Interleukin-4 receptor –3223C→T polymorphism is associated with increased gastric adenocarcinoma risk

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BACKGROUND: Gastric cancer remains one of the most common types of cancer worldwide, with a large geographical variation in incidence and mortality rates. Cytokine polymorphisms are the most studied host polymorphisms and are associated with an increased risk of stomach cancer in many regions, but have not been studied extensively in Eastern European populations.

OBJECTIVE: To investigate the potential association between five cytokine promoter polymorphisms (interleukin [IL] 1β −511C→T [rs16944], IL-4 receptor [IL-4R] −3223C→T [rs2057768], IL-8 −251T→A [rs4073], IL-10 −1082A→G [rs1800896] and tumour necrosis factor-alpha −308G→A [rs1800629]) and susceptibility to gastric adenocarcinoma in a Romanian population.

METHODS: A total of 347 subjects, consisting of 105 patients with gastric adenocarcinoma and 242 controls, were included. All cytokine polymorphisms were genotyped using allele-specific, commercially available probes. Hardy-Weinberg equilibrium in both groups was analyzed using the χ^2 test, and the relationship between targeted polymorphisms and the risk of gastric cancer was estimated using OR and 95% CI.

RESULTS: A significant association between the IL-4R −3223C→T polymorphism and risk of gastric cancer was found. Carriers of the IL-4R −3223TT genotype were at a 2.5-fold increased risk for gastric cancer (OR 2.51 [95% CI 1.08 to 5.84]; P=0.041). Moreover, the presence of the IL-4R −3223TT genotype was associated with an increased risk of noncardia gastric adenocarcinoma (OR 3.08 [95% CI 1.25 to 7.58]; P=0.023). No associations were found among the other polymorphisms.

CONCLUSION: The results suggest that the IL-4R −3223C→T polymorphism may increase the risk of gastric adenocarcinoma, mainly for the noncardia type, in the Romanian population.

Key Words: *Cytokine; Gastric cancer; Genetic predisposition; Promoter polymorphism*

Le polymorphisme du récepteur de l'interleukine 4 –3223 C→T s'associe à une augmentation du risque d'adénocarcinome gastrique

HISTORIQUE : Le cancer gastrique demeure l'un des principaux types de cancer de par le monde, bien que son incidence et son taux de mortalité varient considérablement sur le plan géographique. Les polymorphismes des cytokines sont les polymorphismes de l'hôte les plus étudiés et s'associent à un risque accru de cancer de l'estomac dans de nombreuses régions, mais ils n'ont pas fait l'objet d'études approfondies dans les populations d'Europe de l'Est.

OBJECTIF : Examiner l'association potentielle entre cinq polymorphismes promoteurs des cytokines (interleukine [IL] 1β −511C→T [rs16944], récepteur de l'IL-4 [IL-4R] −3223C→T [rs2057768], IL-8 −251T→A [rs4073], IL-10 −1082A→G [rs1800896] et facteur de nécrose tumorale alpha −308G→A [rs1800629]) et la susceptibilité à l'adénocarcinome gastrique dans une population roumaine.

MÉTHODOLOGIE : Au total, 347 sujets, soit 105 patients ayant un adénocarcinome gastrique et 242 sujets témoins, ont participé à l'étude. Les chercheurs ont procédé au génotypage de tous les polymorphismes des cytokines au moyen de sondes spécifiques à l'allèle offertes sur le marché. Ils ont analysé l'équilibre de Hardy-Weinberg dans les deux groupes au moyen de la méthode du khi-carré et ont évalué le lien entre les polymorphismes ciblés et le risque de cancer gastrique au moyen du RRR et de l'IC 95 %.

RÉSULTATS : Les chercheurs ont constaté une association significative entre le polymorphisme de l'IL-4R −3223C→T et le risque de cancer gastrique. Les porteurs du génotype de l'IL-4R −3223TT risquaient 2,5 fois plus de souffrir d'un cancer gastrique (RRR 2,51 [95 % IC 1,08 à 5,84]; P=0,041). De plus, la présence du génotype de l'IL-4R −3223TT s'associait à un risque plus élevé d'adénocarcinome gastrique ne touchant pas le cardia (RRR 3,08 [95 % IC 1,25 à 7,58]; P=0,023). Les chercheurs n'ont constaté aucun lien entre les autres polymorphismes.

CONCLUSION : D'après les résultats, le polymorphisme de l'IL-4R −3223C→T pourrait accroître le risque d'adénocarcinome gastrique au sein de la population roumaine, notamment s'il ne touche pas le cardia.

Despite advances in surgical treatment and chemotherapy, gastric cancer remains a major global health burden, and is the fourth most common cancer, and the second leading cause of cancer death in men and women worldwide (1).

Interactions of three major factors, including bacterial pathogenicity, host susceptibility and environmental factors, are involved in gastric carcinogenesis (2,3). It is well known that *Helicobacter pylori* infection causes chronic inflammation in the gastric mucosa and, thus, contributes to the transition from normal mucosa to chronic superficial gastritis, which may lead to atrophic gastritis, intestinal metaplasia and, finally, dysplasia/cancer (2,4). *H pylori* infection induces a predominant host inflammatory T helper cell (Th) type 1 response (5) that is mediated by Th1 cells, which consists of the gradual release of proinflammatory cytokines such as interleukin (IL)-1β, tumour necrosis factor-α (TNF-α), IL-8, etc. This response is balanced by an anti-inflammatory reaction mediated by Th2 cytokines, such as IL-10, and IL-4 (6), which limit potentially injurious or excessive inflammatory reactions. Therefore, genetic variations in inflammation-related genes, especially cytokines and their receptors, became potential therapeutic targets in gastric carcinogenesis.

Single nucleotide polymorphisms (SNPs) located within promoters of cytokine genes may affect messenger RNA levels and, consequently, the level of translated cytokine. Thus, SNPs in promoter regions can influence interindividual differences in disease susceptibility by increasing the proinflammatory response (IL-1β –31T→C, IL-1β –511C→T, IL-8 –251T→A, TNF-α –308G→A) or decreasing the anti-inflammatory host reaction (IL-4R –3223C→T, IL-10 –1082A→G, –819C→T and –592C→A). After the first report by El-Omar et al (7), showing that the IL-1β –31T→C polymorphism is a risk factor for gastric cancer (7), many more studies from different

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TABLE 1
Subject characteristics

	Gastric adenocarcinoma (n=105)	Control (n=242)
Male/female, n/n	66/39	152/90
Age, years, mean ± SD	64.22±5.65	60.69±7.94
Location, n		
Cardia	27	
Noncardia	78	
Histological type, n		
Intestinal	60	
Diffuse	44	
Mixed	1	

TABLE 2
Risk of gastric cancer according to genotype

Polymorphism	Gastric cancer (n=105)	Control (n=242)	OR (95%CI)	P
IL-1β −511C→T				
CC	52 (49.5)	110 (45.45)	Reference	–
CT	42 (40.0)	102 (42.15)	0.87 (0.54–1.42)	0.63
TT	11 (10.5)	30 (12.40)	0.78 (0.36–1.67)	0.56
T carriers	53 (50.5)	132 (54.55)	0.89 (0.54–1.34)	0.54
IL-4R −3223C→T				
CC	53 (50.5)	144 (59.5)	Reference	–
CT	40 (38.1)	85 (35.1)	1.28 (0.78–2.09)	0.43
TT	12 (11.4)	13 (5.4)	2.51 (1.08–5.84)	0.041
T carriers	52 (49.5)	98 (40.5)	1.44 (0.91–2.28)	0.17
IL-8 −251T→A				
TT	31 (29.5)	82 (33.9)	Reference	–
TA	54 (51.4)	112 (46.3)	1.27 (0.75–2.16)	0.22
AA	20 (19.1)	48 (19.8)	1.10 (0.57–2.14)	0.4
A carriers	74 (70.5)	160 (66.1)	1.22 (0.74–2.01)	0.82
IL-10 −1082 A→G				
AA	43 (40.9)	89 (36.8)	Reference	–
AG	49 (46.7)	118 (48.8)	0.86 (0.53–1.41)	0.6
GG	13 (12.4)	35 (14.4)	0.77 (0.37–1.60)	0.52
G carriers	62 (59.1)	153 (63.2)	0.84 (0.53–1.34)	0.51
TNF-α −308 G→A				
GG	78 (74.3)	196 (81.0)	Reference	–
GA	26 (24.8)	44 (18.2)	1.49 (0.86–2.58)	0.2
AA	1 (0.9)	2 (0.8)	1.26 (0.11–14.05)	0.9
A carriers	27 (25.7)	46 (19.0)	1.47 (0.86–2.54)	0.2

Data presented n (%) unless otherwise indicated. IL Interleukin; TNF Tumour necrosis factor

countries and regions regarding the association of different cytokine gene polymorphisms have been published, but not from Eastern Europe, where gastric cancer incidence and mortality is the highest in the continent (1).

In Romania, gastric cancer incidence and mortality rates remain high. Gastric cancer is the fifth most common malignancy and the third-ranked cause of cancer death, despite the decreased mortality rates of gastric cancer in many European countries (8). The prevalence of *H pylori* generally ranges from 40% in developed countries to more than 80% in developing countries (9), and was estimated to be 68.5% in the adult population of Romania (10).

Accordingly, we investigated polymorphisms located in the promoter regions of five cytokine genes (IL-1β −511C→T [rs16944], IL-4R −3223C→T [rs2057768], IL-8 −251T→A [rs4073], IL-10 −1082A→G [rs1800896] and TNF-α −308G→A [rs1800629]) in a Romanian population (ie, Eastern European population) to determine whether these polymorphisms are associated with gastric adenocarcinoma susceptibility.

METHODS

Subjects

A total of 347 Romanian subjects were included in the present study: 105 unrelated gastric cancer patients from the Clinical Hospital of Craiova (Craiova, Romania), and 242 age- and sex-matched healthy controls. All subjects underwent upper endoscopy and diagnosis of gastric cancer was made by histological examination of biopsy specimens. Tumours were classified as intestinal or diffuse type according to the classification proposed by Laurén (11). Only *H pylori*-positive patients were selected. *H pylori* infection was evaluated by histological examination, rapid urease test and/or anti-*H pylori* immunoglobulin G quantification. Patients were considered to be infected when at least one of these diagnostic tests was positive. Both control and gastric cancer groups consisted of Romanian individuals of the same ethnic and geographical origins. Individuals with a positive family history of gastric and other types of cancer or inflammatory diseases were excluded.

The Research Ethics Committee of the University of Medicine and Pharmacy of Craiova, Romania, approved the present study and written informed consent was obtained from all subjects.

Genotyping assay

Blood samples were collected from all subjects and genomic DNA was extracted from peripheral blood leukocytes using the Wizard Genomic DNA Purification Kit (Promega, USA) according to the manufacturer's protocol. Polymorphisms were selected on the basis of their previously published involvement in cancer risk and/or functional role. All cytokine polymorphisms were genotyped by allelic discrimination polymerase chain reaction assays (5′ nuclease assay) using predesigned TaqMan SNP Genotyping Assays (Applied Biosystems, USA): IL-1β −511C→T (rs16944, assay C_1839943_10); IL-4R −3223C→T (rs2057768, assay C_2769607_10); IL-8 −251T→A (rs4073, assay C_11748116_10); IL-10 −1082A→G (rs1800896, assay C_1747360_10) and TNF-α −308G→A (rs1800629, assay C_7514879_10). The genotyping assay was performed using the RotorGene 6200 HRM-Corbett Real Time PCR system, and assays were validated and optimized as described on the SNP500 Cancer website (http://snp500cancer.nci.nih.gov). To ensure quality control, DNA samples from case patients and controls were randomly distributed and all samples were blindly genotyped. All samples that did not yield a reliable result in the first round were resubmitted for up to two additional rounds of genotyping. Also included were a negative control sample and three positive controls (homozygous for the wild-type allele, and heterozygous and homozygous for the mutant allele).

Statistical data analysis

The χ^2 test was used to test the distribution of genotypes and allele frequencies for deviations from Hardy-Weinberg equilibrium. The linkage disequilibrium blocks were determined using D′ and r^2 values. Genotype frequencies of SNPs between patients with gastric cancer and the controls were compared using logistic regression, crude and adjusted ORs according to sex and age, and 95% CIs. The homozygous genotype of the common allele was used as the reference group. Recessive and dominant models were also used. All P values were two sided and $P<0.05$ was considered to be statistically significant. All data analysis was performed using SPSS version 17.0 (IBM Corporation, USA).

RESULTS

A total of 105 gastric adenocarcinoma patients and 242 healthy controls were genotyped. Table 1 summarizes the characteristics of gastric cancer patients and controls; there were no differences in distribution with respect to age, sex or ethnicity. Among the gastric cancer cases,

TABLE 3
Risk of cardia and noncardia gastric adenocarcinoma according to genotype

	Noncardia (n=78)		Cardia (n=27)	
Polymorphism	**n (%)**	**OR (95% CI); P**	**n (%)**	**OR (95% CI); P**
IL-1β −511C→T				
CC	39 (50.0)	Reference	13 (48.2)	Reference
CT	30 (38.5)	0.83 (0.48–1.43); 0.6	12 (44.4)	0.99 (0.43–2.28); 0.9
TT	9 (11.5)	0.84 (0.28–1.94); 0.7	2 (7.4)	0.66 (0.12–2.64); 0.5
IL-4R −3223C→T				
CC	36 (46.2)	Reference	17 (63.0)	Reference
CT	32 (41.0)	1.51 (0.87–2.60); 0.2	8 (29.6)	0.80 (0.33–1.93); 0.66
TT	10 (12.8)	3.08 (1.25–7.58); 0.023	2 (7.4)	1.30 (0.27–6.27); 0.8
IL-8 −251T→A				
TT	25 (32.1)	Reference	6 (22.2)	Reference
TA	38 (48.7)	1.11 (0.62–1.98); 0.7	16 (59.3)	1.95 (0.73–5.20); 0.2
AA	15 (19.2)	1.02 (0.49–2.13); 0.9	5 (18.5)	1.42 (0.41–4.91); 0.63
IL-10 −1082 A→G				
AA	34 (43.6)	Reference	9 (33.3)	Reference
AG	36 (46.1)	0.80 (0.46–1.38); 0.47	13 (48.2)	1.09 (0.45–2.66); 0.9
GG	8 (10.3)	1.79 (0.25–1.42); 0.28	5 (18.5)	1.41 (0.44–4.51); 0.6
TNF-α −308 G→A				
GG	57 (73.1)	Reference	21 (77.8)	Reference
GA	20 (25.6)	1.56 (0.85–2.86); 0.2	6 (22.2)	1.27 (0.48–3.34); 0.72
AA	1 (1.3)	1.72 (0.15–19.3); 0.7	0 (0)	–

IL Interleukin; TNF Tumour necrosis factor

60 (57%) were intestinal type, 44 (42%) were diffuse type and one (1%) was mixed type. Based on the site of tumour origin, the gastric cancer group was classified into two subgroups: cardia (n=27) and noncardia (n=78).

Among controls, genotype distributions for each assessed SNP was in Hardy-Weinberg equilibrium. No strong linkage disequilibrium was observed between polymorphisms ($r^2<0.33$). The genotype frequencies for all tested polymorphisms in gastric cancer and control groups are shown in Table 2.

A significant association was observed for the IL-4R –3223C→T polymorphism: subjects with the TT genotype were at a 2.5-fold elevated risk for gastric cancer (OR 2.51 [95% CI 1.08 to 5.84]; P=0.041) when compared with the more frequent CC genotype. In addition, the allele frequencies were significantly different between gastric cancer cases (C [69.5%] and T [30.5%]) and controls (C [77%] and T [23%]) (P=0.043) (data not shown). In a dominant model, carriers of the T allele were not associated with gastric cancer risk (OR 1.44 [95% CI 0.91 to 2.28]).

No significant differences between gastric adenocarcinoma cases and controls were found for IL-1β –511TT (OR 0.78 [95% CI 0.36 to 1.67]), IL-8 –251AA (OR 1.10 [95% CI 0.57 to 2.14]), IL-10 –1082GG (OR 0.84 [95% CI 0.53 to 1.34) and TNF-α –308AA (OR 1.47 [95% CI 0.86 to 2.54]) genotype polymorphisms (Table 2). Also, carriers of the IL-1β –511T, IL-8 –251A, IL-10 –1082G and TNF-α –308A alleles were not associated with an increased risk of gastric cancer in a recessive model or dominant model (data not shown).

Association of these polymorphisms with tumour site and histological type were examined separately. No significant differences were observed between gastric cancer site (noncardia and cardia) or gastric cancer histology (intestinal and diffuse) and controls in a stratified analysis for IL-1β –511C→T, IL-8 –251T→A, IL-10 –1082A→G and TNF-α –308G→A (Tables 3 and 4).

The only association between gastric adenocarcinoma and cytokine polymorphism was found for the IL-4R –3223TT genotype and was restricted to the noncardia subsite (OR 3.08 [95% CI 1.25 to 7.58]; P=0.023) (Table 3).

DISCUSSION

Cytokine polymorphisms are the most studied host genetic variants for association with gastric cancer in many regions, but not in Eastern European populations. Because such a list of candidate genes would be prohibitively extensive, our initial search focused on SNPs located in promoter regions that are most relevant to gastric physiology. We assessed whether genetic variations in five promoter region polymorphisms (three in proinflammatory and two in anti-inflammatory cytokine genes) are associated with gastric cancer risk in the Romanian population.

We found that the IL-4R –3223TT polymorphism increased gastric cancer susceptibility, mainly for the noncardia type (OR 3.08 [95% CI 1.25 to 7.58]; P=0.023). The IL-4R gene encodes the IL-4 receptor, the specific cell surface receptor for the anti-inflammatory cytokine IL-4. In contrast with other cytokines, little data are available in the literature on the role of IL-4 and IL-4R genetic polymorphisms in *H pylori*-induced diseases. The promoter –3223C→T polymorphism has been demonstrated to gradually decrease circulating IL-4R levels (12) and, thus, the presence of IL-4R –3223T allele contributes to impaired cytokine balance by decreasing the anti-inflammatory response. No association was found between IL-4R –3223C→T and any stages of premalignant lesions (intestinal metaplasia and dysplasia) in a gastric precancerous study in South America (13), whereas a European study conducted in Western countries reported a positive association for IL-4R –3223T allele only for noncardia (OR 1.74 [95% CI 1.15 to 2.63]) in a dominant model, but not for all gastric cancer cases (14). The anti-inflammatory cytokine IL-10 gene contains three confirmed bi-allelic promoter polymorphisms (–1082A→G, –819C→T and –592C→A) reported to produce mainly three haplotypes: GCC, ACC and ATA (15). The presence of the –1082A allele is associated with lower IL-10 production both in vitro and in vivo (15,16). We did not detect significant differences for the IL-10 –1082A→G polymorphism between gastric cancer and control groups. Published findings on the IL-10 –1082A→G polymorphism are inconsistent. While some studies revealed a positive association between –1082G (the high producer allele) and gastric cancer risk (17,18), others showed a

TABLE 4
Risk of intestinal and diffuse gastric adenocarcinoma according to genotype

	Intestinal (n=60)		Diffuse (n=44)	
Polymorphism	**n (%)**	**OR (95%CI); P**	**n (%)**	**OR (95%CI); P**
IL-1β −511C→T				
CC	28 (46.7)	Reference	23 (52.3)	Reference
CT	26 (43.3)	1.01 (0.55–1.82); 0.9	16 (36.4)	0.75 (0.38–1.50); 0.45
TT	6 (10.0)	0.79 (0.30–2.07); 0.67	5 (11.4)	0.80 (0.28–2.27); 0.7
IL-4R −3223C→T				
CC	31 (51.7)	Reference	21 (47.7)	Reference
CT	22 (36.7)	1.20 (0.65–2.21); 0.6	18 (40.9)	1.45 (0.73–2.88); 0.34
TT	7 (11.6)	2.05 (0.92–6.78); 0.09	5 (11.4)	2.64 (0.85–8.15); 0.16
IL-8 −251T→A				
TT	15 (25.0)	Reference	16 (36.4)	Reference
TA	31 (51.7)	1.51 (0.77–2.98); 0.23	22 (50.0)	1.01 (0.73–5.20); 0.9
AA	14 (23.3)	1.59 (0.71–3.59); 0.26	6 (13.6)	0.64 (0.24–1.75); 0.37
IL-10 −1082 A→G				
AA	24 (40.0)	Reference	19 (43.2)	Reference
AG	27 (45.0)	0.85 (0.46–1.57); 0.6	21 (47.7)	0.83 (0.42–1.64); 0.6
GG	9 (15.0)	1.79 (0.40–2.25); 0.9	4 (9.1)	0.54 (0.17–1.69); 0.26
TNF-α −308 G→A				
GG	46 (76.7)	Reference	31 (70.5)	Reference
GA	13 (21.7)	1.26 (0.63–2.53); 0.52	13 (29.5)	1.89 (0.90–3.86); 0.1
AA	1 (1.6)	2.13 (0.18–24.1); 0.56	0 (0)	–

IL Interleukin; TNF Tumour necrosis factor

positive association for –1082A (the low producer allele) (19,20). There are also studies with no detected relationship between IL-10 polymorphisms and gastric cancer (21,22).

We did not observe associations for either IL-1β –511T→C or TNF-α 308G→A polymorphisms. IL-β and TNF-α are crucial cytokines in initiating and amplifying the inflammatory responses to *H pylori* infection. In addition, IL-β and TNF-α are potent inhibitors of gastric acid secretion (23). Therefore, increased production of IL-1β and TNF-α in the gastric mucosa would theoretically lead to an enhanced suppression of gastric acid secretion as well as inflammation and, finally, to an increased risk of gastric cancer. Two major IL-1β gene polymorphisms, IL-1β –511T→C and IL-1β –31T→C, in the promoter region are in complete linkage disequilibrium (7) and have been reported to be associated with interindividual differences in IL-1β production. Individuals homozygous for the IL-1β –511T/–31C haplotype were found to produce between two and three times more IL-1β than others haplotypes (24). Controversial results related to the IL-1β –511T→C polymorphism and gastric cancer risk have been reported: some studies found a positive association (7,25,26) whereas others were not able to reproduce it (27,28). The frequency of the IL-1β –511TT genotype in our control group was 12.40%, similar to other Caucasian populations (13% in Scottish and Polish populations, and 14.2% in the Portuguese population) (7,25) and were lower than the Hispanic and Asian populations (approximately 24%) (21,26,28).

The proinflammatory cytokine TNF-α shares many biological properties with IL-1β. Most studies have focused on the TNF-α –308G→A polymorphism, although some other polymorphic positions in the promoter region have already been described (29). The A allele of TNF-α –308G→A polymorphism increases gene expression, with a resulting alteration of the immune response due to the higher production of TNF-α (30). Several studies have examined the association of the TNF-α –308G→A polymorphism and gastric cancer risk. Positive reports in Caucasian populations have been published (19,31), but these findings are in contrast with others studies (27,32).

IL-8 is involved in the recruitment and activation of immune cells in the gastric mucosa, and the presence of the –251A allele has been found to increase its expression (33). Almost all studies reporting a positive association were conducted in populations of Asian origin (17,34,35), whereas negative findings were found in Caucasian populations (36,37). Moreover, the IL8 –251A allele was associated with a significantly reduced risk of noncardia gastric cancer in an *H pylori*-positive group and intestinal type in a European study (14). We did not find any association between the IL-8 –251T→A polymorphism and gastric cancer susceptibility. These results are in accordance with previous studies on Caucasian populations.

Our findings show a consistent association between the IL-4R –3223TT genotype and gastric cancer susceptibility in an Eastern European population, with some differences to associations found in studies conducted in Western European populations. One possible explanation for those differences is that a cytokine allele may be functional only in a specific haplotype context that varies among different ethnic groups and regions. Our results indicate that this association is mainly for the noncardia type, but the small size of these subgroups precludes drawing reliable conclusions.

CONCLUSION

The IL-4R –3223C→T polymorphism increases gastric cancer susceptibility in the Romanian population. Further genome-wide association and genetic functional studies may help to identify the potential role of this polymorphism in human gastric carcinogenesis.

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REFERENCES

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127:2893-917.

2. Crowe SE. Helicobacter infection, chronic inflammation, and the development of malignancy. Curr Opin Gastroenterol 2005;21:32-8.
3. Gonzalez CA, Sala N, Capella G. Genetic susceptibility and gastric cancer risk. Int J Cancer 2002;100:249-60.
4. Correa P. *Helicobacter pylori* and gastric cancer: State of the art. Cancer Epidemiol Biomarkers Prev 1996;5:477-81.
5. Lehmann FS, Terracciano L, Carena I, et al. In situ correlation of cytokine secretion and apoptosis in *Helicobacter pylori*-associated gastritis. Am J Physiol Gastrointest Liver Physiol 2002;283:G481-8.
6. Opal SM, DePalo VA. Anti-inflammatory cytokines. Chest 2000;117:1162-72.
7. El-Omar EM, Carrington M, Chow WH, et al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature 2000;404:398-402.
8. Ferlay J, Parkin DM, Steliarova-Foucher E. Estimates of cancer incidence and mortality in Europe in 2008. Eur J Cancer 2010;46:765-81.
9. Kusters JG, van Vliet AH, Kuipers EJ. Pathogenesis of *Helicobacter pylori* infection. Clin Microbiol Rev 2006;19:449-90.
10. Sporea I, Popescu A, van Blankenstein M, Sirli R, Focsea M, Danila M. The prevalence of *Helicobacter pylori* infection in western Romania. Rom J Gastroenterol 2003;12:15-8.
11. Lauren P. The two histological main types of gastric carcinoma: Diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. Acta Pathol Microbiol Scand 1965;64:31-49.
12. Hackstein H, Hecker M, Kruse S, et al. A novel polymorphism in the 5′ promoter region of the human interleukin-4 receptor alpha-chain gene is associated with decreased soluble interleukin-4 receptor protein levels. Immunogenetics 2001;53:264-9.
13. Kato I, Canzian F, Franceschi S, et al. Genetic polymorphisms in anti-inflammatory cytokine signaling and the prevalence of gastric precancerous lesions in Venezuela. Cancer Causes Control 2006;17:1183-91.
14. Crusius JB, Canzian F, Capella G, et al. Cytokine gene polymorphisms and the risk of adenocarcinoma of the stomach in the European prospective investigation into cancer and nutrition (EPIC-EURGAST). Ann Oncol 2008;19:1894-902.
15. Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An investigation of polymorphism in the interleukin-10 gene promoter. Eur J Immunogenet 1997;24:1-8.
16. Rad R, Dossumbekova A, Neu B, et al. Cytokine gene polymorphisms influence mucosal cytokine expression, gastric inflammation, and host specific colonisation during *Helicobacter pylori* infection. Gut 2004;53:1082-9.
17. Lu W, Pan K, Zhang L, Lin D, Miao X, You W. Genetic polymorphisms of interleukin (IL)-1B, IL-1RN, IL-8, IL-10 and tumor necrosis factor {alpha} and risk of gastric cancer in a Chinese population. Carcinogenesis 2005;26:631-6.
18. Sugimoto M, Furuta T, Shirai N, et al. Effects of interleukin-10 gene polymorphism on the development of gastric cancer and peptic ulcer in Japanese subjects. J Gastroenterol Hepatol 2007;22:1443-9.
19. El-Omar EM, Rabkin CS, Gammon MD, et al. Increased risk of noncardia gastric cancer associated with proinflammatory cytokine gene polymorphisms. Gastroenterology 2003;124:1193-201.
20. Zambon CF, Basso D, Navaglia F, et al. Pro- and anti-inflammatory cytokines gene polymorphisms and *Helicobacter pylori* infection: Interactions influence outcome. Cytokine 2005;29:141-52.
21. Garcia-Gonzalez MA, Lanas A, Quintero E, et al. Gastric cancer susceptibility is not linked to pro-and anti-inflammatory cytokine gene polymorphisms in whites: A nationwide multicenter study in Spain. Am J Gastroenterol 2007;102:1878-92.
22. Savage SA, Abnet CC, Haque K, et al. Polymorphisms in interleukin -2, -6, and -10 are not associated with gastric cardia or esophageal cancer in a high-risk chinese population. Cancer Epidemiol Biomarkers Prev 2004;13:1547-9.
23. Kondo S, Shinomura Y, Kanayama S, et al. Interleukin-1 beta inhibits gastric histamine secretion and synthesis in the rat. Am J Physiol 1994;267:G966-71.
24. Hall SK, Perregaux DG, Gabel CA, et al. Correlation of polymorphic variation in the promoter region of the interleukin-1 beta gene with secretion of interleukin-1 beta protein. Arthritis Rheum 2004;50:1976-83.
25. Machado JC, Pharoah P, Sousa S, et al. Interleukin 1B and interleukin 1RN polymorphisms are associated with increased risk of gastric carcinoma. Gastroenterology 2001;121:823-9.
26. Zhang WH, Wang XL, Zhou J, An LZ, Xie XD. Association of interleukin-1B (IL-1B) gene polymorphisms with risk of gastric cancer in Chinese population. Cytokine 2005;30:378-81.
27. Garza-Gonzalez E, Bosques-Padilla FJ, El-Omar E, et al. Role of the polymorphic IL-1B, IL-1RN and TNF-A genes in distal gastric cancer in Mexico. Int J Cancer 2005;114:237-41.
28. Kato S, Onda M, Yamada S, Matsuda N, Tokunaga A, Matsukura N. Association of the interleukin-1 beta genetic polymorphism and gastric cancer risk in Japanese. J Gastroenterol 2001;36:696-9.
29. Canedo P, Duraes C, Pereira F, et al. Tumor necrosis factor alpha extended haplotypes and risk of gastric carcinoma. Cancer Epidemiol Biomarkers Prev 2008;17:2416-20.
30. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc Natl Acad Sci USA 1997;94:3195-9.
31. Machado JC, Figueiredo C, Canedo P, et al. A proinflammatory genetic profile increases the risk for chronic atrophic gastritis and gastric carcinoma. Gastroenterology 2003;125:364-71.
32. Lee JY, Kim HY, Kim KH, et al. Association of polymorphism of IL-10 and TNF-A genes with gastric cancer in Korea. Cancer Lett 2005;225:207-14.
33. Hull J, Thomson A, Kwiatkowski D. Association of respiratory syncytial virus bronchiolitis with the interleukin 8 gene region in UK families. Thorax 2000;55:1023-7.
34. Lee WP, Tai DI, Lan KH, et al. The −251T allele of the interleukin-8 promoter is associated with increased risk of gastric carcinoma featuring diffuse-type histopathology in the Chinese population. Clin Cancer Res 2005;11:6431-41.
35. Taguchi A, Ohmiya N, Shirai K, et al. Interleukin-8 promoter polymorphism increases the risk of atrophic gastritis and gastric cancer in Japan. Cancer Epidemiol Biomarkers Prev 2005;14:2487-93.
36. Kamangar F, Abnet CC, Hutchinson AA, et al. Polymorphisms in inflammation-related genes and risk of gastric cancer (Finland). Cancer Causes Control 2006;17:117-25.
37. Savage SA, Hou L, Lissowska J, et al. Interleukin-8 polymorphisms are not associated with gastric cancer risk in a Polish population. Cancer Epidemiol Biomarkers Prev 2006;15:589-91.