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Polymorphisms in chemokine and receptor genes and gastric cancer risk and survival in a high risk Polish population

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Abstract

Objective—To examine if genetic variations in chemokine receptor and ligand genes are associated with gastric cancer risk and survival.

Methods—The study included 298 cases and 417 controls from a population-based study of gastric cancer conducted in Warsaw, Poland in 1994–1996. We investigated seven single nucleotide polymorphisms in a chemokine ligand (*CXCL12*) and chemokine receptor (*CCR2*, *CCR5*, *CX3CR1*) genes and one frameshift deletion (*CCR5*) in blood leukocyte DNA in relation to gastric cancer risk and survival. Genotyping was conducted at the NCI Core Genotyping Facility. Odds ratios and 95% confidence intervals were computed using univariate and multivariate logistic regression models. Survival analysis was performed using Cox proportional hazards models.

Results—Gastric cancer risk was not associated with single chemokine polymorphisms. A *CCR5* haplotype that contained the common alleles of IVS1+151 G>T (rs2734648), IVS2+80 C>T (rs1800024) and minor allele of IVS1+246 A>G (rs1799987) was associated with a borderline significantly increased risk (OR = 1.5, 95% CI: 1.0–2.2). For gastric cancer cases, there was a greater risk of death for carriers of the minor alleles of *CCR2* Ex2+241 G>A (rs1799864) (HR = 1.5, 95% CI: 1.1–2.1) and *CCR5* IVS2+80 C>T (rs1800024) (HR = 1.5, 95% CI: 1.1–2.1). Carriers of the *CCR5* minor allele of IVS1+151 G>T (rs2734648) had a decreased risk of death compared to homozygote carriers of the common allele (HR = 0.8, 95% CI: 0.6–1.0).

Conclusions—Our findings do not support an association between gastric cancer risk and single chemokine genetic variation. The observed associations between cancer risk and a CCR5 haplotype and between survival and polymorphisms in *CCR2* and *CCR5* need replication in future studies.

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Keywords

Chemokine; gastric cancer; single nucleotide polymorphisms; survival

Introduction

Gastric cancer is the fourth most common cancer and second most common cause of cancer-related death worldwide [1]. Although there have been dramatic decreases in incidence and mortality in some parts of the world, there is significant geographic variation and incidence rates that remain high in Eastern Europe, Eastern Asia, and Central and South America. Established epidemiologic risk factors include age, male sex, smoking, family history of stomach cancer, and *Helicobacter pylori* infection.

Due to the established role of *H. pylori* infection and inflammation in gastric cancer etiology [2–5], there have been extensive studies on genetic polymorphisms in inflammation-related genes [6–14]. The primary focus of this work has been on cytokines and their regulatory pathways. Chemokines are a family of small cytokine-like proteins that are critical for immune homeostasis and migration of leukocytes [15]. Chemokines and their receptors are classified into four groups based on the N-terminal cysteine structure – CXC, CC, CX3C, and C chemokines [16]. To date, there have been few studies on the genetic variation in chemokine ligand and receptor genes and risk of gastric cancer.

In the chronic phase of *H. pylori* infection, lymphocyte recruitment and extravasation is established via chemokine mediated expression of vascular adhesion molecules (VCAM-1 and ICAM-1) [17]. It has also been shown *in vitro* that γδT cells activated by *H. pylori* urease, IL-7, and IL-1β upregulate CCR2 mRNA in gut mucosa [18]. Chemokine receptors and ligands are also thought to play a role in cancer cell migration, immune evasion, and metastasis formation [15]. Tumor cells both secrete and respond to chemokines. Chemokines can bind to G-protein coupled receptors resulting in directional migration or chemotaxis of tumor cells, potentially contributing to distant metastasis [16,19–22].

We evaluated the risk and survival of gastric cancer associated with seven single nucleotide polymorphisms (SNPs) in chemokine ligand (*CXCL12*) and receptor (*CCR5*, *CCR2*, *CX3CR1*) genes and a 32bp frameshift deletion in *CCR5* in a case–control study of gastric cancer in Warsaw, Poland, a region with one of the highest incidences of gastric cancer worldwide [6,7,9,12,23–25]. These chemokine ligand and receptor genes were chosen based upon prior literature showing a role in the development and progression of cancer, including gastric cancer [16,20,26–28]. Prior research has evaluated expression levels *in vitro* of CCR5, CX3CR1, and CXCL12 in gastric cancer and intestinal epithelium, suggesting a variety of roles in the neoplastic process [20,27–29].

Methods

Study population

Cases and controls were from a population-based study of gastric cancer conducted in Warsaw, Poland, in 1994–1996, as previously described [23]. Cases were Caucasian residents of Warsaw, Poland, aged 21–79 years, who were newly diagnosed with gastric cancer (ICD-9 151) during 1994–1996 and identified by physicians in 22 hospitals in the study area. All cases were confirmed by study pathologists. Controls were randomly selected from a computerized registry of Warsaw residents and frequency-matched to cases by sex and 5-year age groups. The study was approved by the Institutional Review Boards of the U.S. National Cancer Institute and the M. Sklodowska-Curie Memorial Cancer Center

and Institute of Oncology, Warsaw, Poland. Information on adjuvant treatment was collected by asking if the patients had undergone radiation or chemotherapy before the blood draw. Written informed consent was obtained from all participants. Survival information was obtained from death certificates obtained from the Polish Death Certificate Register Office and the Warsaw Cancer Registry. The Warsaw Cancer Registry is updated annually for all registerable cancer cases; the cases in this study were queried in the Polish Death Certificate Register Office for registered date of death.

Detailed personal or proxy interviews were conducted to collect information on tobacco use, alcohol consumption, family history, demographics, medication use, occupational history and diet. Of 464 cases and 480 controls that were recruited to the study, a 30 mL blood sample was obtained from 345 (74.4%) cases and 442 (92.1%) controls. Due to inadequate quality or quantity of DNA, genotyping results for this study were available from peripheral blood leukocyte DNA of 298 (64.2%) cases and 417 (86.9%) controls. Serum levels of IgG antibodies to *H. pylori* and to the cagA protein were determined by antigen-specific ELISA as described previously [30].

Genotyping assays

The SNPs and one frameshift deletion that we examined included *CCR2* [Ex2+241 G>A (rs1799864)], *CCR5* [δ32 (rs333), IVS1+151 G>T (rs2734648), IVS1+246 A>G (rs1799987), IVS2 +80 C>T (rs1800024)], *CXCL12* [Ex5+535 G>A (rs1801157)], and *CX3CR1* [Ex2+754 G>A (rs3732379), Ex2+848 C>T (rs3732378)]. The eight SNPs were selected on the basis of evidence reported in the literature regarding the function and disease association of each SNP and the characteristics of the polymorphisms (non-synonymous and promoter SNPs) [31–34]. Synonymous SNPs in coding and non-coding regions were also selected to improve the coverage of the candidate genes. In addition, we only included SNPs with minor allele frequencies >5% in Caucasians using the publicly available SNP500Cancer database generated by the National Cancer Institute [35] in order to have sufficient statistical power for data analysis. Genotyping was conducted at the NCI Core Genotyping Facility using the TaqMan™ (Applied Biosystem, Inc., Foster City, CA) platform (sequence data and assay conditions can be found at http://snp500cancer.nci.nih.gov). Internal lab quality controls were run for each genotype and included four human DNA controls (Coriell DNA) but no template controls. Approximately 8% blinded quality control samples from two individuals were interspersed with study samples, with greater than 99% concordance.

Statistical analysis

All loci among controls were examined for Hardy–Weinberg equilibrium using the Pearson's chi-square test. Differences of distribution between cases and controls were also assessed using the chi-square test for categorical variables previously found to be associated with gastric cancer in this population (age group, sex, education, and smoking status). Odds ratios (ORs) and 95% confidence intervals (CIs) for the SNPs and frameshift deletion were computed using univariate and multivariate logistic regression models adjusting for age, sex, education, and smoking status. Due to a limited number of cases, odds ratios for the genotypes were calculated comparing the minor allele (homozygous or heterozygous) to the homozygous common allele. Measures of pairwise linkage disequilibrium (D′ and r^2) were estimated using Haploview version 4.2 for haplotype analysis [36]. Haplotype frequencies, odds ratios (ORs), and 95% CIs for haplotypes were estimated using Haplo-Stats version 1.4.4 in *R* [37,38]. Overall survival of gastric cancer cases with chemokine genotyping data were estimated by Kaplan–Meier analysis from date of diagnosis to death. In addition, the proportional hazards assumption was assessed using stratified Kaplan–Meier curves. Cases without a date of death were censored with the date of last contact or 180 months (end of

follow-up). Univariate and multivariate Cox proportional hazard models adjusting for age group, sex, education, smoking status, and pathologic stage were also performed. Statistical analyses were performed using SAS statistical software version 9.2 (SAS Institute, Cary, NC) unless otherwise specified.

Results

As described in prior publications, the cases and controls did not differ with respect to age group or sex, and cases were less educated, more likely to be a current smoker, drink more alcohol, and have a family history of stomach cancer (Table I) [6,7,9,12,23–25]. Lan et al. has also previously shown that there are no statistically significant differences between subjects with or without blood samples, minimizing the effect of selection bias in genotyping results [39]. Among cases, the majority of cancers were classified as intestinal and localized to the distal stomach. The majority of cases had regional and displaced metastasis (39.1% and 35.2%, respectively) compared to localized disease (25.7%), although data were only available for 230 of 298 cases. Similarly, the majority of cases with grade data had moderately (50.0%) or poorly differentiated (40.4%) tumors.

All SNPs analyzed among control subjects were in Hardy–Weinberg equilibrium (HWE) (*p*-values > 0.05). As shown in Table II, we did not observe significant associations of gastric cancer risk with SNPs or the single frameshift deletion investigated in chemokine ligand and receptor genes. A *CCR5* haplotype that contained the common alleles of IVS1+151 G>T (rs2734648), IVS2+80 C>T (rs1800024) and minor allele of IVS1+246 A>G (rs1799987) was associated with a marginal increased risk of gastric cancer (OR = 1.5, 95% CI: 1.0–2.2). Further adjustment for other potential confounding variables, including family history of gastric cancer, alcohol consumption, and *H. pylori* infection status did not affect the results. Stratification by Lauren classification and stage of disease also did not affect the results for all SNPs and the frameshift deletion in *CCR5* (δ32).

Table III shows the hazard ratios for death among gastric cancer cases. Overall survival among gastric cancer cases in our study was 19.2% at 60 months. There was a significantly greater risk of death for carriers of the minor *CCR2* allele Ex2+241 G>A (rs1799864) (HR = 1.5, 95% CI: 1.1–2.1) and the minor *CCR5* allele IVS2+80 C>T (rs1800024) (HR = 1.5, 95% CI: 1.1–2.1). Conversely, greater survival was observed among carriers of the minor *CCR5* allele IVS1+151 G>T (rs2734648) compared to homozygotes of the common allele (HR = 0.8, 95% CI: 0.6–1.0).

Pathological stage was significantly associated with survival (displaced metastasis HR = 5.0, 95% CI: 3.5–7.1; regional metastasis HR = 2.0, 95% CI: 1.4–2.8 compared to a reference of localized disease). However, there was no interaction between stage and any SNPs or the frameshift deletion examined in relation to survival.

Discussion

The primary aim of this study was to evaluate the association of selected chemokine polymorphisms and the risks for gastric cancer and survival of gastric cancer patients. Chemokine ligands and their receptors help control the migration and recruitment of immune effector cells and are thought to play a role in cancer progression, immune evasion, and metastasis [16,19]. Chemokine and chemokine receptor polymorphisms have been shown to be associated with a number of infection or inflammation-related disease states, including coronary artery disease, HIV, and hepatocellular carcinoma [40–43]. However, very little has been published on their role in gastric cancer. Previous work has shown an association between a functional SNP in *CCL22* and *H. pylori* infection related gastric

cancer risk [44]. Liou et al. found an association between a polymorphism in *RANTES* (*CCL5*), the ligand for CCR5, and a reduced risk of gastric cancer in women in a hospital-based case–control study, but no association with *CCR2* V64I genotypes [45].

To date, our study is the largest to evaluate chemokine receptor polymorphisms and risk of gastric cancer. Overall, we found no significant associations with the individual chemokine and chemokine receptor SNPs and frameshift deletion in *CCR5*. However, a haplotype of *CCR5* that contained the common alleles of IVS1+151 G>T (rs2734648), IVS2+80 C>T (rs1800024) and minor allele of IVS1+246 A>G (rs1799987) was modestly associated with increased gastric cancer risk. Strong linkage disequilibrium ($r^2 > 0.9$) was noted among these three SNPs suggesting they may function together to influence the gene function or expression of *CCR5* gene controls. Interestingly, the minor alleles of *CCR5*, IVS1+151 G>T (rs2734648) and IVS2+80 C>T (rs1800024), were associated with increased and decreased survival, respectively. CCR5 and its ligands regulate functions of memory Th1 cells, macrophages, NK cells, and dendritic cells [31]. There is evidence that increased CCR5 expression in gastric T cells infected with *H. pylori* play a role in modulating the gastric immune response [46]. Increased expression of CCR5 in gastric cancer tumor cells has also been associated with poorer prognosis [27]. Our results, if confirmed, allude to the complexity of CCR5 function, and the potential role it may play in gastric cancer development and subsequent risk of death.

Survival analysis among cases also suggested that minor allele carriers of *CCR2* Ex2+241 G>A (rs1799864) might have poorer survival than homozygote carriers of the common allele. Our results differ from one other study which found no association with this polymorphism and survival in gastric cancer, although the number of cases in that study was much smaller [45]. CCR2 is the receptor for CCL2, also known as monocyte chemoattractant protein-1 (MCP-1), and multiple other ligands. The CCR2/CCL2 (MCP-1) axis attracts and activates monocytes, memory T cells, and natural killer (NK) cells [47]. CCR2 presents two isoforms, A and B, and the valine to isoleucine substitution promotes the stability of isoform A which requires a higher concentration of ligand to promote chemotaxis and migration [48,49]. The mutated allele has been shown to have a protective effect in sporadic breast cancer in a case–control study [34]. Coelho et al. has reported an association of increased risk for the progression to high grade squamous intraepithelial lesions in cervical tissue, but a protective effect for this allele in the progression of high grade dysplasia to invasive cervical carcinoma [32,33]. They postulated that decreased CCL2/MCP-1 expression and lower affinity for the stabilized CCR2-A isoform may lessen the infiltration of tumor associated macrophages (TAMs) and their pro-carcinogenic properties [32,33]. However, in advanced gastric cancer, death is frequently due to peritoneal carcinomatosis from malignant ascites which is without effective curative treatment [19]. The pathophysiology of ascites accumulation and subsequent peritoneal metastases is similar in ovarian cancer, in which CCL2/MCP-1 has been shown to be detectable in significant levels in malignant ascites [50,51]. MCP-1 has also been shown to be highly expressed in gastric cancer and associated with tumor vascularity [52]. Although we cannot exclude the possibility of chance contributing to our findings due to the small number of cases, our results could be explained by a mechanism where a stabilized isoform of CCR2 in ascitic fluid would more effectively promote chemotaxis, migration, and subsequent continued promotion of tumorigenesis through recruitment of TAMs.

Strengths of this study include a previously well defined high risk population, high participation rates of cases and controls, and good reproducibility of the genotyping results. This is the largest study to date of chemokine and chemokine receptor polymorphisms and gastric cancer risk and survival. The number of SNPs as well as genes analyzed may not provide a comprehensive coverage of genes examined and a complete picture of chemokine

pathways due to the large family of known chemokines. Also, due to inadequate quality or quantity of DNA, genotyping data were not available for all the cases and controls. As with any genetic association study, the SNPs examined may be in linkage disequilibrium with uninvestigated genetic regions contributing to the results. Although the number of cases in our study is the largest to date to examine chemokine polymorphisms and gastric cancer, there was limited statistical power to detect a haplotype effect. Also, the small number of cases homozygous for minor alleles may have increased the likelihood for false negative or positive associations. We were also unable to detect significant interactions with *H. pylori* status, Lauren's classification, tumor grade, and stage which may have been due to the proportion of cases missing this data. This limitation also applies to the survival analysis, as due to the proportion of cases without this data, survival analysis was not adjusted for these factors.

In summary, other than a single haplotype in *CCR5*, there was no association of gastric cancer risk with SNPs and one frameshift deletion in three chemokine receptor and one chemokine ligand genes. Gastric cancer survival was significantly associated with a functional polymorphism in *CCR2* and two non-functional polymorphisms in *CCR5*. Future studies are needed to confirm our findings as well as investigation of the potential function of these SNPs in tumor behavior, including their role in peritoneal carcinomatosis, metastases, and clinical outcomes.

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Table I

Selected characteristics of gastric cancer cases and controls.

	Cases (n = 298), n (%)	Controls (n = 417), n (%)	p-Value[a]
Gender			
Female	100 (33.6)	147 (35.3)	
Male	198 (66.4)	270 (64.7)	0.64
Age (years)			
≤59	92 (30.9)	123 (29.5)	
60–69	118 (39.6)	164 (39.3)	
≥70	88 (29.5)	130 (31.2)	0.88
Education			
Less than high school	140 (47.0)	158 (37.9)	
High school or technical training	97 (32.6)	124 (29.7)	
Some college/college graduate	61 (20.4)	135 (32.4)	0.002
Smoking status[b]			
Never	85 (28.7)	168 (40.3)	
Ex-smoker	87 (29.4)	133 (31.9)	
Current smoker	124 (41.9)	116 (27.8)	<0.001
Alcohol use[c]			
Non-drinker	104 (35.8)	138 (33.1)	
Former drinker	97 (33.3)	60 (14.4)	
Current drinker	90 (30.9)	219 (52.5)	<0.001
Family history of cancer[d]			
No family history	175 (61.2)	275 (67.1)	
Gastric cancer	35 (12.2)	18 (4.4)	
Other cancer	76 (26.6)	117 (28.5)	<0.001
H. pylori infection status[e,f]			
Negative	50 (16.8)	61 (14.7)	
Positive	248 (83.2)	355 (85.3)	0.44
Lauren classification[g]			
Intestinal	200 (70.9)		
Diffuse	50 (17.7)		
Indeterminate	32 (11.4)		
Tumor localization			
Cardia only	34 (11.4)		
Distal stomach	218 (73.2)		
Combined cardia/distal	35 (11.7)		
Unknown	11 (3.7)		
Stage of disease[h]			
Localized	59 (25.7)		

	Cases (*n* = 298), *n* (%)	Controls (*n* = 417), *n* (%)	*p*-Value[a]
Regional metastasis	90 (39.1)		
Displaced metastasis	81 (35.2)		
Tumor grade[i]			
Well-differentiated	15 (9.6)		
Moderately differentiated	78 (50.0)		
Poorly differentiated	63 (40.4)		

[a] Based on χ^2 test for categorical variables.

[b] Data available for 296 cases.

[c] Data available for 291 cases.

[d] Data available for 286 cases and 410 controls.

[e] Data available for 416 controls.

[f] Negative = tested negative for both IgG antibodies to *H. pylori* and cagA; positive = tested positive for either IgG antibodies to *H. pylori* or cagA antibody, or both.

[g] Data available for 282 cases.

[h] Data available for 230 cases.

[i] Data available for 156 cases.

Table II

Odds ratios (ORs) and 95% confidence intervals (CIs) for gastric cancer risk by chemokine polymorphism, haplotypes, and frameshift deletion (*CCR5*).

	Cases	Controls	OR (95% CI)[a]	OR (95% CI)[b]
CCR2				
rs1799864 G>A				
GG	250	329	1.0 (ref)	1.0 (ref)
A carrier	46	88	0.8 (0.7–1.0)	0.8 (0.7–1.0)
CCR5				
rs333 delta 32				
++	225	331	1.0 (ref)	1.0 (ref)
+−	69	73	1.2 (1.0–1.4)	1.2 (1.0–1.4)
rs2734648 G>T				
GG	133	192	1.0 (ref)	1.0 (ref)
T carrier	162	219	1.0 (0.9–1.2)	1.0 (0.9–1.2)
rs1799987 A>G				
AA	93	142	1.0 (ref)	1.0 (ref)
G carrier	195	259	1.1 (0.9–1.3)	1.1 (0.9–1.3)
rs1800024 C>T				
CC	241	326	1.0 (ref)	1.0 (ref)
T carrier	49	83	0.9 (0.7–1.1)	0.9 (0.7–1.1)
Haplotypes[c]				
GAC	46.9%	49.5%	1.0 (ref)	1.0 (ref)
TGC	32.5%	31.2%	1.1 (0.9–1.4)	1.1 (0.8–1.4)
GGC	11.6%	8.9%	1.4 (1.0–2.0)	1.5 (1.0–2.2)
GAT	8.8%	10.3%	0.9 (0.6–1.3)	0.9 (0.6–1.3)
CX3CR1				
rs3732379 G>A				
GG	142	225	1.0 (ref)	1.0 (ref)
A carrier	153	188	1.1 (1.0–1.3)	1.1 (1.0–1.3)
rs3732378 C>T				
CC	175	265	1.0 (ref)	1.0 (ref)
T carrier	117	144	1.1 (1.0–1.3)	1.1 (0.9–1.3)
Haplotypes[d]				
GC	71.0%	72.9%	1.0 (ref)	1.0 (ref)
AT	21.1%	19.9%	1.1 (0.8–1.4)	1.1 (0.8–1.4)
AC	7.8%	7.2%	1.1 (0.7–1.7)	1.1 (0.7–1.6)
CXCL12				
rs1801157 G>A				
GG	193	258	1.0 (ref)	1.0 (ref)
A carrier	99	156	0.9 (0.8–1.1)	0.9 (0.8–1.1)

[a] Unadjusted.

[b] Adjusted for age, sex, smoking status, education.

[c] Haplotypes with >1% frequency based on rs2724648, rs1799987, and rs1800024.

[d] Haplotypes with >1% frequency based on rs3732379 and rs3732378.

Table III

Hazard ratio for death among patients with gastric cancer.

Chemokine polymorphism	No. of deaths	Person-years	Hazard ratio (95% CI)[a]	Hazard ratio (95% CI)[b]
CCR2				
rs1799864 G>A				
GG	219	9812.4	1.0	1.0
A carrier	43	1287.6	1.4 (1.0–1.9)	1.5 (1.1–2.1)
CCR5				
rs333 delta 32				
++	195	8766.9	1.0	1.0
+−	65	2244.6	1.2 (0.9–1.6)	1.2 (0.9–1.6)
rs2734648 G>T				
GG	126	3720.5	1.0	1.0
T carrier	135	7378.6	0.7 (0.5–0.9)	0.8 (0.6–1.0)
rs1799987 A>G				
AA	87	2922.5	1.0	1.0
G carrier	167	8103.8	0.8 (0.6–1.0)	0.9 (0.7–1.2)
rs1800024 C>T				
CC	210	9642.4	1.0	1.0
T carrier	46	1374.5	1.4 (1.0–1.9)	1.5 (1.1–2.1)
CX3CR1				
rs3732379 G>A				
GG	125	5557.4	1.0	1.0
A carrier	136	5539.2	1.1 (0.9–1.4)	1.2 (0.9–1.5)
rs3732378 C>T				
CC	157	6575.7	1.0	1.0
T carrier	101	4503.8	1.0 (0.8–1.3)	1.0 (0.8–1.3)
CXCL12				
rs1801157 G>A				
GG	173	6791.9	1.0	1.0
A carrier	85	4290.4	0.9 (0.7–1.1)	1.0 (0.8–1.4)

[a] Unadjusted.

[b] Adjusted for age, sex, smoking status, education, and stage of disease.