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• GASTRIC CANCER •

GSTT1, *GSTM1* and *CYP2E1* genetic polymorphisms in gastric cancer and chronic gastritis in a Brazilian population

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Abstract

AIM: To test the hypothesis that, in the Southeastern Brazilian population, the *GSTT1*, *GSTM1* and *CYP2E1* polymorphisms and putative risk factors are associated with an increased risk for gastric cancer.

METHODS: We conducted a study on 100 cases of gastric cancer (GC), 100 cases of chronic gastritis (CG), and 150 controls (C). Deletion of the *GSTT1* and *GSTM1* genes was assessed by multiplex PCR. *CYP2E1/Pst*I genotyping was performed using a PCR-RFLP assay.

RESULTS: No relationship between *GSTT1/GSTM1* deletion and the *c1/c2* genotype of *CYP2E1* was observed among the three groups. However, a significant difference between CG and C was observed, due to a greater number of *GSTT1/GSTM1* positive genotypes in the CG group. The *GSTT1* null genotype occurred more frequently in Negroid subjects, and the *GSTM1* null genotype in Caucasians, while the *GSTM1* positive genotype was observed mainly in individuals with chronic gastritis infected with *H pylori*.

CONCLUSION: Our findings indicate that there is no obvious relationship between the *GSTT1*, *GSTM1* and *CYP2E1* polymorphisms and gastric cancer.

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INTRODUCTION

In Brazil, gastric cancer occupies the fifth position, with an estimates of 20 640 new cases and 11 145 deaths in 2003, as a consequence of late diagnosis, and also of its high recurrence rate[1]. Gastric carcinogenesis is a multi-step process, involving both genetic and environmental factors[2]. Among the latter, the most outstanding are dietary factors[3], smoking[4]; drinking[5]; *Helicobacter pylori* infection[6], and the occurrence of previous gastric injuries[7]. Correa[8] and Stemmermann[9] suggested, in separate studies, a general hypothesis of pre-cancerous sequences for gastric carcinogenesis, especially for the intestinal types, namely superficial gastritis, chronic atrophic gastritis, intestinal metaplasia, dysplasia, and cancer.

Over the last decades, several studies have revealed the participation of polymorphisms in metabolic and DNA repair enzymes, that might confer different degrees of susceptibility to cancer. Among these metabolic enzyme polymorphisms, the most outstanding are those of cytochrome P-450 (CYPs), glutathione-S-transferases (GSTs), and N-acetyltransferases (NATs)[10-12].

Concerning the superfamily of metabolic enzymes, both *CYP2E1* gene, a member of the cytochrome P-450 superfamily, and the *GSTT1* and *GSTM1* genes, that catalyze the conjugation reaction of glutathione with electrophilic compounds, exhibit polymorphisms which have been considered as potentially important modifiers of the individual risk for environmentally induced cancers, including cancer of stomach[13-17]. Subjects with null *GSTT1* and *GSTM1* have a decreased capability of detoxifying some carcinogens, among wich N-nitrous compounds are involved in stomach carcinogenesis[14].

Previous studies have shown inconclusive or controversial findings on associations between polymorphism of these genes and cancer susceptibility, due to the different types of cancer investigated and the diverse ethnic origin of the populations studied. Increased risk for oral[18], nasopharyngeal[19], and pulmonary[20] cancer was observed in carriers of the rare allele *CYP2E1*, while an increased risk for esophageal cancer was observed in carriers of the common allele[21]. On the other hand, *GSTT1* and *GSTM1* null genotypes have been linked to an increased risk for cancer of the lung, bladder and colon, and other specific sites[11,17].

The relationship between *GSTT1*, *GSTM1* and *CYP2E1* gene polymorphisms and the risk for gastric cancer is not obvious. Many investigations were conducted on Asian populations[22-36]. The Brazilian population is characterized by heterogeneous ethnic groups, emphasizing the need to investigate the frequency of these metabolizing genes and their association with gastric cancer.

We performed a case-control study to evaluate the association between the *GSTT1*, *GSTM1* and *CYP2E1* polymorphisms in patients from the Brazilian Southeast with gastric cancer or chronic gastritis, a lesion that increases the risk for gastric cancer by 10%[37]. We also explored the potential interactions between the *GSTT1*, *GSTM1* and *CYP2E1* polymorphisms and demographic risk factors.

MATERIALS AND METHODS

Subjects

We conducted a simultaneous case-control study for gastric cancer and chronic gastritis. The case groups comprised 100 patients with histopathologically confirmed diagnosis of gastric

adenocarcinoma (73 men and 27 women) with a mean age of 60 years (ranging from 28 to 93 years), and 100 patients with histopathologically confirmed diagnosis of chronic gastritis (54 men and 46 women) with a mean age of 53 years (ranging from 19 to 86 years), respectively. These subjects were recruited from the "Hospital de Base", São José do Rio Preto, SP, and from the Pio XII Foundation, Barretos, SP, Brazil. The pathological diagnoses of gastric cancer and chronic gastritis were made according to criteria proposed by Lauren[38] and the Sidney classification[39], respectively. *H pylori* infection was histologically established by Giemsa staining. The control group consisted of 150 healthy volunteers (90 men and 60 women), with a mean age of 54 years (ranging from 20 to 93 years), with no previous history of gastric disease, matched to the patients with respect to age, gender and ethnicity. Most controls were blood donors. Epidemiological data on the study population were collected through a standard interviewer-administered questionnaire, which included questions about current and past occupation, ethnicity, life-long smoking habits and alcohol consumption, and family history of cancer.

The human subject protocol was approved by the Research Ethics Committee of the IBILCE-UNESP, and written informed consent was obtained from all subjects.

Blood sampling and DNA extraction

Whole blood was collected and put into EDTA-coated tubes. Lymphocytes were isolated, transferred to tubes, and assigned a unique identifier code. DNA was then extracted using a non-organic extraction procedure, and stored at -20 ℃ until use for genotyping[40].

Cenotype analysis

The *GSTT1* and *GSTM1* genes were determined simultaneously in a single assay, using a PCR multiplex protocol, where part of exon 7 of the constitutional gene *CYP1A1* was co-amplified as an internal control.

PCR was performed in 25 μL reaction buffer containing 0.5 mmol/L of dNTPs, 2.0 mmol/L of $MgCl_2$, 12.5 pmol of each primer, about 150 ng DNA, and 1.25 U of thermostable Taq DNA polymerase, using a programmable thermocycler. The primers used for *GSTM1* were 5'- GAACTCCCTGAAAAGCTA AGC and 5'- GTTGGGGCTCAAATATACGGTGG. The primers used for *GSTT1* were 5'-TTCCTTACTGGTCCTCACATCTC and 5'TCACCGGATCAGGCCAGCA. The primers used for *CYP1A1* were 5'GAACTGCCACTTCAGCTGTCT and 5'CAGCTGCATT TGGAAGTGCTC.

PCR conditions were 94 ℃ for 5 min, followed by 40 denaturation cycles of 2 min at 94 ℃, 1 min annealing at 59 ℃, and 1 min extension at 72 ℃. The PCR products were then analyzed by electrophoresis on ethidium bromide-stained 20 g/L agarose gel.

The presence or absence of *GSTT1* and *GSTM1* genes was detected by the presence or absence of a band at 480 bp and at 215 bp, respectively. A band at 312 bp (CYP1A1) was documented successful amplification[41].

This technique could not distinguish between heterozygote and homozygote positive genotypes, but it could conclusively identify the null genotypes.

PCR-RFLP was performed to investigate the *CYP2E1*c2* allele. PCR was used to amplify the transcription regulation region of *CYP2E1* that includes the *Pst*I enzyme recognition site. PCR was performed in 25 μL reaction buffer containing 0.28 mmol/L of dNTPs, 1.5 mmol/L of $MgCl_2$, 10 pmol of each primer, about 200 ng DNA, and 1.5 units of thermostable Taq DNA polymerase, using a programmable thermocycler. The *CYP2E1* primers were 5' CCAGTCGACTCTACATTGTCA and 5' TTCATTCTGTCTTCTAACTGG. After 5 min of pretreatment at 94 ℃, 35 denaturation cycles of 1 min at 94 ℃, 30 s annealing at 60 ℃, and 1 min extension at 72 ℃ were performed. After amplification, the PCR products were subjected to restriction digestion by enzyme *Pst*I for 16 h at 37 ℃. The PCR-RFLP fragments were then analyzed by electrophoresis on ethidium bromide-stained 20 g/L agarose gel[42].

All the experiments included positive and negative controls for each studied polymorphism.

Statistical analysis

Statistical analyses were performed using Statidisk, Statistica, Minitab Release 10.1 computer software programs. The probability level (*P*) of 0.05 was used as significance criterion . Student' s *t*-test and ANOVA *F*-test tests were used to compare continuous variables between the groups. Chi-square test or Fisher's exact test was utilized as appropriate to compare the groups with regard to genotype frequencies and putative risk factors such as gender, ethnicity, smoking, drinking, *H pylori* infection, occupational pesticide exposure, and histological type of adenocarcinoma. In order to investigate gene-environment interactions, we also calculated the odds ratios (OR) and their 95% confidence intervals (95% CI), according to combinations of the *GSTT1*, *GSTM1* and *CYP2E1* polymorphisms with putative risk factors.

RESULTS

Figure 1 (PCR) and Figure 2 (PCR-PFLP) show the genotype analysis results. Table 1 shows the frequency distributions of the *GSTT1* and *GSTM1* genotypes among the groups. With respect to the genotype frequencies, considering the combinations between the *GSTT1*/*GSTM1* genes, no statistically significant differences were observed between the gastric cancer and chronic gastritis patients (*P*=0.189), nor between gastric cancer patients and controls (*P*=0.448). However, a significant difference (*P*=0.048) was observed between chronic gastritis patients and controls, due to a higher frequency of combination *GSTT1*/*GSTM1* positive genotypes in the chronic gastritis patients.

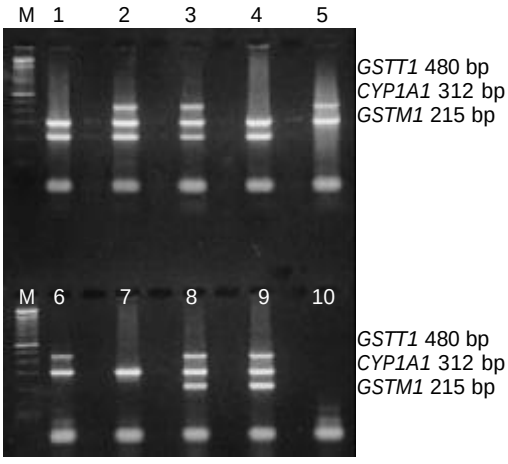

Figure 1 Polymerase chain reaction of the *GSTT1* and *GSTM1* genes. Lanes M: molecular weight maker; Lanes 1 and 4 : patients homozygously null for *GSTT1*; Lanes 2, 3, 8 and 9: patients with positive *GSTT1* and *GSTM1* genotypes; Lanes 5 and 6: patients homozygously null for *GSTM1*; Lane 7: patient homozygously null for *GSTT1*and *GSTM1*; Lane 10 negative control.

The associations of the different genotypes with demographic risk factors (gender, ethnicity, smoking, drinking, pesticide-exposure, *H pylori* infection and histological type of gastric cancer) in each group evidenced that the *GSTT1* null genotype occurred more frequently in Negroid controls (*P*=0.003), and

Table 1 *GSTT1* and *GSTM1* genotype frequencies among gastric cancer (GC) and chronic gastritis (CG) patients and controls (C)

Groups	*n*	*GSTT1* Null (%)	*GSTM1* Null (%)	*GSTT1/GSTM1*			
				+/+ (%)	+/0 (%)	0/+ (%)	0/0 (%)
GC	100	17 (17.0)	47 (47.0)	43 (43.0)	40 (40.0)	10 (10.0)	7 (7.0)
CG	100	12 (12.0)	38 (38.0)	57 (57.0)	31 (31.0)	5 (5.0)	7 (7.0)
C	150	28 (18.6)	62 (41.3)	64 (42.7)	58 (38.7)	24 (16.0)	4 (2.6)

+/+=presence of *GSTT1* and *GSTM1*, +/0=presence of *GSTT1* and absence of *GSTM1*, 0/+=absence of *GSTT1* and presence of *GSTM1*, 0/0=absence of *GSTT1* and *GSTM1*.

Table 2 Associations of *GSTT1* and *GSTM1* genotypes with demographic risk factors in gastric cancer (GC) and chronic gastritis (CG) cases and controls (C)

Groups	Categories	Genotype			
		GSTM1 positive (%)	*GSTM1* null (%)	*GSTT1* positive (%)	*GSTT1* null (%)
C	Ethnicity				
	Caucasian	75 (55.6)	60 (44.4)	114 (84.4)	21 (15.6)
	Negroid	13 (86.7)	2 (13.3)	8 (53.3)	7 (46.7)
		P=0.020		P=0.003	
GC	Ethnicity				
	Caucasian	42 (46.3)	45 (51.7)	73 (83.9)	14 (16.1)
	Negroid	11 (84.6)	2 (15.4)	10 (76.9)	3 (23.1)
		P=0.017		P=0.690	
CG	*H pylori* Infection				
	No	43 (71.7%)	17 (28.3%)	54 (90.0%)	6 (10.0%)
	Yes	19 (48.7%)	20 (51.3%)	34 (87.2%)	5 (12.8%)
		P=0.032		P=0.748	

the *GSTM1* null genotype in Caucasian controls (P=0.020) and gastric cancer patients (P=0.017). The *GSTM1* positive genotype was observed mainly in chronic gastritis cases with *H pylori* infection (P=0.032) (Table 2).

The frequencies of the *GSTT1* and *GSTM1* polymorphisms were compared among the groups by χ^2 tests and estimated OR (data not shown), according to the pattern of the gastric cancer risk factors represented by gender, ethnicity, smoking, drinking, pesticide-exposure, and *H pylori* infection. Thus, comparing the GC and CG groups, multivariate analysis revealed that smoking was not associated with increased OR's for stomach cancer in the *GSTM1* positive subjects (1.54, 95% CI=0.71-3.33), whereas it was associated with elevated OR's in the *GSTM1* null subjects (2.7, 95% CI=1.04-7.14).

Table 3 shows the frequency distributions of *CYP2E1* genotypes among the groups. The frequencies of *CYP2E1* (*c1/c2*) variant genotypes in the gastric cancer, chronic gastritis and control groups were 11.0%, 9.0% and 10.7%, respectively. The rare homozygous genotype (*c2/c2*) was not found. The results showed no statistical difference (P=0.878) between the groups, nor was there any relationship with the investigated etiological factors, according to the χ^2 test and estimated OR's (data not shown).

Table 3 *CYP2E1* genotype frequencies among gastric cancer (GC) and chronic gastritis (CG) patients and controls (C)

Groups	Genotype	
	c1/c1 (%)	*c1/c2* (%)
GC	89 (89.0)	11 (11.0)
CG	91 (91.0)	9 (9.0)
C	134 (89.3)	16 (10.7)

c1/c1=homozygote for the common allele, *c1/c2*=heterozygote.

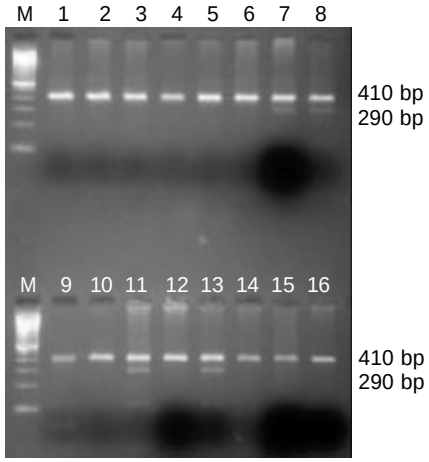

Figure 2 PCR-RFLP of *CYP2E1*/PstI. Lanes M: molecular weight maker; Lanes 1, 2, 3, 4, 5, 6, 10 ,12, 14, 15 and 16 patients homozygote for the common allele of *CYP2E1/Pst*I; Lanes 7, 8, 9, 11 and 13 heterozygote for the rare allele of *CYP2E1/Pst*I.

DISCUSSION

Interindividual differences in the cellular mechanisms of activation and detoxification of carcinogenic chemicals could confer different degrees of susceptibility to cancer[43]. However, the results have not always been consistent, due to a number of possible reasons for anomalous findings, such as interaction between environmental and genetic factors, which is a complicating factor that needs to be taken into account[32].

GSTT1, *GSTM1* and *CYP2E1* genetic polymorphisms have shown pronounced interethnic variations[10]. Brazil is a large country with a very heterogeneous population, resulting from the cross-mating of the native population with immigrants from

Europe, Africa and Asia. Therefore, descriptive studies of the frequencies of genetic polymorphisms in the Brazilian population could be useful in verifing genetic variability in relation to xenobiotic metabolism, since this variability may influence cancer susceptibility. This is the first study that simultaneously evaluated the *GSTT1*, *GSTM1* and *CYP2E1* polymorphisms in Brazilian patients with gastric cancer and chronic gastritis.

The *GSTM1* genotype was absent in 35-65% of individuals[44], while *GSTT1* was deleted in 10-65% of the human population[17]. The prevalence of the *CYP2E1 c2* allele was shown to be 2-8% in both Caucasians and African Americans[13], but higher in Asian populations, ranging from 17 to 26%[45].

The frequencies of *GSTT1* (18.6%) and *GSTM1* (41.3%) null genotypes and the CYP2E21/*Pst*I (10.7%) polymorphism observed in the control group were not different from other studies in Brazilian populations[46-49]. We showed that the frequency of the *GSTT1* null genotype was higher in Negroid subjects, and that the *GSTM1* null genotype was higher in Caucasians. Other studies described similar results in Brazilian[46,50] and also in American populations[51].

Although studies of the *GSTT1* and *GSTM1* polymorphisms were performed previously, their association with gastric cancer susceptibility has not been established. Most of them showed no association between the *GSTT1* null genotype and risk for gastric cancer[25,32,36,52,53]. However, two others suggested that the *GSTT1* null genotype might confer an increased risk for gastric cancer[39,54].

The correlation between the *GSTM1* null genotype and gastric cancer appeared to be more consistent[22,25,27,28,30,53]. On the other hand, other authors failed to demonstrate any statistically significant difference in the *GSTM1* polymorphism distribution of gastric cancer patients and controls[24,26,32,36,52,55,56].

Several case-control studies also failed to find a significant association between the *CYP2E1*/*Pst*I polymorphism and gastric cancer[23,24,26,34]. However, while Nishimoto *et al*.[51] observed that the rare variant *c2/c2* was associated with a reduced risk for gastric cancer in non-Japanese Brazilians, Wu *et al*.[35] observed that the distribution of the *c2/c2* genotype, detected by *Pst*I or *Rsa*I digestion, differed significantly between gastric cancer cases and controls. These authors suggested that the *CYP2E1* genotype could be a determinant of gastric cancer. The reason for these inconsistent results is not clear, but one problem is the lack of sufficient investigation of the gene-environment interactions. Thus, Cai *et al*.[31] and Gao *et al*.[33] suggested that gene-environment interactions between the *CYP2E1* polymorphism and smoking might have the potential to alter the susceptibility for cancer development in the stomach.

Our current data corroborate the hypothesis of there being no association between *GSTT1* and *GSTM1* deletions and *CYP2E1*/*Pst*I polymorphism with gastric cancer and chronic gastritis. However, smoking raised the OR's for stomach cancer in *GSTM1* null subjects. Our findings suggest that *GSTM1* null carriers may be more susceptible to the action of tobacco with regard to stomach cancer. Polycyclic aromatic hydrocarbons and N-nitrosamines found in cigarette smoke are potential human carcinogens. Thus, a deficiency of the detoxifying enzymes may affect the metabolic fates of these chemicals and raise cancer risk in subjects with a *GSTM1* null genotype. Cai *et al*.[30] reported an increased frequency of the *GSTM1* null genotype in smokers with gastric cancer, that may modulate tobacco-related gastric carcinogenesis .

We also observed that a *GSTM1* positive genotype was more prevalent in chronic gastritis patients with *H pylori* infection. In the multi-step carcinogenesis of the stomach, chronic gastritis preceded the formation of gastric cancer, and a great proportion of the clinical tumors occurred in connection with advanced forms of this pathology[57]. *H pylori* has been reported to be a Class I human carcinogen[58], and chronic *H pylori* infection was shown to increase the risk for gastric carcinoma from 2.8 to 9 fold[59-64]. Ng *et al*.[27] observed that the absence of the *GSTM1* enzyme might increase the risk of developing gastric cancer in patients with *H pylori* infection. Thus, chronic gastritis patients with *H pylori* infection, but with a *GSTM1* positive genotype, might benefit from a protective effect and exhibit a smaller predisposition to developing gastric cancer.

In this study, no association between the *CYP2E1*/*Pst*I polymorphism and overall risk for gastric cancer was observed. Different from the study of Nishimoto *et al*.[51] in a Brazilian population, the rare variant *c2/c2* was not observed. Moreover, the risk for gastric cancer as related to demographic risk factors was also not affected by the *CYP2E1*/*Pst*I polymorphisms.

In conclusion, the present work does not show any obvious relationship between *GSTT1*, *GSTM1* and *CYP2E1* polymorphisms and the development of gastric cancer in a Brazilian population. However, smoking and the *GSTM1* null genotype may be associated with an increased OR for stomach cancer. We emphasize that studies with negative findings also need to be reported, so as to avoid a publication bias leading to an overestimate of positive findings. We also suggest that the investigation of a greater number of biometabolism genes associated with DNA repair genes might bring a broader view of the process.

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