

Polymorphisms in inflammation-related genes and risk of gastric cancer (Finland)

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Abstract *Helicobacter pylori* infection is an important risk factor for gastric cancer, but <3% of carriers of this organism will ever develop gastric cancer. Since inflammation plays a significant role in gastric carcinogenesis, it has been suggested that polymorphisms in genes involved in inflammatory response may partly explain why only a subgroup of patients infected with *H. pylori* develop gastric cancer. We compared relative frequencies of 17 single nucleotide polymorphisms (SNPs) in eight inflammation-related genes between 112 gastric cancer patients and 208 controls. Cases and controls were selected from a large cohort of Finnish male smokers who were recruited into the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study. The studied SNPs were *IL-1A* (−889 C/T), *IL-1B* (−511 C/T and −31 T/C), *IL-6* (−174 G/C and −597 G/A), *IL-8* (−251 T/A, +396 T/G and +781 C/T), *IL-8RA*

(*Ex2* +860 G/C), *IL-8RB* (*Exon 3* +1235 T/C, *Exon 3* +811 C/T, and *Exon 3* +1010 G/A), *IL-10* (−819 C/T, −592 C/A, −1082 A/G), and *TNF A* (−308 G/A, −238 G/A). We found no statistically significant association between any of these SNPs, or the number of pro-inflammatory polymorphisms, with risk of gastric cancer. Our results do not support the hypothesis that polymorphisms in genes involved in the inflammatory response confer differences in gastric cancer risk among different individuals.

Keywords Cytokine · Gastric cancer · Genetic · Interleukin · Polymorphism

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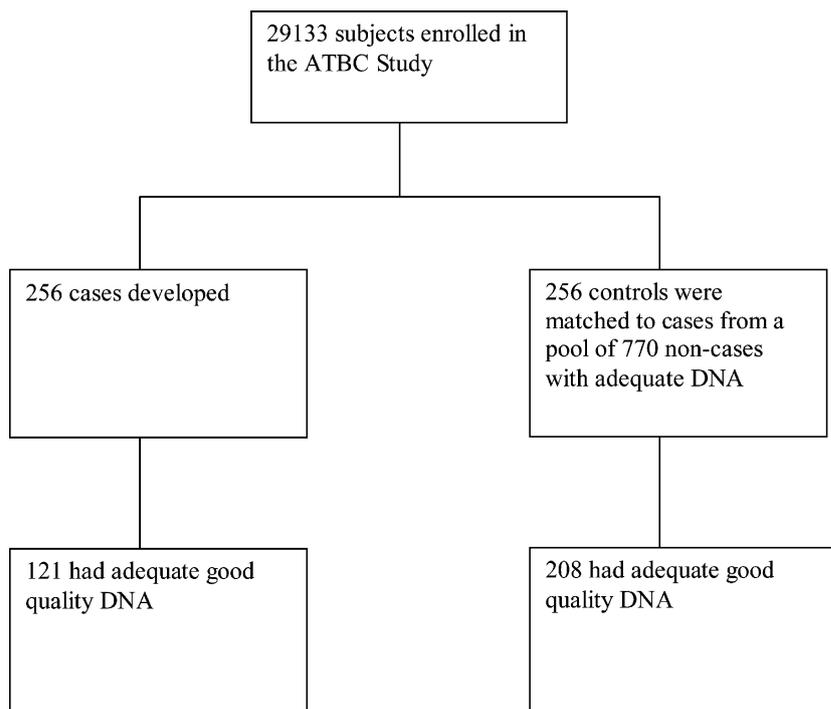
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Introduction

Although *Helicobacter pylori* infection is the strongest identified risk factor for distal gastric cancers (GC), only <3% of *H. pylori* carriers will ever develop GC [1]. *H. pylori* and other important environmental or dietary risk factors only partially explain the incidence patterns of GC [2]. Therefore, it has been suggested that genetic polymorphisms in humans may contribute to variation in risk of GC [3].

Inflammation is an essential part of the carcinogenic process in gastric cancer [4, 5]. Chronic superficial gastritis is one of the early phases of progression to intestinal-type GC [6], the predominant histological type of gastric cancer. Therefore, stronger inflammatory response by the host has been hypothesized to increase the GC risk. Some recent well-designed studies have suggested that stronger pro-inflammatory genetic profiles are associated with higher

Fig. 1. Case and control selection flow diagram



GC risk [7–11]. Most notably, one of these studies suggested risk of gastric non-cardia cancer increased with the number of pro-inflammatory polymorphisms, and individuals with three pro-inflammatory polymorphisms had a more than 20-fold increase in risk of this cancer [9]. However, studies in some other populations have failed to replicate these results [11–18], and further investigation of this issue in different populations is needed.

The current study was conducted to examine the association of polymorphisms in several genes including interleukins (*IL*), interleukin receptors (*ILR*), and tumor necrosis factor α (*TNF-A*) with risk of GC in a group of Finnish males. Associations of these polymorphisms with the major anatomic (non-cardia) and histologic (intestinal type) subsets of GC were also examined separately.

Material and methods

Study population

The cases and controls in this study were selected from the participants of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study, a randomized cancer prevention study conducted in Southern Finland. Between 1985 and 1988, 29,133 eligible male smokers aged 50–69 years were recruited into this study. The trial ended in 1993, but the participants are still followed as a cohort. Details of study design and methods have been published [19].

The cases in the current evaluation were all incident gastric adenocarcinomas that were diagnosed in the

ATBC study, from its initiation to 30 April 1999, and had enough DNA (>150 ng) for genotyping. Incident cancer cases were primarily identified via the Finnish Cancer Registry, which provides almost 100% case coverage [20]. Diagnosis of GC cases (ICD9-code 151) was confirmed by review of hospital records and histopathological specimens. Cases were classified as cardia cancer if they involved the esophagogastric junction, and as non-cardia cancer if they did not. The study was approved by the institutional review boards of both the National Public Health Institutes in Finland and the National Institutes of Health in the United States.

A total of 256 GC cases were diagnosed by 30 April 1999 and all were initially selected as cases for this study. Of these only 121 were found to have enough DNA for genotyping analysis in this study; the rest either did not have whole blood samples for DNA extraction, or blood samples did not yield adequate quantities of DNA. Among the 121 cases with adequate DNA, 112 were adenocarcinomas and nine were other types of cancer. Only the adenocarcinomas were included as cases in this study.

A total of 256 non-cancer subjects that were age-matched (3 months) to the initially selected GC cases were selected as controls. To maximize the number of controls with adequate DNA samples and also for logistical purposes, these controls were selected from a pool of 770 ATBC study participants that had remained cancer free until 1999 and already had DNA extracted for other studies. However, only 208 controls were found to have adequate DNA for genotyping analysis (Figure 1).

Analysis of several factors showed that cases with DNA ($n=121$) were similar to all cases ($n=256$). Mean age was 59 years in all cases versus 58.5 years in those with DNA; 63% of all cases versus 64% cases with DNA were urban; median smoking duration was 37.2 years in all cases versus 36.7 years in those with DNA; and 93% of all non-cardia cancers versus 91% of non-cardia cancers with DNA were *H. pylori* positive.

Genotyping

In all, eight genes were genotyped for 17 single nucleotide polymorphisms (SNPs). These SNPs were *IL-1A* (−889 C/T), *IL-1B* (−511 C/T and −31 T/C), *IL-6* (−174 G/C and −597 G/A), *IL-8* (−251 T/A, +396 T/G and +781 C/T), *IL-8RA* (Ex2 +860 G/C), *IL-8RB* (Exon 3 +1235 T/C, Exon 3 +811 C/T, and Exon 3 +1010 G/A), *IL-10* (−819 C/T, −592 C/A, −1082 A/G), and *TNF A* (−308 G/A, −238 G/A). A complete list of SNPs and the corresponding dbSNP number is shown in Table 1.

Genotyping was performed in the US National Cancer Institute Core Genotyping Facility (NCI-CGF), Gaithersburg, MD, using MGBEclipse™ (*IL8-RB* Exon 3 +811 C/T) or Taqman™ (all other SNPs) platforms (Table 1). MGBEclipse™ and Taqman™ methods, including primers, probe sequences, and cycling conditions are available at the NCI-CGF website (<http://snp500cancer.nci.nih.gov>). All assays were validated (100% concordance) using 102 sequenced Coriell DNA samples (information available on the NCI-CGF website). Three Coriell controls (one homozygous allele 1, one homozygous allele 2, and one heterozygote) and a no template control were used on the genotyping assay plates with the sample DNA.

Background data collection

At study entry, all participants completed a questionnaire on background characteristics and habits and gave a fasting serum sample, which was stored at −70 °C. Diet was assessed using a self-administered food use questionnaire with 276 food items [21].

H. pylori serum assays

Serum was evaluated for IgG antibodies to whole-cell (WC) and CagA *H. pylori* antigen by enzyme-linked immunosorbent assay (ELISA), as described elsewhere [22]. Experienced technicians who were unaware of subjects' case-control status performed these assays. An optical density ratio either ≥ 1.0 for whole-cell antibodies or ≥ 0.35 for CagA antigen was considered a positive *H. pylori* test. *H. pylori* was positive in 79% of the controls, 91% of the non-cardia cancer cases, and 68% of the cardia cancer cases.

Statistical analysis

Means and standard deviations (SD) of age and smoking duration were calculated and were compared between cases and controls using *t*-test. Educational level and urban/rural status were compared between cases and controls using χ^2 -square test. For cases, the proportions of anatomic and histologic subtypes of GC were calculated.

Percentages of each of the three possible genotypes were calculated for each SNP. These percentages were tested for Hardy-Weinberg Equilibrium (HWE). Tajima's *D'* was calculated as a measure of linkage disequilibrium (LD)

Table 1 Number and percentages of cases and controls successfully genotyped for each SNP

	dbSNP ID	Genotyping platform ^a	No. of controls (%) ^b	No. of cases (%) ^b
<i>IL-1A</i> −889 C/T	rs1800587	Taqman	204 (98%)	111 (99%)
<i>IL-1B</i> −511 C/T	rs16944	Taqman	165 (79%)	104 (92%)
<i>IL-1B</i> −31 T/C	rs1143627	Taqman	207 (100%)	112 (100%)
<i>IL-6</i> −174 G/C	rs1800795	Taqman	152 (73%)	103 (92%)
<i>IL-6</i> −597 G/A	rs1800797	Taqman	203 (98%)	110 (98%)
<i>IL-8</i> −251 T/A	rs4073	Taqman	207 (100%)	112 (100%)
<i>IL-8</i> +396 T/G	rs2227307	Taqman	208 (100%)	111 (99%)
<i>IL-8</i> +781 C/T	rs2227306	Taqman	208 (100%)	111 (99%)
<i>IL-8RA</i> Ex2+860 G/C	rs2234671	Taqman	208 (100%)	112 (100%)
<i>IL-8RB</i> Ex3+1235 T/C	rs1126579	Taqman	208 (100%)	112 (100%)
<i>IL-8RB</i> Ex3+811 C/T	rs2230054	MGBEclipse	161 (77%)	102 (91%)
<i>IL-8RB</i> Ex3+1010 G/A	rs1126580	Taqman	192 (92%)	109 (97%)
<i>IL-10</i> −819 C/T	rs1800871	Taqman	152 (73%)	98 (88%)
<i>IL-10</i> −592 C/A	rs1800872	Taqman	208 (100%)	112 (100%)
<i>IL-10</i> −1082 A/G	rs1800896	Taqman	205 (99%)	112 (100%)
<i>TNF</i> −308 G/A	rs1800629	Taqman	208 (100%)	112 (100%)
<i>TNF</i> −238 G/A	rs361525	Taqman	208 (100%)	112 (100%)

^a Taqman and MGBEclipse methods are fully described at <http://snp500cancer.nci.nih.gov>

^b All percentages are rounded to the closest integer

between pairs of SNPs in genes in which at least three SNPs were assayed (i.e., for SNPs in *IL-8*, *IL-8RB*, and *IL-10*).

Distributions of genotypes were compared between cases and control using chi-square tests with 2 degrees of freedom. Using logistic regression, crude and adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated to assess the strength of association of the heterozygote and the less common homozygote genotypes with gastric cancer; the more common homozygous genotype served as the reference group. Adjustment was done for serum positivity to *H. pylori*, the strongest risk factor for GC in this population [23]. We also tested for interactions between each SNP and *H. pylori*, and between each SNP and other important risk factors of non-cardia cancer in this population (low intake of fruits, vitamin C, tocopherols, and lycopene) [24].

It has been suggested that the number of pro-inflammatory genotypes may increase the risk of gastric non-cardia cancer beyond a multiplicative model [9]. Therefore, as an *a priori* hypothesis, we compared the number of pro-inflammatory genotypes in *IL-B -511 T+*, *IL-10 -592 A+*, and *TNF-A -308 G+*, the genotypes that were tested in previous studies [9], between all GC cases and controls, and also between non-cardia cancers and controls using the chi-square test with 3 degrees of freedom. Logistic regression was used to calculate the ORs and 95% CIs for the risk associated with the number of pro-inflammatory genotypes.

Haplotypes were constructed for genes in which at least three SNPs were assayed, i.e., for *IL-8*, *IL-8RB*, and *IL-10*. We limited haplotype analysis to the three genes in which at least three polymorphic sites had been genotyped, because estimating haplotypes with fewer than three SNP was not accurate. Haplotypes were estimated using PHASE version 2.0 software [25, 26]. Distributions of diplotypes (combinations of two haplotypes for each individual) were compared between cases and controls using chi-square test.

Throughout the analyses, two-sided *p*-values <0.05 were considered as statistically significant. All statistical analyses were performed using STATA version 8.0 software (Stata Corporation, College Station, TX).

Power calculation

The number of cases and controls were 112 and 208, respectively. Assuming $\alpha=0.05$ and prevalence of the less frequent allele in controls=0.2, this study had 94% power to detect an odds ratio of 2.5. This number was calculated using PS[®] power and sample size calculation software (Du Pont and Plummer, available online) and verified using manual calculation.

Results

A total of 112 gastric adenocarcinoma cases and 208 controls were enrolled in this study. Mean (SD) ages of cases and controls at study baseline were 58.5 (4.9) and 59.0 (5.0) years, respectively ($p=0.43$). Mean (SD) of smoking duration was 36.7 (10.0) and 37.5 (8.4) in case and controls, respectively ($p=0.42$). There was no significant difference in the level of attained education ($p=0.53$) or urban versus rural residence between cases and control ($p=0.99$).

Of the total 112 GC subjects, 30 (27%) were classified as cardia cancer and 82 (73%) as non-cardia cancer cases. The histology of the tumors was classified as intestinal in 72 (64%), as diffuse in 22 (20%), and as undetermined or mixed type in 18 (16%) of the case subjects.

Table 1 shows the number of cases and controls who were successfully genotyped for each SNP. Twelve of the 17 SNPs were successfully genotyped in nearly all cases and controls. However, for five SNPs, *IL-1B -511*, *IL-6 -174*, *IL-8RB Exon 3 +811* and *+1010*, and *IL-10 -819* this proportion was lower. Thirteen SNPs were in HWE, but four (*IL1A -889*, *IL-1B -511*, *IL-6 -174*, and *IL-6 -597*) were not.

In *IL-8*, *D*'s for linkage between $-251T$ and $+396T$, $-251T$ and $+781C$, and $+396T$ and $+781T$ were 0.76, 0.52, and 0.52, respectively. All three *IL-8RB* SNPs were in almost complete LD; *D*'s for linkage between $+1235T$ and $+811C$, $+1235T$ and $+1010G$, and $+811C$ and $+1010G$ were 0.98, 0.98, and 0.99, respectively. In *IL-10*, $-819C$ and $-592C$ were in almost complete LD ($D' \cong 1.0$), while these two were essentially unlinked to $-1082 A$ ($D'=-0.05$).

Table 2 compares the distribution of genotypes for each SNP between cases and controls. No significant difference was observed between cases and controls for any of these SNPs. Separate comparisons between gastric non-cardia cases and controls, and between intestinal type cancer cases and controls also did not reveal any statistically significant differences.

Table 3 shows the ORs and 95% CIs for the association between SNPs examined in this study and risk of gastric cancer. No significant association was observed for any of these SNPs. Adjustment for *H. pylori* serum positivity, the strongest known risk factor for GC in this population, made essentially no difference. Tests of interactions between *H. pylori* and SNPs in non-cardia cancers did not yield statistically significant results. Tests of interaction between SNPs and intake of fruits, vitamin C, tocopherols, and lycopene were non-significant, except for the interaction between *TNF-A -238* and fruit intake ($p=0.045$).

Table 4 compares the distribution of the number of pro-inflammatory genotypes (0–3) between controls, all GC

Table 2 Distribution of genotypes for each SNP by case/control status

	Genotype	Controls n (%) ^a	All cases n (%) ^a	<i>p</i> -value ^b	Non-cardia cases n (%) ^a	<i>p</i> -value ^b	Intestinal type cancer cases n (%) ^a	<i>p</i> -value ^b
<i>IL-1A</i> -889 C/T	CC	93 (46%)	47 (42%)	0.32	32 (40%)	0.20	28 (39%)	0.64
	CT	100 (49%)	53 (48%)		40 (49%)		38 (54%)	
	TT	11 (5%)	11 (10%)		9 (11%)		5 (7%)	
<i>IL-1B</i> -511 C/T	CC	70 (42%)	42 (40%)	0.67	31 (40%)	0.32	32 (48%)	0.74
	CT	63 (38%)	45 (43%)		36 (47%)		24 (36%)	
	TT	32 (19%)	17 (16%)		10 (13%)		11 (16%)	
<i>IL-1B</i> -31 T/C	TT	83 (40%)	44 (49%)	0.92	33 (40%)	0.56	33 (46%)	0.66
	TC	86 (42%)	49 (44%)		38 (46%)		26 (36%)	
	CC	38 (18%)	19 (17%)		11 (13%)		13 (18%)	
<i>IL-6</i> -174 G/C	GG	51 (34%)	21 (21%)	0.05	18 (23%)	0.15	14 (21%)	0.10
	GC	58 (38%)	54 (52%)		39 (51%)		35 (52%)	
	CC	43 (28%)	27 (26%)		20 (26%)		18 (27%)	
<i>IL-6</i> -597 G/A	GG	61 (30%)	25 (23%)	0.15	18 (23%)	0.11	16 (23%)	0.18
	GA	86 (42%)	59 (54%)		45 (56%)		39 (55%)	
	AA	56 (28%)	26 (24%)		17 (21%)		16 (23%)	
<i>IL-8</i> -251 T/A	TT	72 (35%)	42 (38%)	0.82	27 (33%)	0.78	26 (36%)	0.98
	TA	111 (54%)	56 (50%)		43 (52%)		38 (53%)	
	AA	24 (12%)	14 (13%)		12 (15%)		8 (11%)	
<i>IL-8</i> +396 T/G	TT	72 (35%)	42 (38%)	0.77	27 (33%)	0.77	26 (37%)	0.95
	TG	112 (54%)	55 (50%)		43 (52%)		37 (52%)	
	GG	24 (12%)	14 (13%)		12 (15%)		8 (11%)	
<i>IL-8</i> +781 C/T	CC	81 (39%)	47 (42%)	0.82	29 (36%)	0.74	29 (41%)	0.87
	CT	105 (50%)	52 (47%)		41 (51%)		36 (51%)	
	TT	22 (11%)	12 (11%)		11 (14%)		6 (8%)	
<i>IL-8RA</i> Ex2 +860 G/C	GG	187 (90%)	99 (88%)	0.46	72 (88%)	0.51	66 (92%)	0.69
	GC	19 (9%)	13 (12%)		10 (12%)		6 (8%)	
	CC	2 (1%)	0 (0%)		0 (0%)		0 (0%)	
<i>IL-8RB</i> Ex3 +1235 T/C	TT	66 (32%)	36 (32%)	0.78	23 (28%)	0.79	24 (33%)	0.96
	TC	108 (52%)	61 (54%)		46 (56%)		36 (50%)	
	CC	34 (16%)	15 (13%)		13 (16%)		12 (17%)	
<i>IL-8RB</i> Ex 3 +811 C/T	CC	58 (36%)	48 (47%)	0.12	34 (45%)	0.26	31 (48%)	0.25
	CT	78 (48%)	45 (44%)		34 (45%)		27 (42%)	
	TT	25 (16%)	9 (9%)		7 (9%)		7 (11%)	
<i>IL-8RB</i> Ex3 +1010 G/A	GG	84 (44%)	51 (47%)	0.88	35 (44%)	0.88	30 (43%)	0.88
	GA	85 (44%)	46 (42%)		33 (42%)		30 (43%)	
	AA	23 (12%)	12 (11%)		11 (14%)		10 (14%)	
<i>IL-10</i> -819 C/T	CC	80 (53%)	58 (59%)	0.58	43 (59%)	0.21	34 (55%)	0.96
	CT	62 (41%)	35 (36%)		29 (40%)		24 (38%)	
	TT	10 (7%)	5 (5%)		1 (1%)		4 (6%)	
<i>IL-10</i> -592 C/A	CC	109 (52%)	68 (61%)	0.32	49 (60%)	0.17	42 (58%)	0.61
	CA	82 (39%)	38 (34%)		31 (38%)		26 (36%)	
	AA	17 (8%)	6 (5%)		2 (2%)		4 (6%)	
<i>IL-10</i> -1082 A/G	AA	72 (35%)	38 (34%)	0.42	26 (32%)	0.35	23 (32%)	0.72
	AG	96 (47%)	47 (42%)		35 (43%)		33 (46%)	
	GG	37 (18%)	27 (24%)		21 (26%)		16 (22%)	
<i>TNF</i> -308 G/A	GG	154 (74%)	86 (77%)	0.36	65 (79%)	0.12	56 (78%)	0.12
	GA	52 (25%)	23 (21%)		14 (17%)		13 (18%)	
	AA	2 (1%)	3 (3%)		3 (4%)		3 (4%)	
<i>TNF</i> -238 G/A	GG	203 (97%)	106 (95%)	0.17	77 (94%)	0.12	68 (94%)	0.12
	AG	5 (2%)	6 (5%)		5 (6%)		4 (6%)	

^a All percentages are rounded to the closest integer. Due to rounding, percentages may not add up to 100%

^b *p*-values are obtained from chi-square test with two degrees of freedom

cases, and non-cardia cancer cases. The three pro-inflammatory genotypes included in this analysis were *IL-1B* -511 T+, *IL-10* -592A+, and *TNF-A* -308A+. The distribution of the number of pro-inflammatory genotypes was not statistically different between cases and controls, and

increasing the number of pro-inflammatory genotypes did not increase gastric cancer risk.

Table 5 compares the distributions of diplotypes between cases and controls. No statistically significant difference in these distributions was observed between cases and controls.

Table 3 Crude and adjusted ORs (95% CIs) for the association between genotypes and risk of gastric cancer

	Genotype	Unadjusted OR (95% CI)	Adjusted OR (95% CI) ^a
<i>IL-1A</i> - 889 C/T	CC	Ref	Ref
	CT	1.05 (0.65–1.70)	1.10 (0.67–1.80)
	TT	1.98 (0.80–4.90)	1.98 (0.79–4.92)
<i>IL-1B</i> - 511 C/T	CC	Ref	Ref
	CT	1.19 (0.69–2.04)	1.25 (0.72–2.17)
	TT	0.89 (0.44–1.79)	0.82 (0.39–1.67)
<i>IL-1B</i> - 31 T/C	TT	Ref	Ref
	TC	1.07 (0.65–1.78)	1.08 (0.65–1.81)
	CC	0.94 (0.49–1.83)	0.87 (0.44–1.71)
<i>IL-6</i> - 174 G/C	GG	Ref	Ref
	GC	2.16 (1.16–4.02)	2.33 (1.23–4.41)
	CC	1.45 (0.73–2.91)	1.54 (0.76–3.11)
<i>IL-6</i> - 597 G/A	GG	Ref	Ref
	GA	1.67 (0.95–2.96)	1.78 (0.99–3.18)
	AA	1.13 (0.59–2.19)	1.15 (0.59–2.24)
<i>IL-8</i> - 251 T/A	TT	Ref	Ref
	TA	0.86 (0.52–1.44)	0.87 (0.52–1.44)
	AA	1.00 (0.37–2.72)	0.92 (0.42–2.00)
<i>IL-8</i> + 396 T/G	TT	Ref	Ref
	TG	0.84 (0.53–1.42)	0.85 (0.51–1.41)
	GG	1.00 (0.46–2.14)	0.92 (0.42–2.00)
<i>IL-8</i> + 781 C/T	CC	Ref	Ref
	CT	0.85 (0.52–1.39)	0.86 (0.52–1.41)
	TT	0.94 (0.42–2.07)	0.86 (0.38–1.93)
<i>IL-8RA</i> Ex2 + 860 G/C	GG	Ref	Ref
	GC	1.29 (0.61–2.72)	1.31 (0.62–2.78)
	CC	–	–
<i>IL-8RB</i> Ex3 + 1235 T/C	TT	Ref	Ref
	TC	1.04 (0.62–1.73)	1.02 (0.60–1.71)
	CC	0.81 (0.39–1.68)	0.86 (0.41–1.79)
<i>IL-8RB</i> Ex 3 + 811 C/T	CC	Ref	Ref
	CT	0.70 (0.41–1.18)	0.70 (0.41–1.20)
	TT	0.44 (0.19–1.02)	0.44 (0.19–1.05)
<i>IL-8RB</i> Ex3 + 1010 G/A	GG	Ref	Ref
	GA	0.89 (0.54–1.46)	0.92 (0.55–1.53)
	AA	0.86 (0.39–1.87)	0.95 (0.43–2.09)
<i>IL-10</i> - 819 C/T	CC	Ref	Ref
	CT	0.78 (0.46–1.32)	0.75 (0.43–1.29)
	TT	0.69 (0.22–2.12)	0.77 (0.24–2.42)
<i>IL-10</i> - 592 C/A	CC	Ref	Ref
	CA	0.74 (0.46–1.21)	0.74 (0.45–1.21)
	AA	0.57 (0.22–1.50)	0.63 (0.23–1.70)
<i>IL-10</i> - 1082 A/G	AA	Ref	Ref
	AG	0.93 (0.55–1.57)	0.92 (0.54–1.58)
	GG	1.38 (0.73–2.60)	1.45 (0.76–2.76)
<i>TNF</i> - 308 G/A	GG	Ref	Ref
	GA	0.79 (0.45–1.38)	0.73 (0.41–1.29)
	AA	2.69 (0.44–16.39)	2.49 (0.41–15.24)
<i>TNF</i> - 238 G/A	GG	Ref	Ref
	AG	2.30 (0.69–7.70)	2.43 (0.72–8.23)

SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; Ref, reference group

^a Adjusted for *H. pylori* seropositivity

Discussion

The role of immune mechanisms and their genetic determinants in the etiology of GC has long been suspected [6, 27]. Further motivation to work on the association between inflammation-related genes and GC risk was generated by two interesting studies from El-Omar and colleagues. The first study showed a 2–3-fold increase in risk of GC in

subjects with pro-inflammatory *IL-1B* genotypes [7], and the second study showed a more than 20-fold increase in GC risk associated with the presence of three pro-inflammatory genotypes in several inflammation-related genes [9]. The associations between pro-inflammatory *IL-1B* genotypes and GC were confirmed in some, but not all, subsequent studies. While some studies showed an increased risk, others found no change or even a reduced risk.

Table 4 Frequencies and odds ratios of the number of pro-inflammatory polymorphisms^a in gastric cancer cases and controls

Number of pro-inflammatory polymorphisms	Controls n (%) ^b	All cases n (%) ^b	OR (95% CI)	<i>p</i> -value ^c	Non-cardia cases n (%) ^a	OR (95% CI)	<i>p</i> -value ^c
0	24 (15%)	19 (18%)	Ref	0.44	13 (17%)	Ref	0.54
1	76 (46%)	53 (51%)	0.88 (0.43–1.77)		41 (53%)	1.02 (0.47–2.22)	
2	53 (32%)	24 (23%)	0.57 (0.26–1.23)		18 (23%)	0.66 (0.28–1.57)	
3	12 (7%)	8 (8%)	0.84 (0.29–2.47)		5 (6%)	0.77 (0.22–2.66)	

^a Pro-inflammatory genotypes included were *IL-1B* –511 T+, *IL-10* –592 A+, and *TNF-A* –308A+

^b All percentages are rounded to the closest integer. Due to rounding, percentages may not add up to 100%

^c *p*-values are obtained from chi-square test with three degrees of freedom

We conducted this study to see if pro-inflammatory genotypes increase the risk of GC in a group of Finnish subjects. In total, 17 SNPs in eight genes were examined. Selection of polymorphisms in this study was based on biologic properties of the genes and previously published literature.

IL-1s are a cluster of genes whose products have an important role in controlling inflammatory reactions, as well as controlling the acidity of the stomach [7, 8, 28]. To our knowledge, only one previous study has examined the association between *IL-1A* –889 polymorphisms and GC and found no association [17]. Two polymorphisms in *IL-1B*, –511 C/T and –31 T/C, have been extensively studied; –511T and –31C are associated with higher inflammatory response. While some studies have shown 2–3-fold increase in risk of GC with pro-inflammatory variants of *IL-1B* [7–9, 29–31], others have found no association or even a reduced risk [11, 13–18, 32].

IL-6 is a pleiotropic cytokine with both pro- and anti-inflammatory properties [33, 34], and its levels are increased in patients infected with *H. pylori*. Two previous studies, one in a US population [9] and the other in a Chinese population [35], have explored the association between *IL-6* (–174 G/C) and GC risk, and neither one found an association.

Interleukin-8 (IL-8) is a multifunctional cytokine that can stimulate the division of endothelial cells [36]. *IL-8* has

three SNPs at positions –251, +396, and +781, and –251A is associated with an increased production of IL-8 [37]. Four SNPs have been identified in *IL-8* receptor genes [38] and were examined in this study. A previous study that examined the association of these polymorphisms with gastric cardia cancer risk found a twofold risk associated with the –251A and +396G variant forms, but no significant association with *IL-8* receptor polymorphisms [38].

Interleukin-10 (IL-10) is an anti-inflammatory cytokine, which is involved in down-regulating cell-mediated and cytotoxic inflammatory responses [39]. *IL-10* has three confirmed biallelic polymorphisms in the gene promoter region: –1082 A/G, –819 C/T, and –592 C/A. Presence of –1082A is associated with lower production of IL-10 *in vitro* and *in vivo* [40–42], and accordingly stronger inflammatory response. Data regarding the effect of *IL-10* –1082 on GC risk has been conflicting. While Wu and colleagues found a positive association between –1082G (the high producer allele) and GC risk [11], El-Omar showed a positive association between –1082A (the low producer allele) and gastric non-cardia cancer [9]. Two studies that examined the association between *IL-10* polymorphisms and gastric cardia cancer did not find an association [9, 35].

Tumor Necrosis Factor-Alpha (TNF- α), another pro-inflammatory cytokine, is a central mediator of the immune

Table 5 Diplotype distributions in gastric cancer cases and controls

	Diplotype	Controls n (%) ^a	All cases n (%) ^a	<i>p</i> -value ^b
<i>IL-8</i> –251, +396, +781	TTC/TTC	46 (22%)	24 (21%)	0.74
	TTC/other	120 (57%)	69 (62%)	
	Other/other	42 (20%)	19 (17%)	
<i>IL-8RB</i> +1235, +811, +1010	TCG/TCG	66 (32%)	36 (32%)	0.58
	TCG/other	105 (50%)	61 (54%)	
	Other/other	37 (18%)	15 (13%)	
<i>IL-10</i> –819, –592, –1082	CCA/CCA	37 (18%)	27 (24%)	0.37
	CCA/other	99 (48%)	47 (42%)	
	Other/other	72 (35%)	38 (34%)	

^a All percentages are rounded to the closest integer. Due to rounding, percentages may not add up to 100%

^b *p*-values are obtained from chi-square test with two degrees of freedom

response and shares many biological activities with IL-1 [43, 44]. Two well-defined polymorphisms, -308 G/A and -238 G/A , have been reported in the promoter region of *TNF-A*, and -308A is associated with an increased production of *TNF- α* [43]. Several studies have examined the association between *TNF-A -308A* and GC risk. Jang et al. [43], Wu et al. [11, 32], Garza-Gonzalez et al. [31], and Perri et al. [18] did not find an association. In contrast, El-Omar et al. [9] and Machado et al. [10] found an almost two-fold increase in risk of GC in subjects with this polymorphism. The association between *TNF-A -238* and GC has also been studied. Wu et al. [32] did not find an association, but Jang et al. [43] found an inverse association.

We did not find an association between any of the 17 polymorphisms studied in the abovementioned eight inflammation-related genes and risk of GC. Although this study had modest numbers of GC, lack of association is unlikely to be due to low power. For most of these SNPs, this study had >90% power to detect an odds ratio of 2.5 or higher. Furthermore, distributions of genotypes were strikingly similar in cases and controls, which suggests that even with larger sample sizes it would be unlikely to find a significant difference between these two groups. We did not correct *p*-values for the number of comparisons; had we done so, it would have been even less likely to find significant results.

We tested for multiple interactions between inflammation-related SNPs and environmental risk factors of non-cardia cancer in this population, i.e., *H. pylori*, and low intake of fruit, vitamin C, tocopherols, and lycopene. The only significant interaction was that between low fruit consumption and *TNF-A -238* ($p=0.045$). We attributed this significant term to multiple testing and did not further pursue interaction. However, we acknowledge that we did not have adequate power to test for interactions; interaction analysis may require over 1000 cases of GC, and probably needs pooling of several studies.

It has been suggested that pro-inflammatory polymorphisms only play a role in the etiology of specific subsets of GC, for example gastric non-cardia cancer (as opposed to gastric cardia cancer) [9], or intestinal type gastric cancers (as opposed to diffuse type cancers). Therefore, we analyzed the data separately for non-cardia cancers and intestinal type cancers. The results remained similar. However, there was a limited sample size for each of these subgroups. Previous studies have also suggested that higher numbers of pro-inflammatory genotypes will incrementally increase the risk of gastric non-cardia cancer [9]. We examined this association with a group of polymorphisms similar to the ones tested in previous studies and found no association. We also examined the association of specific haplotypes with GC risk and found no association.

There is no doubt that the original studies that found a positive association between pro-inflammatory polymorphisms had strong designs. However, several studies in various populations, including the current study, did not replicate the initial findings. The reasons for these differences are unclear to us. Investigating several aspects of the previous studies did not help in finding common features between studies that found positive association for individual SNPs and those that did not. For example, for the association between *IL-1B -511 T* and GC risk, although positive associations were generally found in larger studies, the second largest study showed a null association. Similarly, although most positive associations were from Western and most null associations were from East-Asian studies, counter-examples can be found. These findings may implicate dominance of different etiologic factors in different populations.

The effects of inflammatory polymorphisms might have been masked by smoking. However, smokers constitute a large proportion of most populations, and therefore a large proportion of the subjects in some of the previous studies. Furthermore, many studies conducted within the ATBC study population have shown that risk factors of most cancers are not significantly different from those seen in other populations. For gastric non-cardia cancer, for example, age, *H. pylori*, and low fruit and vitamin C were all strong risk factors [23, 24].

In summary, we examined the association between several inflammation-related polymorphisms and risk of GC. Positive associations between pro-inflammatory genotypes and higher risk of GC, previously reported in other populations, were not replicated among Finnish male smokers. These findings may implicate different etiologies in different populations.

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