Mol Biol Rep (2012) 39:7617–7625
DOI 10.1007/s11033-012-1596-x

IL-1ra anti-inflammatory cytokine polymorphism is associated with risk of gastric cancer and chronic gastritis in a Brazilian population, but the TNF-β pro-inflammatory cytokine is not

J. Garcia Oliveira · M. Cristina Duarte · A. Elizabete Silva

Received: 21 June 2011 / Accepted: 31 January 2012 / Published online: 12 February 2012

Abstract Genetic polymorphisms in genes that codify inflammatory cytokines have been associated with gastric carcinogenesis. This study evaluated polymorphisms *IL-1RN* VNTR and *TNFB*+252A/G in a population from Southeast Brazil with regard to the risk of chronic gastritis and gastric cancer and the presence of an association of gastric lesions with risk factors such as gender, age, smoking, drinking and *Helicobacter pylori* infection. In this case–control study, polymorphism at *IL-1RN* VNTR was investigated using the allele-specific polymerase chain reaction method, while the polymerase chain reaction–restriction fragment length polymorphism technique was used to identify the *TNFB*+252A/G genotype in 675 Brazilian individuals [229 with chronic gastritis (CG), 200 with gastric cancer (GC) and 246 healthy individuals as controls (C)]. Multiple logistic regression analysis (log-additive, dominant, and recessive models) have not showed association of the genotype frequencies for the SNP *TNFB*+252A/G with risk of CG or GC. However, as for *IL-1RN* VNTR it was observed significant differences in all three analysis models, with higher values of OR in recessive model, both in the GC group (OR = 3.04, 95% CI = 1.41–6.56, $p < 0.01$) and CG (OR = 2.32, 95% CI = 1.10–4.90, $p = 0.02$) compared to the C group. In addition, the multiple logistic regression showed also an association with risk factors such as male gender, older age and alcohol intake regarded GC group. So, our results indicated that the *IL-1RN**2 allele may increase the risk of gastric cancer and precancerous lesions in the Southeast Brazilian population, reinforcing the importance of host genetic factors in the susceptibility to gastric cancer and the participation of cytokines in both the inflammation and the carcinogenic process.

Keywords Genetic polymorphism · Inflammation · *IL-1RN* · *TNF-β* · Gastric carcinogenesis

J. G. Oliveira · M. C. Duarte · A. E. Silva (✉)
Departamento de Biologia, Campus São José do Rio Preto, UNESP, São Paulo State University, Rua Cristóvão Colombo, 2265, São José do Rio Preto, SP 15054-000, Brazil
e-mail: anabete@ibilce.unesp.br

J. G. Oliveira
e-mail: kjuliana21@yahoo.com.br

M. C. Duarte
e-mail: duartebio@hotmail.com

Introduction

The relationship between inflammation and cancer is well established, mainly in the gastrointestinal tract [1]. Many of the factors involved in chronic inflammation and immune response play a role in cell proliferation, stimulation of angiogenesis and inhibition of apoptosis [2]. Gastric cancer is a model of multifactorial disease resulting from environmental and genetic factors, following a chronic inflammation of the gastric mucosa induced by *Helicobacter pylori* infection [3].

H. pylori is the most important risk factor for gastric cancer, triggering chronic inflammation of the stomach and leading to stepwise development of the malignancy [4]. It is widely accepted that chronic *H. pylori* infection induces gastric atrophy and hypochlorhydria, which are precursors of all pathophysiological changes of gastric carcinogenesis [5].

Although *H. pylori* infection is widespread throughout the world's human population, only about 1–2% of exposed individuals progress to gastric cancer [6]. Thus, besides virulence factors of the bacterium, host genetic

patterns, mainly those that regulate the immunological and inflammatory response, may also contribute to a neoplastic progression [7]. Genetic polymorphisms in genes that codify pro- and anti-inflammatory cytokines have been associated with an increase in the synthesis of those interleukins and have emerged as a hypochlorhydria-determining factor for cancer susceptibility [8, 9].

The interleukin-1 (*IL-1*) gene cluster on chromosome 2q contains three related genes, *IL-1A*, *IL-1B* and *IL-1RN*, which encode, respectively, the pro-inflammatory cytokines IL-1α and IL-1β, as well as their endogenous receptor antagonist (IL-1ra) [10]. IL-1ra, encoded by the *IL-1RN* gene, is an anti-inflammatory cytokine that competitively binds to IL-1 receptors but does not elicit a response, thereby modulating the potentially damaging effects of IL-1 [11, 12]. The *IL-1RN* gene has penta-allelic 86 bp variable number of tandem repeats (VTNR) in the second intron, resulting in a short allele (IL-1RN*2, with two repeats) or long alleles (IL-1RN*L, with three to six repeats) [13].

The short allele is associated with chronic inflammation, autoimmune conditions and increased secretion of IL-1β [9]. In 2000, El-Omar et al. [10] reported an increased risk for developing gastric cancer in individuals having the *IL-1RN**2/*2 genotypes. This finding was subsequently confirmed in other population studies [7, 8, 11, 14, 15], but the data are still controversial [16, 17].

In addition, genetic polymorphisms in pro-inflammatory cytokines such as TNF-β are known to be related to several autoimmune, infectious and neoplastic diseases [18–21]. Tumor necrosis factor β (TNF-β) or lymphotoxin-α (LT-α) is encoded by the same gene cluster for TNFA, located in tandem within the MHC complex on chromosome 6p21.3 [22]. TNF-β is a lymphokine that also plays an important role in the regulation of the immune response as part of the cytokine network. It is mainly produced by stimulated T-cells, B and natural killer-cell lymphocytes [23].

An increased level of TNF-β expression in vitro has been shown for the Single Nucleotide Polymorphism (SNP) *TNF-B*+252A/G [24]. Also, there are studies showing that the *TNF-B*+252G-carriage is associated with a high serum concentration of C-reactive protein, a biomarker of inflammation [25, 26]. Therefore, this polymorphism has been investigated as a potential factor of susceptibility to gastric cancer [27].

The aim of this study was to determine if there is an association between anti- and pro-inflammatory cytokines polymorphisms, such as the *IL-1RN* VTNR and SNP of *TNF-B*+252A/G and the risk of chronic gastritis and gastric cancer in a population from Southeast Brazil, and whether gastric lesions are associated with risk factors such as gender, age, smoking, alcohol intake and *H. pylori* infection.

Methods

Population

The Brazilian National Research Ethics Committee approved this work, and written informed consent was obtained from all individuals participating.

This was a case–control study on chronic gastritis and gastric cancer, in which a total of 675 DNA samples extracted from peripheral blood leukocytes were genotyped. It was used DNA samples stored in our laboratory collected between the periods 2002 and 2004 and used in previous studies [28, 29] and the current period (2007–2010).

All subjects of case groups were recruited from the Service of Endoscopy or Surgery Center, Hospital de Base, Medical School, Sao Jose do Rio Preto-SP, Brazil. In cases groups were included, incident cases recruited from the moment that started the sample collection in those years, with participation rate about 90% of patients interviewed. Recurrent malignancies were excluded.

The chronic gastritis groups (CG) comprised 229 individuals (113 men and 116 women) with a mean age of 52.3 $\pm$ 14.5 years (range 19 –86 years). All had a histopathologically confirmed diagnosis of chronic gastritis, mainly nonatrophic, but also was included gastritis atrophic and with metaplasia area (Sydney System) [30], but individuals who presented specific gastritis types such as autoimmune and ischemic were excluded.

The gastric cancer group (GC) comprised 200 individuals (156 men and 44 women) with a mean age of 61.7 $\pm$ 12.5 years (range 28–93 years), and with a histopathologically confirmed diagnosis of intestinal or diffuse gastric cancer (Lauren's classification) [31]. Information on *H. pylori* infection was obtained when available from the patients' medical records (95 with gastric cancer and 177 with chronic gastritis). The diagnosis was established using the Giemsa staining technique or the urease test, performed at the respective Pathology Service.

The cancer-free control group (C) was composed of 246 healthy individuals (126 men and 120 women), with a mean age of 55.5 $\pm$ 17.7 years (range 20–93 years), blood donors recruited from the Blood Bank of Base Hospital, Medical School, Sao Jose do Rio Preto-SP, Brazil. Individuals were selected without diagnoses of cancer according to government guidelines for donated blood that tests for 20 related diseases (http://www.hemonline.com.br/portarias/rdc153/index frame.htm), and no previous history of gastric disease. The participation rate was about 100% of subjects interviewed.

Epidemiological data on the study population were collected using a standard interviewer-administered questionnaire, with questions about current and past occupation,

smoking habits, alcohol intake, and family history of cancer. Considering the ethnic admixture of the evaluated Brazilian population, the study subjects were visually classified for ethnicity, based on their appearance, as Caucasians (90%) or Negroids (10%). Smokers were defined as individuals who consumed at least 100 cigarettes during their lifetime, and alcohol consumers were those who drank more than four drinks per week [32].

IL-1RN and *TNFB*+252A/G genotyping

About 5 mL of whole blood were collected from all study participants in sterile EDTA-coated vacutainer tubes. DNA was extracted as described in a previous report [33] and stored at −20°C until use.

IL-1RN polymorphisms were investigated using the allele-specific polymerase chain reaction (PCR) method [10], and the PCR–RFLP (restriction fragment length polymorphism) technique was employed to identify the *TNF-β* genotype [21], in both cases and control groups. In brief, the assay was carried out in a total reaction volume of 25 μL, containing 2.5 μL 10× PCR buffer, 2 μL dNTPs (1.25 μmol/L, Invitrogen, Carlsbad, CA, USA), 0.5 μL $MgCl_2$ (25 mmol/L), 1.25 μL of each primer (25 mmol/L, Sigma–Aldrich, St. Louis, MO, USA), 15.3 μL dH_2O, 2 μL DNA (100 ng/μL), and 0.2 μL Taq DNA polymerase (5 U/μL, Invitrogen, Carlsbad, CA, USA). The PCR procedure for *IL-1RN* was as follows: an initial denaturation step at 94°C for 3 min, 35 amplification cycles at 94°C for 45 s, 61°C for 45 s, and 72°C for 1.5 min, followed by a final elongation cycle at 72°C for 7 min. The size of the amplified fragments is shown in Table 1. Alleles 1, 3, 4 and 5 were grouped together and named long alleles (L) and analyzed separately from the short allele 2 (two repeats). So, the genotypes were further classified as L/L, L/2, and 2/2. PCR for the *TNF-β* polymorphism was performed as follows: an initial denaturation step at 94°C for 5 min, 35 amplification cycles at 94°C for 1 min, 65°C for 1 min, and 72°C for 1 min, followed by a final elongation cycle at 72°C for 7 min. Then, 10 μL of PCR products were digested with 0.5 μL of the specific enzyme *Nco*I (5 U/μL) in a 10 μL volume containing 2.5 μL 10× buffer 1 (New England Biolabs, MA, USA) and 7.0 μL dH_2O (Table 1). The products were then electrophoresed on a 1.5% agarose gel (Invitrogen, Carlsbad, CA, USA) and visualized by ethidium bromide staining. Random samples were selected and performed their duplicates by others researchers of the laboratory for quality control, but there were not discrepancies, and the concordance rate was 98%.

Statistical analysis

Hardy–Weinberg equilibrium for *TNF-β* polymorphism was assessed using Chi-square test. Multiple logistic regression analysis was conducted using the following models: log-additive (major allele homozygotes vs heterozygotes vs minor allele homozygotes), dominant (major allele homozygotes vs heterozygotes + minor allele homozygotes), and recessive (major allele homozygotes + heterozygotes vs minor allele homozygotes). The age, gender, smoking, drinking and *H. pylori* infection as covariates were adjusted to obtain statistical significance in both the gastric cancer and the chronic gastritis groups for two polymorphisms. Statistical analyses were performed using the GraphPad Instat, SPSS (11.5 version) and SNPStats (http://bioinfo.iconcologia.net/index.php) computer software programs [34]. A probability level (p) of less than 0.05 was adopted as significance criterion.

Results

The samples obtained from the 675 subjects in the GC, CG and C groups were genotyped for the *IL-1RN* VNTR and *TNF-β*+252A/G polymorphisms. The genotype and allele frequency distributions of the *TNF-β*+252A/G polymorphism were in agreement with the Hardy–Weinberg equilibrium, in both the case and the control groups (data not shown).

Table 1 Primer sequences, restriction enzyme and fragment sizes for *IL1RN* and *TNFB* gene polymorphisms

Genes	Primers	Enzyme T°/Time	Fragment (bp)
IL1RN	F: 5′-CCCCTCAGCAACACTCC-3′		Allele 1 = 4 repetitions (410)
	R: 5′-GGTCAGAAGGGCAGAGA-3′		Allele 2 = 2 repetitions (210)
			Allele 3 = 5 repetitions (500)
			Allele 4 = 3 repetitions (325)
			Allele 5 = 6 repetitions (595)
TNFB+252A/G	F: 5′-CTCCTGCACCTGCTGCCTGGATC-3′	*Nco*I 37°C, 1 h	A/A: 235 + 133
	R: 5′-GAAGAGACGTTCAGGTGGTGTCAT-3′		A/G: 368 + 235 + 133
			G/G: 368

bp base pairs, T^o temperature

Genotype frequencies and logistic regression analysis adjusted for age, gender, smoking and drinking in all three analysis models (log-additive, dominant, and recessive models for rare alleles) of both polymorphisms in the gastric cancer, chronic gastritis and control group (GC vs C and CG vs C) are presented in Table 2.

In the GC group, the genotype frequencies of *IL1RN* VNTR were 40.5% for the L/L genotype, 44.5% for L/2 and 15.0% for 2/2. In the CG group, the genotype frequencies were 52.0, 37.5 and 10.5%, respectively, while in the C group, these frequencies were respectively 64.2, 31.3 and 4.5%. As showed in the Table 2, the 2/2 genotype was associated with risk of gastric cancer and chronic gastritis in all 3 models evaluated, evidencing higher values of OR in the recessive model (OR = 3.04, 95% CI = 1.41–6.56, $p < 0.01$ and OR = 2.32, 95% CI = 1.10–4.90, $p = 0.02$, respectively). However, when compared both case groups there was a statistically significant difference in genotype frequencies only in the log-additive and dominant models (OR = 1.42, 95% CI = 1.04–1.95, $p = 0.02$ and OR = 1.70, 95% CI = 1.11–2.62, $p = 0.01$, respectively) (data not showed in Table 2). In contrast, for *TNF*-β+252A/G, no significant difference was found among the three groups evaluated (Table 2).

We evaluated the combined effect of the two polymorphisms studied and the combination at least one variant allele *IL1-RN**2 with TNF-β+252A/G+G/G remained the risk for GC (OR = 3.69; 95% CI = 2.13–6.41; $p < 0.01$) and CG (OR = 2.34; 95% CI = 1.42–3.86; $p < 0.01$) in relation to control group (Table 3).

The multiple logistic regression for risk factors showed that in the GC group male gender (OR = 2.27, 95% CI = 1.42–3.62, $p < 0.01$), age above 60 years (OR = 1.70, 95% CI = 1.10–2.61, $p < 0.01$) and alcohol intake

Table 2 Genotype frequencies and logistic regression analysis of *IL1RN*-VNTR and *TNF*β polymorphisms in the gastric cancer (GC), chronic gastritis (CG) and control (C) groups (GC vs C, CG vs C)

Polymorphisms	Genotype	Controls *n* (%)	Patients	
			GC *n* (%)	CG *n* (%)
IL-1RN-VNTR	L/L[a]	158 (64.2)	81 (40.5)	119 (52.0)
	L/2	77 (31.3)	89 (44.5)	86 (37.5)
	2/2[b]	11 (4.5)	30 (15.0)	24 (10.5)
Log-additive				
OR (95% CI)			2.22 (1.59–3.11)	1.58 (1.17–2.14)
p			<0.01	<0.01
Dominant				
OR (95% CI)			2.66 (1.74–4.07)	1.67 (1.14–2.44)
p			<0.01	<0.01
Recessive				
OR (95% CI)			3.04 (1.41–6.56)	2.32 (1.10–4.90)
p			<0.01	0.02
TNFB+252A/G	A/A[a]	126 (51.2)	86 (43.0)	103 (45.0)
	A/G	93 (37.8)	97 (48.5)	95 (41.5)
	G/G[b]	27 (11.0)	17 (8.5)	31 (13.5)
Log-additive				
OR (95% CI)			1.16 (0.85–1.59)	1.22 (0.93–1.60)
p			0.35	0.15
Dominant				
OR (95% CI)			1.41 (0.93–2.12)	1.27 (0.88–1.84)
p			0.10	0.20
Recessive				
OR (95% CI)			0.77 (0.38–1.57)	1.37 (0.78–2.41)
p			0.48	0.27

Logistic regression analysis adjusting for age, gender, smoking and drinking habits

CI confidence interval, *L* alleles 1, 3, 4 e 5, *n* number of samples, *OR* odds ratio, *p* probability

[a] Reference group for dominant model, [b] reference group for recessive model

Table 3 Combined effect of *IL-1RN* (L/L, L/2+2/2 genotypes) and *TNFB*+252A/G (A/A, A/G+G/G genotypes) polymorphisms on risk of gastric cancer (GC) and chronic gastritis (CG)

Risk genotype		Groups			OR (95% CI), *p*		
IL-1RN	*TNFB+252*	C ($n = 246$)	GC ($n = 200$)	CG ($n = 229$)	GC x C	CG x C	GC x CG
L/L	A/A	79	34	59	1.00 (reference)	1.00 (reference)	1.00 (reference)
L/L	A/G + G/G	78	48	49	1.43 (0.83–2.45), 0.22	0.84 (0.511.37), 0.53	1.7 (0.95–3.03), 0.08
L/2+2/2	A/A	45	48	44	2.47 (1.39–4.39), <0.01	1.30 (0.76–2.23), 0.34	1.89 (1.05–3.40), 0.03
L/2+2/2	A/G+G/G	44	70	77	3.69 (2.13–6.41), <0.01	2.34 (1.42–3.86), <0.01	1.57 (0.92–2.68), 0.10

CI confidence interval, *OR* odds ratio, *L* alleles 1, 3, 4 e 5, *n* number of samples, *p* probability

Table 4 Distribution of risk factors, genotypes of *IL-1RN* and *TNFB*+252A/G polymorphisms, OR and 95% confidence intervals (CI) between gastric cancer (GC) and chronic gastritis (CG) with control (C) group

Variables	F (GC/C) %	OR (95% CI)	*p*	F (CG/C) %	OR (95% CI)	*p*
Gender						
F	22.0/48.8	Reference	<0.01	50.7/48.8	Reference	0.79
M	78.0/51.2	2.27 (1.42–3.62)		49.3/51.2	0.95 (0.64–1.39)	
Age (years)						
<60	39.5/56.9	Reference	<0.01	54.5/45.5	<53 Reference	0.03
≥60	60.5/43.1	1.70 (1.10–2.61)		45.5/54.5	≥53 0.66 (0.45–0.96)	
Smoking						
Nonsmokers	29.5/36.6	Reference	0.20	44.5/36.6	Reference	<0.01
Smokers	70.5/63.4	0.72 (0.44–1.19)		55.5/63.4	0.58 (0.38–0.87)	
Alcohol						
Nondrinkers	43.5/74.4	Reference	<0.01	68.1/74.4	Reference	<0.01
Drinkers	56.5/25.6	3.09 (1.91–5.02)		31.9/25.6	1.81 (1.16–2.83)	
IL-1RN						
L/L	40.5/64.2	Reference	<0.01	52.0/64.2	Reference	<0.01
L/2+2/2	59.5/35.8	2.53 (1.66–3.80)		48.0/35.8	1.79 (1.22–2.62)	
TNFB+252						
A/A	45.0/51.2	Reference	0.13		Reference	0.20
A/G+G/G	55.0/48.8	1.38 (0.90–2.09)			1.27 (0.87–1.84)	

Logistic regression analysis adjusting for age, gender, smoking, drinking habits

CI confidence interval, *OR* odds ratio, *L* alleles 1, 3, 4 e 5, *p* probability

(OR = 3.09, 95% CI = 1.91–5.02, $p < 0.01$) were associated with greater susceptibility to the development of this neoplasm (Table 4). While the comparisons between gastritis and control groups showed that only alcohol intake was associated with increased risk of chronic gastritis (OR = 1.81, 95% CI = 1.16–2.83, $p < 0.01$), but age above 53 years (OR = 0.66, 95% CI = 0.45–0.96, $p = 0.03$) and smoking (OR = 0.58, 95% CI = 0.38–0.87, $p < 0.01$) were negatively associated with the development of gastritis.

Because the reduced number of cases tested for *H. pylori* infection (95 with gastric cancer and 177 with gastritis), the Table 5 shows another multiple logistic regression analysis between both case groups also adjusted for the other risk factors. There was no statistically significant association for *H. pylori* (OR = 1.39, 95% CI = 0.78–2.48, $p = 0.25$, Table 5). There was also no association of both polymorphisms when addressed separately in *H. pylori* positive and *H. pylori* negative subjects within a group of gastric cancer and gastritis (data not shown).

Discussion

Studies on cytokine gene polymorphisms have generated further evidence to support the role of inflammation in

Table 5 Distribution of risk factors, genotypes of *IL-1RN* and *TNFB*+252A/G polymorphisms, OR and 95% confidence intervals (CI) between gastric cancer (GC) and chronic gastritis (CG) groups tested for *H. pylori*

Variables	F (GC/CG) %	OR (95% CI)	*p*
Gender			
F	23.1/49.1	Reference	0.76
M	76.9/50.9	0.92 (0.63–1.34)	
Age (years)			
<55	33.6/61.0	Reference	<0.01
≥55	66.4/39.0	3.17 (2.07–4.84)	
Smoking			
Nonsmokers	24.3/43.0	Reference	0.25
Smokers	75.7/57.0	0.68 (0.35–1.31)	
Alcohol			
Nondrinkers	39.0/66.2	Reference	0.04
Drinkers	61.0/39.0	1.64 (1.01–2.68)	
H. pylori			
Negative	56.8/46.6	Reference	0.25
Positive	43.2/53.4	1.39 (0.78–2.48)	
IL1RN			
L/L	46.3/55.3	Reference	0.11
L/2+2/2	53.7/44.7	0.63 (0.35–1.11)	
TNFB+252			
A/A	43.1/46.3	Reference	0.71
A/G+G/G	56.9/53.7	0.90 (0.51–1.58)	

Results for 95 samples in the GC group and 177 samples in the CG group. Logistic regression analysis adjusting for age, gender, smoking, drinking habits, *H. pylori* infection, *CI* confidence interval, *OR* odds ratio, *L* alleles 1, 3, 4 e 5, *p* probability

gastrointestinal carcinogenesis, mediated by an array of pro- and anti-inflammatory cytokines. Polymorphisms of these genes are associated with differences in gastric mucosal cytokine mRNA level, which result in an individual final clinical outcome [35]. Considering this scenario, we investigated the effect of polymorphisms of two cytokine genes, one anti-inflammatory (*IL-1RN* VNTR) and the other pro-inflammatory (*TNF-β*+252A/G), on the risk of developing GC and CG in a population from Southeast Brazil.

Our results demonstrated that in this population there was an association of the *IL-1RN**2 allele variant and of the combined effect of polymorphisms *IL1-RN**2 and *TNF-β*+252*G with susceptibility to GC and CG. The presence of polymorphism *TNF-β*+252A/G alone was not associated with risk of any of the gastric lesions studied.

The results of our study add to the current controversy about the role of *IL-1RN* VNTR polymorphism in the gastric carcinogenesis risk, recently addressed in three meta-analysis studies [12, 36, 37]. The first one, by Kamangar et al. [36] observed that the overall associations between *IL-1RN* pro-inflammatory polymorphisms and gastric cancer were null, even in studies conducted in Western countries. The second meta-analysis, reported by Peleteiro et al. [37] showed that allele *IL-1RN**2 was associated with increase of the risk of gastric precancerous lesions, supporting a role for this polymorphism in the early stages of gastric carcinogenesis. The last meta-analysis mentioned, by Xue et al. [12] observed that allele *IL-1RN**2 was significantly associated with an increased risk of developing gastric carcinoma and even more significantly with non-cardia gastric carcinoma or with intestinal-type gastric carcinoma. However, this association was evident among Caucasians, but not among Asians or Hispanics. These discrepancies may be due to the low frequency of the risk allele in some populations, such as the Asians.

Rocha et al. [38] conducted an assessment study on cytokine polymorphisms in an admixed population of Southeast Brazil (Minas Gerais state) with a background of approximately 33% Portuguese, 33% Amerindian and 33% African ancestry. They demonstrated an association between the polymorphic allele *IL-1RN**2 and the development of gastric cancer, and also an association with the cagA-positive *H. pylori* status. However, they found a low 2/2 homozygous frequency (3.6%) compared to our study (15.0%) and to studies conducted in European Caucasian (28%) and Asian populations (0.4%) [39]. More recently, another study [7] performed in a population of northern Brazil, with a strong genetic contribution of Portuguese (50%) and Amerindians (40%) but low African ancestry (10%), also showed a positive association between allele *IL-1RN**2 and gastric ulcer and gastric adenocarcinoma and CagA + *H. pylori* infection. In this study, the frequency of homozygous 2/2 was about 6% [7]. However, the 2/2 homozygous frequency in the control group of the present study (4.5%) is more similar than those reported by Rocha et al. (3.9%) [38], but discrepant of Asian population (0.4%) [39].Those results indicate that the genotype frequencies of *IL-1RN* are related to the ethnic composition of the population studied.

In the present study, focusing on another population of Southeast Brazil (São Paulo state), the Caucasian ethnicity was around 90% for the control and cases groups. These data when compared with other epidemiological studies in the Southeast (84.1%), Central-Western (87.2%), Northern (60%) and Southern (94.0%) in Brazilian population demonstrated a variation of the frequency of Caucasians in healthy individuals in different Brazilian regions [40–42]. Because of the broad geographic extension of Brazil, there is variation in population composition among different regions that dates back to its colonization by Europeans and Africans, which eventually got admixed with the native Amerindians [43], and consequently there are differences in genotype distribution.

The polymorphic allele *IL1-RN**2 has been linked to an increased production of the pro-inflammatory cytokine IL1-β, which causes a chronic inflammatory process and reduced production of gastric acid (hypochlorhydria) [44]. The reactive oxygen species and nitric oxide, products derivatives of the inflammatory process, are known as mutagens, whereas hypochlorhydria can favor infections by bacteria, thus increasing the production of carcinogens. The DNA damage caused by this cascade of factors may be amplified by increased cell division inherent to the inflammatory process. Thus, genotypes that enhance the production of pro-inflammatory cytokines may be related to the process of gastric carcinogenesis [45].

In addition, about the importance of pro-inflammatory cytokines, that can also modify the immune/inflammatory responses upon *H. pylori* infection, and influence gastric carcinogenesis, we assessed the *TNF-*β+252A/G polymorphism. Although we found a higher frequency of genotypes A/G and G/G in groups GC and CG compared to controls, the difference was not statistically significant. Similarly, no association was found by Lee et al. [46] in the Korean population, and Guo et al. [21] also found no relationship between the polymorphic allele and the groups of gastric cardiac adenocarcinoma and squamous cell carcinoma of the esophagus. However, a recent study in the Japanese population showed association of genotypes G/G and A/G with an increased risk of noncardia gastric cancer of the diffuse type and attributed this association to the ability of the 252G allele to increase the production of pro-inflammatory cytokines such as TNF-β [25]. These discrepant results may be linked to ethnicity, among others factors.

When analyzing the influence of *H. pylori* infection on the risk for the gastric lesions evaluated, no association was observed. However, several studies have found this association between *IL-1RN* polymorphisms and gastric lesions risk in the presence of the bacterium *H. pylori* [11, 47, 48]. Furthermore, Rocha et al. and Melo-Barbosa et al. [7, 38] found a strong association of this polymorphism and infection by *H. pylori* CagA+ strains with the development of gastric lesions and adenocarcinoma. This influence of *H. pylori* infection is well defined in the progression of gastric cancer in some populations. Individuals with pro-inflammatory genotypes overexpress gastric IL-1β in response to *H. pylori* infection, leading to increased inflammation, gastric atrophy, hypochlorhydria and the development of gastric carcinoma [8]. However, in the present study, we did not find this association. This discrepancy might be due to the small number of cases with the available information in their medical records for *H. pylori* infection (95 with gastric cancer and 177 with chronic gastritis). Despite the relevance of the study, also should be considered some limitations that did not enable the analysis of association by cancer histological type and anatomical site.

Other risk factors such as male gender, old age and alcohol intake were found to be significantly associated with the risk of gastric cancer. According to the data of the Brazilian National Cancer Institute (INCA), the highest incidence of GC occurs in men around age 70, and about 65% of patients diagnosed with this type of cancer are over 50 years old. In Brazil, GC is *the* third in incidence among men and fifth in women, so our results are consistent with the epidemiological data reported in the literature [49]. Another risk factor well established in the literature in relation to gastric carcinogenesis is the excessive consumption of alcohol. The oxidation of ethanol generates acetaldehyde, which primarily has direct carcinogenic and mutagenic effects. This metabolite interferes with many sites of synthesis and DNA repair, which can lead to tumor development [32].

Conclusions

In conclusion, our findings do not evidence any association of the *TNF-*β+252A/G polymorphism alone with susceptibility to gastric lesions, but indicate a significant role of the *IL-1RN**2 allele for both GC and CG in the Southeast Brazilian population evaluated. Thus, they show the importance of polymorphisms in genes for anti-inflammatory factors in gastric carcinogenesis, reinforcing the contribution of the host genetic factors in the development of gastric cancer.

Acknowledgments This study was supported by the Brazilian agencies FAPESP, CNPq and CAPES.

References

1. Ferrone C, Dranoff G (2010) Dual roles for immunity in gastrointestinal cancers. J Clin Oncol 28:4045–4051. doi:10.1200/JCO.2010.27.9992
2. McNamara D, El-Omar E (2008) H*elicobacter pylori* infection and the pathogenesis of gastric cancer: a paradigm for host-bacterial interactions. Dig Liver Dis 40:504–509. doi:10.1016/j.dld.2008.02.031
3. Correa P (1988) A human model of gastric carcinogenesis. Cancer Res 48:3554–3560
4. Hussein NR (2010) Helicobacter pylori and gastric cancer in the Middle East: a new enigma? World J Gastroenterol 16:3226–3234. doi:10.3748/wjg.v16.i26.3226
5. Hold GL, Rabkin CS, Chow WH, Smith MG, Gammon MD, Risch HA, Vaughan TL, McColl KE, Lissowska J, Zatonski W, Schoenberg JB, Blot WJ, Mowat NA, Fraumeni JF Jr, El-Omar EM (2007) A functional polymorphism of toll-like receptor 4 gene increases risk of gastric carcinoma and its precursors. Gastroenterology 132:905–912. doi:10.1053/j.gastro.2006.12.026

6. Scholte GH, van Doorn LJ, Cats A, Bloemena E, Lindeman J, Quint WG, Meuwissen SG, Kuipers EJ (2002) Genotyping of *Helicobacter pylori* in paraffin-embedded gastric biopsy specimens: relation to histological parameters and effects on therapy. Am J Gastroenterol 97:1687–1695. doi:10.1111/j.1572-0241.2002.05775.x
7. Melo Barbosa HP, Martins LC, Dos Santos SE, Demachki S, Assumpção MB, Aragão CD, de Oliveira Corvelo TC (2009) Interleukin-1 and TNF-alpha polymorphisms and *Helicobacter pylori* in a Brazilian Amazon population. World J Gastroenterol 15:1465–1471. doi:10.3748/wjg.15.1465
8. El-Omar EM (2001) The importance of interleukin 1b in *Helicobacter pylori* associated disease. Gut 48:743–747. doi:10.1136/gut.48.6.743
9. Perez–Perez GI, Garza-Gonzalez E, Portal C, Olivares AZ (2005) Role of cytokine polymorphisms in the risk of distal gastric cancer development. Cancer Epidemiol Biomarkers Prev 14:1869–1873. doi:10.1158/1055-9965.EPI-04-0889
10. 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 JF Jr, Rabkin CS (2000) Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature 404:398–402. doi:10.1038/35006081
11. Feng Y, Zhang J, Dai L, Zhang J, Wang P, Zang J, Li Y, Wang K (2008) Inflammatory cytokine gene polymorphisms in gastric cancer cases' and controls' family members from Chinese areas at high cancer prevalence. Cancer Lett 270:250–259. doi:10.1016/j.canlet.2008.05.013
12. Xue H, Lin B, Ni P, Xu H, Huang G (2010) Interleukin-1B and interleukin-1 RN polymorphisms and gastric carcinoma risk: a meta-analysis. J Gastroenterol Hepatol 25:1604–1617. doi:10.1111/j.1440-1746.2010.06428.x
13. Vamvakopoulos JE, Taylor CJ, Morris-Stiff GJ, Green C, Metcalfe S (2002) The interleukin-1 receptor antagonist gene: a single-copy variant of the intron 2 variable number tandem repeat (VNTR) polymorphism. Eur J Immunogenet 29:337–340. doi:10.1046/j.1365-2370.2002.00319.x
14. Machado JC, Pharoah P, Sousa S, Carvalho R, Oliveira C, Figueiredo C, Amorim A, Seruca R, Caldas C, Carneiro F, Sobrinho-Simões S (2001) Interleukin 1B and interleukin 1RN polymorphisms are associated with increased risk of gastric carcinoma. Gastroenterology 121:823–829. doi:10.1053/gast.2001.28000
15. Garza-González E, Bosques-Padilla FJ, El-Omar E, Hold G, Tijerina-Menchaca R, Maldonado-Garza HJ, Pérez-Pérez GI (2005) Role of the polymorphic IL-1B, IL-1RN and TNF-A genes in distal gastric cancer in Mexico. Int J Cancer 114:237–241. doi:10.1002/ijc.20718
16. Lee SG, Kim B, Choi W, Lee I, Choi J, Song K (2003) Lack of association between pro-inflammatory genotypes of the interleukin-1 (IL-1B-31C/and IL-1RN*2/*2) and gastric cancer/duodenal ulcer in Korean population. Cytokine 21:167–171. doi:10.1016/S1043-4666(03)00032-2
17. Wu MS, Wu CY, Chen CJ, Lin MT, Shun CT, Lin JT (2003) Interleukin-10 genotypes associate with the risk of gastric carcinoma in Taiwanese Chinese. Int J Cancer 104:617–623. doi:10.1002/ijc.10987
18. Havemose-Poulsen A, Sørensen LK, Stoltze K, Bendtzen K, Holmstrup P (2005) Cytokine profiles in peripheral blood and whole blood cell cultures associated with aggressive periodontitis, juvenile idiopathic arthritis, and rheumatoid arthritis. J Periodontol 76:2276–2285. doi:10.1902/jop.2005.76.12.2276
19. Kohaar I, Tiwari P, Kumar R, Nasare V, Thakur N, Das BC, Bharadwaj M (2009) Association of single nucleotide polymorphisms (SNPs) in TNF-LTA locus with breast cancer risk in Indian population. Breast Cancer Res Treat 114:347–355. doi:10.1007/s10549-008-0006-5
20. Liang WB, Lv ML, Su XW, Gao LB, Fang WL, Luo HB, Zhang L (2010) Association of tumor necrosis factor gene polymorphisms with susceptibility to dilated cardiomyopathy in a Han Chinese population. DNA Cell Biol 29:625–628. doi:10.1089=dna.2010.1044
21. Guo W, Wang N, Li Y, Zhang JH (2005) Polymorphisms in tumor necrosis factor genes and susceptibility to esophageal squamous cell carcinoma and gastric cardiac adenocarcinoma in a population of high incidence region of North China. Chin Med J 118:1870–1878
22. Makhatadze NJ (1998) Tumor necrosis factor locus: genetic organisation and biological implications. Hum Immunol 59:571–579. doi:10.1016/S0198-8859(98)00056-1
23. Ghosh J, Joshi G, Pradhan S, Mittal B (2010) Investigation of TNFA 308G>A and TNFB 252G>A polymorphisms in genetic susceptibility to migraine. J Neurol 257:898–904. doi:10.1007/s00415-009-5430-x
24. Bouma G, Crusius JB, Oudkerk PM, Kolkman JJ, von Blomberg BM, Kostense PJ, Giphart MJ, Schreuder GM, Meuwissen SG, Pena AS (1996) Secretion of tumour necrosis factor a and lymphotoxin a in relation to polymorphisms in the TNF genes and HLA-DR alleles: relevance for inflammatory bowel disease. Scand J Immunol 43:456–463
25. Suzuki G, Izumi S, Hakoda M, Takahashi N (2004) LTA 252G allele containing haplotype block is associated with high serum C-reactive protein levels. Atherosclerosis 176:91–94. doi:10.1016/j.atherosclerosis.2003.12.013
26. Clarke R, Xu P, Bennett D, Lewington S, Zondervan K, Parish S, Palmer A, Clark S, Cardon L, Peto R, Lathrop M, Collins R (2006) Lymphotoxin-alpha gene and risk of myocardial infarction in 6,928 cases and 2,712 controls in the ISIS case–control study. PLoS Genet 2:e107. doi:10.1371/journal.pgen.0020107
27. Suzuki G, Cullings H, Fujiwara S, Matsuura S, Kishi T, Ohishi W, Akahoshi M, Hayashi T, Tahara E (2009) LTA 252GG and GA genotypes are associated with diffuse-type noncardia gastric cancer risk in the Japanese population. Helicobacter 14:571–579. doi:10.1111/j.1523-5378.2009.00694.x
28. Colombo J, Rossit AR, Caetano A, Borim AA, Wornrath D, Silva AE (2004) GSTT1, GSTM1 and CYP2E1 genetic polymorphisms in gastric cancer and chronic gastritis in a Brazilian population. World J Gastroenterol 10:1240–1245
29. Duarte MC, Colombo J, Rossit AR, Caetano A, Borim AA, Wornrath D, Silva AE (2005) Polymorphisms of DNA repair genes XRCC1 and XRCC3, interaction with environmental exposure and risk of chronic gastritis and gastric cancer. World J Gastroenterol 11:6593–65600
30. Dixon MF, Genta RM, Yardley JH, Correa P (1996) Classification and grading of gastritis: the updated Sydney system. Am J Surg Pathol 20:1161–1181. doi:00000478-199610000-00001
31. Lauren P (1965) 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 64:31–49
32. Ahrendt SA, Chown JT, Yang SC, Wu L, Zhang M-J, Jen J (2000) Alcohol consumption and cigarette smoking increase the frequency of p53 mutations in nomsmall cell lung cancer. Cancer Res 60:3155–3159
33. Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1225
34. Solé X, Guinó E, Valls J, Iniesta R, Moreno V (2006) SNPStats: a web tool for the analysis of association studies. Bioinformatics 22:1928–1929. doi:10.1093/bioinformatics/btl268

35. Sugimoto M, Yamaoka Y, Furuta T (2010) Influence of interleukin polymorphisms on development of gastric cancer and peptic ulcer. World J Gastroenterol 16:1188–1200. doi:10.3748/wjg.v16.i10.1188
36. Kamangar F, Cheng C, Abnet CC, Rabkin CS (2006) Interleukin-1B polymorphisms and gastric cancer risk–a meta-analysis. Cancer Epidemiol Biomarkers Prev 15:1920–1928. doi:10.1158/1055-9965.EPI-06-0267
37. Peleteiro B, Lunet N, Carrilho C, Durães C, Machado JC, La Vecchia C, Barros H (2010) Association between cytokine gene polymorphisms and gastric precancerous lesions: systematic review and meta-analysis. Cancer Epidemiol Biomarkers Prev 19:762–776. doi:10.1158/1055-9965.EPI-09-0917
38. Rocha GA, Guerra JB, Rocha AM, Saraiva IE, da Silva DA, de Oliveira CA, Queiroz DM (2005) IL1RN polymorphic gene and cagA-positive status independently increase the risk of noncardia gastric carcinoma. Int J Cancer 115:678–683. doi:10.1002/ijc.20935
39. Furuta T, El-Omar EM, Xiao F, Shirai N, Takashima M, Sugimura H (2002) Interleukin 1beta polymorphisms increase risk of hypochlorhydria and atrophic gastritis and reduce risk of duodenal ulcer recurrence in Japan. Gastroenterology 123:92–105. doi:10.1053/gast.2002.34156
40. Pincerati MR, Dalla-Costa R, Pavoni DP, Petzl-Erler ML (2010) Genetic polymorphisms of the T-cell coreceptors CD28 and CTLA-4 in Afro- and Euro-Brazilians. Int J Immunogenet 37:253–261. doi:10.1111/j.1744-313X.2010.00917.x
41. Trevilatto PC, de Souza Pardo AP, Scarel-Caminaga RM, De Brito RB Jr, Alvim-Pereira F, Alvim-Pereira CC, Probst CM, Garlet GP, Sallum AW, Line SR (2011) Association of IL1 gene polymorphisms with chronic periodontitis in Brazilians. Arch Oral Biol 56:54–62. doi:10.1016/j.archoralbio.2010.09.004
42. Santos NP, Ribeiro-Rodrigues EM, Ribeiro-Dos-Santos AK, Pereira R, Gusmão L, Amorim A, Guerreiro JF, Zago MA, Matte C, Hutz MH, Santos SE (2010) Assessing individual interethnic admixture and population substructure using a 48-insertion-deletion (INSEL) ancestry-informative marker (AIM) panel. Hum Mutat 31:184–190. doi:10.1002/humu.21159
43. Salzano FM, Freire-Maia N (1970) The origins.In Problems in human biology: a study of Brazilian populations, 1st edn. Wayne State university Press, Detroit, pp 38–45
44. Kumar S, Kumar A, Dixit VK (2009) Evidences showing association of interleukin-1B polymorphisms with increased risk of gastric cancer in an Indian population. Biochem Biophys Res Commun 387:456–460. doi:10.1016/j.bbrc.2009.07.033
45. Hong S, Lee HJ, Kim SJ, Hahm KB (2010) Connection between inflammation and carcinogenesis in gastrointestinal tract: focus on TGF-beta signaling. World J Gastroenterol 16:2080–2093. doi:10.3748/wjg.v16.i17.2080
46. Lee SG, Kim B, Yook JH, Oh ST, Lee I, Song K (2004) TNF/LTA polymorphisms and risk for gastric cancer/duodenal ulcer in the Korean population. Cytokine 28:75–82. doi:10.1016/j.cyto.2004.06.009
47. Al-Moundhri MS, Alkindy M, Al-Nabhani M, Al-Bahrani B, Burney IA, Al-Habsi H, Ganguly SS, Tanira M (2009) Combined polymorphism analysis of glutathione S-transferase M1/G1 and interleukin-1B (IL-1B)/interleukin 1-receptor antagonist (IL-1RN) and gastric cancer risk in an Omani Arab Population. J Clin Gastroenterol 43:152–156. doi:10.1097/MCG.0b013e31815853fa
48. Crusius JB, Canzian F, Capellá G, Pena AS, Pera G, Sala N, Agudo A, Rico F, Del Giudice G, Palli D, Plebani M, Boeing H, Bueno-de-Mesquita HB, Carneiro F, Pala V, Save VE, Vineis P, Tumino R, Panico S, Berglund G, Manjer J, Stenling R, Hallmans G, Martínez C, Dorronsoro M, Barricarte A, Navarro C, Quirós JR, Allen N, Key TJ, Binghan S, Caldas C, Linseisen J, Kaaks R, Overvad K, Tjønneland A, Büchner FC, Peeters PH, Numans ME, Clavel-Chapelon F, Trichopoulou A, Lund E, Jenab M, Rinaldi S, Ferrari P, Riboli E, González CA (2008) Cytokine gene polymorphisms and the risk of adenocarcinoma of the stomach in the European prospective investigation into cancer and nutrition (EPIC-EURGAST). Ann Oncol 19:1894–1902. doi:10.1093/annonc/mdn400
49. INCA—National Institute of Cancer (2011) Incidence and mortality ratios by cancer in Brazil. http://www.inca.org.br. Accessed 15 June 2011