Null Results in Brief

Interleukin-8 Polymorphisms Are Not Associated with Gastric Cancer Risk in a Polish Population

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Introduction

Inflammation seems to play a critical role in the development of many types of cancer, including gastric, colorectal, and bladder cancers (1-6). The presence of chronic inflammation has been well described in gastric cancer, the fourth most common cancer diagnosis and second most common cause of cancer death worldwide (4, 7-9). Genetic variants of several pathways critical for the inflammatory response have been studied and a number of single nucleotide polymorphisms (SNPs) in several genes from different pathways have been associated with gastric cancer risk, including *IL1, IL1RN, IL1B, TNF, IL6, IL8*, and *IL10* (10-17).

Interleukin-8 (*IL8*), a potent chemokine, may play a role in gastric cancer pathogenesis. Gastric cancer specimens have increased IL-8 protein levels, and many gastric cancer cell lines express high levels of *IL8* mRNA and protein (16, 18, 19). SNPs in the *IL8* promoter (−251 A>T, rs4073) and a linked SNP in intron 1 (IVS +230 G>T, rs2227307) were associated with increased risk for gastric cardia adenocarcinoma in a high-risk Chinese population (14). The A allele of the *IL8* −251 A>T SNP (rs4073) was associated with increased risk for gastric ulcer and gastric cancer in *Helicobacter pylori*–infected Japanese individuals (13). Increased IL-8 protein production was seen in individuals with the *IL8* −251 A allele after lipopolysaccharide stimulation of whole blood (20). The A allele also seems to increase transcriptional activity *in vitro* in response to IL1-1β and tumor necrosis factor-α stimulation (13).

Central and Eastern European populations, including Poland, show a high incidence and mortality of stomach cancer among Caucasians (21). Because IL-8–mediated inflammation and *IL8* SNPs may play a role in gastric cancer etiology, we studied genetic variation in *IL8* in a population-based case-control study of gastric cancer in individuals from Warsaw, Poland (22).

Materials and Methods

Study Population. As previously described (22), cases consisted of residents of Warsaw, Poland, ages 21 to 79 years, newly diagnosed with gastric cancer between March 1994 and April 1996. All cases were gastric adenocarcinoma and pathologic slides were reviewed in a standardized fashion. Controls were frequency matched to cases by sex and 5-year age groups and randomly selected from a computerized registry of Warsaw residents. Written informed consent was obtained from all participants, and the Institutional Review Boards at the U.S. National Cancer Institute, Bethesda, MD and the Cancer Center and M. Sklodowska-Curie Institute of Oncology, Warsaw, Poland approved the study.

Genotype Assays. Genomic DNA from 288 cases and 430 controls was extracted from buffy coats by standard methods. Of the cases, 211 were from the distal stomach, 32 were from the cardia only, and 35 were distal and cardia, and location within the stomach was unknown in 10 cases. Four SNPs in *IL8* were genotyped, *IL8* −251 A>T (also annotated as *IL8* −351, rs4073), *IL8* IVS1 +230 G>T (rs2227307), *IL8* IVS1 −240 C>T (rs2227306), and *IL8* Ex1 −65 C>T (rs2227538), by either Taqman Assays (Applied Biosystems, Foster City, CA) or MGB Eclipse Assays (Epoch Biosciences, Bothell, WA) at the National Cancer Institute's Core Genotyping Facility. Details on assay design and conditions are available at http://snp500cancer.nci.nih.gov/ (23).

Statistical Analyses. Analysis of the case-control genotype data was conducted with contingency tables using additive, dominant (one or two copies of variant allele necessary for risk), and recessive (two copies of variant allele necessary for risk) genetic models in SAS v8.02 software. We also examined whether the association between the SNPs and cancer risk was modified by other risk factors, including age, smoking, and site of tumor origin. Haplotypes were constructed, and a case-control permutation test was done using PHASE v2.1 (24). HaploStats v1.1.1 (25) was used to construct haplotypes and determine the global score *P*, haplotype frequencies, and odds ratios. Hardy-Weinberg equilibrium was determined for all loci in controls by χ^2.

Results

Allele frequencies and *P* values using codominant, dominant, and recessive models are shown in Table 1. *IL8* Ex1 −65 C>T was monoallelic in this study population. *IL8* −251 A>T, *IL8* IVS1 +230 G>T, and *IL8* IVS1 −240 C>T were in Hardy-Weinberg equilibrium in controls. These three SNPs in *IL8* do not seem to be associated with risk for gastric cancer in this study of individuals from Warsaw, Poland. When only distal stomach cases were analyzed, there was no association between genotype and risk for disease. The low frequency of gastric cardia cases precluded individual analyses. Analyses for association based on age, smoking status, or *H. pylori*

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Table 1. Variation in *IL8* and gastric cancer risk

IL8 SNP	Genotype	Controls (%)	Cases (%)	Codominant χ^2 *P*	Dominant *P* value, OR (95% CI)	Recessive *P*, OR (95% CI)
−251 A>T rs4073	TT	106 (24.8)	71 (24.7)	0.964	0.993, 1.00 (0.71-1.42)	0.801, 0.96 (0.68-1.34)
	AT	205 (47.9)	140 (48.8)			
	AA	117 (27.3)	76 (26.5)			
IVS1 +230 G>T rs2227307	GG	102 (23.7)	74 (25.8)	0.576	0.314, 0.84 (0.60-1.18)	0.529, 0.90 (0.63-1.26)
	GT	207 (48.1)	142 (49.5)			
	TT	121 (28.1)	71 (24.7)			
IVS1 −240 C>T rs2227306	CC	133 (31.1)	80 (27.8)	0.580	0.344, 0.85 (0.61-1.19)	0.458, 0.87 (0.61-1.25)
	CT	204 (47.7)	140 (48.6)			
	TT	91 (21.3)	68 (23.6)			

NOTE: Cases were compared with controls using codominant, variant dominant, and variant recessive models. The number of cases and controls and *P*s are shown.
Abbreviations: OR, odds ratio, CI, confidence interval.

infection was not statistically significant. Haplotypes of *IL8* −251 A>T, *IL8* IVS1 +230 T>G, and *IL8* IVS1 −240 C>T constructed with PHASEv2.1 and HaploStats v1.1.1 did not show a statistically significant differences between controls and total cases, tumor site, or other risk factors.

Discussion

Because the *IL8* −251 A>T SNP seems to effect promoter function (13, 20), it is an important candidate for studies of genetic variation, inflammation, and disease risk. Genetic variation in the three *IL8* SNPs studied (*IL8* −251 A>T, rs4073; *IL8* IVS1 +230 T>G, rs2227307; and *IL8* IVS1 −240 C>T, rs2227306) does not seem to be a risk factor for gastric cancer in this study of individuals from Poland. This study of 288 cases and 430 controls is somewhat larger than previous studies of gastric cancer and *IL8* polymorphisms (13, 14). Ohyauchi et al. found an association between the A allele of the *IL8* −251 A>T SNP in 212 *H. pylori*–positive gastric cancer (majority noncardia) patients from Japan and two control groups (n = 244 and n = 102; ref. 13). Increased risk for gastric cardia cancer was seen in Chinese individuals (90 cases and 454 controls) who carried the A allele of the *IL8* −251 A>T SNP and the G allele of the linked *IL8* IVS1 +230T>G (14) but noncardia gastric cancer, and *H. pylori* were not evaluated. *IL8* −251 A>T SNP has also been extensively studied in relation to other cancers. Table 2 shows a summary of association studies that investigated the *IL8* −251 A>T SNP in a variety of cancers in different parts of the world. Although *IL8* −251 A>T has not been consistently associated with cancer risk, it may contribute to cancer risk in certain populations.

Due to the lack of consistent association of the *IL8* −251 A>T allele and cancer risk, it is important to take into consideration environmental factors and population characteristics. The allele frequencies of the *IL8* −251 A>T SNP differ between ethnic groups, perhaps in response to differential selective pressure from geographic infectious diseases. The dbSNP database (build 124, http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=4073) illustrates the differences in frequencies of the A and T alleles (Table 3). Differences between individuals of African descent (African/African American) and those of European descent (Caucasian) are most notable from the PGA-UW-FHCRC and SNP500Cancer panels. However, there also seem to be significant differences in the Kyugen data of between the CEPH parents (European descent) and those from East Asia, with an A allele frequency of 42% and 34%, respectively. In addition, there are other cytokine genes with significant genetic variation between different ethnic groups (30). It is possible that population-specific genetic variation in different genes in the same or related pathways could also contribute to cancer risk.

The lack of association of *IL8* SNPs in our study of gastric cancer risk in Poland may be due to differences in allele frequencies between ethnic groups, disease etiology, and/or sample size limitations. Like the other studies described (13, 14), our study of genetic variation in *IL8* in gastric cancer in Poland is somewhat limited by sample size. Significantly larger case-control studies of genetic variation in *IL8* and its role as a risk factor in inflammation and cancer are needed.

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Table 2. Summary of published studies of the *IL8* −251 A>T (rs4073) polymorphism and cancer risk

Study	Location	Study design	Disease type	No. cases	No. controls
Campa et al. (26)	Norwegian, Caucasians	Case-Control	Non–small cell lung cancer	250	214
Landi et al. (27)	Spain	Case-Control	Colorectal cancer	377	326
Leibovici et al. (5)	Texas, Caucasians	Case-Control	Bladder cancer	519	505
McCarron et al. (28)	London, Caucasians	Case-Control	Prostate cancer	247	263
Ohyauchi et al. (13)	Japan	Case-Control	*H. pylori*–positive gastric cancer	212	244
Savage et al. (14)	North-Central China	Cohort	Gastric cardia adenocarcinoma	90	454
Savage et al. (14)	North-Central China	Cohort	Esophageal squamous cell cancer	131	454
van der Kuyl et al. (29)	The Netherlands	Case-Control*	AIDS-related Kaposi's sarcoma	84	69

Abbreviations: NS, not significant; OR, odds ratio; 95% CI, 95% confidence interval.
*Study consisted of 153 HIV-infected men, 84 with AIDS-related Kaposi's sarcoma, and 69 with an AIDS-defining event other than Kaposi's sarcoma.

Table 3. Frequency of *IL8* −251 A>T (rs4073) SNP in different populations from dbSBP database, build 124 (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=4073)

Submitter	Population	TT (%)	TA (%)	AA (%)	T Allele frequency, %	A Allele frequency, %
PGA-UW-FHCRC	European, n = 22	10 (45.5)	9 (40.9)	3 (13.6)	65.9	34.1
	African, n = 24	1 (4.2)	9 (37.5)	14 (58.3)	22.9	77.1
SNP500CANCER	African/African American, n = 24	2 (8.3)	6 (25.0)	16 (66.7)	46.0	54.0
	Caucasian*, n = 31	13 (41.9)	9 (29.0)	9 (29.0)	56.5	43.5
	Hispanic, n = 23	6 (26.1)	12 (52.2)	5 (21.7)	52.2	47.8
	Pacific Rim, n = 23	8 (34.8)	8 (34.8)	7 (30.4)	52.2	47.8
KYUGEN †	CEPH parents, n = 78	NA	NA	NA	58.0	42.0
	East Asia, n = 100	NA	NA	NA	66.0	34.0

Abbreviation: NA, not available.
*Caucasian individuals from the SNP500CANCER population were not in Hardy-Weinberg Equilibrium.
†Allele frequency data only was available for the KYUGEN data.

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Table 2. Summary of published studies of the *IL8* −251 A>T (rs4073) polymorphism and cancer risk (Cont'd)

OR (95% CI)			
TT	TA	AA	TA+AA
Reference	0.83 (0.52-1.32) P = NS	0.91 (0.51-1.61) P = ns	
Reference		0.70 (0.50-0.99), P = 0.043	
Reference			1.23 (0.88-1.72), P = NS
0.66 (0.44-0.99), P = 0.04	NS	NS	
Reference	2.02 (1.37-2.97), P = 0.005	1.89 (0.81-4.40), P = 0.003	
Reference	1.11 (0.65-1.92), P = 0.048	1.96 (1.03-3.75), P = 0.048	
Reference	0.74 (0.47-1.18), P = 0.57	0.97 (0.54-1.75), P = 0.57	
0.49 (0.25-0.97), P = 0.038			Reference