Short Communication

Variants of the *IL8* and *IL8RB* Genes and Risk for Gastric Cardia Adenocarcinoma and Esophageal Squamous Cell Carcinoma

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Abstract

The population of Linxian in north central China is at high risk for gastric cardia adenocarcinoma (GCC) and esophageal squamous cell carcinoma (ESCC), and chronic inflammation may contribute to this risk. Interleukin-8 (*IL8*), a potent chemoattractant, has three well-characterized single nucleotide polymorphisms (SNP), one (−251) of which alters transcriptional activity. Four well-described SNPs in the two *IL8* receptors, *IL8RA* and *IL8RB*, have been associated with inflammation. We conducted a case-cohort study in the Nutrition Intervention Trials (Linxian, China) to assess the association between these SNPs and incident GCC (n = 90) and ESCC (n = 131). *IL8*, *IL8RA*, and *IL8RB* SNPs were analyzed using a multiplex assay system, haplotypes were constructed, and risks were estimated using Cox proportional hazards models. The homozygous variants of *IL8* −251 and +396 were associated with 2-fold increased relative risks for GCC, but the highest risk observed was for the AGT/AGC haplotype of *IL8* −251/+396/+781 (relative risk, 4.14; 95% confidence interval, 1.31-13.1). Variation within *IL8* was not associated with ESCC. Few subjects had variation at the *IL8RA* SNP and no significant associations were observed for *IL8RB* SNPs or haplotypes with either GCC or ESCC. We conclude that variation in *IL8* seems to increase the risk for GCC but not ESCC in this high-risk population. These variants could confer an altered *IL8* expression pattern or interact with environmental factors to increase the risk for inflammation and GCC. (Cancer Epidemiol Biomarkers Prev 2004;13(12):2251–7)

Introduction

Malignancies of the gastrointestinal tract are increasing worldwide, but the rates and sites vary according to geographic regions. Overall, gastric cancer is the fourth most common cancer diagnosis in the world and the second most common cause of cancer death (1, 2). The highest incidence of gastric cancer is found in Japan, South America, and eastern Europe in the range of 30 to 85 cases per 100,000 men and 15 to 40 cases per 100,000 women. Regions associated with low risk, including Israel, the United States, and Kuwait, have rates of 4 to 8 cases per 100,000 men and 2 to 4 cases per 100,000 women (3). Risk factors that contribute to the development of gastric cancer include diets high in salt and nitrates, cigarette smoking, *Helicobacter pylori* infection, and chronic inflammation (4-6). Gastric cardia adenocarcinoma (GCC) differs from gastric noncardia cancer (GNCC) in that it arises more proximally in the stomach and is associated with a younger age at presentation (1, 7, 8). Esophageal cancer incidence also varies significantly based on geographic location and worldwide is the fourth most common gastrointestinal malignancy and the sixth leading cause of cancer death (9). In developed countries, the main risk factors for esophageal squamous cell carcinoma (ESCC) are cigarette smoking and ethanol consumption, whereas in developing countries the risk factors are less well understood but likely include inadequate nutrition and excess carcinogen exposure.

The population of Linxian, a county in north central China, is at very high risk for both ESCC and GCC; the combined age standardized incidence rate there is >125 per 100,000 per year (10). Worldwide, GCC represents 5% to 10% of all gastric cancers, but it is the predominant form in Linxian (1). The cause of these extraordinarily high rates in Linxian is most likely multifactorial. Previous studies suggest that age, family history (5, 11, 12), low levels of antioxidants (13, 14), and tooth loss (15) are associated with higher risk of ESCC and GCC in this

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population. Tobacco and alcohol use, the leading risk factors for ESCC in Western countries, have only a minor role in this population (16).

Inflammation has been postulated to contribute to the development of cancer, including GNCC, and may also play an important role in GCC (17-19). The gastric cardia is often inflamed, although this association and GCC risk has not been as well studied (20). There is growing evidence that differences in inflammatory responses could be a consequence of genetic variation, leading to chronic inflammation and possibly malignant transformation (18, 19, 21, 22). Genetic variants of several pathways critical for the inflammatory response have been studied, and several genes from different pathways have been associated with either or both GNCC and esophageal cancer, including *IL1*, *IL1RN*, *IL1B*, *TNF*, *IL6*, and *IL10* (21, 23-27). The role of *IL8* and *IL8* receptor polymorphisms in GCC and ESCC has not been well studied.

Interleukin-8 (IL-8), a member of the CXC chemokine family, functions as a potent chemoattractant for neutrophils and lymphocytes (28, 29). Many gastric cancer cell lines express high levels of *IL8* mRNA and protein (30, 31). IL-8 protein levels in gastric cancer specimens were increased 10-fold when compared with normal gastric tissues (25). IL-8 interacts with two closely related receptors, encoded by *IL8RA* (CXCR-1) and *IL8RB* (CXCR-2). These receptors are 77% similar at the protein level, but the former is unique for IL-8, whereas the latter binds additional ligands. Gastric cancer cell lines also express both IL-8RA and IL-8RB protein (31). The *IL8* gene (4q13-q21) contains four exons, three introns, a proximal promoter region, and three well-characterized single nucleotide polymorphisms (SNP) and common haplotypes of the promoter and 5′ region [in the proximal promoter at −251 T/A (from start of transcription), intron 1 SNPs at +396 T/G and +781 C/T; refs. 32, 33]. Functional studies using lipopolysaccharide-stimulated IL-8 production in whole blood suggested a trend toward increased IL-8 production in individuals with the variant A allele at *IL8* −251 (32). Four SNPs have been identified in the *IL8* receptors (34, 35). There is a nonsynonymous *IL8RA* SNP in exon 2 at +2607 (serine to threonine), whereas the three SNPs in *IL8RB* do not predict a change in function (synonymous SNP in exon 3 of *IL8RB* and two in the 3′ untranslated area of exon 3 at +1208 and +1440).

We hypothesized that genetic variation in *IL8* and/or its receptors could contribute to the risk for either GCC or ESCC in the high-risk Linxian population. Using a stratified case-cohort design, we determined individual genotypes for *IL8*, *IL8RA*, and *IL8RB* SNPs, as well as informative haplotypes in *IL8* and *IL8RB*, and evaluated the relation of these genetic variants to risk for GCC and ESCC.

Materials and Methods

Trial Description. Between 1986 and 1991, the National Cancer Institute and the Cancer Institute of the Chinese Academy of Medical Sciences completed the Nutrition Intervention Trials in Linxian. The study design, methods, and results have been reported previously (36-38). Two randomized, double-blind, placebo-controlled trials were conducted. The smaller enrolled 3,318 adults ages 40 to 69 years who had esophageal squamous dysplasia on cytologic examination. These subjects were randomized to receive either multivitamin or placebo for 6 years. The larger of the two trials enrolled 29,584 adults ages 40 to 69 years from the general population and randomized them to receive one of four vitamin/mineral combinations or placebo for 5.25 years. All individuals in both trials continue to be followed as a cohort.

Participant Selection. At the end of the Linxian intervention trials in 1991, ~6,000 individuals who were alive and cancer free were selected for a blood sampling study. We were able to extract DNA with a yield of >1.5 μg from RBCs on 4,005 of these individuals (36). The subjects for this study were selected from this group, in accord with a stratified case-cohort design (39-41). We selected all subjects diagnosed with ESCC (n = 131) and GCC (n = 90) between May 1991 and May 1996 plus an age- and sex-stratified random sample of all eligible subjects without regard to case status (n = 454, including 421 noncases). The random sample serves as the reference group for risk estimates and hereafter is called the subcohort. The six strata were defined by sex and by three age categories (<50, 50-59, and ≥60 years). In each stratum, the control to site-specific case ratio was >2:1. *H. pylori* status was not available for the subjects in this study.

Variable Definition. Disease classifications were based on monthly end point surveillance (16, 37, 38) and by interview and examination of all living participants, or their next of kin, in May 1996 (>99% response rate). An international end point review committee of U.S. and Chinese experts reviewed all cancer diagnoses from the 1991 to 1996 period. Gastric cancers were defined as cardia cancers if they were in the proximal 3 cm of the stomach. These methods of assessment were identical to those used during the trial period.

Genotyping. Investigators blinded to all patient identifiers and information did the genotype analysis. Genomic DNA was amplified by PCR with MJ Research model PTC-225 thermal cyclers (Waltham, MA) under the following conditions: 5 ng of genomic DNA, 0.2 μmol/L of each primer, 200 μmol/L of each deoxynucleotide triphosphate, 2 mmol/L $MgCl_2$, 0.5 units AmpliTaq Gold DNA polymerase (ABI-Perkin-Elmer, Foster City, CA), and the manufacturer's buffer. Primers and annealing temperatures are listed in Table 1. The PCR reaction for the *IL8* SNPs included one PCR reaction with two products spanning the regions of interest. Amplicons for *IL8RA* and *IL8RB* were generated separately. After individual amplification of each SNP, samples were pooled and a 15-μL aliquot was incubated for 60 minutes at 37°C with shrimp alkaline phosphatase (5 units) and exonuclease (1 unit). Enzymes were inactivated by incubation at 75°C for 15 minutes.

A single base extension (SBE) technology using specific primers was done according to the manufacturer's directions (ABI Prism SNaPshot multiplex system from Applied Biosystems, Foster City, CA) with the following modifications: 2 μL of reaction mix, 3 μL of pooled PCR product, primers (0.15-0.6 μmol/L), and

Table 1. Primer sequences

Name (direction)	dbSNP identifier	Use	Primer sequence* (5′ → 3′)	Annealing temperature (°C)
IL8-1 (forward)	N/A	PCR	GGCTGGCTTATCTTCACCATC	58
IL8-1 (reverse)	N/A	PCR	GCCAACCTGAGTCATCACACTTCC	
IL8-2 (forward)	N/A	PCR	CACATCTTTCTGACCTACAGCG	58
IL8-2 (reverse)	N/A	PCR	AAGTTCTTTAGCCCTCCTTGGC	
IL8RA (forward)	N/A	PCR	TGACACAGCCAAATGGCGG	60
IL8RA (reverse)	N/A	PCR	ACCTTCCACACACAACCTCAGG	
IL8RB (forward)	N/A	PCR	GTCCTTTGGCTTCATCGTG	64
IL8RB (reverse)	N/A	PCR	TCACACCATTTTACAATCCCC	
IL8 −251 A/T (forward)	rs4073	SBE	gactgactgactgactTATCTAGAAATAAAAAAGCCTACA	N/A
IL8 +396 G/T (reverse)	rs2227307	SBE	ctgactgactTTACGTTAAATATATGCCTGCTAC	N/A
IL8 +781 C/T (reverse)	rs2227306	SBE	AAAACAGACATAACTGACAACATTGAAC	N/A
IL8RA +2607 G/C (reverse)	rs1805038	SBE	ctgactgactgactgactgactGACCCAGGTGATCCAGGAGA	N/A
IL8RB +785 C/T (forward)	rs3883989	SBE	actgactgactgactgactgactgactTGTCGTCCTCATCTTCCTGCT	N/A
IL8RB +1208 C/T (forward)	rs1801032	SBE	tgactgactgactgactgactgactgactgactCCCCATTGTGGTCACAGGAAG	N/A
IL8RB +1440 G/A (forward)	rs1126580	SBE	CAGGCTGGCCAACGGG	N/A

NOTE: Abbreviation: N/A, not applicable.
*Lowercase bases indicate nonannealing sequences.

water to make a 10-μL reaction. Samples were amplified using 25 cycles of 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 30 seconds. After the initial SNaPshot reactions were generated, they were treated for 60 minutes at 37°C with 5 units of shrimp alkaline phosphatase followed by a 15-minute, 75°C enzyme inactivation step. A 0.5-μL amount of sample was mixed with 9 μL of high dye formamide and 0.5 μL of size standard and then denatured. Samples were run on the ABI Prism 3100 genetic analyzer according to the manufacturer's instructions. Analysis was done using ABI Prism GeneScan version 3.7 and Genotyper software version 3.7. Any uncertain genotypes were duplicated and verified manually. Ten percent to 20% of samples in the analysis plates were included as blind duplicates and verified.

Statistical Analysis. Pearson correlation coefficients, Hardy-Weinberg equilibrium tests, and D and D' calculations were determined in the randomly selected subcohort. Throughout the article, all P values reported are two sided.

Relative risks (RR) and 95% confidence intervals (95% CI) were estimated using the case-cohort estimator for the Cox proportional hazards models (39-42). Risk estimates were determined using subjects homozygous for the most prevalent genotype as the reference group. All estimates came from models stratified on the six sex-age sampling strata. Additional stratum-specific age terms for continuous age were used to adjust for variation within age strata. All models were also adjusted for smoking (ever versus never), drinking (any ethanol in the previous 12 months), and trial. We also tested whether a history of cancer in first-degree relatives confounded these estimates and found that it did not. Intervention group assignment in the underlying trials could not confound the association between genotype and cancer because the treatment group assignments were allocated randomly. Although effect modification by treatment group assignment is a possibility, we had insufficient power to test this hypothesis. Nested models were compared using score tests. We tested the proportional hazards assumption for each main effect (genotype) using a time-dependent covariate (Genotype × Follow-up time). This test was nonsignificant ($P > 0.05$) in all cases.

Haplotypes were constructed using PHASE (version 1.0) software (43). All *IL8* and *IL8RB* haplotypes were either known (homozygous at each site) or estimated by PHASE. Only individuals with information for each genotype were included in the haplotype analysis. The two inferred haplotypes were cross-tabulated and haplotype categories were created for each gene. RR and 95% CI were estimated as above using indicator variables for each haplotype. Persons carrying two copies of the most frequent haplotype were chosen *a priori* as the reference group.

Results

Summary of Case-Cohort Characteristics and Genotypes. Case-cohort subject characteristics are shown in Table 2. All three groups were similar with regard to age, sex, smoking, and alcohol consumption. Genotype frequencies for all SNPs studied in the three subject groups are shown in Table 3. The *IL8RA* SNP at +2607 was infrequent in this population, with an allele frequency of ~1%. There were no significant associations seen between age, sex, smoking, or alcohol consumption and the *IL8*, *IL8RA*, or *IL8RB* SNPs. Six of the seven SNPs were in Hardy-Weinberg equilibrium within the population subcohort; the exception was the *IL8RB* +1208 SNP.

Table 2. Linxian Nutrition Intervention Trials case-cohort subject characteristics

	Subcohort	ESCC	GCC
n	454	131	90
Age, median (y)	58.8	57.4	60.4
Male (%)	55.5	50.4	41.1
Smoking (%)	39.3	36.6	47.8
Drinking (%)	26.4	22.1	23.3
General Population Trial (%)*	63.7	41.2	46.7
Follow-up time, median (y)	4.6	3.2	3.0

*The Nutrition Intervention Trial was composed of two trials, the General Population Trial and the Dysplasia Trial (see Materials and Methods).

***IL8* Polymorphisms.** RRs were determined for individuals with GCC and ESCC using the most frequent genotype as the reference. This analysis suggested that individuals homozygous for the variant allele *IL8* −251AA were at a 2-fold increased risk (RR, 1.96; 95% CI, 1.03-3.75) for GCC when compared with the individuals who were TT (Table 4A). Individuals with the variant genotype of *IL8* +396 GG were also at a 2-fold increased risk (RR, 2.06; 95% CI, 1.09-3.89) for GCC. The variant for *IL8* +781 had a similar RR for GCC, but this association was not statistically significant. The *IL8* genotypes were not significantly associated with ESCC in this population.

The highest risk suggested in this study was for the *IL8* −251/+396/+781 AGT/AGC haplotype, which conferred a RR (95% CI) for GCC of 4.14 (1.31-13.1; Table 4B). Individuals homozygous for all three variant alleles in *IL8* (AGT/AGT) had a RR (95% CI) of 1.77 (0.84-3.72). These two comparisons taken together suggest that the *IL8* −251/+396 AG haplotype may be particularly important. Analysis of haplotypes using just *IL8* −251/+396 showed a RR (95% CI) of 2.12 (1.11-4.04; $P = 0.019$) for the AG/AG haplotype, whereas the TT/AT haplotype had a similar result (RR, 2.15; 95% CI, 0.71-6.48; $P = 0.019$) but was too infrequent (3.4%) to be certain (Table 4C).

Analysis of the *IL8* SNPs in the ESCC cases did not show increased risk for the development of cancer. Neither individual genotypes nor *IL8* haplotypes constructed with *IL8* −251/+396/+781 or with *IL8* −251/+396 were associated with ESCC risk (Table 4).

The *IL8RA* Gene. Analysis of the *IL8RA* +2607 G/C SNP in exon 2 (serine-to-threonine change) showed a variant allele frequency of only 0.01 in this Chinese population, precluding risk estimation. This SNP showed an allele frequency of 0.10 in 74 Centre d'Etude du Polymorphisme Humain African Americans, whereas 91 Caucasian counterparts showed an allele frequency of only 0.01. In SNP500 samples (http://snp500cancer.nci.nih.gov), the following variant allele frequencies were observed in each ethnic group: African Americans, 0.0; Caucasians, 0.0; Hispanic, 0.22; and Pacific Rim, 0.0, confirming that this is a rare SNP in individuals of Chinese ancestry.

The *IL8RB* Gene. Analysis of the *IL8RB* +785 T/C SNP showed that only 0.5% of this population was homozygous for the variant C allele (Table 3). RRs were calculated for each *IL8RB* SNP using the most common genotype as the reference group, but no associations were observed for either GCC or ESCC (Table 5A).

Haplotype analysis was done using the *IL8RB* +785 and +1440 SNPs only because the *IL8RB* +1208 C/T SNP was not in Hardy-Weinberg equilibrium in this population. No associations were found for these haplotypes (Table 5B).

Discussion

The population in the Linxian region of north central China is at especially high risk for the development of GCC and ESCC compared with most other regions of the world. Environmental factors have been shown to contribute to the development of these cancers in this population (13, 15). The role of chronic inflammation in the development of GNCC has been well shown (17); it is present but has not been as well studied in GCC (20). It is possible that a slight alteration in response to an inflammatory stimulus due to genetic variation in the context of additional environmental risk factors in the Linxian population could be enough to increase risk for developing cancer. In this context, we chose to investigate IL8, an important chemoattractant, which contributes to the inflammatory response complementary to the IL-1 and tumor necrosis factor pathways. Prior studies have implicated IL8 in GNCC, and inflammation most likely plays a role in both GNCC and GCC (25, 30, 31, 43). It has also been suggested that the −251 T/A variant of *IL8* alters expression of the gene (32, 33). For these reasons, this study was conducted to determine if variation within the genes of the *IL8* pathway might confer additional cancer risk in a population at high risk.

Table 3. *IL8* and *IL8RB* genotype frequencies among study participants

	Subcohort*			GCC			ESCC		
	WT	Ht	Hv	WT	Ht	Hv	WT	Ht	Hv
IL8 −251 T/T T/A A/A	147 (34.3)	207 (48.3)	75 (17.5)	26 (29.6)	39 (44.3)	23 (26.1)	48 (37.2)	55 (42.6)	26 (20.2)
IL8 +396 T/T G/T G/G	152 (37.8)	181 (45.0)	69 (17.2)	29 (33.7)	33 (38.4)	24 (27.9)	49 (39.5)	50 (40.3)	25 (20.2)
IL8 +781 C/C C/T T/T	167 (41.1)	177 (43.6)	62 (15.3)	28 (32.9)	41 (48.2)	16 (18.8)	53 (42.1)	51 (40.5)	22 (17.5)
IL8RB +785 T/T T/C C/C	346 (82.6)	71 (17.0)	2 (0.5)	71 (79.8)	17 (19.1)	1 (1.1)	108 (83.7)	21 (16.3)	0 (0.0)
IL8RB +1208 C/C C/T T/T	202 (48.7)	154 (37.1)	59 (14.2)	56 (62.9)	22 (24.7)	11 (12.4)	61 (48.4)	45 (35.7)	20 (15.9)
IL8RB +1440 G/G G/A A/A	241 (57.8)	149 (35.7)	27 (6.5)	45 (51.7)	36 (41.4)	6 (6.9)	77 (61.6)	39 (31.2)	9 (7.2)

NOTE: Genotype frequencies are presented as *n* (%). Abbreviations: WT, wild-type (the most frequent allele in our population); Ht, heterozygous; Hv, homozygous variant.

*The subcohort was tested by χ^2 to determine if the population meets the assumption of Hardy-Weinberg equilibrium for each: *IL8* −251, $P = 0.89$; *IL8* +396, $P = 0.23$; *IL8* +781, $P = 0.19$; *IL8RB* +785, $P = 0.42$; *IL8RB* +1208, $P = 0.001$; *IL8RB* +1440, $P = 0.54$.

Table 4. Variation within the *IL8* gene and risk for GCA and ESCC

A

Genotype	GCC			ESCC		
	RR (95% CI)*	$P_{(2\ df)}$†	$P_{(1\ df)}$‡	RR (95% CI)	$P_{(2\ df)}$†	$P_{(1\ df)}$‡
IL8 −251						
T/T	1.00 (reference)	0.068	0.048	1.00 (reference)	0.36	0.57
A/T	1.11 (0.65-1.92)			0.74 (0.47-1.18)		
A/A	1.96 (1.03-3.75)			0.97 (0.54-1.75)		
IL8 +396						
T/T	1.00 (reference)	0.022	0.036	1.00 (reference)	0.30	0.28
G/T	0.99 (0.57-1.72)			0.76 (0.47-1.21)		
G/G	2.06 (1.09-3.89)			0.95 (0.52-1.73)		
IL8 +781						
C/C	1.00 (reference)	0.17	0.059	1.00 (reference)	0.57	0.45
C/T	1.43 (0.84-2.43)			0.85 (0.54-1.34)		
T/T	1.76 (0.88-3.54)			1.02 (0.55-1.87)		

B

−251/+396/+781 Haplotype§	Subcohort frequency (%)	GCC		ESCC	
		RR (95% CI)	$P_{(5\ df)}$†	RR (95% CI)	$P_{(5\ df)}$†
TTC/TTC	135 (35.3)	1.00 (reference)	0.034	1.00 (reference)	0.61
TTC/AGT	155 (40.5)	1.25 (0.70-2.26)		0.83 (0.50-1.37)	
AGT/AGT	57 (14.9)	1.77 (0.84-3.72)		1.02 (0.53-1.94)	
TTC/AGC	18 (4.7)	0.29 (0.04-2.30)		0.43 (0.12-1.63)	
AGT/AGC	9 (2.4)	4.14 (1.31-13.1)		0.67 (0.14-3.25)	
TTC/ATT	9 (2.4)	1.94 (0.48-7.78)		0.70 (0.14-3.43)	

C

−251/+396 Haplotype§	Subcohort frequency (%)	RR (95% CI)	$P_{(3\ df)}$†	RR (95% CI)	$P_{(3\ df)}$†
TT/TT	143 (31.9)	1.00 (reference)	0.019	1.00 (reference)	0.14
TT/AG	217 (48.4)	0.96 (0.55-1.67)		0.65 (0.41-1.04)	
AG/AG	73 (16.3)	2.12 (1.11-4.04)		1.02 (0.56-1.84)	
TT/AT	15 (3.4)	2.15 (0.71-6.48)		1.43 (0.50-4.07)	

*RRs and 95% CIs were generated from models stratified on age and sex with additional adjustment from continuous age variables for each strata and variables for smoking, drinking, and trial.

†This *P* value comes from the score test for the addition of all the genotype/haplotype indicator variables to the base model simultaneously. It assesses the overall association between genotype/haplotype and cancer. 2, 3, or 5 *df* denote a *P* global.

‡This *P* value comes from the score test for the addition on a single variable for genotype, where Wt = 0, Hz = 1, and Hv = 2. 1 *df* denotes a *P* trend.

§Two haplotypes were inferred for each subject using PHASE software (see Materials and Methods). Haplotypes were constructed only if genotype data were available at all loci for each individual. Individuals with two copies of the most frequent haplotype were set as the reference group and the RRs and 95% CIs associated with other diploid states were estimated using the same models as described in footnote *.

This study suggests that genetic variation within the inflammatory chemokine *IL8* gene is associated with the risk for GCC in the Linxian population. The less common genotypes of *IL8* (−251A, +396G, and +781T) were associated with a 2-fold increased risk for GCC, whereas the risk for the AGT/AGC haplotype of *IL8* −251/+396/+781 was 4-fold. This haplotype occurred in only 9% of our population but may still be of importance and should be investigated in a larger study. The RR for the AGT/AGT haplotype was 1.8 and further analysis of the two SNPs (*IL8* −251/+396) within the haplotype suggest an effect for the *IL8* −251/+396 AG/AG haplotype, although it is possible that the *IL8* +781 T/C SNP in the haplotypes could influence risk. Moreover, specific haplotypes of *IL8* −251/+396 SNPs were more strongly associated with GCC, illustrating the importance of coinheritance of the risk alleles.

The *IL8* receptor genes, *IL8RA* and *IL8RB*, are critical for *IL8* ligand binding and subsequent signaling. In this study, we observed that genetic variation at the *IL8RA* +2607 G/C SNP was rare in this population. The observation that the *IL8RB* +1208 SNP is not in Hardy-Weinberg equilibrium raises interesting questions about the recent evolutionary history of this variant, but conclusions are beyond the scope of this study. Variation within the *IL8RB* gene at the three SNPs studied was not associated with GCC or ESCC in this study, suggesting that variation within the *IL8* inflammatory molecule itself may be more important than variation within its receptor, a possibility that merits further investigation. This study suggests that variants in the *IL8* gene may play an important role in the development of GCC in the Linxian population but not in the development of ESCC. This may reflect substantive differences in the etiologies

Table 5. Variation within the *IL8RB* gene and risk for GCC and ESCC

A

Genotype	GCC			ESCC		
	RR (95% CI)*	$P_{(2\ df)}$†	$P_{(1\ df)}$‡	RR (95% CI)	$P_{(2\ df)}$†	$P_{(1\ df)}$‡
IL8RB +785						
T/T	1.00 (reference)	0.58	0.43	1.00 (reference)	0.44	0.23
T/C	1.11 (0.61-2.01)			0.81 (0.46-1.43)		
C/C	2.61 (0.21-32.21)			~0		
IL8RB +1208						
C/C	1.00 (reference)	0.035	0.041	1.00 (reference)	0.60	0.37
C/T	0.51 (0.30-0.87)			0.87 (0.55-1.37)		
T/T	0.65 (0.32-1.34)			0.94 (0.51-1.75)		
IL8RB +1440						
G/G	1.00 (reference)	0.40	0.22	1.00 (reference)	0.34	0.47
G/A	1.35 (0.83-2.20)			0.90 (0.57-1.41)		
A/A	1.28 (0.49-3.33)			1.56 (0.67-3.61)		

B

+785/+1440 Haplotype	Subcohort frequency (%)	GCC		ESCC	
		RR (95% CI)	$P_{(3\ df)}$†	RR (95% CI)	$P_{(3\ df)}$†
TG/TG	211 (47.3)	1.00 (reference)	0.22	1.00 (reference)	0.61
TG/TA	143 (32.1)	1.70 (1.00-2.89)		1.03 (0.64-1.64)	
TG/CG	65 (14.6)	1.60 (0.82-3.14)		1.01 (0.55-1.84)	
TA/TA	27 (6.1)	1.59 (0.59-4.26)		1.71 (0.73-4.01)	

*RRs and 95% CIs were generated from models stratified on age and sex with additional adjustment from continuous age variables for each strata and variables for smoking, drinking, and trial.
†This *P* value comes from the score test for the addition of all the genotype/haplotype indicator variables to the base model simultaneously. It assesses the overall association between genotype/haplotype and cancer. 2, 3, or 5 *df* denote a *P* global.
‡This *P* value comes from the score test for the addition on a single variable for genotype where Wt = 0, Hz = 1, and Hv = 2. 1 *df* denotes a *P* trend.
§Two haplotypes were inferred for each subject using PHASE software (see Materials and Methods). Haplotypes were constructed only if genotype data were available at all loci for each individual. Individuals with two copies of the most frequent haplotype were set as the reference group and the RR and 95% CI associated with other diploid states were estimated using the same models as described in footnote *.

of the two malignancies under study. Typically, ESCC is not associated with inflammation, whereas the gastric cardia is often inflamed (20).

Although this study was relatively small (n = 90 GCC, n = 131 ESCC, and n = 454 subcohort), it is one of the largest studies analyzing GCC, ESCC, non–case-controls, and inflammatory cytokines to date. As with any preliminary study, our findings may represent false-positive results (44) and additional studies in this and other appropriate populations are warranted to confirm these findings. This study provides intriguing results that suggest the role of variation in an important inflammatory pathway, *IL8*, in a high-risk population. The lack of association of ESCC risk with *IL8* variants further suggests that the *IL8* association with GCC may be specific for that cancer. Participants in the Nutrition Intervention Trials continue to be followed up, and as additional cases of GCC and ESCC are collected, further analysis with larger numbers of cases will be possible.

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