Role of Cytokine Gene Polymorphisms in Gastric Cancer Risk in Chile

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Abstract. *Aim: To assess the role of pro- and anti-inflammatory polymorphisms in gastric cancer susceptibility. Patients and Methods: We genotyped 12 polymorphisms in eight cytokine genes (Interleukin-1β -IL1B-, IL8, IL17A, IL17F, IL32, tumor necrosis factor-α –TNF-, IL1RN, IL10) in a case–control study of 147 patients with gastric cancer and 172 controls. Results: Single polymorphism analysis revealed an association between the IL10 -592C>A single nucleotide polymorphism and cases with moderately- or well-differentiated tumors [AA vs. GG, odds ratio (OR)=3.01; 95% confidence interval (CI)=1.08-8.50]. We further analyzed gene–gene interactions using a combined attribute network implemented in multifactor dimensionality reduction software. The analysis revealed an interaction between IL8 -251A>T and IL32 rs28372698 SNPs among cases with moderately- or well-differentiated tumors. Homozygosity for both IL8 -251T and IL32 T alleles increases the odds for developing gastric cancer up to 2.63-fold (OR=2.63; 95% CI=1.15-6.03). This association was higher compared to the homozygosity for the IL8-251 T allele alone (OR=1.11; 95% CI=0.51-2.43) or the IL32 T allele alone (OR=1.21; 95% CI=0.54-2.72). Conclusion: These findings suggest that IL10 -592C>A increases the odds for developing gastric cancer. An interaction between IL8 -251A>T and IL32 rs28372698 SNPs is also proposed.*

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Key Words: Gastric cancer, polymorphisms, SNP, cytokine, interleukin, interaction.

Gastric cancer (GC) is the sixth most common cancer and the third leading cause of death due to cancer worldwide (1). Mortality due to GC varies according to world region, from 15.2 [age-standardized rate (ASR)] per 100,000 in East Asia to 2.3 (ASR per 100,000) in North America. Chile is the 16th higher mortality country from GC in both sexes (15.0 ASR per 100,000) and the 11th top country for mortality from GC in men (23.1 ASR per 100,000); therefore, Chile has one of the highest GC mortality rates worldwide. Risk factors for GC include high salt intake, low fruit and vegetable consumption, smoking, *Helicobacter pylori* infection, and genetic factors (2).

Much attention has been attributed on the role of inflammation-related polymorphisms after El-Omar *et al.* proposed that functional polymorphisms in the interleukin-1 beta gene (*IL1B*) and a variable number of tandem repeats (VNTR) located in the interleukin-1 receptor antagonist gene (*IL1RN*) are associated with a two- to three-fold increased GC risk (3). Since that time, many studies have been published analyzing the contribution of polymorphisms on *IL1B, IL1RN, IL8, IL10*, and tumor necrosis factor-α (*TNF*) to GC susceptibility (4-7), with conflicting results explained, in part, by ethnic differences, as well as both tumor localization and histological type. Other polymorphisms which have gained attention recently are rs2275913 (-197G>A) of *IL17A* (also known as *IL17*) gene, and rs763780 (p.His161Arg) of *IL17F* gene. Both belong to the IL17 family of cytokines produced by the cluster of differentiation 4 positive ($CD4^+$) effector T-helper 17 (Th17) cell subset. Th17 cells accumulate in the tumor microenvironment of gastric cancer, and IL17 expression is related to tumor progression features such as infiltration, lymph vascular invasion, and lymph node involvement (8, 9).

In the past decade, IL32 has been described as a pro-inflammatory cytokine produced by T-cells, natural killer (NK) cells, and epithelial cells (10) involved in diseases with an

inflammatory background. Recently, Ishigami *et al*. found that IL32 is expressed in gastric adenocarcinoma and concluded that its expression is associated with tumor depth, lymph node metastasis, and lymphatic and venous invasion, proposing that this cytokine is a prognostic marker (11). In general, little is known regarding the role of *IL32* genetic variation in cancer susceptibility. A recent article by Platinga *et al*. found that the promoter polymorphism rs28372698 has functional consequences for mRNA expression and is associated with thyroid carcinoma (12).

In the present study, we assessed the role of polymorphisms in *IL1B, IL1RN, IL8 IL10, TNF, IL17A, IL17F*, and *IL32* genes in GC susceptibility.

Patients and Methods

Patients. A total of 147 individuals with preoperative diagnosis of gastric adenocarcinoma were recruited between December 2010 and October 2013 from different hospitals located in Santiago de Chile: University of Chile Clinical Hospital, Salvador Hospital, Barros Luco Trudeau Hospital, and San Juan de Dios Hospital. Patients were invited to donate a blood sample for a genetic study regarding GC susceptibility. Only cases with a histopathological postoperative diagnosis of gastric adenocarcinoma were included. In all cases, the tumor was located distally from the cardia. Cases which met criteria for genetic syndromes including GC were excluded. Clinicopathological features of included patients are shown in Table I.

The sample of 172 controls (55 men and 117 women) with a mean age of 43.5±16.1 (range=20 to 82) years was obtained from individuals who underwent endoscopy, requested by their physician, at the Department of Gastroenterology at the University of Chile Clinical Hospital. Participants were invited to donate a blood sample for a genetic study regarding gastric cancer susceptibility, participating as healthy controls. Those with evidence of peptic or duodenal ulcer or endoscopic evidence suggestive of premalignant lesions (such as atrophic gastritis or intestinal metaplasia) were excluded. Both cases and controls declared Chilean ancestry dating from at least two generations.

Ethical approval. This study was approved by the Ethical Committee of the following institutions: University of Chile School of Medicine (#023/2011), University of Chile Clinical Hospital (#029/2011), Metropolitan South-Santiago Public Health Agency (#MK523B-118), Metropolitan East-Santiago Public Health Agency (#24/01/2012), and Metropolitan West-Santiago Public Health Agency (#236/2009). All participants gave their written informed consent. The study was performed in accordance with the Declaration of Helsinki.

Genotyping. Genomic DNA was isolated from peripheral blood leukocytes according to the method described by Chomczynski and Sacchi (13). Genotyping of SNPs was carried out by TaqMan SNP assay according to the manufacturer's protocol (Applied Biosystems, Foster City, CA, USA). The assays were used for the following SNPs studied: C_9546517_10 (rs1143634, *IL1B* 3954C>T); C_1839944_10 (rs1143627, *IL1B* -31 T>C); C_183943_10 (rs16944, *IL1B* -511C>T); C_1839941_10 (rs1143623, *IL1B* -1473 G>C); C_11748116_10 (rs4073, *IL8* -251 T>A); C__15879983_10 (rs2275913, IL-17 -197G>A); C___2234166_10 (rs763780, *IL17F* p.His161Arg); C___7514879_10 (rs1800629, *TNF* -308G>A); C___1747363_10 (rs1800872, IL10 -592 C>A); C___1747360_10 (rs1800896, *IL10* -1082A>G); C__64281225_10 (rs28372698, IL32). Genotyping of *IL1RN* 86-bp VNTR polymorphism was carried out by PCR according to El-Omar *et al*. (3).

Table I. *Clinicopathological characteristics of patients with gastric cancer.*

Variable	Gastric cancer (%)
TNM stage	
IA	18 (12.2%)
IB	12 (8.2%)
IIA	12 (8.2%)
IIB	8 (5.4%)
IIIA	19 (12.9%)
IIIB	28 (19.0%)
IIIC	39 (26.5%)
IV	11 (7.5%)
Borrmann	
0	26 (17.7%)
I	6 (4.1%)
II	14 (9.5%)
III	67 (45.6%)
IV	26 (17.7%)
V	8 (5.4%)
Histological type	
Poorly-differentiated with signet ring cells	41 (27.9%)
Poorly-differentiated no signet ring cells	31 (21.1%)
Poorly to moderately-differentiated	18 (12.2%)
Moderately-differentiated	36 (24.5%)
Moderately- to well-differentiated	6 (4.1%)
Well-differentiated	15 (10.2%)
Tumor size	
<5 cm	52 (35.4%)
≥5 cm	95 (64.6%)

Statistical analyses. The Hardy–Weinberg equilibrium assumption was assessed in the control sample using a goodness-of-fit chi-square test (HWChisq function included in Hardy-Weinberg package v1.4.1, available from <http:cran.r-project.org/ package=HardyWeinberg>). Fisher's exact test (oddsratio.fisher function included in epitools package v0.5-6, available from <cran.r-project.org/ package=epitools>) or Pearson's Chi-squared test for independence (chisq.test function) was used to test the association of genotypes and alleles in cases and controls. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate the strength of the association in cases and controls (oddsratio.fisher function included in epitools package v0.5-6). A two-sided p-value <0.05 was used as the criterion for significance. Haplotype estimation and linkage disequilibrium (LD) measures were carried out using UNPHASED v 3.1.6 software, which uses a maximum likelihood approach (14). A combined attribute network implemented in multifactor dimensionality reduction (MDR) 3.0.2 (<http://sourceforge.net/projects/mdr/>) was performed to detect pair of genes which potentially interact. All statistical analyses were performed using the R statistical environment (available at <http://www.r-project.org/>), unless otherwise indicated.

Results

All 319 participants included in the present study were successfully genotyped for the 11 SNPs and the *IL1RN* VNTR polymorphism. Genotypic frequencies in the control group were in agreement with those expected under the Hardy–Weinberg equilibrium. There were no statistically significant differences in genotypic frequencies between men and women ($p>0.371$) and between those aged more or less than 50 years ($p>0.455$) in the control group. Genotypic frequencies for each analyzed polymorphism are described in Table II.

Table III summarizes the association of each polymorphism with GC. No association was observed for the analyzed polymorphisms except for the *IL10* -592C>A polymorphism. Homozygosity for the minor allele of *IL10* -592C>A increased the odds for developing GC up to 2.43-fold (AA *vs*. GG: OR=2.43, 95% CI=1.01-6.15, $p=0.04$). Association was higher in cases with moderately or well-differentiated tumors (AA *vs*. GG: OR=3.01, 95% CI=1.08-8.50, $p=0.02$) than in cases with poorly-differentiated tumors (AA *vs*. GG: OR=1.83, 95% CI=0.54-5.80, $p=0.26$).

A total of three haplotypes were estimated for the *IL1B* loci for the entire sample cohort, which is consistent with the strong linkage disequilibrium observed for the three analyzed SNPs belonging to the *IL1B* loci (*IL1B* -511T>C/*IL1B* -31 C>T: D'=1.0, $r^2=0.93$; *IL1B* -31C>T/IL1B +3954C>T: D'=0.90, $r^2=0.18$; IL1B -511T>C/*IL1B* +3954C>T: D'=0.90, $r^2=0.16$). The distribution of haplotype frequencies was not significantly different between the control and GC samples ($p=0.69$, Pearson's chi-squared test for independence).

Only three haplotypes were estimated for the *IL10* loci: G-C, A-C, and A-A; haplotype G-A was not observed in our sample. Both polymorphisms (*IL10* -1082A>G and *IL10* -592C>A) were in strong LD (D'=1.0, $r^2=0.17$). No statistically significant differences were seen in the haplotype frequency distribution between the GC and control samples ($p=0.51$, Pearson's chi-squared test for independence).

We finally asked whether there were gene–gene interactions among the analyzed polymorphisms. To address this issue, we performed a combined attribute network implemented in MDR 3.0.2 in order to find candidate gene–gene interactions. Information gain (IG) values between pairs of polymorphisms were used. IG is a measure of the benefit of considering two polymorphisms as one unit (a pair) compared to considering them separately (a measure of synergistic interaction). We consider values of IG>0.5% as being suggestive of synergistic interaction. Only the pair *IL8* -251A>T and IL32 rs28372698 A>T gave an IG value >0.5%. The frequency of individuals homozygous for both *IL8*-251T and *IL32* rs28372698 T alleles was higher in the whole GC sample (17.0%) than in controls (9.3%) (OR=1.99, 95% CI=0.97-4.19, $p=0.05$) (Table IV). When we considered well- or moderately-differentiated GC cases, the association reached statistical significance (OR=2.63, 95% CI=1.15-6.03, $p=0.01$). Therefore, homozygosity for both *IL8* -251T and *IL32* rs28372698 T alleles increases the odds for developing GC up to 2.63-fold. The association was higher compared to the effect of being homozygous for the *IL8*-251 T allele alone (OR=1.11, 95% CI=0.1-2.43, $p=0.86$) or the *IL32* rs28372698 T allele alone (OR=1.21, 95% CI=0.54-2.72, $p=0.70$). This result led us to propose that *IL8* -251T and *IL32* rs28372698 T alleles act synergistically to increase the risk for developing well- to moderately differentiated GC.

Discussion

Since the finding of the association of pro-inflammatory polymorphisms with GC by El-Omar *et al*. (3), much effort has been made to associate diverse pro- and anti-inflammatory polymorphisms with GC in different ethnicities. In the present study, we interrogated 12 polymorphisms in order to find associations with GC in the Chilean population.

We did not detect any association with GC for the following polymorphisms: *IL1B* -511C>T, *IL1B* -31T>C, *IL1B* +3954C>T, *IL1RN* VNTR, *IL8* -251A>T, *IL-17* -197G>A, *IL17F* His161Arg, *TNF* -308G>A, *IL10*-1082A>G, and *IL32* rs28372698 A>T. In 2010, Xue *et al*. published a meta-analysis of 18 studies of *IL1B* -511, 21 studies analyzing *IL1B* -31, 10 of *IL1B* +3454, and 20 of *IL1RN* VNTR (15). They did not find statistically significant associations for the three polymorphisms analyzed with GC overall. However, after stratifying for ethnicity, they found a slightly higher association for carrying -511 T among Caucasians (n=8 studies) (OR=1.33, 95% CI=1.04-1.71, $p=0.02$) compared to Asians (n=8 studies) (OR=1.13, 95% CI=0.97-1.31, $p=0.01$). The tendency was the same for *IL1RN**2. Six studies on Caucasian subjects analyzing +3454 revealed no association with GC (OR=1.00, 95% CI=0.86-1.16, $p=0.98$). When histological type was considered, the association of -511 and *IL1RN* was higher among cases with intestinal-type (-511: OR=1.55, 95% CI=1.05-2.28, $p=0.03$; *IL1RN*: OR=1.66, 95% CI=1.23-2.25, $p=0.001$) than in cases with diffuse-type carcinoma (n=5) (-511: OR=1.09, 95% CI=0.84-1.40, $p=0.53$; *IL1RN*: OR=1.11, 95% CI=0.78-1.58, $p=0.56$). Our results show that the association for -511 tended to be slightly higher among cases with well- or moderately-differentiated tumors (OR=1.36, 95% CI=0.67-2.92, $p=0.41$) than among cases with poorly differentiated tumors (OR=1.01, 95% CI=0.51-2.07, $p=1.00$).

A meta-analysis published by Xu *et al*. reported different frequencies for Asians *vs*. Caucasians *IL1B* -511 T allele (4). Our *IL1B* -511T frequency finding falls into the distribution of frequencies described for the Asian population. Zeng *et al*. studied *IL1B* polymorphisms in two Chinese regions: one with a high GC prevalence (Sanxi, mortality rate 23.6/100,000) and another with low GC prevalence (Guangdong, mortality rate 7.39/100,000), and found that the frequency of -511T was higher in the high-prevalence region (0.51) than in the low-

Table II. *Genotypic and allelic frequencies of the analyzed polymorphisms*.

	n (%)			
	Controls	All GC cases	Well- or moderately-differentiated tumor	Poorly-differentiated tumor
IL1B +3954C>T (rs1143634) HWE *p*=0.90				
CC	122 (70.9%)	111 (75.5%)	59 (78.7%)	52 (72.2%)
CT	46 (26.7%)	31 (21.1%)	13 (17.3%)	18 (25.0%)
TT	4 (2.3%)	5 (3.4%)	3 (4.0%)	2 (2.8%)
T Allele	54 (0.16)	41 (0.14)	19 (0.13)	22 (0.15)
IL1B -31T>C (rs1143627) HWE *p*=0.90				
TT	36 (20.9%)	31 (21.1%)	14 (18.7%)	17 (23.6%)
TC	87 (50.6%)	72 (49.0%)	36 (48.0%)	36 (50.0%)
CC	49 (28.5%)	44 (29.9%)	25 (33.3%)	19 (26.4%)
C Allele	185 (0.54)	160 (0.54)	86 (0.57)	74 (0.51)
IL1B -511C>T (rs16944) HWE *p*=0.75				
CC	41 (23.8%)	31 (21.1%)	14 (18.7%)	17 (23.6%)
CT	83 (48.3%)	72 (49.0%)	36 (48.0%)	36 (50.0%)
TT	48 (27.9%)	44 (29.9%)	25 (33.3%)	19 (26.4%)
T Allele	179 (0.52)	160 (0.54)	86 (0.57)	74 (0.51)
IL1RN				
11	74 (43.0%)	64 (43.5%)	32 (42.7%)	33 (45.8%)
12	74 (43.0%)	57 (38.8%)	30 (40.0%)	27 (37.5%)
22	21 (12.2%)	20 (13.6%)	9 (12.0%)	11 (15.3%)
13	2 (1.2%)	2 (1.4%)	2 (2.7%)	0 (0.0%)
14	1 (0.6%)	2 (1.4%)	0 (0.0%)	2 (2.8%)
23	0 (0.0%)	2 (1.4%)	2 (2.7%)	0 (0.0%)
LL	77 (44.8%)	68 (46.3%)	34 (45.3%)	35 (48.6%)
L2	74 (43.0%)	59 (40.1%)	32 (42.7%)	27 (37.5%)
22	21 (12.2%)	20 (13.6%)	9 (12.0%)	11 (15.3%)
2 Allele	116 (0.34)	99 (0.34)	50 (0.33)	49 (0.34)
IL8 -251A>T (rs4073) HWE *p*=0.73				
AA	26 (15.1%)	22 (15.0%)	11 (14.7%)	11 (15.3%)
AT	78 (45.3%)	56 (38.1%)	28 (37.3%)	28 (38.9%)
TT	68 (39.5%)	69 (46.9%)	36 (48.0%)	33 (45.8%)
T Allele	214 (0.62)	194 (0.66)	100 (0.67)	94 (0.65)
IL10 -592C>A (rs1800872) HWE *p*=0.10				
CC	78 (45.3%)	55 (37.4%)	28 (37.3%)	27 (37.5%)
CA	83 (48.3%)	73 (49.7%)	35 (46.7%)	38 (52.8%)
AA	11 (6.4%)	19 (12.9%)	12 (16.0%)	7 (9.7%)
A Allele	105 (0.31)	111 (0.38)	59 (0.39)	52 (0.36)
IL10 -1082A>G (rs1800896) HWE *p*=0.91				
AA	88 (51.2%)	79 (53.7%)	43 (57.3%)	36 (50.0%)
GA	71 (41.3%)	54 (36.7%)	25 (33.3%)	29 (40.3%)
GG	13 (7.6%)	14 (9.5%)	7 (9.3%)	7 (9.7%)
A Allele	247 (0.72)	212 (0.72)	111 (0.74)	101 (0.70)
IL17 -197G>A (rs2275913) HWE *p*=0.91				
GG	105 (61.0%)	103 (70.1%)	54 (72.0%)	49 (68.1%)
GA	59 (34.3%)	36 (24.5%)	16 (21.3%)	20 (27.8%)
AA	8 (4.7%)	8 (5.4%)	5 (6.7%)	3 (4.2%)
A Allele	75 (0.22)	52 (0.18)	26 (0.17)	26 (0.18)
IL17F c.482A>G, p.His161Arg (rs763780)				
TT	160 (93.0%)	136 (92.5%)	71 (94.7%)	65 (90.3%)
TC	12 (7.0%)	11 (7.5%)	4 (5.3%)	7 (9.7%)
CC	0 (0.0%)	0 (0.0%)	(0.0%)	(0.0%)
C Allele	12 (0.03)	11 (0.04)	4 (0.03)	7 (0.05)
IL32 rs28372698 HWE *p*=0.19				
TT	59 (34.3%)	54 (36.7%)	34 (45.3%)	20 (27.8%)
TA	91 (52.9%)	76 (51.7%)	32 (42.7%)	44 (61.1%)
AA	22 (12.8%)	17 (11.6%)	9 (12.0%)	8 (11.1%)
A Allele	135 (0.39)	110 (0.37)	50 (0.33)	60 (0.42)

Table II. *Continued*

Table II. *Continued*

	n (%)			
	Controls	All GC cases	Well- or moderately-differentiated tumor	Poorly-differentiated tumor
TNF -308G>A (rs1800629)				
GG	147 (85.5%)	128 (87.1%)	68 (90.7%)	60 (83.3%)
GA	23 (13.4%)	18 (12.2%)	7 (9.3%)	11 (15.3%)
AA	2 (1.2%)	1 (0.7%)	0 (0.0%)	1 (1.4%)
A Allele	27 (0.08)	20 (0.07)	7 (0.05)	13 (0.09)

HWE: Hardy-Weinberg equilibrium.

prevalence region (0.34), concluding that the high frequency of the -511T allele among inhabitants of the high-prevalence region could explain the high incidence (16). Zabaleta *et al*. also proposed that differences in frequencies of pro-inflammatory alleles among populations may contribute to differences in cancer incidence (17). Other studies conducted in Hispanic countries with GC mortality rates as high as in Chile have reported a -511T allelic frequency similar to those obtained in our study: 0.58 in Peru (18), and 0.52 in Costa Rica (19). Taken together, despite the failure to detect an association of *IL1B* polymorphisms with GC, the high frequency of pro-inflammatory *IL1B* polymorphisms could contribute to explaining the high incidence of GC observed in Chile.

Neither IL8 -251A>T nor *TNF* -308G>A were significantly associated with GC in our study. A recent meta-analysis confirms a previous observation that -251A>T (rs4073) polymorphism is not significantly associated overall with GC in Caucasians; nevertheless, it is significantly associated with GC overall in Asians (OR=1.32, 95% CI=1.09-1.99). A meta-analysis published recently reports that *TNF* -308G>A AA genotype is associated with GC (7). Nevertheless, we found fewer than five AA homozygotes in GC as well as in controls, a fact that is consistent with the -308A frequency described in the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP) for Latino populations. Therefore, we were not able to test the association of this genotype with GC.

Much attention has been paid to the role of Th-17 response in gastric carcinogenesis. We examined two SNPs: rs2275913 (-197G>A) of the *IL17A* gene (also known as *IL17*), and rs763780 (p.H161A) on the *IL17F* gene. Both belong to the IL17 family of cytokines produced by the $CD4^+$ effector Th17 cell subset. We did not find an association for either SNP with GC. There is evidence of a functional effect of rs2275913 (-197G>A). Stimulated peripheral mononucleated cells from donors harboring the A allele showed higher IL17 production than those with the GG genotype (20). Six studies have investigated the association between this polymorphism and GC among Chinese (21-23), Japanese (24, 25), and Iranian (26) populations. All but one of those studies (21) showed that the AA genotype increases the risk of GC 2- to 3-fold. To the best of our knowledge, no association studies of rs2275913 with GC have been conducted among Caucasians or Latino populations. Therefore, it is possible that the risk conferred by the AA genotype could be restricted to Asian and Middle-Eastern populations.

IL17F rs763780 (p.H161A) polymorphism has been categorized as a risk factor for many diseases with an inflammatory background, the majority of them studied in Asian populations. In particular, studies conducted in China (22, 23) and Japan (25) found an association of the rs763780 minor allele with GC. Intriguingly, studies conducted in Caucasian or mixed populations have failed to associate this polymorphism with disease (27-30). Therefore, as proposed for SNP -197G>A, it is possible that the risk conferred by *IL17F* rs763780 could be restricted to Asian populations.

To the best of our knowledge, no other study has analyzed the role of *IL32* rs28372698 in any type of cancer, except those published by Platinga *et al*. (12), who found an association with epithelial cell-derived thyroid carcinoma. IL32 expression in gastric adenocarcinoma has been linked to the inflammatory response occurring in the gastric mucosa (11,31); consequently, it could be hypothesized that rs28372698 is associated with GC. Our results failed to detect any significant association with GC. Nevertheless, there may be a non-significant association with moderately/well-differentiated GC (TT *vs*. TA+AA: OR=1.58, 95% CI=0.88-2.86, p=0.12). Further studies are required to determine the role of IL32 rs28372698 in GC risk.

A statistically significant association of SNP *IL10* -592C>A was detected. We found that the AA genotype increases the odds for developing GC up to 3-fold. A meta-analysis published by Xue *et al*. reveals that the AA genotype is associated with a reduced risk of developing gastric cancer among Asians (6); meanwhile, among Caucasians the results are conflicting. The discrepancy could be due to the markedly different frequencies for allele A: 0.7 in Asians, 0.2 in Europeans, and 0.5 in Africans. Our finding is consistent with those published by El-Omar *et al*. (32) and Zambon *et al*. (33). In the former, conducted on a sample from the United States, only the AA genotype was

Table III. *Odds ratios (95% confidence intervals) for gastric cancer in relation to the analyzed polymorphisms.*

	All GC cases	Well- or moderately-differentiated tumor	Poorly-differentiated tumor
IL1B +3954C>T (rs1143634)			
CT+TT *vs*. CC	0.79 (0.46-1.34), *p*=0.38	0.66 (0.32-1.30), *p*=0.27	0.94 (0.48-1.79), *p*=0.48
TT *vs*. CT+CC	1.47 (0.31-7.54), *p*=0.74	1.74 (0.25-10.5), *p*=0.44	1.19 (0.11-8.54), *p*=1.00
TT *vs*. CC	1.37 (0.29-7.10) *p*=0.74	1.55 (0.22-9.47), *p*=0.68	1.17 (0.10-8.47), *p*=1.00
T Allele	0.87 (0.55-1.38), *p*=0.58	0.78 (0.42-1.40), *p*=0.41	0.97 (0.54-1.70), *p*=1.00
IL1B -31C>T (rs1143627)			
CC+TC *vs*. TT	0.99 (0.56-1.77), *p*=1.00	1.15 (0.56-2.49), *p*=0.73	0.86 (0.43-1.77), *p*=0.73
CC *vs*. TC+TT	1.07 (0.64-1.79), *p*=0.81	1.56 (0.82-2.97), *p*=0.45	0.90 (0.46-1.73), *p*=0.88
CC *vs*. TT	1.04 (0.53-2.06), *p*=1.00	1.31 (0.56-3.13), *p*=0.56	0.82 (0.35-1.94), *p*=0.69
C Allele	1.00 (0.72-1.39), *p*=0.87	1.14 (0.76-1.72), *p*=0.49	0.90 (0.60-1.35), *p*=0.69
IL1B -511T>C (rs16944)			
CT+TT *vs*. CC	1.17 (0.67-2.07), *p*=0.59	1.36 (0.67-2.92), *p*=0.41	1.01 (0.51-2.07), *p*=1.00
TT *vs*. CT+CC	1.10 (0.66-1.85), *p*=0.71	1.29 (0.69-2.40), *p*=0.45	0.92 (0.47-1.79), *p*=0.88
TT *vs*. CC	1.21 (0.62-2.36), *p*=0.64	1.52 (0.66-3.61), *p*=0.33	0.95 (0.41-2.24), *p*=1.00
T Allele	1.10 (0.80-1.52), *p*=0.58	1.23 (0.83-1.86), *p*=0.28	0.97 (0.65-1.47), *p*=0.92
IL1RN			
L2+22 *vs*. LL	0.94 (0.59-1.50), *p*=0.88	0.97 (0.55-1.75), *p*=1.00	0.88 (0.49-1.58), *p*=0.68
22 *vs*. L2+ LL	1.13 (0.55-2.30), *p*=0.74	0.98 (0.37-2.38), *p*=1.00	1.27 (0.52-2.96), *p*=0.54
22 *vs*. LL	1.07 (0.51-2.29), *p*=0.86	0.97 (0.35-2.50), *p*=1.00	1.15 (0.45-2.83), *p*=0.83
2 Allele	1.00 (0.71-1.41), *p*=1.00	0.98 (0.64-1.50), *p*=1.00	0.99 (0.64-1.52), *p*=1.00
IL8 -251A>T (rs4073)			
TT+AT *vs*. AA	1.01 (0.55-1.87), *p*=1.00	1.03 (0.46-2.47), *p*=1.00	0.99 (0.44-2.36), *p*=1.00
TT *vs*. AT+AA	1.35 (0.85-2.16), *p*=0.21	1.41 (0.79-2.53), *p*=0.26	1.29 (0.71-2.34), *p*=0.39
TT *vs*. AA	1.19 (0.59-2.45), *p*=0.61	1.25 (0.52-3.14), *p*=0.69	1.14 (0.48-2.90), *p*=0.84
T Allele	1.17 (0.85-1.65), *p*=0.36	1.21 (0.80-1.86), *p*=0.36	1.14 (0.75-1.76), *p*=0.53
IL10 -592C>A (rs1800872)			
AA+CA *vs*. CC	1.39 (0.86-2.23), *p*=0.17	1.39 (0.77-2.54), *p*=0.27	1.38 (0.76-2.54), *p*=0.32
AA *vs*. CA+CC	2.17 (0.94-5.24), *p*=0.05	2.77 (1.06-7.34), *p*=0.03	1.57 (0.49-4.67), *p*=0.42
AA *vs*. CC	2.43 (1.01-6.15), *p*=0.04	3.01 (1.08-8.50), *p*=0.02	1.83 (0.54-5.80), *p*=0.26
A Allele	1.38 (0.98-1.94), *p*=0.06	1.47 (0.97-2.24), *p*=0.06	1.28 (0.83-1.97), *p*=0.24
IL10 -1082A>G (rs1800896)			
GG+AG *vs*. AA	0.90 (0.56-1.44), *p*=0.65	0.78 (0.43-1.39), *p*=0.41	1.05 (0.58-1.89), *p*=0.89
GG *vs*. AG+AA	1.26 (0.52-3.02), *p*=0.55	1.26 (0.41-3.57), *p*=0.62	1.31 (0.42-3.74), *p*=0.61
GG *vs*. AA	1.19 (0.49-2.96), *p*=0.68	1.10 (0.35-3.23), *p*=1.00	1.31 (0.40-3.90), *p*=0.60
G Allele	0.98 (0.69-1.41), *p*=1.00	0.89 (0.56-1.41), *p*=0.66	1.08 (0.69-1.69), *p*=0.74
IL17 -197G>A (rs2275913)			
AA+GA *vs*. GG	0.67 (0.41-1.10), *p*=0.09	0.61 (0.32-1.14), *p*=0.11	0.74 (0.39-1.36), *p*=0.31
AA *vs*. GA+GG	1.17 (0.38-3.71), *p*=0.80	1.46 (0.36-5.28), *p*=0.54	0.89 (0.15-3.86), *p*=1.00
AA *vs*. GG	1.02 (0.32-3.25), *p*=1.00	1.21 (0.30-4.45), *p*=0.77	0.80 (0.13-3.54), *p*=1.00
A Allele	0.77 (0.51-1.16), *p*=0.20	0.70 (0.41-1.16), *p*=0.27	0.79 (0.46-1.32), *p*=0.39
IL17F p.His161Arg (rs763780)			
AG *vs*. AA	1.08 (0.42-2.76), *p*=1.00	0.75 (0.17-2.59), *p*=0.78	1.43 (0.46-4.16), *p*=0.44
G Allele	1.07 (0.42-2.71), *p*=1.00	0.76 (0.18-2.56), *p*=0.79	1.41 (0.46-3.99), *p*=0.45
IL32 rs28372698 T>A			
TT+TA *vs*. AA	1.12 (0.54-2.35), *p*=0.86	1.07 (0.45-2.80). *p*=1.00	1.17 (0.47-3.21), *p*=0.83
TT *vs*. TA+AA	1.11 (0.68-1.81), *p*=0.72	1.58 (0.88-2.86), *p*=0.12	0.73 (0.38-1.40), *p*=0.36
TT *vs*. AA	1.18 (0.54-2.65), *p*=0.71	1.40 (0.54-3.88), *p*=0.52	0.93 (0.33-2.82), *p*=1.00
T Allele	1.08 (0.77-1.51), *p*=0.68	1.29 (0.85-1.98), *p*=0.22	0.90 (0.60-1.37), *p*=0.68
TNF -308G>A (rs1800629)			
AA+AG *vs*. GG	0.87 (0.43-1.74), *p*=0.75	0.61 (0.21-1.53), *p*=0.30	1.17 (0.50-2.62), *p*=0.70
A Allele	0.86 (0.45-1.63), *p*=0.65	0.57 (0.21-1.40), *p*=0.25	1.16 (0.53-2.42), *p*=0.72

found to increase the risk of noncardia GC (OR=2.4, 95% CI=1.1-5.1). Zambon *et al*. also reported that the AA genotype was over-represented among non-cardia GC cases from Italy. It should be noted that all GC cases included in the present study are classified as non-cardia GC. According to Rad *et al*. (34), gastric mucosa from Europeans harboring the AA genotype

Table IV. *Association of combined genotypes of interleukins IL8 -251A>T and IL32 rs28372698 A>T with gastric cancer.*

IL8 -251A>T	*IL32* rs28372698 A>T	Controls (%)	Cases (%)	OR (95% CI)	Well- or moderately-differentiated tumor (%)	OR (95%-CI)
AA+AT	AA+AT	61 (35.5%)	49 (33.3%)	1 (Reference)	21 (28.0%)	1 (Reference)
TT	AA+AT	52 (30.2%)	44 (29.9%)	1.05 (0.59-1.89), p=0.89	20 (26.7%)	1.11 (0.51-2.43), p=0.86
AA+AT	TT	43 (25.0%)	29 (19.7%)	0.84 (0.44-1.60), p=0.65	18 (24.0%)	1.21 (0.54-2.72), p=0.70
TT	TT	16 (9.3%)	25 (17.0%)	(a) 1.94 (0.88-4.35), p=0.10 (b) 1.99 (0.97-4.19), p=0.05	16 (21.3%)	(a) 2.87 (1.13-7.40), p=0.02 (b) 2.63 (1.15-6.03), p=0.01

OR:Odds ratio; CI: Confidence interval; (a): *versus* IL8 AA+AT / IL32 AA+AT; (b): *versus* non IL8 TT/IL32 TT.

secreted significantly less IL10 (measured as mRNA amount) as compared to those with the GG genotype. In an animal model, mice lacking IL10 expression developed gastritis at higher rates as compared to wild-type mice (35,36), a finding consistent with the fact that IL10 is considered as a potent anti-inflammatory cytokine (37). Taken together, the aforementioned studies in rodents support the findings of El-Omar *et al*. and Zambon *et al*., and those from the present study, suggesting that the AA genotype of IL10 -592C>A increases GC risk.

Finally, we asked whether there are gene–gene interactions between the polymorphisms analyzed. A potential synergistic interaction was observed between *IL8* -251A>T and *IL32* rs28372698. The analysis of moderately- or well-differentiated GC cases showed that homozygosity for both minor alleles had a higher OR (OR=2.87) than homozygosity for only the minor allele of *IL8* -251A>T (OR=1.11) or *IL32* rs28372698 (OR=1.21). Sakitani *et al*. observed a reduced expression of *IL8* in AGS cells knocked-down for *IL32*, a situation that was reversed by re-expression of IL32 (31). They concluded that IL32 functions as an intracellular regulator of IL8. Taken together, the results from Sakitani *et al*. support the possible synergy observed in our study between *IL8* -251A>T and *IL32* rs28372698. Additional evidence from further studies conducted in different populations is needed to confirm our finding.

In summary, we performed a case–control study with several polymorphisms of pro- and anti-inflammatory genes in GC cases. We detected significant association only for the *IL10* polymorphism, possibly due to differences in allelic frequency among populations. Our results support a possible synergistic interaction between *IL8* -251A>T and *IL32* rs28372698 SNPs, a finding that has not been previously reported and needs further analysis in other populations.

Acknowledgements

We would especially like to thank Paula Pino and Solange Cortes for their support in the inclusion of patients. Grant support was provided by Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT) #11110199, and Program U-INICIA VID 2011 University of Chile #11/03.

References

1 Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D and Bray F: GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 11, 2012. Available at http://globocan.iarc.fr.

2 Guggenheim DE and Shah MA: Gastric cancer epidemiology and risk factors. J Surg Oncol *107*: 230-236, 2013.

3 El-Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, Herrera J, Lissowska J, Yuan CC, Rothman N, Lanyon G, Martin M, Fraumeni JFJ and Rabkin CS: Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature *404*: 398-402, 2000.

4 Xu J, Yin Z, Cao S, Gao W, Liu L, Yin Y, Liu P and Shu Y: Systematic review and meta-analysis on the association between IL1B polymorphisms and cancer risk. PLoS ONE *8*: e63654, 2013.

5 Cheng D, Hao Y, Zhou W and Ma Y: Positive association between interleukin-8 -251A > T polymorphism and susceptibility to gastric carcinogenesis: a meta-analysis. Cancer Cell Int *13*: 100, 2013.

6 Xue H, Wang Y, Lin B, An J, Chen L, Chen J and Fang J: A meta-analysis of interleukin-10 -592 promoter polymorphism associated with gastric cancer risk. PLoS ONE *7*: e39868, 2012.

7 Zhu F, Zhao H, Tian X and Meng X: Association between tumor necrosis factor-α rs1800629 polymorphism and risk of gastric cancer: a meta-analysis. Tumour Biol *35*: 1799-1803, 2014.

8 Maruyama T, Mimura K, Izawa S, Shiba S, Watanabe M, Kawaguchi Y, Fujii H and Kono K: Immunonutritional diet modulates natural killer cell activation and Th17 cell distribution in patients with gastric and esophageal cancer. Nutrition *27*: 146-152, 2011.

9 Iida T, Iwahashi M, Katsuda M, Ishida K, Nakamori M, Nakamura M, Naka T, Ojima T, Ueda K, Hayata K, Nakamura Y and Yamaue H: Tumor-infiltrating CD4+ Th17 cells produce IL17 in tumor microenvironment and promote tumor progression in human gastric cancer. Oncol Rep *25*: 1271-1277, 2011.

10 Dinarello CA and Kim S: IL32, a novel cytokine with a possible role in disease. Ann Rheum Dis *65(Suppl 3)*: iii61-iii64, 2006.

11 Ishigami S, Arigami T, Uchikado Y, Setoyama T, Kita Y, Sasaki K, Okumura H, Kurahara H, Kijima Y, Harada A, Ueno S and Natsugoe S: IL32 expression is an independent prognostic marker for gastric cancer. Med Oncol *30*: 472, 2013.

12 Plantinga TS, Costantini I, Heinhuis B, Huijbers A, Semango G, Kusters B, Netea MG, Hermus ARMM, Smit JWA, Dinarello CA, Joosten LAB and Netea-Maier RT: A promoter polymorphism in human interleukin-32 modulates its expression and influences the risk and the outcome of epithelial cell-derived thyroid carcinoma. Carcinogenesis *34*: 1529-1535, 2013.
13 Chomczynski P and Sacchi N: The single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: twenty-something years on. Nat Protoc *1*: 581-585, 2006.
14 Dudbridge F: Likelihood-based association analysis for nuclear families and unrelated subjects with missing genotype data. Hum Hered *66*: 87-98, 2008.
15 Xue H, Lin B, Ni P, Xu H and Huang G: Interleukin-1B and interleukin-1RN polymorphisms and gastric carcinoma risk: a meta-analysis. J Gastroenterol Hepatol *25*: 1604-1617, 2010.
16 Zeng Z, Hu P, Hu S, Pang R, Chen M, Ng M and Sung JJY: Association of interleukin 1B gene polymorphism and gastric cancers in high and low prevalence regions in china. Gut *52*: 1684-1689, 2003.
17 Zabaleta J, Schneider BG, Ryckman K, Hooper PF, Camargo MC, Piazuelo MB, Sierra RA, Fontham ETH, Correa P, Williams SM and Ochoa AC: Ethnic differences in cytokine gene polymorphisms: potential implications for cancer development. Cancer Immunol Immunother *57*: 107-114, 2008.
18 Gehmert S, Velapatiño B, Herrera P, Balqui J, Santivañez L, Cok J, Vargas G, Combe J, Passaro DJ, Wen S, Meyer F, Berg DE and Gilman RH: Interleukin-1 beta single-nucleotide polymorphism's C allele is associated with elevated risk of gastric cancer in *Helicobacter pylori*-infected peruvians. Am J Trop Med Hyg *81*: 804-810, 2009.
19 Alpízar-Alpízar W, Pérez-Pérez GI, Une C, Cuenca P and Sierra R: Association of interleukin-1B and interleukin-1RN polymorphisms with gastric cancer in a high-risk population of Costa Rica. Clin Exp Med *5*: 169-176, 2005.
20 Espinoza JL, Takami A, Onizuka M, Kawase T, Sao H, Akiyama H, Miyamura K, Okamoto S, Inoue M, Ohtake S, Fukuda T, Morishima Y, Kodera Y, Nakao S: A single nucleotide polymorphism of *IL17* gene in the recipient is associated with acute GVHD after HLA-matched unrelated BMT. Bone Marrow Transplant *46*: 1455-1463, 2011.
21 Wu X, Zeng Z, Chen B, Yu J, Xue L, Hao Y, Chen M, Sung JJY and Hu P: Association between polymorphisms in interleukin-17A and interleukin-17F genes and risks of gastric cancer. Int J Cancer *127*: 86-92, 2010.
22 Qinghai Z, Yanying W, Yunfang C, Xukui Z and Xiaoqiao Z: Effect of interleukin-17A and interleukin-17F gene polymorphisms on the risk of gastric cancer in a chinese population. Gene *537*: 328-332, 2014.
23 Zhang X, Zheng L, Sun Y and Zhang X: Analysis of the association of interleukin-17 gene polymorphisms with gastric cancer risk and interaction with *Helicobacter pylori* infection in a Chinese population. Tumour Biol *35*: 1575-1580, 2014.
24 Arisawa T, Tahara T, Tsutsumi M and Shibata T: Influence of IL17A polymorphisms on the aberrant methylation of DAPK and CDH1 in non-cancerous gastric mucosa. BMC Med Genet *13*: 59, 2012.
25 Shibata T, Tahara T, Hirata I and Arisawa T: Genetic polymorphism of interleukin-17A and -17F genes in gastric carcinogenesis. Hum Immunol *70*: 547-551, 2009.
26 Rafiei A, Hosseini V, Janbabai G, Ghorbani A, Ajami A, Farzmandfar T, Azizi MD, Gilbreath JJ and Merrell DS: Polymorphism in the interleukin-17A promoter contributes to gastric cancer. World J Gastroenterol *19*: 5693-5699, 2013.
27 Saraiva AM, Alves e Silva MRM, Correia Silva JDF, da Costa JE, Gollob KJ, Dutra WO and Moreira PR: Evaluation of IL17A expression and of *IL17A, IL17F* and *IL23R* gene polymorphisms in brazilian individuals with periodontitis. Hum Immunol *74*: 207-214, 2013.
28 Navratilova Z, Gallo J, Mrazek F and Petrek M: Genetic variation in key molecules of the Th-17 immune response is not associated with risk for prosthetic joint infection in a Czech population. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub *156*: 248-252, 2012.
29 Durães C, Machado JC, Portela F, Rodrigues S, Lago P, Cravo M, Ministro P, Marques M, Cremers I, Freitas J, Cotter J, Tavares L, Matos L, Medeiros I, Sousa R, Ramos J, Deus J, Caldeira P, Chagas C, Duarte MA, Gonçalves R, Loureiro R, Barros L, Bastos I, Cancela E, Moraes MC, Moreira MJ, Vieira AI and Magro F: Phenotype-genotype profiles in Crohn's disease predicted by genetic markers in autophagy-related genes (GOIA study II). Inflamm Bowel Dis *19*: 230-239, 2013.
30 Paradowska A, Maśliński W, Grzybowska-Kowalczyk A and Łacki J: The function of interleukin 17 in the pathogenesis of rheumatoid arthritis. Arch Immunol Ther Exp (Warsz) *55*: 329-334, 2007.
31 Sakitani K, Hirata Y, Hayakawa Y, Serizawa T, Nakata W, Takahashi R, Kinoshita H, Sakamoto K, Nakagawa H, Akanuma M, Yoshida H, Maeda S and Koike K: Role of interleukin-32 in *Helicobacter pylori*-induced gastric inflammation. Infect Immun *80*: 3795-3803, 2012.
32 El-Omar EM, Rabkin CS, Gammon MD, Vaughan TL, Risch HA, Schoenberg JB, Stanford JL, Mayne ST, Goedert J, Blot WJ, Fraumeni JFJ and Chow W: Increased risk of noncardia gastric cancer associated with proinflammatory cytokine gene polymorphisms. Gastroenterology *124*: 1193-1201, 2003.
33 Zambon C, Basso D, Navaglia F, Falda A, Belluco C, Fogar P, Greco E, Gallo N, Farinati F, Cardin R, Rugge M, Di Mario F and Plebani M: Increased risk of noncardia gastric cancer associated with proinflammatory cytokine gene polymorphisms. Gastroenterology *126*: 382-384, 2004.
34 Rad R, Dossumbekova A, Neu B, Lang R, Bauer S, Saur D, Gerhard M and Prinz C: Cytokine gene polymorphisms influence mucosal cytokine expression, gastric inflammation, and host specific colonisation during *Helicobacter pylori* infection. Gut *53*: 1082-1089, 2004.
35 Sutton P, Kolesnikow T, Danon S, Wilson J and Lee A: Dominant nonresponsiveness to *Helicobacter pylori* infection is associated with production of interleukin 10 but not gamma interferon. Infect Immun *68*: 4802-4804, 2000.
36 Ismail HF, Fick P, Zhang J, Lynch RG and Berg DJ: Depletion of neutrophils in IL-10(–/–) mice delays clearance of gastric Helicobacter infection and decreases the Th1 immune response to Helicobacter. J Immunol *170*: 3782-3789, 2003.
37 Mosser DM and Zhang X: Interleukin-10: new perspectives on an old cytokine. Immunol Rev *226*: 205-218, 2008.

Received March 24, 2014
Revised May 20, 2014
Accepted May 21, 2014