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BRIEF ARTICLE

Polymorphisms of the *TLR2* and *TLR4* genes are associated with risk of gastric cancer in a Brazilian population

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Abstract

AIM: To investigate toll-like receptor 2 (*TLR2*) -196 to -174 del, and *TLR4* (+896A/G rs4986790 and +1196C/T rs4986791) polymorphisms at risk of chronic gastritis and gastric cancer in a Brazilian population and association of gastric lesions with risk factors such as smoking, alcohol intake and *Helicobacter pylori* infection.

METHODS: In this case-control study, polymorphism at *TLR2* -196 to -174 del was investigated by using the allele-specific polymerase chain reaction (PCR) method, while the PCR-restriction fragment length polymorphism technique was carried out to identify the *TLR4* (rs4986790 and rs4986791) genotypes in 607 Brazilian individuals (208 with chronic gastritis-CG, 174 with gastric cancer-GC and 225 controls -C).

RESULTS: The single nucleotide polymorphisms *TLR4*+1196C/T was not associated with risk of chronic gastritis or gastric cancer and the homozygous genotypes *TLR4*+896GG and *TLR4*+1196TT were absent in the studied population. However, the frequency of *TLR2* -196 to -174 ins/del + del/del and *TLR4*+896AG genotypes was significantly higher ($P < 0.01$ and $P = 0.01$, respectively) in the cancer group (33.4% and 11.5%, respectively) than in the control group (16.9% and 4.5%, respectively). It was also observed that the G-C haplotype of the *TLR4*+896A/G+1196C/T ($P = 0.02$) and the combination of variant alleles of the *TLR2*/*TLR4*+896G ($P = 0.02$) are associated with susceptibility to gastric cancer. In addition, the multiple logistic regression showed that male gender [odds ratio (OR) = 2.70; 95% CI: 1.66-4.41; $P < 0.01$], alcohol intake (OR = 2.93; 95% CI: 1.76-4.87; $P < 0.01$), *TLR2* -196 to -174 del (OR = 2.64; 95% CI: 1.56-4.44; $P < 0.01$) and *TLR4*+896G (OR = 3.19; 95% CI: 1.34-7.61; $P < 0.01$) polymorphisms were associated with a higher susceptibility to developing this neoplasm.

CONCLUSION: Our data indicate that *TLR2* -196 to -174 del and *TLR4*+896G may increase the risk of gastric cancer in a Brazilian population.

Key words: Polymorphisms; Toll-like receptor 2; Toll-like receptor 4; Gastric cancer; Gastritis

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INTRODUCTION

Gastric cancer is one of the most serious health problems in many countries, including Brazil, which ranks third in incidence and mortality, and with an estimated incidence in 2010 of about 21 500 new cases, with an incidence of 14.25 per 100 000 males and 7.70 per 100 000 females[1]. The *Helicobacter polori* (*H. pylori)* is the major etiological risk factor for this malignancy, which progresses through a multi-step process, developing from gastritis, to gastric atrophy, intestinal metaplasia, dysplasia, and finally to carcinoma[2]. It is widely accepted that chronic *H. pylori* infection induces a gastric atrophy and hypochlorhydria, which are precursors of all pathophysiological changes of gastric carcinogenesis[3]. However, colonization with *H. pylori* can lead to various outcomes. Nearly all *H. pylori* positive subjects have chronic gastritis, and only 1%-2% development of stomach cancer among infected[4]. Hence, other factors are likely to be involved in gastric tumorigenesis such as host genetic factors, as well as the diversity of *H. pylori* virulence genes.

Host genetic factors, as polymorphisms in inflammatory and immune response genes, are mainly related to the recognition of the bacteria by the immune system and the variation in the level of cytokine response[5]. Among host factors, several inflammatory proteins including cytokines, growth factors, and chemokines have been known to control immune response against *H. pylori* infection[6,7]. Therefore, many studies have focused on the analyses of polymorphisms in genes associated with the inflammatory response in the gastric mucosa and risk for malignancy[8-11]. Other mediators also have polymorphic variants that modulate the innate immune response pattern, as the toll-like receptors (TLRs), which provide first line of host defense against harmful pathogens[12].

Among the TLRs, it has been reported that the TLR2, lipoproteins bacterial receptor and the TLR4, the lipopolysaccharide (LPS) receptor, are involved in the response to infection by *H. pylori* on gastric epithelial cells[3,13-15]. Both the TLR2 and the TLR4 promote transcription of genes involved in immune activation including nuclear factor kappa B (*NF-κB)* and also mintage-activated protein (*MAP*) kinase pathways[16]. *TLR4* is up regulated in gastric epithelial cell lines infected with *H. pylori* and in macrophages and expression of TLR4 protein has been demonstrated in chronic active gastritis, in precancerous lesions, and also in gastric tumor cells. TLR2 activates NF-κB in epithelial cells, in response to *H. pylori* infection, causing the expression of interleukin (IL)-8, macrophage inflammatory protein-3α and growth-regulated oncogene alpha[7]. Thus, it is conceivable that functionally relevant polymorphisms in *TLR* genes can alter the host immune response to pathogens as infection induced by *H. pylori*.

Single nucleotide polymorphisms (SNPs) in *TLR2* have been associated with susceptibility to various infectious and inflammatory diseases such as leprosy[17], increased risk of Gram-negative sepsis[18], asthma[19], recurrent bacterial infections[20] and sporadic colorectal cancer susceptibility[21]. The specific polymorphism *TLR2* -196 to -174 del/del genotype has been reported to show decreased transcriptional activity of the *TLR2* gene[22]. Such fact aroused the interest for this polymorphism, and previous studies in the Japanese population demonstrated its association with increased susceptibility to non-cardia gastric cancer[23] and intestinal metaplasia[24].

Likewise, the *TLR4* presents some polymorphisms implicated in increased susceptibility to various diseases such as atherosclerosis[25], asthma[3], malaria[26], and also infection with the *H. pylori* associated with gastric cancer and its precursors[27]. Two SNPs in *TLR4*+896A/G (rs4986790) and +1196C/T (rs4986791) have received special attention in some studies, although the results are still controversial[6,28,29].

Therefore, the aim of this study was to evaluate the influence of the 22-bp nucleotide deletion -196 to –174 del, in the promoter region of the gene *TLR2* and +896A/G and +1196C/T polymorphisms (Asp299Gly and Thr399Ile) respectively in *TLR4* gene on the risk of chronic gastritis and gastric cancer in a Brazilian population and whether there is an association of gastric lesions with risk factors such as smoking, alcohol intake and *H. pylori* infection.

MATERIALS AND METHODS

Subjects

This was a case-control study on chronic gastritis and gastric cancer, in which a total of 607 DNA samples from peripheral blood leukocytes were genotyped. The case groups comprised 208 individuals (102 men and 106 women) with a histopathologically confirmed diagnosis of chronic gastritis - CG (Sidney System)[30], with a mean age of 52.8 + 14.5 years (range 19 to 84 years), and 174 individuals (134 men and 40 women) with a histopathologically confirmed diagnosis of gastric cancer - GC (Lauren's classification)[31], with a mean age of 62.2 ± 12.2 years (range 28 to 93 years). All subjects were recruited from the Hospital de Base in São José do Rio Preto, SP, and from the Pio XII Foundation in Barretos, SP, Brazil. *H. pylori* infection was histologically established either by the Giemsa staining technique or by the urease test, performed at the Pathology Services of the Hospital de Base and the Pio XII Foundation. Results of *H. pylori* infection were obtained for the available cases. The cancer-free control group (C) with no previous history of gastric disease was composed of 225 healthy individuals (112 men and 113 women), mainly blood donors, with a mean age of 56.5 + 18.1 years (range 20 to 93 years). 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. All the individuals were ethnically classified on their visual appearance as Caucasians in the three groups evaluated. The few cases of African descent were excluded from the study (about 10%).

Table 1 Primer sequences, restriction enzymes and fragment sizes for toll-like receptor 2 and toll-like receptor 4 gene polymorphisms and interleukin-1β gene

Genes	Primers	Enzyme T°/time	Fragment (bp)	Ref.
TLR2 *del* -196 to -174	F: 5′-CACGGAGGCAGCGAGAAA-3′ R: 5′-CTGGGCCGTGCAAAGAAG-3′	-	286 ins/ins: 286 ins/del: 286, 264 del/del: 264	[24]
TLR4+896A/G rs4986790	F: 5′-AGCATACTTAGACTACCACCTCGATG 3′ R: 5′-GTTGCCATCCGAAATTATAAGAAAAG 3′	*Bst*XI 37 ℃, 1 h	131 A/A: 131 A/G: 131, 108 G/G: 108	[34]
TLR4+1196C/T rs4986791	F: 5′-GGTTGCTGTTCTCAAAGTGATTTTGGGAGAA-3′ R: 5′-ACCTGAAGACTGGAGAGTGAGTTAAATGCT-3′	*Hin*fI 37 ℃, 1 h	407 C/C: 407 C/T: 407, 378 T/T: 378	[33]
IL1-β	F: CATGTGACCTGCTCGTCAGT R: CCCTAGGGATTGAGTCCACA	*Hin*fI 37 ℃, 1 h	370 195, 175	[47]
TLR2	F: 5′-CACGGAGGCAGCGAGAAA-3′ R: 5′-CTGGGCCGTGCAAAGAAG-3′	*Bst*XI 37 ℃, 1 h	286 188, 98	[24]

TLR: Toll-like receptor; IL: Interleukin; T°: Temperature.

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

Genotyping

About 5 mL of whole blood were collected from all study participants in sterile EDTA-coated vacutainers. DNA was extracted according to a previous report[32], and stored at -20 ℃ until use for genotyping.

Polymorphism at *TLR2* -196 to -174 del was investigated using the allele-specific polymerase chain reaction (PCR) method[24], and the PCR-restriction fragment length polymorphism (RFLP) technique was carried out in order to identify the *TLR4* (rs4986790 and rs4986791) genotype[33,34] in cases and control groups. In brief, the procedure was carried in a total reaction volume of 25 μL, containing 2.5 μL 10 × PCR buffer, 2 μL deoxyribonucleotide triphosphatess (1.25 μmol/L), 0.5 μL $MgCl_2$ (25 mmol/L), 1.25 μL of each primer (25 mmol/L, Sigma-Aldrich, United States), 15.3 μL dH_2O, 2 μL DNA (100 ng/μL), and 0.2 μL Taq DNA polymerase (5 U/μL, Invitrogen, United States). PCR for *TLR2* -196 to -174 *del* was as follows: initial denaturation step at 95 ℃ for 5 min, amplification was carried out by 35 cycles at 95 ℃ for 30 s, at 60 ℃ for 40 s, and at 72 ℃ for 40 s, followed by a final elongation cycle at 72 ℃ for 7 min (Table 1). PCR for both *TLR4* polymorphisms were as follows: after an initial denaturation step at 94 ℃ for 3 min, amplification was carried out by 30 cycles at 94 ℃ for 30 s, at 62 ℃ for 30 s, and at 72 ℃ for 30 s, followed by a final elongation cycle at 72 ℃ for 7 min. Then, 10 μL of *TLR4* +896 A/G and *TLR4* +1196 C/T polymorphism PCR products were digested with 0.5 μL (5 U/μL) of the *Bst*XI and *Hin*fI specific enzymes, respectively in a 10 μL volume including 2.5 μL 10 × buffer 1 (New England Biolabs, United States) and 7.0 μL dH_2O (Table 1). The products were then electrophoresed on a 3% agarose 1000 (Invitrogen, United States) gel, to allow detection by ethidium bromide staining.

In order to confirm the veracity of the results, one confirmed polymorphic case was used as positive control for every RFLP procedure, to attest the good functioning of the restriction enzyme. For the *TLR4*+896A/G (rs4986790) and +1196C/T (rs4986791) polymorphisms, we also used other fragments of the *TLR2* and *IL-1β* gene that was known to have the enzyme recognition site to verify the correct functioning of the enzymes *Bst*XI and *Hin*fI (Table 1), considering we did not detect any polymorphic homozigous subjects.

Statistical analysis

Fisher's exact test was used to compare the groups regarding genotype and allele frequencies, and the chi-square test for determining Hardy-Weinberg equilibrium. Multiple logistic regression models were used to determine the effects of the variables in gastric cancer and chronic gastritis. The models included age (reference: < 61 and 53 years old - median of the groups), gender (reference: female), smoking habits (reference: nonsmokers), drinking habits (reference: nondrinkers), and *H. pylori* infection (reference: *H. pylori*-negative). The results are shown as odds ratio (OR), showing 95% CI. ORs were calculated using a dominant model due to low frequency of polymorphic homozygous (i.e., combining heterozygous and homozygous for the minor allele *vs* homozygous for the major allele) for all SNPs. The haplotype frequencies of *TLR4* were inferred by Haploview program (4.0 version). Statistical analyses were performed using the GraphPad Instat, and SPSS (11.5 version) computer software programs. A probability level (*P*) of less that 0.05 was adopted as a significance criterion.

RESULTS

The samples of the 607 subjects were genotyped for the *TLR2* -196 to -174 *del* and *TLR4* (+896A/G rs4986790

Table 2 Genotype and allele frequencies of toll-like receptor 2 (-196 to -174 *del*) and toll-like receptor 4 (rs4986790 and rs4986791) polymorphisms in gastric cancer, chronic gastritis and control groups

Genotypes/alleles	GC *n* = 174 (%)	C *n* = 225(%)	CG *n* = 208 (%)
TLR2 -196 to -174			
ins/ins	116 (66.6)	189 (84.0)	160 (76.9)
del/ins	50 (28.7)	34 (15.1)	41 (19.7)
del/del	8 (4.7)	2 (0.9)	7 (3.4)
OR (95% CI)	2.62 (1.63-4.22)		1.57 (0.97-2.54)
P value	< 0.01		0.06
Alleles			
ins	81.0	91.5	87.0
del	19.0	8.5	13.0
OR (95% CI)	2.53 (1.65-3.88)		1.65 (1.06-2.55)
P value	< 0.01		0.02
TLR4 +896A/G (rs4986790)			
AA	154 (88.5)	215 (95.5)	187 (89.9)
AG	20 (11.5)	10 (4.5)	21 (10.1)
GG	0	0	0
OR (95% CI)	2.79 (1.27-6.13)		2.41 (1.10-5.25)
P value	0.01		0.02
Alleles			
A	94.0	97.0	95.0
G	6.0	3.0	5.0
OR (95% CI)	2.68 (1.23-5.81)		2.33 (1.08-5.02)
P value	0.01		0.02
TLR4 +1196C/T (rs4986791)			
CC	165 (94.8)	219 (97.3)	202 (97.1)
CT	9 (5.2)	6 (2.7)	6 (2.9)
TT	0	0	0
OR (95% CI)	1.99 (0.69-5.70)		1.08 (0.34-3.41)
P value	0.28		1.00
Alleles			
C	98.0	99.0	98.0
T	2.0	1.0	2.0
OR (95% CI)	1.96 (0.69-5.57)		1.08 (0.34-3.38)
P value	0.29		1.00

OR: Odds ratio; GC: Gastric cancer; CG: Chronic gastritis; C: Control.

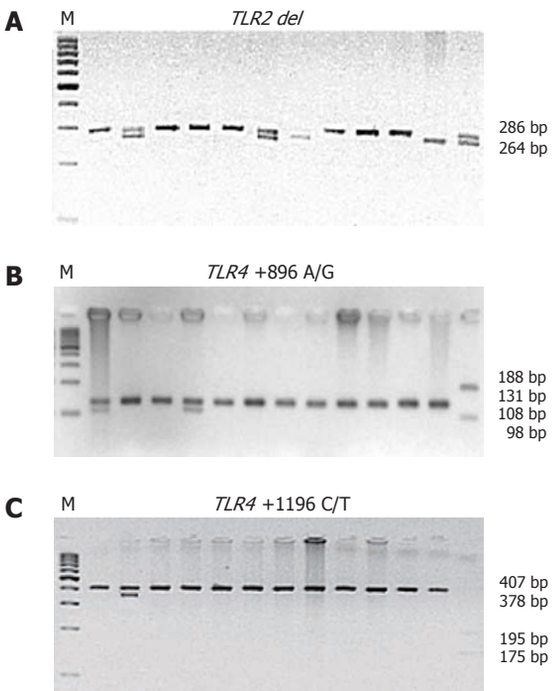

Figure 1 Eletrophoretic pattern of fragments generated by polymerase chain reaction-allele specific and polymerase chain reaction-restriction fragment length polymorphism for the polymorphisms. A: Toll-like receptor (*TLR*)2 -196 to -174 del: ins/ins = 286 bp; ins/del = 286 + 264 bp; del/del = 264 bp; B: *TLR4* + 896A/G: A/A = 131 bp; A/G = 131 + 108 bp and positive control of enzyme *BstXI*, *TLR2* gene fragment of 286 bp with the enzyme cutting site: 188 + 98 bp (last lane); C: *TLR4* + 1196 C/T: C/C = 407 bp; C/T: 407 + 378 bp and positive control of enzyme *Hinf*I, interleukin-1β gene fragment of 370 bp with the enzyme cutting site: 195 + 175 bp (last lane). M: Molecular weight marker of 100 bp.

Table 3 Toll-like receptor 4 haplotype frequency distribution between gastric cancer, chronic gastritis and control groups

Haplotypes *TLR4* +896/+1196	GC (%)	C (%)	χ^2	*P* value	CG (%)	C (%)	χ^2	*P* value
A-C	91.4	95.7	6.802	< 0.01	94.0	95.7	1.361	0.24
G-C	63.0	31.0	5.247	0.02	46.0	31.0	1.598	0.20
A-T	21.0	11.0	1.461	0.22	NF	NF	NF	NF

Haplotype G-T not found. NF: Not found; GC: Gastric cancer; CG: Chronic gastritis; C: Control.

and +1196C/T rs4986791). The genotype and allele frequencies for these polymorphisms are presented in Table 2. The genotype and allele frequency distribution of the three polymorphisms complied with Hardy-Weinberg equilibrium in both cases and control groups (data not shown). The banding patterns of these SNPs are represented in Figure 1.

For *TLR2* -196 to -174 del, the genotype (ins/del and del/del) and allele (del) frequencies were increased statistically ($P < 0.01$) in gastric cancer group (33.4% and 19% respectively) than in control group (16.0% and 8.5% respectively). In addition, among the groups of chronic gastritis (13.0%) and control (8.5%), the allele frequencies (del) was statistically significant ($P = 0.02$).

Similarly, for *TLR4*+896A/G (rs4986790), the genotypes (A/G) and allele (G) frequencies were increased statistically in gastric cancer group (11.5% and 6.0%, respectively; $P = 0.01$) and chronic gastritis (10.1% and 5.0%, respectively; $P = 0.02$) than in control group (4.5% and 3%, respectively). This results due to the higher frequency of polymorphic allele (*TLR4* +896G) in the gastric cancer and chronic gastritis groups.

In contrast, for *TLR4*+1196C/T (rs4986791), no significant difference was found between gastric cancer and control group (Table 2). Homozygous genotypes *TLR4*+896GG and *TLR4*+1196TT were absent in the studied population. We also compared the genotype and allele frequencies between gastric cancer and gastritis groups and no significant difference was found for the polymorphisms studied (data not shown).

The *TLR4* haplotype analysis (Table 3) demonstrated higher frequency of both wild alleles (haplotype A-C) in control subjects compared with gastric cancer (95.7% and 91.4%, respectively; $P < 0.01$). However, the opposite was observed for frequency of variant haplotype

Table 4 Combined effect of toll-like receptor 2 (-196 to -174 *del*) and toll-like receptor 4 (rs4986790 and rs4986791) polymorphisms on risk of gastric cancer and chronic gastritis

Risk genotype	Groups								
	GC (n = 174)	C (n = 225)	OR (95% CI) P value	CG (n = 208)	C (n = 225)	OR (95% CI) P value	GC (n =174)	CG (n = 225)	OR (95% CI) P value
Neither	113	187	1.00 (reference)	160	187	1.00 (reference)	113	160	1.00 (reference)
TLR2 ins/del or del/del/*TLR4*+896 AG	10	4	4.13 (1.26-13.50) 0.02	11	4	3.21 (1.00-10.29) 0.06	10	11	1.28 (0.52-3.13) 0.64
TLR2 ins/del or del/del/*TLR4*+1196 CT	4	1	6.61 (0.73-59.99) 0.07	1	1	1.16 (0.07-18.84) 1.00	4	1	5.66 (0.62-51.37) 0.16
TLR4+896 AG/+1196 CT	1	1	1.65 (0.10-26.73) 1.00	3	1	3.50 (0.36-34.05) 0.34	1	3	0.47 (0.04-4.59) 0.64

C: Control group; OR: Odds ratio; GC: Gastric cancer; CG: Chronic gastritis; TLR: Toll-like receptor.

Table 5 Distribution of risk factors, genotypes of toll-like receptor 2 (-196 to -174 *del*) and toll-like receptor 4 (rs4986790 and rs4986791), and odds ratios for gastric cancer, chronic gastritis and control groups

Variables	F (GC/C) %	OR (95% CI)	P value	F (CG/C) %	OR (95% CI)	P value
Gender						
Female	23/49.7	Reference	< 0.01	51.0/50.3	Reference	0.96
Male	77/50.3	2.70 (1.66-4.41)		49.0/49.7	0.99 (0.66 -1.48)	
Age (yr)						
< 61	40.9/56.0	Reference	0.30	52.8/43.5	(< 53 yr) Reference	0.03
⩾ 61	59.1/44.0	1.26 (0.81-1.98)		47.2/56.5	(⩾ 53 yr) 0.64 (0.43-0.95)	
Smoking						
Nonsmokers	30.5/34.3	Reference	0.13	44.8/34.3	Reference	0.01
Smokers	69.5/65.7	0.67(0.39-1.13)		55.2/65.7	0.57 (0.37-0.87)	
Alcohol						
Nondrinkers	46.6/74.3	Reference	< 0.01	68.2/74.3	Reference	0.06
Drinkers	53.4/25.7	2.93 (1.76-4.87)		31.8/25.7	1.54 (0.97-2.44)	
TLR2						
ins/ins	66.6/84.0	Reference	< 0.01	76.9/84.0	Reference	0.18
ins/del	33.4/16.0	2.64 (1.56-4.44)		23.1/16.0	1.39 (0.84 -2.28)	
del/del						
TLR4 +896A/G (rs4986790)						
AA	88.5/95.5	Reference	< 0.01	89.9/95.5	Reference	0.04
AG	11.5/4.5	3.19 (1.34-7.61)		10.1/4.5	2.29 (1.02-5.13)	
TLR4 +1196C/T (rs4986791)						
CC	94.8/97.3	Reference	0.63	97.1/97.3	Reference	0.91
CT	5.2/2.7	1.33 (0.41-4.33)		2.9/2.7	0.93 (0.28-3.05)	

F: Frequency of individual (%); OR: Odds ratio; GC: Gastric cancer; CG: Chronic gastritis; C: Control; TLR: Toll-like receptor.

G-C, which was higher in the gastric cancer group compared with control group (63.0% and 31.0%, respectively; P = 0.02).

In another statistical analysis, which evaluated the combined effect between the three polymorphisms (*TLR2* -196 to -174 del, *TLR4*+896A/G and +1196C/T), the combination of variant alleles of the polymorphisms *TLR2* ins/del and del/del with *TLR4* +896 AG showed a higher risk of gastric cancer compared to healthy individuals (OR = 4.13; 95% CI: 1.26-13.50; P = 0.02). The other combinations of variant alleles did not show any significant difference (Table 4). The combination of the three variant alleles was found in only one individual of the group of chronic gastritis (data not shown).

The potential associations between the distributions of *TLR2* -196 to -174 del and *TLR4* (+896A/G and +1196C/T) genotypes adjusting for risk factors for gastric cancer and chronic gastritis in comparison of control group are presented in Table 5.

In the gastric cancer group, the multiple logistic regression shows that male gender (OR = 2.7; 95% CI: 1.66-4.41; P < 0.01), alcohol intake (OR = 2.93; 95% CI: 1.76-4.87; P < 0.01), *TLR2* -196 to -174 ins/del+del/del (OR = 2.64; 95% CI: 1.56-4.44; P < 0.01) and *TLR4* +896AG (OR = 3.19; 95% CI: 1.34-7.61; P < 0.01) were associated with a higher susceptibility to developing this neoplasm. The comparison between gastritis and control group showed that only *TLR4*+896AG polymorphism was associated with risk of chronic gastritis (OR = 2.29; 95% CI: 1.02-5.13; P = 0.04), while age above 53 years (OR = 0.64; 95% CI: 0.43-0.95; P = 0.03) and smoking (OR = 0.57; 95% CI: 0.37-0.87; P = 0.01) were negatively associated with the development of gastritis. In another multiple logistic regression analysis considering also the individuals tested for *H. pylori* infection (95 with gastric cancer and 177 with gastritis), there was no statistically

significant association (OR = 0.73, 95% CI: 0.29-1.78; $P = 0.49$, data not shown in Table 5). There was also no association of three polymorphisms with *H. pylori* when evaluated negative and positive individuals within a group of gastric cancer and gastritis (data not shown).

DISCUSSION

TLRs participate in *H. pylori* bacterium recognition in gastric mucosa, and SNPs in TLRs are associated with impaired immune response, inducing a potent inflammatory response. Therefore, it is relevant to carry out studies on host genetic factors that can be associated with susceptibility of gastric diseases. Hence, we investigated whether *TLR2* -196 to –174 del and *TLR4* (+896A/G rs4986790 and +1196C/T rs4986791) polymorphisms affect the risk of developing gastric cancer and chronic gastritis in a Brazilian population. Our results have demonstrated for the first time in this population, an association of *TLR2* -196 to -174 del and of *TLR4*+896 G polymorphisms with susceptibility to gastric cancer. The polymorphism *TLR4*+1196 T was not associated with risk to the gastric lesions evaluated, and the homozygous genotypes *TLR4*+896GG and *TLR4*+1196TT were absent in the studied population.

Some studies that investigated the association of *TLR2* -196 to -174 del polymorphisms at risk of developing diseases related to an inflammatory process have shown conflicting results. For instance, del allele or del/del genotype of *TLR2* -196 to-174 polymorphism was significantly associated with cervical cancer susceptibility[35] and risk of non-cardia gastric cancer in a Japanese population, but not for gastritis, gastric ulcer and duodenal ulcer[23], while *TLR2* -196 to -174 ins allele was associated with more severe intestinal metaplasia in older patients[24]. However, Wang *et al*[36] failed to show association of *TLR2* -196 to -174 del/del and ins/del carriers with ulcerative colitis.

The -196 to -174 del polymorphism in *TLR2* gene located on chromosome 4, causes a 22-bp nucleotide deletion that alters the promoter activity of gene. The *TLR2* del/del genotype is reported to show decreased transcriptional activity this gene[22]. In our study, we observed significantly higher frequencies of genotypes *TLR2* ins/del and del/del in the gastric cancer group compared to the healthy individuals, emphasizing its role in the gastric carcinogenesis.

The *TLR4* gene is mapped on chromosome 9 and consists of three exons. In exon 3, two non-synonymous SNPs *TLR4*+896A/G and +1196C/T allows the substitution of amino acids Asp299Gly and Thr399Ile, respectively. In the analysis by Haploview, the frequencies of *TLR4* G-C (299Gly-399Thr) and G allele were higher in patients with gastric cancer indicating an association of this haplotype with increased risk of gastric cancer to its carriers. The substitution of Asp299Gly amino acids disrupt the normal structure of the extracellular region of the TLR4 and may cause decreased ligand recognition or protein interaction, and decreased responsiveness to lipopolysaccharide, disrupting transport of *TLR4* to the cell membrane[13,37]. This change leads to an exaggerated inflammatory response with severe tissue destruction, likely due to a failure in stimulating regulatory cells and production of IL-10 cytokine[38]. Arbour *et al*[13] were the first to report that individuals having either the Asp-299Gly and/or Thr399Ile polymorphisms had a blunted response towards inhaled LPS. Thus, during the cascade of progression of gastric carcinogenesis, the subjects with this polymorphism can have an increased risk of severe inflammation followed by development of hypochlorhydria and gastric atrophy, which are regarded as important precursor alterations of gastric cancer[12].

Both SNPs in *TLR4* are presented in about 10% of Caucasian and African populations and are reported to have a positive correlation with susceptibility to infectious diseases, whereas studies in Asian populations have shown the absence of these polymorphisms[26,39]. However, in our study with a Southeastern Brazilian population, we found that both SNPs *TLR4* (+896A/G and +1196C/T) were present in heterozygous in about 4.5% to 11.5% and 2.7% to 5.2% respectively in the gastric cancer and control groups. Other studies in the Brazilian population found similar frequencies of heterozygous *TLR4*+896A/G polymorphism in Chagas disease (5.6%), ulcerative colitis (7.1%) and Crohn's disease (7%)[40,41]. But, to the best of our knowledge, there are not studies on *TLR2* and *TLR4* polymorphisms in gastric cancer of Brazilian population.

With regards to *TLR4*+896A/G and +1196C/T polymorphisms, Garza-Gonzalez *et al*[29] showed no association with the risk of gastric cancer in the Mexican population, while Trejo de la O *et al*[7] observed that both SNPs in *TLR4* had an association with duodenal ulcer and gastric cancer also in Mexican patients. Yet, other studies have demonstrated association with only one of these polymorphisms and risk of gastric cancer and precancerous lesions, either *TLR4*+1196C/T (Thr399Ile)[6,42] or *TLR4*+896A/G (Asp299Gly) polymorphisms[3] are associated with susceptibility to gastric carcinogenesis. In addition, in this study in Caucasian population[3], homozygous polymorphic *TLR4*+896 GG was not found, corroborating our results.

Concerning *H. pylori* infection, we evaluated the association between *TLR2* and *TLR4* polymorphisms in the case groups with the available information in their medical records (95 with gastric cancer and 177 with gastritis) and no association was found. The reduced number of samples available for statistical analysis may have harmed these results. Rad *et al*[8] studied the role of various TLRs (2/4/7 and 9) in response to *H. pylori* using mice mutants lacking these receptors. The results demonstrated the importance of the TLR2 in response to this bacterium, unlike the TLR4. TLR4-receptor lacking mice had little or no change in response to *H. pylori* compared with controls. Although the role that the effect of polymorphism in *TLR2* in the activity of this receptor in

gastric cells is not fully understood, this deletion is likely to alter their activity. Since *TLR2* has an important role in immune response against the *H. pylori* bacterium, the change of its function becomes relevant in carcinogenesis of the stomach[8].

Besides the influence of the *H. pylori*, other risk factors as gender, age, smoking and alcohol intake were analyzed. There are statistically significant results for male gender and alcohol intake in the gastric cancer group compared with the control group. According to the National Cancer Institute the highest incidence of gastric cancer occurs in men around age 70 years, and about 65% of patients diagnosed with this type of cancer were over 50 years[1]. Another risk factor well established in the literature in relation to gastric carcinogenesis is the excessive consumption of alcohol[43-45]. Really, ethanol oxidation generates acetaldehyde, which presents carcinogenic effects, since it interferes with many DNA synthesis and repair sites, leading to tumor development[46].

In conclusion, our findings indicate a significant role of both *TLR2* -196 to -174 del and *TLR4*+896G (Asp299Gly) polymorphic variant with susceptibility to gastric cancer in the Southeastern Brazilian population evaluated, whereas no association was observed for *TLR4* +1196T polymorphism. Thus, it is feasible to highlight that host genetic factors as the interaction of polymorphisms in genes of toll-like receptors can play an important role in gastric carcinogenesis.

COMMENTS

Background

Gastric cancer (GC) has high rate of incidence and mortality in Brazilian population. Thus is important to establish host genetic factors, as polymorphisms in genes related with inflammatory and immune response associated with higher risk of development of this neoplasia. In this study, authors have shown, for the first time association of toll-like receptor (*TLR)2* -196 to -174 *del* and *TLR4*+896AG (Asp299Gly) polymorphisms with gastric cancer in a sample of Brazilian population.

Research frontiers

Epidemiological studies on association of polymorphisms with susceptibility to disease as cancer frequently present conflicting results, possibly due to different factors as ethnicity, sample number, population sub-sampling, which can contribute to this discrepancy. Thus, are relevant studies in different populations that help clarify these aspects.

Innovations and breakthroughs

In this study it was possible to make the combined analysis of three polymorphisms (*TLR2* -196 to -174 *del*, *TLR4*+896A/G and +1196C/T) and to show that the combination of single nucleotide polymorphisms *TLR2 ins/del and del/del* with *TLR4* +896 AG led to a higher risk of gastric cancer.

Applications

Considering the high incidence of gastric cancer in the Brazilian population, the data show that carriers of polymorphisms in genes involved with immune response as *TLR2 ins/del* and *del/del* and *TLR4* +896 AG, together with other genetic and environmental factors constitute a risk group to gastric carcinogenesis.

Peer review

This is a cross sectional study on the role of the TLR2 and TLR4 polymorphisms in GC in a Brazilian population. The main point that needs to be further clarified is the ethnic composition of the population. An important observation is that the TLR4 variants are at similar frequencies on the African and Caucasian populations.

REFERENCES

1 **INCA**. National Institute of Cancer: Incidence and mortality ratios by cancer in Brazil. Available from: URL: http://www.inca.org.br

2 **Correa P**. A human model of gastric carcinogenesis. *Cancer Res* 1988; **48**: 3554-3560

3 **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, El-Omar EM. A functional polymorphism of toll-like receptor 4 gene increases risk of gastric carcinoma and its precursors. *Gastroenterology* 2007; **132**: 905-912

4 **Scholte GH**, van Doorn LJ, Cats A, Bloemena E, Lindeman J, Quint WG, Meuwissen SG, Kuipers EJ. Genotyping of Helicobacter pylori in paraffin-embedded gastric biopsy specimens: relation to histological parameters and effects on therapy. *Am J Gastroenterol* 2002; **97**: 1687-1695

5 **Garza-González E**, Bosques-Padilla FJ, El-Omar E, Hold G, Tijerina-Menchaca R, Maldonado-Garza HJ, Pérez-Pérez GI. Role of the polymorphic IL-1B, IL-1RN and TNF-A genes in distal gastric cancer in Mexico. *Int J Cancer* 2005; **114**: 237-241

6 **Achyut BR**, Ghoshal UC, Moorchung N, Mittal B. Association of Toll-like receptor-4 (Asp299Gly and Thr399Ileu) gene polymorphisms with gastritis and precancerous lesions. *Hum Immunol* 2007; **68**: 901-907

7 **Trejo-de la O A**, Torres J, Pérez-Rodríguez M, Camorlinga-Ponce M, Luna LF, Abdo-Francis JM, Lazcano E, Maldonado-Bernal C. TLR4 single-nucleotide polymorphisms alter mucosal cytokine and chemokine patterns in Mexican patients with Helicobacter pylori-associated gastroduodenal diseases. *Clin Immunol* 2008; **129**: 333-340

8 **Rad R**, Ballhorn W, Voland P, Eisenächer K, Mages J, Rad L, Ferstl R, Lang R, Wagner H, Schmid RM, Bauer S, Prinz C, Kirschning CJ, Krug A. Extracellular and intracellular pattern recognition receptors cooperate in the recognition of Helicobacter pylori. *Gastroenterology* 2009; **136**: 2247-2257

9 **Kumar S**, Kumar A, Dixit VK. Evidences showing association of interleukin-1B polymorphisms with increased risk of gastric cancer in an Indian population. *Biochem Biophys Res Commun* 2009; **387**: 456-460

10 **Melo Barbosa HP**, Martins LC, Dos Santos SE, Demachki S, Assumpção MB, Aragão CD, de Oliveira Corvelo TC. Interleukin-1 and TNF-alpha polymorphisms and Helicobacter pylori in a Brazilian Amazon population. *World J Gastroenterol* 2009; **15**: 1465-1471

11 **Partida-Rodríguez O**, Torres J, Flores-Luna L, Camorlinga M, Nieves-Ramírez M, Lazcano E, Perez-Rodríguez M. Polymorphisms in TNF and HSP-70 show a significant association with gastric cancer and duodenal ulcer. *Int J Cancer* 2010; **126**: 1861-1868

12 **El-Omar EM**, Ng MT, Hold GL. Polymorphisms in Toll-like receptor genes and risk of cancer. *Oncogene* 2008; **27**: 244-252

13 **Arbour NC**, Lorenz E, Schutte BC, Zabner J, Kline JN, Jones M, Frees K, Watt JL, Schwartz DA. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet* 2000; **25**: 187-191

14 **Maeda S**, Akanuma M, Mitsuno Y, Hirata Y, Ogura K, Yoshida H, Shiratori Y, Omata M. Distinct mechanism of Helicobacter pylori-mediated NF-kappa B activation between gastric cancer cells and monocytic cells. *J Biol Chem* 2001; **276**: 44856-44864

15 **Ding SZ**, Torok AM, Smith MF, Goldberg JB. Toll-like receptor 2-mediated gene expression in epithelial cells during Helicobacter pylori infection. *Helicobacter* 2005; **10**: 193-204

16 **Seya T**, Shime H, Ebihara T, Oshiumi H, Matsumoto M. Pattern recognition receptors of innate immunity and their application to tumor immunotherapy. *Cancer Sci* 2010; **101**: 313-320

17 **Kang TJ**, Chae GT. Detection of Toll-like receptor 2 (TLR2) mutation in the lepromatous leprosy patients. *FEMS Immunol Med Microbiol* 2001; **31**: 53-58

18 **Schröder NW**, Morath S, Alexander C, Hamann L, Hartung T, Zähringer U, Göbel UB, Weber JR, Schumann RR. Lipoteichoic acid (LTA) of Streptococcus pneumoniae and Staphylococcus aureus activates immune cells via Toll-like receptor (TLR)-2, lipopolysaccharide-binding protein (LBP), and CD14, whereas TLR-4 and MD-2 are not involved. *J Biol Chem* 2003; **278**: 15587-15594

19 **Eder W**, Klimecki W, Yu L, von Mutius E, Riedler J, Braun-Fahrländer C, Nowak D, Martinez FD. Toll-like receptor 2 as a major gene for asthma in children of European farmers. *J Allergy Clin Immunol* 2004; **113**: 482-488

20 **Kutukculer N**, Yeniay BS, Aksu G, Berdeli A. Arg753Gln polymorphism of the human toll-like receptor-2 gene in children with recurrent febrile infections. *Biochem Genet* 2007; **45**: 507-514

21 **Boraska Jelavić T**, Barisić M, Drmic Hofman I, Boraska V, Vrdoljak E, Peruzović M, Hozo I, Puljiz Z, Terzić J. Microsatelite GT polymorphism in the toll-like receptor 2 is associated with colorectal cancer. *Clin Genet* 2006; **70**: 156-160

22 **Noguchi E**, Nishimura F, Fukai H, Kim J, Ichikawa K, Shibasaki M, Arinami T. An association study of asthma and total serum immunoglobin E levels for Toll-like receptor polymorphisms in a Japanese population. *Clin Exp Allergy* 2004; **34**: 177-183

23 **Tahara T**, Arisawa T, Wang F, Shibata T, Nakamura M, Sakata M, Hirata I, Nakano H. Toll-like receptor 2 -196 to 174del polymorphism influences the susceptibility of Japanese people to gastric cancer. *Cancer Sci* 2007; **98**: 1790-1794

24 **Tahara T**, Arisawa T, Wang F, Shibata T, Nakamura M, Sakata M, Hirata I, Nakano H. Toll-like receptor 2 (TLR) -196 to 174del polymorphism in gastro-duodenal diseases in Japanese population. *Dig Dis Sci* 2008; **53**: 919-924

25 **Kiechl S**, Wiedermann CJ, Willeit J. Toll-like receptor 4 and atherogenesis. *Ann Med* 2003; **35**: 164-171

26 **Mockenhaupt FP**, Cramer JP, Hamann L, Stegemann MS, Eckert J, Oh NR, Otchwemah RN, Dietz E, Ehrhardt S, Schröder NW, Bienzle U, Schumann RR. Toll-like receptor (TLR) polymorphisms in African children: common TLR-4 variants predispose to severe malaria. *J Commun Dis* 2006; **38**: 230-245

27 **Lorenz E**, Frees KL, Schwartz DA. Determination of the TLR4 genotype using allele-specific PCR. *Biotechniques* 2001; **31**: 22-24

28 **Wu MS**, Cheng TY, Shun CT, Lin MT, Chen LC, Lin JT. Functional polymorphisms of CD14 and toll-like receptor 4 in Taiwanese Chinese with Helicobacter pylori-related gastric malignancies. *Hepatogastroenterology* 2006; **53**: 807-810

29 **Garza-Gonzalez E**, Bosques-Padilla FJ, Mendoza-Ibarra SI, Flores-Gutierrez JP, Maldonado-Garza HJ, Perez-Perez GI. Assessment of the toll-like receptor 4 Asp299Gly, Thr399Ile and interleukin-8 -251 polymorphisms in the risk for the development of distal gastric cancer. *BMC Cancer* 2007; **7**: 70

30 **Dixon MF**, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol* 1996; **20**: 1161-1181

31 **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

32 **Miller SA**, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; **16**: 1215

33 **Lachheb J**, Dhifallah IB, Chelbi H, Hamzaoui K, Hamzaoui A. Toll-like receptors and CD14 genes polymorphisms and susceptibility to asthma in Tunisian children. *Tissue Antigens* 2008; **71**: 417-425

34 **Soares SC**, Abé-Sandes K, Nascimento Filho VB, Nunes FM, Silva WA. Genetic polymorphisms in TLR4, CR1 and Duffy genes are not associated with malaria resistance in patients from Baixo Amazonas region, Brazil. *Genet Mol Res* 2008; **7**: 1011-1019

35 **Pandey S**, Mittal RD, Srivastava M, Srivastava K, Singh S, Srivastava S, Mittal B. Impact of Toll-like receptors [TLR] 2 (-196 to -174 del) and TLR 4 (Asp299Gly, Thr399Ile) in cervical cancer susceptibility in North Indian women. *Gynecol Oncol* 2009; **114**: 501-505

36 **Wang F**, Tahara T, Arisawa T, Shibata T, Nakamura M, Fujita H, Iwata M, Kamiya Y, Nagasaka M, Takahama K, Watanabe M, Hirata I, Nakano H. Genetic polymorphisms of CD14 and Toll-like receptor-2 (TLR2) in patients with ulcerative colitis. *J Gastroenterol Hepatol* 2007; **22**: 925-929

37 **Schröder NW**, Schumann RR. Non-LPS targets and actions of LPS binding protein (LBP). *J Endotoxin Res* 2005; **11**: 237-242

38 **Higgins SC**, Lavelle EC, McCann C, Keogh B, McNeela E, Byrne P, O'Gorman B, Jarnicki A, McGuirk P, Mills KH. Toll-like receptor 4-mediated innate IL-10 activates antigen-specific regulatory T cells and confers resistance to Bordetella pertussis by inhibiting inflammatory pathology. *J Immunol* 2003; **171**: 3119-3127

39 **Cheng PL**, Eng HL, Chou MH, You HL, Lin TM. Genetic polymorphisms of viral infection-associated Toll-like receptors in Chinese population. *Transl Res* 2007; **150**: 311-318

40 **Queiroz DM**, Oliveira AG, Saraiva IE, Rocha GA, Rocha AM, das Graças Pimenta Sanna M, Guerra JB, Dani R, Ferrari Mde L, Castro LP. Immune response and gene polymorphism profiles in Crohn's disease and ulcerative colitis. *Inflamm Bowel Dis* 2009; **15**: 353-358

41 **Ramasawmy R**, Cunha-Neto E, Fae KC, Borba SC, Teixeira PC, Ferreira SC, Goldberg AC, Ianni B, Mady C, Kalil J. Heterozygosity for the S180L variant of MAL/TIRAP, a gene expressing an adaptor protein in the Toll-like receptor pathway, is associated with lower risk of developing chronic Chagas cardiomyopathy. *J Infect Dis* 2009; **199**: 1838-1845

42 **Santini D**, Angeletti S, Ruzzo A, Dicuonzo G, Galluzzo S, Vincenzi B, Calvieri A, Pizzagalli F, Graziano N, Ferraro E, Lorino G, Altomare A, Magnani M, Graziano F, Tonini G. Toll-like receptor 4 Asp299Gly and Thr399Ile polymorphisms in gastric cancer of intestinal and diffuse histotypes. *Clin Exp Immunol* 2008; **154**: 360-364

43 **Doll R**, Forman D, La Vecchia C. Alcoholic beverages and cancers of the digestive tract and larynx. In: MacDonald I, editor. Health Issues Related to Alcohol Consumption. 2nd ed. Bodmin Cornwall: MPG Books, 1999: 351-393

44 **Seitz HK**, Matsuzaki S, Yokoyama A, Homann N, Väkeväinen S, Wang XD. Alcohol and cancer. *Alcohol Clin Exp Res* 2001; **25**: 137S-143S

45 **Pöschl G**, Seitz HK. Alcohol and cancer. *Alcohol Alcohol* 2004; **39**: 155-165

46 **Jelski W**, Szmitkowski M. Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) in the cancer diseases. *Clin Chim Acta* 2008; **395**: 1-5

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