups were also seen when comparing haplotype distributions. Table 2 shows the allelic combination for *IL1RN, IL1B-511, IL1B-31* and *IL1B + 3954*. We found differences based on ethnicity in expected haplotype frequencies of more than 10% for 2-T-C-C, L-C-T-T and L-T-C-C. African-American newborns were more than three times as likely to carry the

Table 1 Relation between ethnicity and *IL1B, IL1RN, IL10* and *TNF* gene polymorphisms in newborns from Louisiana, 2005

Genotypes	Ethnic group (n = 593)		Two-sided P value[a]
	Caucasians (n = 299)	African-Americans (n = 294)	
	n (%)	n (%)	
IL1B-511	n = 289	n = 291	
Genotype			
C/C	124 (42.9)	58 (19.9)	<0.001
C/T	130 (45.0)	155 (53.3)	
T/T	35 (12.1)	78 (26.8)	
T carriers	165 (57.1)	233 (80.1)	
Alleles			
Allele C	378 (65.4)	271 (46.6)	<0.001
Allele T	200 (34.6)	311 (53.4)	
IL1B-31	n = 283	n = 284	
Genotype			
T/T	125 (44.2)	49 (17.3)	<0.001
T/C	119 (42.0)	135 (47.5)	
C/C	39 (13.8)	100 (35.2)	
C carriers	158 (55.8)	235 (82.7)	
Alleles			
Allele C	197 (34.8)	335 (59.0)	<0.001
Allele T	369 (65.2)	233 (41.0)	
IL1B + 3954	n = 286	n = 289	
Genotype			
C/C	183 (64.0)	224 (77.5)	0.002
C/T	92 (32.2)	57 (19.7)	
T/T	11 (3.8)	8 (2.8)	
T carriers	103 (36.0)	65 (22.5)	
Alleles			
Allele C	458 (80.0)	505 (87.4)	0.001
Allele T	114 (20.0)	73 (12.6)	
IL1RN	n = 293	n = 289	
Genotype			
L/L[b]	148 (50.5)	240 (83.0)	<0.001
L/2[b]	119 (40.6)	45 (15.6)	
2/2	26 (8.9)	4 (1.4)	
2 carrier	145 (49.5)	49 (17.0)	
Alleles			
Allele L	415 (70.8)	525 (90.8)	<0.001
Allele 2	171 (29.2)	53 (9.2)	
IL10-1082	n = 277	n = 284	
Genotype			
GG	65 (23.5)	37 (13.0)	<0.001
GA	140 (50.5)	130 (45.8)	
AA	72 (26.0)	117 (41.2)	
A carriers	212 (76.5)	247 (87.0)	
Alleles			
Allele G	270 (48.7)	204 (35.9)	<0.001
Allele A	284 (51.3)	364 (64.1)	
IL10-592	n = 283	n = 282	
Genotype			
CC	161 (56.9)	111 (39.4)	<0.001
CA	112 (39.6)	125 (44.3)	
AA	10 (3.5)	46 (16.3)	
A carriers	122 (43.1)	171 (60.6)	
Alleles			
Allele C	434 (76.7)	347 (61.5)	<0.001
Allele A	132 (23.3)	217 (38.5)	
TNF-308	n = 277	n = 277	
Genotype			
GG	186 (67.1)	208 (75.1)	0.062
GA	83 (30.0)	66 (23.8)	
AA	8 (2.9)	3 (1.1)	
A carriers	91 (32.9)	69 (24.9)	
Alleles			
Allele G	455 (82.1)	482 (87.0)	0.025
Allele A	99 (17.9)	72 (13.0)	

[a] P values for difference from chi-square or Fisher's exact tests

[b] L represents any long allele (1, 3, 4 or 5)

haplotype L-T-C-C relative to the reference haplotype, L-C-T-C (OR: 3.57, 95% CI: 2.52, 5.06). It is of note that L-T-C-C includes two risk-associated alleles. Almost all of the *IL1* haplotypes were significantly different between African-Americans and Caucasians in their distributions relative to the reference haplotype. The distribution of the haplotypes of the *IL10* gene polymorphisms was also significantly different between ethnic groups (Table 3; $P < 0.001$). African-American newborns were approximately two times more likely to carry the haplotype A-A than Caucasians, relative to G-C haplotype (OR: 2.28, 95% CI: 1.69, 3.08). The A-A haplotype includes both risk-associated alleles.

The LD values (D') are shown in Fig. 1. The overall pattern of LD differed slightly between the two populations, with Caucasians having D' values greater than or equal to those in African-Americans for all comparisons of polymorphisms. In African-Americans, but not in Caucasians, *IL1B-31* and *IL1B + 3954* were in complete linkage equilibrium. The two SNPs in the *IL10* gene promoter (*-1082G/A* and *-592C/T*) were in complete LD in both ethnic groups (data not shown).

Table 2 *IL1B* and *IL1RN* haplotype frequency by ethnicity in newborns from Louisiana

Haplotypes[a]	Ethnic group		Pairwise odds ratio (95% CI)
	Caucasians *n* (%)	African-Americans *n* (%)	
L-C-T-C	218 (39.2)	185 (33.0)	1.0 (reference)
L-T-C-C	70 (12.6)	212 (37.9)	3.57 (2.52–5.06)
2-T-C-C	102 (18.3)	36 (6.4)	0.42 (0.26–0.65)
L-C-T-T	86 (15.5)	24 (4.3)	0.33 (0.19–0.55)
2-C-T-C	46 (8.2)	10 (1.8)	0.26 (0.11–0.53)
L-C-C-C	2 (0.4)	40 (7.1)	23.6 (5.94–203.0)
L-T-C-T	6 (1.1)	40 (7.1)	7.86 (3.20–23.1)
Others	26 (4.7)	13 (2.4)	0.59 (0.27–1.23)

[a] Allelic combination in each haplotype is in the following order: *IL1RN, IL1B-511, IL1B-31* and *IL1B + 3954*

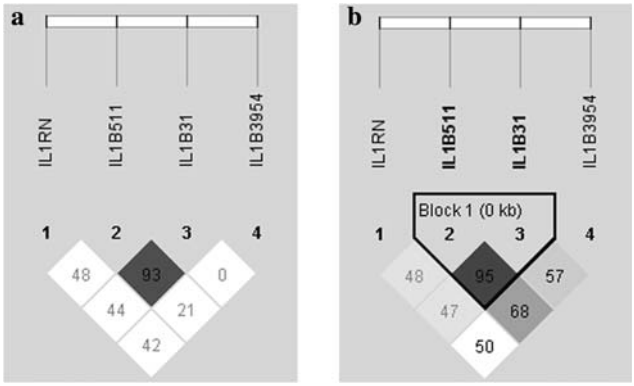

Fig. 1 Haplotype block structure and LD across the *IL1B* region (defined by 3 *IL1B* SNPs and the *IL1RN* VNTR) in **a** African-Americans and **b** Caucasians. Haplotype blocks are shown in *brackets* with their size and SNPs included in each *block*. *Dark squares* without a number indicate a D' value of 1 for the pairwise comparison. The number in each square represents D' if it does not equal 1. *White squares* indicate strong evidence for historical recombination, while *gray squares* represent little evidence for strong LD or recombination

Discussion

The allele frequencies we found are consistent with those of previous works in which the distribution of polymorphic alleles for *IL1B* and *IL10* genes between Caucasians and African-Americans were evaluated in selected groups, mainly women [19, 22, 41, 42]. However, ours is a population-based sample, which provides a better estimate, and our study evaluates the *IL1* markers in a haplotype context, thereby providing a more comprehensive assessment of ethnic patterns of genetic variation in these genes and *TNF*.

In our study, we found that African-American newborns had higher frequencies of alleles *IL1B-511T* and *IL1B-31C* than Caucasians. Since both of these alleles have been previously associated with increased cancer risk, these data may be important in explaining disparities. Such a conclusion is partially supported by a recent meta-analysis of 25 studies strongly supporting an association of *IL1B-511T* with gastric cancer in Caucasians, but not in Asians [7]. The association of *IL1B-31C* was not confirmed by this meta-analysis, probably due to the relatively small number of studies analyzed for this SNP (even though this SNP is in nearly complete linkage disequilibrium with the SNP at position -511; [7]). The discrepancy between Asian and Caucasian populations may be in part related to the higher allele frequency of the risk-associated polymorphisms in Asian populations. Such high frequencies can prevent the detection of statistically significant relative risks. Consistent with this interpretation is a Chinese study in regions of high and low risk of gastric cancer that detected a significant association of *IL1B-511T* and gastric cancer in only the lower risk population [63]. A recent population-based study in Honduras is also consistent with this explanation, because this population has both gastric cancer risks and *IL1B-511T* allele frequencies that are very high [38]. In this study, no significant association of *IL1B-511T* and gastric cancer was found. Unfortunately, few, if any studies have examined the relationship of *IL1B-511T* (or *IL1B-31C*) and cancer risk in populations of African descent.

Association studies do not prove that *IL1B-511T* or *IL1B-31C* are functional in promoting gastric cancer, as has been suggested in previous reports. Rather, recent in vitro studies evaluating haplotypes containing these SNPs have shown that haplotypes containing them, rather than the individual polymorphisms, affect regulation of *IL1B*. Using in vitro studies of transcription, Chen et al. [9] demonstrated that the C allele at position -31 (*IL1B-31C*) could decrease or increase promoter activity compared to the T allele, depending on the variant found at the nearby -511 SNP. Similarly, the promoter activity was increased by a T allele at position -3737 (*IL1B-3737T*), but only when positions -31 and -511 had C and T alleles, respectively [9]. A functional activity of this haplotype was demonstrated by Hall et al. [18] in peripheral blood mononuclear cells

Table 3 Haplotype analysis for the *IL10* gene polymorphisms

Haplotypes[a]	Ethnic group		Pairwise odds ratio (95% CI)
	Caucasians *n* (%)	African-Americans *n* (%)	
G-C	265 (48.0)	194 (34.4)	1.0 (reference)
A-A	125 (22.7)	209 (37.0)	2.28 (1.69–3.08)
A-C	159 (28.8)	153 (27.2)	1.31 (0.97–1.77)
G-A	3 (0.5)	8 (1.4)	3.64 (0.86–21.53)

[a] Allelic combination in each haplotype is in the following order: *IL10-1082* and *IL10-592*

(PBMC) from patients with reumathoid arthritis (RA) and healthy controls. PBMCs from homozygous individuals for the *IL1B-511T/-31C/+3454C* haplotype produced between two and three times more IL1β after 24 h of stimulation with lypopolysaccharide (LPS) when compared with PBMC from individuals carrying either just one copy of the same haplotype or any other haplotype. Additional support for the role of *IL1B* haplotypes in LPS-induced IL1β secretion by PBMC was provided by Wen et al. [59].

Chen et al. [9] also found that haplotypes containing *IL1B-511T* and *IL1B-31C* (combinations previously associated with increased transcription of *IL1B)* were found in 56.7% of African-Americans and 33.7% of Caucasians studied. These percentages are similar to our findings in the Louisiana population. The results of Chen et al. [9], in conjunction with ours, support the idea that haplotypes rather than single polymorphisms should be assessed. In addition, our haplotype analysis of the *IL1B* polymorphisms provided results consistent with the frequently observed tendency of persons of African descent to have less linkage disequilibrium and smaller haplotype blocks in their genomes than Caucasians [15].

We found that Caucasians had a higher frequency of *IL1B + 3954T*, an allele that was associated in African-American and Caucasian populations in New Orleans with the presence of multi-focal atrophic gastritis (MAG), a type of chronic gastric inflammation considered to be pre-neoplastic [62]. However, this SNP was not associated with gastric cancer in a meta-analysis that examined the small number of existing studies [7]. While the reasons for this paradox are not clear, only a minority of MAG lesions progress to gastric cancer, suggesting an indirect relationship. Therefore, risk factors for MAG and gastric cancer are likely to be correlated, but not identical.

We found that the African-American newborns had significantly higher frequencies of *IL10-1082A* and *IL10-592A* alleles. As the *IL10-1082A/-592A* haplotype is associated with a lower production of IL-10, an inhibitor of proinflammatory mediators, it is reasonable that it would be associated with increased risk of cancer. In support of this idea, it was demonstrated that *H. pylori*-infected individuals carrying the *ATA* haplotype of the *IL10* gene expressed significantly lower amounts of mRNA for this protein at the gastric mucosa, as compared to individuals carrying the *GCC* haplotype [45]. To our knowledge, no meta-analysis has yet been performed to assess this association with gastric cancer risk in a variety of populations.

In addition to higher frequencies of *IL1B + 3954T*, Caucasian newborns had higher frequencies of *IL1RN* VNTR allele 2, and *TNF-308A*. The IL1 receptor antagonist is an inhibitor of IL1β effects [12, 17], but the presence of allele 2 of the *IL1RN* gene is associated with increased IL1β production [24, 47]. The association of allele 2 of the *IL1RN* VNTR may be affected by linkage disequilibrium with *IL1B-511*. In both black and white populations, *IL1B-511* and *IL1RN* VNTR were in partial linkage disequilibrium. This concept is further supported by a previous report showing that, even though the levels of IL1β in the gastric mucosa of *H. pylori*-infected individuals is higher in individuals carrying the *IL1RN*2* allele, these levels are further increased with the simultaneous carrying of the *IL1B-511T* (*IL1B-511T/IL1RN*2* haplotype) [45].

Even though the *TNF-308* polymorphism has not been consistently associated with increased protein production [6, 54, 57], some studies have associated this polymorphism with cancer risk [14, 34, 51]. More studies are needed, particularly meta-analyses and haplotype analyses, to evaluate this association across populations.

Even though racial disparities in risk for cancer have been well documented [40, 44], the basis for this observation is not clear. One potential contributor to these disparities may be a differential distribution of genetic variants that affect the expression of cytokines and other inflammatory mediators that change the risk of different types of cancer [21, 23, 27, 29, 31]. However, several studies have failed to replicate previously published associations or have shown opposite results [2, 23, 27, 29, 30, 31, 37, 61]. One possible explanation is that different populations have different genetic structures and that these differences change the genetic risk of previously published genetic variants. Similarly, the pattern of linkage disequilibrium may differ among populations, and the effect of a single SNP may be dependent on the promoter haplotype context [9, 55]. Our data certainly support the idea that differential distribution of variation in candidate genes exists in African-Americans and Caucasians, providing increased evidence for these genes as potential risk factors in cancer susceptibility.

In addition to genetic susceptibility, other factors are likely to contribute to the elevated incidence of gastric cancer in African-Americans. For example, we reported in a study of 172 African-Americans and 97 Caucasians in New Orleans that 55.2% of the African-Americans were positive for *H. pylori*, compared to 20.6% of the Caucasians ($P = 0.0002$) [62]. In addition, lower socio-economic status and residential crowding are identified risk factors for the elevated incidence of *H. pylori* infection in a variety of populations [8, 16].

In summary, our findings and the supportive functional data on these polymorphisms, although not proof of altered disease susceptibility, indicate that the differences in the incidence of cancer observed between African-Americans and Caucasians may be at least partially explained by the genetic differences we describe. The cytokine polymorphism with the strongest evidence for association with cancer, *IL1B-511T*, has a higher allele

frequency in African-Americans, a population with elevated cancer risk in Louisiana.

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References

1. Alpizar-Alpizar W, Perez-Perez GI, Une C, Cuenca P, Sierra R (2005) Association of interleukin-1B and interleukin-1RN polymorphisms with gastric cancer in a high-risk population of Costa Rica. Clin Exp Med 5:169–176
2. Balasubramanian SP, Azmy IA, Higham SE, Wilson AG, Cross SS, Cox A, Brown NJ, Reed MW (2006) Interleukin gene polymorphisms and breast cancer: a case control study and systematic literature review. BMC Cancer 6:188
3. Barber MD, Powell JJ, Lynch SF, Fearon KC, Ross JA (2000) A polymorphism of the interleukin-1 beta gene influences survival in pancreatic cancer. Br J Cancer 83:1443–1447
4. Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21:263–265
5. Beales IL, Calam J (1998) Interleukin 1 beta and tumour necrosis factor alpha inhibit acid secretion in cultured rabbit parietal cells by multiple pathways. Gut 42:227–234
6. Brinkman BM, Zuijdeest D, Kaijzel EL, Breedveld FC, Verweij CL (1995) Relevance of the tumor necrosis factor alpha (TNF alpha) -308 promoter polymorphism in TNF alpha gene regulation. J Inflamm 46:32–41
7. Camargo MC, Mera R, Correa P, Peek RM Jr, Fontham ETH, Goodman KJ, Piazuelo MB, Sicinschi LA, Zabaleta J, Schneider BG (2006) Interleukin-1B and Interleukin 1 receptor antagonist gene polymorphisms and gastric cancer: a meta-analysis. Cancer Epidemiol Biomarkers Prev 15:1674–1687
8. Camargo MC, Yepez MC, Ceron C, Guerrero N, Bravo LE, Correa P, Fontham ET (2004) Age at acquisition of *Helicobacter pylori* infection: comparison of two areas with contrasting risk of gastric cancer. Helicobacter 9:262–270
9. Chen H, Wilkins LM, Aziz N, Cannings C, Wyllie DH, Bingle C, Rogus J, Beck JD, Offenbacher S, Cork MJ, Rafie-Kolpin M, Hsieh CM, Kornman KS, Duff GW (2006) Single nucleotide polymorphisms in the human interleukin-1B gene affect transcription according to haplotype context. Hum Mol Genet 15:519–529
10. Coussens LM, Werb Z (2002) Inflammation and cancer. Nature 420:860–867
11. Crawley E, Kay R, Sillibourne J, Patel P, Hutchinson I, Woo P (1999) Polymorphic haplotypes of the interleukin-10 5′ flanking region determine variable interleukin-10 transcription and are associated with particular phenotypes of juvenile rheumatoid arthritis. Arthritis Rheum 42:1101–1108
12. Dripps DJ, Brandhuber BJ, Thompson RC, Eisenberg SP (1991) Interleukin-1 (IL-1) receptor antagonist binds to the 80-kDa IL-1 receptor but does not initiate IL-1 signal transduction. J Biol Chem 266:10331–10336
13. El-Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, Herrera J, Lissowska J, Yuan CC, Rothman N, Lanyon G, Martin M, Fraumeni JF Jr, Rabkin CS (2000) Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature 404:398–402
14. El-Omar EM, Rabkin CS, Gammon MD, Vaughan TL, Risch HA, Schoenberg JB, Stanford JL, Mayne ST, Goedert J, Blot WJ, Fraumeni JF Jr, Chow WH (2003) Increased risk of noncardia gastric cancer associated with proinflammatory cytokine gene polymorphisms. Gastroenterology 124:1193–1201
15. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D (2002) The structure of haplotype blocks in the human genome. Science 296:2225–2229
16. Goodman KJ, Correa P (1995) The transmission of *Helicobacter pylori*: a critical review of the evidence. Int J Epidemiol 24:875–887
17. Granowitz EV, Clark BD, Mancilla J, Dinarello CA (1991) Interleukin-1 receptor antagonist competitively inhibits the binding of interleukin-1 to the type II interleukin-1 receptor. J Biol Chem 266:14147–14150
18. Hall SK, Perregaux DG, Gabel CA, Woodworth T, Durham LK, Huizinga TW, Breedveld FC, Seymour AB (2004) Correlation of polymorphic variation in the promoter region of the interleukin-1 beta gene with secretion of interleukin-1 beta protein. Arthritis Rheum 50:1976–1983
19. Hassan MI, Aschner Y, Manning CH, Xu J, Aschner JL (2003) Racial differences in selected cytokine allelic and genotypic frequencies among healthy, pregnant women in North Carolina. Cytokine 21:10–16
20. Havranek E, Howell WM, Fussell HM, Whelan JA, Whelan MA, Pandha HS (2005) An interleukin-10 promoter polymorphism may influence tumor development in renal cell carcinoma. J Urol 173:709–712
21. Hefler LA, Ludwig E, Lebrecht A, Zeillinger R, Tong-Cacsire D, Koelbl H, Leodolter S, Tempfer CB (2002) Polymorphisms of the interleukin-1 gene cluster and ovarian cancer. J Soc Gynecol Investig 9:386–390
22. Hoffmann SC, Stanley EM, Cox ED, DiMercurio BS, Koziol DE, Harlan DM, Kirk AD, Blair PJ (2002) Ethnicity greatly influences cytokine gene polymorphism distribution. Am J Transplant 2:560–567
23. Hu Z, Shao M, Chen Y, Zhou J, Qian J, Xu L, Ma H, Wang X, Xu Y, Lu D, Shen H (2006) Allele 2 of the interleukin-1 receptor antagonist gene (IL1RN*2) is associated with a decreased risk of primary lung cancer. Cancer Lett 236:269–275
24. Hurme M, Santtila S (1998) IL-1 receptor antagonist (IL-1Ra) plasma levels are co-ordinately regulated by both IL-1Ra and IL-1beta genes. Eur J Immunology 28:2598–2602
25. Hwang IR, Kodama T, Kikuchi S, Sakai K, Peterson LE, Graham DY, Yamaoka Y (2002) Effect of interleukin 1 polymorphisms on gastric mucosal interleukin 1beta production in *Helicobacter pylori* infection. Gastroenterology 123:1793–1803
26. IARC (1994) IARC monograph on the evaluation of carcinogenic risks to humans:schistosomes, liver flukes and *Helicobacter pylori*. IARC 61:1–241
27. Kato S, Onda M, Yamada S, Matsuda N, Tokunaga A, Matsukura N (2001) Association of the interleukin-1 beta genetic polymorphism and gastric cancer risk in Japanese. J Gastroenterol 36:696–699
28. Kroeger KM, Carville KS, Abraham LJ (1997) The -308 tumor necrosis factor-alpha promoter polymorphism effects transcription. Mol Immunol 34:391–399
29. Lee JY, Kim HY, Kim KH, Kim SM, Jang MK, Park JY, Lee JH, Kim JH, Yoo JY (2005) Association of polymorphism of IL-10 and TNF-A genes with gastric cancer in Korea. Cancer Lett 225:207–214
30. Lee KM, Park SK, Hamajima N, Tajima K, Choi JY, Noh DY, Ahn SH, Yoo KY, Hirvonen A, Kang D (2006) Genetic polymorphisms of interleukin-1 beta (IL-1B) and IL-1 receptor antagonist

(IL-1RN) and breast cancer risk in Korean women. Breast Cancer Res Treat 96:197–202
31. Lee SG, Kim B, Choi W, Lee I, Choi J, Song K (2003) Lack of association between pro-inflammatory genotypes of the interleukin-1 (IL-1B -31 C/+ and IL-1RN *2/*2) and gastric cancer/duodenal ulcer in Korean population. Cytokine 21:167–171
32. Liu J, Zhai X, Jin G, Hu Z, Wang S, Wang X, Qin J, Gao J, Ma H, Wang X, Wei Q, Shen H (2006) Functional variants in the promoter of interleukin-1beta are associated with an increased risk of breast cancer: a case-control analysis in a Chinese population. Int J Cancer 118:2554–2558
33. Liu K, Muse SV (2005) PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinformatics 21:2128–2129
34. Machado JC, Figueiredo C, Canedo P, Pharoah P, Carvalho R, Nabais S, Castro AC, Campos ML, Van Doorn LJ, Caldas C, Seruca R, Carneiro F, Sobrinho-Simoes M (2003) A proinflammatory genetic profile increases the risk for chronic atrophic gastritis and gastric carcinoma. Gastroenterology 125:364–371
35. Machado JC, Pharoah P, Sousa S, Carvalho R, Oliveira C, Figueiredo C, Amorim A, Seruca R, Caldas C, Carneiro F, Sobrinho-Simoes M (2001) Interleukin 1B and interleukin 1RN polymorphisms are associated with increased risk of gastric carcinoma. Gastroenterology 121:823–829
36. Mege JL, Meghari S, Honstettre A, Capo C, Raoult D (2006) The two faces of interleukin 10 in human infectious diseases. Lancet Infect Dis 6:557–569
37. Michaud DS, Daugherty SE, Berndt SI, Platz EA, Yeager M, Crawford ED, Hsing A, Huang WY, Hayes RB (2006) Genetic polymorphisms of interleukin-1B (IL-1B), IL-6, IL-8, and IL-10 and risk of prostate cancer. Cancer Res 66:4525–4530
38. Morgan DR, Dominguez RL, Keku TO, Heidt PE, Martin CF, Galanko JA, Omofoye OA, Sandler RS (2006) Gastric cancer and the high combination prevalence of host cytokine genotypes and *Helicobacter pylori* in Honduras. Clin Gastroenterol Hepatol 4:1103–1111
39. Moss SF, Blaser MJ (2005) Mechanisms of disease: Inflammation and the origins of cancer. Nat Clin Pract Oncol 2:90–97
40. National Cancer Institute (2006) State cancer profiles, http://www.statecancerprofiles.cancer.gov
41. Ness RB, Haggerty CL, Harger G, Ferrell R (2004) Differential distribution of allelic variants in cytokine genes among African-Americans and white Americans. Am J Epidemiol 160:1033–1038
42. Nguyen DP, Genc M, Vardhana S, Babula O, Onderdonk A, Witkin SS (2004) Ethnic differences of polymorphisms in cytokine and innate immune system genes in pregnant women. Obstet Gynecol 104:293–300
43. Nikolova PN, Pawelec GP, Mihailova SM, Ivanova MI, Myhailova AP, Baltadjieva DN, Marinova DI, Ivanova SS, Naumova EJ (2007) Association of cytokine gene polymorphisms with malignant melanoma in Caucasian population. Cancer Immunol Immunother 56:371–379
44. Parkin DM, Bray F, Ferlay J, Pisani P (2005) Global cancer statistics, 2002. CA Cancer J Clin 55:74–108
45. Rad R, Dossumbekova A, Neu B, Lang R, Bauer S, Saur D, Gerhard M, Prinz C (2004) Cytokine gene polymorphisms influence mucosal cytokine expression, gastric inflammation, and host specific colonisation during *Helicobacter pylori* infection. Gut 53:1082–1089
46. Rad R, Prinz C, Neu B, Neuhofer M, Zeitner M, Voland P, Becker I, Schepp W, Gerhard M (2003) Synergistic effect of *Helicobacter pylori* virulence factors and interleukin-1 polymorphisms for the development of severe histological changes in the gastric mucosa. J Infect Dis 188:272–281
47. Santtila S, Savinainen K, Hurme M (1998) Presence of the IL-1RA allele 2 (IL1RN*2) is associated with enhanced IL-1beta production in vitro. Scand J Immunol 47:195–198
48. Schottenfeld D, Beebe-Dimmer J (2006) Chronic inflammation: a common and important factor in the pathogenesis of neoplasia. CA Cancer J Clin 56:69–83
49. Seifart C, Plagens A, Dempfle A, Clostermann U, Vogelmeier C, von Wichert P, Seifart U (2005) TNF-alpha, TNF-beta, IL-6, and IL-10 polymorphisms in patients with lung cancer. Dis Markers 21:157–165
50. Shacter E, Weitzman SA (2002) Chronic inflammation and cancer. Oncology (Williston. Park) 16:217–226 see also 229
51. Shih CM, Lee YL, Chiou HL, Chen W, Chang GC, Chou MC, Lin LY (2006) Association of TNF-alpha polymorphism with susceptibility to and severity of non-small cell lung cancer. Lung Cancer 52:15–20
52. Sicinschi LA, Lopez-Carrillo L, Camargo MC, Correa P, Sierra RA, Henry RR, Chen J, Zabaleta J, Piazuelo MB, Schneider BG (2006) Gastric cancer risk in a Mexican population: role of *Helicobacter pylori* CagA positive infection and polymorphisms in interleukin-1 and -10 genes. Int J Cancer 118:649–657
53. Snoussi K, Strosberg AD, Bouaouina N, Ben AS, Chouchane L (2005) Genetic variation in pro-inflammatory cytokines (interleukin-1beta, interleukin-1alpha and interleukin-6) associated with the aggressive forms, survival, and relapse prediction of breast carcinoma. Eur Cytokine Netw 16:253–260
54. Stuber F, Udalova IA, Book M, Drutskaya LN, Kuprash DV, Turetskaya RL, Schade FU, Nedospasov SA (1995) -308 tumor necrosis factor (TNF) polymorphism is not associated with survival in severe sepsis and is unrelated to lipopolysaccharide inducibility of the human TNF promoter. J Inflamm 46:42–50
55. Terry CF, Loukaci V, Green FR (2000) Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. J Biol Chem 275:18138–18144
56. Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV (1997) An investigation of polymorphism in the interleukin-10 gene promoter. Eur J Immunogenet 24:1–8
57. Uglialoro AM, Turbay D, Pesavento PA, Delgado JC, McKenzie FE, Gribben JG, Hartl D, Yunis EJ, Goldfeld AE (1998) Identification of three new single nucleotide polymorphisms in the human tumor necrosis factor-alpha gene promoter. Tissue Antigens 52:359–367
58. Ward E, Jemal A, Cokkinides V, Singh GK, Cardinez C, Ghafoor A, Thun M (2004) Cancer disparities by race/ethnicity and socioeconomic status. CA Cancer J Clin 54:78–93
59. Wen AQ, Wang J, Feng K, Zhu PF, Wang ZG, Jiang JX (2006) Effects of haplotypes in the interleukin 1beta promoter on lipopolysaccharide-induced interleukin 1beta expression. Shock 26:25–30
60. Witkin SS, Gerber S, Ledger WJ (2002) Influence of interleukin-1 receptor antagonist gene polymorphism on disease. Clin Infect Dis 34:204–209
61. Wu MS, Wu CY, Chen CJ, Lin MT, Shun CT, Lin JT (2003) Interleukin-10 genotypes associate with the risk of gastric carcinoma in Taiwanese Chinese. Int J Cancer 104:617–623
62. Zabaleta J, Camargo MC, Piazuelo MB, Fontham E, Schneider BG, Sicinschi LA, Ferrante W, Balart L, Correa P, Ochoa AC (2006) Association of interleukin-1beta gene polymorphisms with precancerous gastric lesions in African-Americans and Caucasians. Am J Gastroenterol 101:163–171
63. Zeng ZR, Hu PJ, Hu S, Pang RP, Chen MH, Ng M, Sung JJ (2003) Association of interleukin 1B gene polymorphism and gastric cancers in high and low prevalence regions in China. Gut 52:1684–1689
64. Zhang WH, Wang XL, Zhou J, An LZ, Xie XD (2005) Association of interleukin-1B (IL-1B) gene polymorphisms with risk of gastric cancer in Chinese population. Cytokine 30:378–381