Molecular evaluation of glutathione S transferase family genes in patients with sporadic colorectal cancer

Gabriela Helena Rodrigues-Fleming, Glaucia Maria de Mendonça Fernandes, Anelise Russo, Patrícia Matos Biselli-Chicote, João Gomes Netinho, Érika Cristina Pavarino, Eny Maria Goloni-Bertollo

AIM
To evaluate the association between polymorphisms...
in glutathione S transferases (GSTs) and the risk of sporadic colorectal cancer (SCRC), tumor progression and the survival of patients.

METHODOLOGY
A case-control study of 970 individuals from the Brazilian population was conducted (232 individuals from the case group with colorectal cancer and 738 individuals from the control group without a history of cancer). PCR multiplex and PCR-RFLP techniques were used to genotype the GST polymorphisms. The tumors were categorized according to the TNM classification: tumor extension (T), affected lymph nodes (N), and presence of metastasis (M). Logistic regression, multiple logistic regression and survival analysis were used to analyze the data. The results are presented in terms of odds ratio (OR) and 95% confidence interval (CI). The level of significance was set at 5% (P ≤ 0.05).

RESULTS
Age equal to or over 62 years (OR = 8.79; 95%CI: 5.90-13.09, P < 0.01) and female gender (OR = 2.91; 95%CI: 1.74-4.37; P < 0.01) were associated with increased risk of SCRC. Analysis of the polymorphisms revealed an association between the GSTM1 polymorphisms and a risk of SCRC (OR = 1.45; 95%CI: 1.06-2.00; P = 0.02), as well as between GSTTI and a reduced risk of the disease (OR = 0.65; 95%CI: 0.43-0.98; P = 0.04). An interaction between the presence of the wild-type allele of GSTP1 and the GSTM1 polymorphism and tobacco consumption on risk of SCRC (OR = 2.33; 95%CI: 1.34-4.05; P = 0.05) was observed. There was an association between the GSTM1 null genotype and the presence of advanced tumors (OR = 2.33; 95%CI: 1.23-4.41; P = 0.009), as well as increased risk of SCRC in the presence of a combination of GSTTI non-null/GSTM1 null genotypes (OR = 1.50; 95%CI: 1.03-2.19; P = 0.03) and GSTTI non-null/GSTM1 null/GSTP1 Val* (OR = 1.85; 95%CI: 1.01-3.36; P = 0.04). Combined GSTTI non-null/GSTM1 null genotypes (OR = 2.40; 95%CI: 1.19-4.85; P = 0.01) and GSTTI non-null/GSTM1 null/GSTP1 Val* (OR = 2.92; 95%CI: 1.05-8.12; P = 0.04) were associated with tumor progression. Polymorphisms were not associated with the survival of patients with SCRC.

CONCLUSION
Females aged 62 years or older are more susceptible to SCRC. Polymorphisms of GSTTI and GSTM1 null genotypes modulated the susceptibility to SCRC in the population studied.

Key words: Colorectal neoplasms; Smoking; Alcohol; Glutathione S transferase; Genetic polymorphisms

© The Author(s) 2018. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Sporadic colorectal cancer (SCRC) is the third most common cancer worldwide and includes malignancies that occur in the colon and rectum. Age greater than 60 years, smoking, and alcohol habits are some of the risk factors for SCRC. Detoxification and elimination of carcinogens contained in tobacco and alcohol require metabolic activation mediated by enzymes that metabolize the xenobiotics (XME). Polymorphisms in genes such as GSTP1, GSTTI, and GSTM1 that encode enzymes involved in XMEs may be related to important processes in colorectal carcinogenesis.

INTRODUCTION
Colorectal cancer is the third most frequent cancer worldwide[12] and the fifth most frequent type of cancer in Brazil[2]. Estimates for the year 2018 in Brazil are 17380 new cases for men and 18980 new cases for women[2].

Sporadic colorectal cancer (SCRC) develops from adenomas in the colon and rectum walls, of varying sizes, and can change to dysplasia, triggering the development of cancer[2-4]. SCRC is a multifactorial disease, influenced by genetic factors, such as mutations or polymorphisms in genes that participate in pathways responsible for regulating cell growth, including tumor suppressor genes and proto-oncogenes[5,6]. Other related factors are age, gender, environmental factors, and lifestyle habits such as smoking and alcohol consumption[7]. Genetic factors may influence the effect of the environment on predisposition to the disease. Therefore, the incidence of SCRC varies among populations[5,8,9].

There are many genes encoding enzymes responsible for the metabolism of xenobiotics, in which detoxification occurs. Some of the major genes involved in phase II are the cytosolic glutathione S transferase (GST) superfamily, including GST mi (GSTM1), theta (GSTTI), and pi (GSTP1) [10,11]. These catalyze the conjugation of structurally different by-products of oxidative stress and xenobiotics to glutathione (GSH), which leads to the elimination of toxic substances from the cells and the protection of important cellular components such as nucleic acids and proteins[12]. GST gene expression varies between different tissues and cell types[13].

In addition to being very common in the general population, the complete absence of GSTTI and/or GSTM1 may alter their expression or the activity of the protein itself[14]. In general, GSTP1 appears to be highly expressed in proliferating cells compared with differentiated cells. In addition, many of the GSTs are overexpressed in various neoplastic and higher
levels are observed in aggressive cancer cells\textsuperscript{15}. The change in the GSTP1 gene also significantly alters the enzymatic activity\textsuperscript{16,17}, influencing the detoxification of carcinogens, causing DNA damage, and exerting an indirect effect on the risk of cancer development\textsuperscript{18}.

Therefore, the objectives of this study were to evaluate the association of epidemiological risk factors and these polymorphisms with the development of SCRC, the interaction between these polymorphisms and both smoking and alcohol habits, and the association between the polymorphisms and clinical-histopathological parameters and survival among patients with SCRC.

**MATERIALS AND METHODS**

**Approval and consent**

The study was approved by the Ethics Committee-Medical School of Sao Jose do Rio Preto - FAMERP (No. 012/2012). The 970 individuals who agreed to participate in the study signed a consent form. The variables analyzed included gender, age, ethnicity, profession, smoking, alcohol consumption, and personal and familial history of cancer.

**Study populations**

The case group consisted of 232 (112 men and 120 women) patients from the Department of Coloproctology of the Base Hospital of Sao Jose do Rio Preto who received the clinical and/or histopathological diagnosis of SCRC between 2010 and 2016. The exclusion criterion was previous treatment with chemotherapy and/or radiotherapy. The control group included 738 (370 men and 368 women) blood donors from the Blood Center of Sao Jose do Rio Preto. The exclusion criterion for controls was personal and family history of cancer in at least three previous generations. Individuals who had smoked at least 100 cigarettes throughout their lives were considered smokers, and those who drank more than four servings of alcohol per week (one serving corresponded to 30 mL of liquor, a 102-mL glass of wine containing 12% alcohol, or a 340-mL can of beer) were considered alcohol consumers\textsuperscript{19,20}. SCRC was categorized according to TNM classification: tumor extension (T), affected lymph nodes (N), and presence of metastasis (M)\textsuperscript{21}.

**Molecular analysis**

Analysis of the GSTT1 and GSTM1 polymorphisms was performed using the polymerase chain reaction (PCR) multiplex technique, with the CYP1A1 gene as the internal positive control of amplification\textsuperscript{22}. PCR products were analyzed on 1.5% agarose gel stained with red gel. The restriction enzyme digestion was performed using BsmAI. The results and genotyping were performed after 2.0% agarose gel electrophoresis stained with red gel. The presence of 91 and 85 bp bands corresponded to the GG polymorphic genotype; the 176, 91, and 85 bp bands corresponded to the heterozygous genotype AG; and the 176 bp band corresponded to the wild-type AA genotype.

**Statistical analysis**

Descriptive statistics included mean values, standard deviation for continuous data, and percentage for categorical data. The Hardy-Weinberg equilibrium (HWE) was evaluated using the chi-square test through the BioEstat Program version 5.0. The binary logistic regression model, using the Minitab/Windows-Version 12.22 program, was used to evaluate the association of age, gender, smoking, and drinking habits with SCRC, and to evaluate the association between SCRC and clinical-histopathological parameters. Binary multiple logistic regression, adjusted for age, gender, and smoking and drinking habits, was used to evaluate the association between the genetic models of the polymorphisms and the development of SCRC using the SNPStats program (available at: http://bioinfo.iconcologia.net/SNPStats_web). The effect of the polymorphisms was evaluated in the models as (1) codominant (heterozygous vs wild-type homozygous and polymorphic homozygous vs wild-type homozygous); (2) dominant (heterozygous + polymorphic homozygous vs wild-type homozygous); (3) recessive (homozygous polymorphic vs wild-type homozygous + heterozygous); (4) overdominant (heterozygous vs wild-type homozygous + polymorphic homozygous); or (5) additive (polymorphic homozygous with 2 + heterozygous vs wild-type homozygous). The SNPStats program was used to evaluate the interaction between the polymorphisms and smoking habit, adjusted for age, gender, and alcohol consumption, and to evaluate the interaction between polymorphisms and alcohol consumption, adjusted for age, gender, and smoking, in SCRC risk. The effect of the polymorphisms on the overall survival time of SCRC patients was analyzed by the Kaplan-Meier curve and log rank test using the StatsDirect version 2.7.2 program. The results are presented in terms of odds ratio (OR) and 95% confidence interval (CI). For all statistical analyses the level of significance was set at 5% (\(P < 0.05\)).

**RESULTS**

**Sociodemographic data**

Table 1 presents the demographic data of SCRC patients and controls. Age equal to or above 62 years (OR = 8.79; 95%CI: 5.90-13.09; \(P < 0.01\)) and female gender (OR = 2.91; 95%CI: 1.74-4.37; \(P < 0.01\)) were associated with a risk of SCRC. The genotypic frequencies of GSTP1 Ile105Val polymorphism were observed in the HWE in both groups (Case: \(P = 1\), Control: \(P = 0.29\)).
Table 1 Sociodemographic characteristics, risk factors, and polymorphisms GSTT1, GSTM1, GSTP1 A313G in patients with colorectal cancer and controls n (%)

| Variables       | Case (n = 232) | Control (n = 738) | OR† (95%CI) |
|-----------------|----------------|-------------------|-------------|
| Gender          |                |                   |             |
| Male            | 112 (48)       | 370 (50)          | 1.00        |
| Female          | 120 (52)       | 368 (50)          | 2.91 (1.94-4.37)* |
| Age [yr (mean) ± SD] |              |                   |             |
| < 62            | 112 (49)       | 621 (84)          | 1.00        |
| ≥ 62            | 120 (51)       | 117 (16)          | 8.79 (5.90-13.09)* |
| Smoking Habit   |                |                   |             |
| Non-smoker      | 130 (56)       | 465 (63)          | 1.00        |
| Smoker          | 102 (44)       | 273 (37)          | 1.45 (0.98-2.14) |
| Alcohol Consumption |            |                   |             |
| Non-drinker     | 132 (57)       | 395 (54)          | 1.00        |
| Drinker         | 100 (43)       | 343 (46)          | 1.28 (0.85-1.91) |
| GSTP1           |                |                   |             |
| Codominant      | A/A            | 227 (43.7)        | 1.00        |
|                 | A/G            | 224 (43.2)        | 1.06 (0.73-1.54) |
|                 | G/G            | 68 (13.1)         | 0.88 (0.48-1.59) |
| Dominant        | A/A            | 227 (43.7)        | 1.00        |
|                 | A/G-G/G        | 292 (56.3)        | 1.02 (0.71-1.45) |
| Recessive       | A/A-A/G        | 451 (86.9)        | 1.00        |
|                 | G/G            | 68 (13.1)         | 0.85 (0.48-1.50) |
| Overdominant    | A/A-G/G        | 295 (56.8)        | 1.00        |
|                 | A/G            | 224 (43.2)        | 1.09 (0.76-1.55) |
| Additive        | -              | -                 | 0.97 (0.75-1.27) |
| GSTT1           | +/+            | 573 (77.6)        | 1.00        |
|                 | 0/0            | 165 (22.4)        | 0.65 (0.43-0.98)* |
| GSTM1           | +/+            | 365 (52.2)        | 1.00        |
|                 | 0/0            | 333 (47.8)        | 1.45 (1.06-2.00) |

†OR adjusted for age, gender, and alcohol and smoking habits and polymorphisms; *P < 0.05 vs control. OR: Odds ratio.

In the present study, we observed that individuals with advanced age (≥ 62 years) were more susceptible to SCRC, which is consistent with previous reports where old age was considered to be an etiological factor for this tumor type [2,24]. In terms of gender, women are more susceptible to SCRC. Other studies have observed a similar trend in gender among patients with SCRC and the control group [25-27]. An increase in the number of cases among women due to an increase in cigarette smoking and alcohol consumption has been observed [28,29]. It is important to note that the group

## Discussion

In the present study, we observed that individuals with advanced age (≥ 62 years) were more susceptible to SCRC, which is consistent with previous reports where old age was considered to be an etiological factor for this tumor type [2,24]. In terms of gender, women are more susceptible to SCRC. Other studies have observed a similar trend in gender among patients with SCRC and the control group [25-27]. An increase in the number of cases among women due to an increase in cigarette smoking and alcohol consumption has been observed [28,29]. It is important to note that the group
Table 2 Interaction between polymorphisms in the genes GSTP1, GSTT1, and GSTM1 and smoking or alcohol habits on the risk of sporadic colorectal cancer

| Tobacco consumption | Non-smoker | Control | OR (95%CI) | Case | Control | OR (95%CI) | Non-smoker | Control | OR (95%CI) | Case | Control | OR (95%CI) |
|---------------------|------------|---------|-----------|-----|---------|-----------|------------|---------|-----------|-----|---------|-----------|
| GSTP1 A/A           | 136        | 116     | 1.00      |     | 85      | 94        | 1.00       |         |           | 85  | 94      | 1.00       |
| GSTP1 A/A/G/G       | 80         | 52      | 1.00      |     | 45      | 45        | 1.00       |         |           | 45  | 45      | 1.00       |
| GSTP1 A/G/G/G       | 210        | 179     | 1.00      |     | 103     | 103       | 1.00       |         |           | 103 | 103     | 1.00       |
| GSTP1 G/G/G/G       | 52         | 119     | 1.00      |     | 56      | 56        | 1.00       |         |           | 56  | 56      | 1.00       |
| GSTM1 +/+           | 231        | 154     | 1.00      |     | 147     | 147       | 1.00       |         |           | 147 | 147     | 1.00       |
| GSTM1 0/0           | 200        | 111     | 1.00      |     | 110     | 110       | 1.00       |         |           | 110 | 110     | 1.00       |
| GSTT1 0/0           | 200        | 111     | 1.00      |     | 110     | 110       | 1.00       |         |           | 110 | 110     | 1.00       |
| GSTT1 0/0           | 200        | 111     | 1.00      |     | 110     | 110       | 1.00       |         |           | 110 | 110     | 1.00       |

OR: Odds ratio. P < 0.05 vs control. GSTP1 Ile105Val polymorphism was in equilibrium in all the case and control groups. The result was similar to that observed in a previous study.

Smoking and drinking habits were not associated with SCRC in the present study. In the Tunisian population, the GSTP1 gene polymorphism was associated with SCRC, corroborating other investigations in the case and control groups. This association was not observed in the Bulgarian and Chinese populations.

The GSTP1 Ile105Val polymorphism results in an alteration of the amino acid sequence of the protein and a consequent reduction in enzymatic activity and inefficient detoxification. However, although the GSTP1 null genotype was associated with increased risk of SCRC in this study, the level of expression of this gene may be an important factor, which is not dependent on this genetic change. A hepatocellular carcinoma (HCC) study found that increased GSTP1 gene expression in vivo and in vitro resulted in reduced cell proliferation in tumor cells, inhibition of Akt phosphorylation, and cell cycle disruption in G1/S by increasing p21 and p27 cell cycle inhibitors. High GSTP1 expression was also associated with better prognosis in patients with HCC. In addition, hypermethylation of GSTP1 has been observed in several types of cancers.
exposure to environmental factors and the population heterogeneity. It has been observed that the effect of GST polymorphisms, when combined, may increase the risk of SCRC two- or threefold\[26,47,48\]. The present study demonstrated that combinations of GSTT1 non-null/GSTM1 null genotypes and GSTT1 non-null/GSTP1 Val* (presence of at least one polymorphic allele) are associated with an increased risk of SCRC and tumor progression. These findings corroborate the results of individual analyses of polymorphisms, which indicate the influence of the GSTT1 non-null genotype on SCRC because the null genotype was associated with a reduced risk of the disease.

In the Indian population, an association between the GSTM1 null/GSTT1 null genotypes and the combination of GSTM1 null/GSTT1 null/GSTP1 Val* and the risk of SCRC was observed\[25\]. This result was also observed in a study by Vlaykova et al\[41\] in the Bulgarian population. A study in the Turkish population found an association between the GSTT1 null/GSTM1 non-null genotypes and GSTT1 null/GSTM1 non-null/GSTP1 Ile* (wild-type homozygote) and SCRC\[38\]. Cong et al\[45\] observed an increased risk in the presence of GSTT1/GSTM1 genotypes, whereas the combination of GSTT1 non-null/GSTM1 null genotypes resulted in a significant reduced risk of SCRC, differing from the findings of this and other studies. On the other hand, other studies that analyzed the effect of the combined genotypes GSTT1/GSTM1 did not find an association with the risk of SCRC\[26,47,48\]. Several studies have evaluated the potential association between SCRC and the combined genotypes of these polymorphisms. The observed results vary, indicating the importance of studying the effects of the genotypic combination in SCRC.

In the present study, a significant interaction between the presence of the wild-type allele of GSTP1 Ile105Val polymorphism and smoking habit on the risk of SCRC was demonstrated. Differing from the results of the present study, a study in the Chinese population found no interaction between the GSTP1 Ile105Val and smoking habit or drinking habit on the risk of SCRC\[38\]. The literature is sparse in terms of studies evaluating the interaction between risk factors and the GSTP1 Ile105Val polymorphism in the development of SCRC. The biological relevance of this finding is unclear as the presence of at least one polymorphic allele of the GSTP1 gene combined with the nullity of GSTM1 and the presence of the GSTT1 allele were associated with increased risk of SCRC. In addition, smoking habit was not associated with this tumor type in the present study.

With regard to the GSTT1 and GSTM1 polymorphisms, this study did not find an association between smoking or drinking habits and the risk of SCRC. These results are in accordance with two other studies in a Korean and Japanese population\[46,48\]. The study by Piao et al\[49\] did not show a relationship between drinking habit and the GSTT1 and GSTM1 null genotypes on the risk of SCRC. However, a study in Singapore found an increased risk for smokers carrying at least two null genotypes that caused low enzyme activity\[38\]. The controversial results regarding these polymorphisms may suggest that other genes involved in the metabolism of xenobiotics may be more relevant in the development of SCRC, such as polymorphisms in genes acting on phase I xenobiotic metabolism\[27,50\]. Although the polymorphisms studied change in order to reduce or eliminate the enzymatic activity, other genes can also act, compensating for the detoxification of the

---

**Table 3** Distribution of the clinical-histopathological parameters in relation to the polymorphisms in the genes GSTP1, GSTT1, and GSTM1 in patients with colorectal cancer n (%)

| Models  | Genotypes | Tumor progression (TNM) (n = 201) | Primary site |
|---------|-----------|---------------------------------|-------------|
|         |           | Non-advanced                    | Advanced     | OR\(^1\) | 95%CI | Colon | Rectum | OR\(^1\) | 95%CI |
| GSTT1   | Codominant| +/+                             | 47 (78)      | 1.00    | -     | 42 (66) | 65 (46) | 1.00 |
|         |           | 0/0                             | 13 (22)      | 0.57    | (0.26-1.27) | 13 (14) | 27 (19) | 1.47 |
| GSTM1   |            | +/+                             | 34 (56)      | 1.00    | -     | 45 (69) | 55 (39) | 1.00 |
|         |           | 0/0                             | 26 (43)      | 2.33    | (1.23-4.41) | 46 (50) | 86 (61) | 1.49 |

\(^1\)OR adjusted for age, gender, alcohol and smoking habits and polymorphisms; \(^*\)P < 0.05 vs control. OR: Odds ratio.
Table 4  Association between the double combined \textit{GSTT1}/\textit{GSTM} genotypes and triple combined \textit{GSTT1}/\textit{GSTM}/\textit{GSTP1}, colorectal cancer, tumor progression, and primary site, adjusted for gender, age, smoking, and alcohol consumption

|                      | Colorectal cancer | Tumor progression (TNM) (n = 198) | Primary site |
|----------------------|------------------|----------------------------------|--------------|
|                      | Case             | Control                          | Non-advanced | Advanced | OR\(^1\) 95%CI | Colon | Rectum | OR\(^1\) 95%CI |
| Double combination of genotypes | n = 738          |                                  | (n = 60)     | (n = 138) |               |       |        |               |
| \textit{GSTT1} GT\textit{M1} |                  |                                  |             |          |               |       |        |               |
| (+) (+)              | 68               | 303                              | 1.00        | 26       | 42             | 1.00  | 34     | 34             | 1.00 |
| (-) (-)              | 59               | 270                              | 1.50        | 21       | 76             | 2.40  | 36     | 61             | 1.67 | (0.88-3.38) |
| (+) (+)              | 19               | 82                               | 1.00        | 8        | 11             | 0.74  | 7      | 12             | 1.70 | (0.90-3.94) |
| (-) (-)              | 34               | 83                               | 0.61        | 5        | 9              | 1.20  | 4      | 10             | 2.60 | (0.72-9.46) |
| Triple combination of genotypes | n = 519          |                                  |             |          |               |       |        |               |
| \textit{GSTT1} \textit{GSTM1} \textit{GSTP1} |                  |                                  |             |          |               |       |        |               |
| (+) (+) Val* Ile/Ile | 32               | 96                               | 1.00        | 12       | 20             | 1.00  | 16     | 10             | 1.00 |
| (-) (+) Val* Ile/Ile | 30               | 126                              | 1.13        | 14       | 22             | 0.93  | 12     | 17             | 1.81 | (0.67-4.92) |
| (+) (+) Val* Ile/Ile | 9                | 34                               | 0.90        | 3        | 6              | 1.07  | 2      | 7              | 4.57 | (0.80-26.24) |
| (+) (-) Val* Ile/Ile | 42               | 86                               | 1.52        | 8        | 22             | 1.78  | 11     | 19             | 2.58 | (0.94-6.79) |
| (-) (-) Val* Ile/Ile | 55               | 98                               | 1.85        | 10       | 42             | 2.92  | 19     | 33             | 2.06 | (0.83-5.15) |
| (-) (-) Val* Ile/Ile | 9                | 23                               | 1.27        | 3        | 5              | 1.25  | 1      | 7              | 5.47 | (0.92-32.60) |
| (-) (-) Val* Ile/Ile | 5                | 34                               | 1.27        | 1        | 3              | 1.01  | 1      | 2              | 1.88 | (0.27-13.33) |

\(^1\)OR adjusted for age, gender, alcohol and smoking habits and polymorphisms; \(^1\)P < 0.05 vs control; \^{*}Ile/Val ou Val/Ile. OR: Odds Ratio.

Substances present in tobacco and alcohol.

With regard to the clinical-histopathological parameters of SCRC, some studies have shown that low activity GST genotypes can be associated with more aggressive tumors and survival in colorectal cancer patients\(^{[51,52]}\). An association between the \textit{GSTM1} null genotype and the presence of advanced tumors has been observed. One study demonstrated an association between aggressive tumors and the presence of the \textit{GSTT1} null genotype\(^{[47]}\). However, other studies that evaluated the same polymorphisms did not find an association between the polymorphic genotypes and the clinical-histopathological parameters of SCRC\(^{[3,27,42,49]}\). This biological relationship between GST and progression is still not well described. However, a possible explanation for this is that GSTs play important roles in the regulation of genes related to activation of cellular maintenance, proliferation and apoptosis evasion. Thus, GSTs interact with tumor necrosis factor (TNF) receptor associated factor 2 (TRAF2) and decrease signal transduction from receptors in the TNF alpha-like (TNF-α) and c-Jun NH2-terminal kinase (JNK kinase) pathways\(^{[12,53,54]}\).

No association between polymorphisms and the primary sites of SCRC were identified in the present study. In accordance with these findings, the study by Vlaykova \textit{et al}\(^{[4]}\) did not find an association between polymorphisms of \textit{GSTT1} and \textit{GSTM1} null genotypes and the primary site. However, Wang \textit{et al}\(^{[41]}\), observed an increased risk of rectal cancer in the presence of the \textit{GSTM1} null genotype, while the \textit{GSTT1} null genotype was associated with a risk of colon cancer.

It is worth noting that predisposition to SCRC is multifactorial and results from the interaction between allelic variants of low-penetrance genes and environmental factors such as advanced age, eating habits, and smoking and drinking habits\(^{[2,55,56]}\). Therefore, the findings regarding the modulation of susceptibility to SCRC in the presence of the polymorphisms analyzed, regardless of smoking or drinking habits, reinforce the influence of these polymorphisms on the etiology of SCRC, even though they do not influence patient survival. These results may contribute to the understanding of the mechanisms involved in colorectal carcinogenesis.

In conclusion, females with advanced age are more susceptible to SCRC. The presence of the \textit{GSTM1} null genotype is associated with an increased risk of SCRC. The \textit{GSTM1} null genotype is associated with tumor progression. The combination of \textit{GSTT1}/\textit{GSTM1} and \textit{GSTT1}/\textit{GSTM1}/\textit{GSTP1} genotypes are associated with...
Polymorphisms in the coding genes GSTP1, GSTT1, and GSTM1 may alter enzymatic activity. This change can lead to DNA damage and deregulation of the mechanisms involved in colorectal cancer.

Research background
Colorectal cancer is the third most common cancer worldwide and develops on the inner walls of the colon and rectum. Genetic and environmental factors may increase the risk of colorectal cancer via the metabolism of carcinogens. Therefore, the evaluation of polymorphisms in genes involved in this process may help to modulate the development of colorectal cancer. Polymorphisms in genes encoding GSTP1, GSTT1, and GSTM1 may alter enzymatic activity. This change can lead to DNA damage and deregulation of the mechanisms involved in colorectal cancer.

Research motivation
Polymorphisms in the coding genes GSTP1, GSTT1, and GSTM1 have been studied in terms of susceptibility to diseases such as cancer. However, the literature presents conflicting results. Therefore, several studies are needed to assess and confirm the role of factors that influence changes in metabolic processes related to colorectal cancer.

Research objectives
The main objective of this study was to evaluate the influence of polymorphisms in the GSTP1, GSTT1 and GSTM1 genes on the risk of colorectal cancer. The data showed that carriers of polymorphisms in the GSTM1 genes and the combination of GSTT1 non-null/GSTM1 null genotypes and GSTT1 non-null/GSTM1 null/GSTP1 Val* (with the presence of at least one polymorphic allele) constitute a risk group for sporadic colorectal cancer (SCRC), and polymorphisms in the GSTM1 gene and the GSTT1 non-null/GSTM1 null combinations, GSTT1 non-null/GSTM1 null/GSTP1 Val* increase the aggressiveness of the tumor. Thus, considering the high incidence of this cancer, it is important to understand the factors that lead to carcinogenesis in order to develop preventive and therapeutic strategies for the management of cancer.

Research methods
This case-control study evaluated 970 individuals, 232 cases and 738 controls, using multiplex polymerase chain reaction (PCR) and polymerase chain reaction-restriction fragment chain reaction (PCR-RFLP) polymorphism. Demographics are tabulated by percentage. The binary logistic regression model was used to evaluate the association of age, gender, smoking and eating habits with SCRC, and to evaluate the association of the Hardy-Weinberg equilibrium (HWE) with the Chi-square test. Multiple binary logistic regression, adjusted for age, gender and smoking and alcohol habits, was also used to evaluate the association between the genetic models of the polymorphisms and the development of SCRC. The dominant genotypic model was used to assess the interaction between polymorphisms and smoking habits, adjusted for age, gender, and ethnicity, and to evaluate the interaction of polymorphisms and alcohol consumption, adjusted for age, gender and smoking, on the risk of SCRC. In addition, the Kaplan-Meier curve was used to assess the overall survival of patients with SCRC.

Table 5  Polymorphisms GSTT1, GSTM1, and GSTP1 in relation to overall survival of colorectal cancer patients

| Polymorphisms | Survival (5 yr) |
|---------------|---------------|
| GSTT1         |               |
| Positive      | 64            |
| Negative      | 68            |
| GSTM1         |               |
| Positive      | 67            |
| Negative      | 63            |
| GSTP1 A134G   |               |
| AA            | 61            |
| AG            | 70            |
| GG            | 63            |

*p < 0.05 vs control.

an increased risk of SCRC and tumor progression. Polymorphisms are not associated with the overall survival rate of SCRC patients.

Research results
The data showed that carriers of polymorphisms in the GSTM1 genes and the combination of GSTT1 non-null/GSTM1 null genotypes and GSTT1 non-null/GSTM1 null/GSTP1 Val* (with the presence of at least one polymorphic allele) constitute a risk group for SCRC, and polymorphisms in the GSTM1 gene and the GSTT1 non-null/GSTM1 null combinations, GSTT1 non-null/GSTM1 null/GSTP1 Val* increase the aggressiveness of the tumor. Thus, considering the high incidence of this cancer, it is important to understand the factors that lead to carcinogenesis in order to develop preventive and therapeutic strategies for the management of cancer.

Research conclusions
Similar studies have not previously been performed in the Brazilian population. Therefore, this work is unprecedented in this population. In addition, we emphasize the importance of the association between female gender and susceptibility to SCRC as well as the survival analysis associated with the polymorphisms studied, which have not been extensively studied in the literature. Polymorphisms in the GSTP1, GSTT1 and GSTM1 genes were involved in carcinogenesis and the poor prognosis of SCRC. In the Brazilian population it was observed that females with advanced age were more susceptible to SCRC. The presence of the GSTM1 null genotype, and the combination of GSTT1/GSTM1 and GSTT1/GSTM1/GSTP1 genotypes are associated with an increased risk of SCRC and tumor progression.

This study provides a perspective on biomarkers of GSTs related to the prognosis of SCRC that has not been extensively studied in other populations, especially the Brazilian population. These data may contribute to clinical practice. Another interesting fact was the association between females, age over 60 years and the risk of SCRC. Menopausal women (estrogen reduction) were also shown to be more susceptible to SCRC. Polymorphisms in the genes involved in the xenobiotic metabolism pathway are associated with the development and poor prognosis of SCRC.

In this study, statistical analyses were widely used, and unlike other studies, multiple logistic regression was performed to evaluate the interactions between the polymorphisms studied and variables. In addition, survival was assessed by Kaplan Meier analysis. These analyses are extremely relevant in studies involving population genetic polymorphisms.

An association between some polymorphisms of xenobiotic metabolism and the development and progression of SCRC was observed. Advanced age and female gender were associated with the development of SCRC and polymorphisms in the genes involved in the xenobiotic metabolism pathway were associated with the development and poor prognosis of SCRC. This study may contribute to GSTs being used as diagnostic and prognostic biomarkers for SCRC. These data together with the findings of other studies may contribute to the development of treatment strategies for SCRC.

Research perspectives
This study demonstrated the importance of population studies with a large sample size in research on polymorphisms. Thus, we intend to expand the sample size to validate the results and include more polymorphisms related to the xenobiotic pathways in order to better understand the roles of these pathways in SCRC carcinogenesis. Research methods such as real-time PCR, are important in order to accurately quantify the presence of polymorphisms.

REFERENCES
1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11. Lyon, France: International Agency for Research on Cancer; 2013. Available from: URL: http://globoCAN.iarc.fr
2. Instituto Nacional do Câncer. Ministério da Saúde; 2018. Accessed February 20, 2018. Available from: URL: http://www.inca.gov.br
Glutathione S-transferase family genes in CRC

3 Oines M, Helsingin LM, Bretthauer M, Emlisson L. Epidemiology and risk factors of colorectal polyps. Best Pract Res Clin Gastroenterol 2017; 31: 419-424 [PMID: 28842051 DOI: 10.1016/j.bpcg.2017.07.004]

4 Jess JR. Classification of colorectal cancer based on correlation of clinical, morphological and molecular features. Histopathology 2007; 50: 113-130 [PMID: 17204026 DOI: 10.1111/j.1365-2559.2006.02549.x]

5 Bhalla A, Zulfiqar M, Bluth MH. Molecular Diagnostics in Colorectal Cancer: Advancements and Applications for 2018. Clin Lab Med 2018; 38: 311-342 [PMID: 29776633 DOI: 10.1016/j.cll.2018.02.008]

6 Huang D, Sun W, Zhou Y, Li P, Chen F, Chen H, Xia D, Xu E, Lai M, Wu Y, Zhang H. Mutations of key driver genes in colorectal cancer progression and metastasis. Cancer Metastasis Rev 2018; 37: 173-187 [PMID: 29323254 DOI: 10.1007/s10555-017-9726-5]

7 Marley AR, Nan H. Epidemiology of colorectal cancer. Int J Mol Epidemiol Genet 2016; 7: 105-114 [PMID: 2776617]

8 Gorukmez O, Yakut T, Gorukmez O, Sag SO, Topak A, Sahinturk S, Kanat O. Glutathione S-transferase T1, M1 and P1 Genetic Polymorphisms and Susceptibility to Colorectal Cancer in Turkey. Asian Pac J Cancer Prev 2016; 17: 3855-3859 [PMID: 27646429]

9 Nascimento H, Coy CS, Teori MT, Boin IF, Góes JR, Costa FF, Lima CS. Possible influence of glutathione S-transferase GSTT1 null genotype on age of onset of sporadic colorectal adenocarcinoma. Dis Colon Rectum 2003; 46: 510-515 [PMID: 12682546 DOI: 10.1016/S1053-2127(02)00176-7]

10 Shen X, Wang J, Yan X, Ren X, Wang F, Chen X, Xu Y. Predictive value of GSTP1 Ile105Val polymorphism in clinical outcomes of chemotherapy in gastric and colorectal cancers: a systematic review and meta-analysis. Cancer Chemother Pharmacol 2016; 77: 1285-1302 [PMID: 27154175 DOI: 10.1007/s00280-016-3047-1]

11 Ramsay EE, Dilda PJ. Glutathione S-conjugates as prodrugs to target drug-resistant tumors. Front Pharmacol 2014; 5: 181 [PMID: 25157254 DOI: 10.3389/fphar.2014.00181]

12 Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. Annu Rev Pharmacol Toxicol 2005; 45: 51-88 [PMID: 15822171 DOI: 10.1146/annurev.pharm.tox.45.120403.095857]

13 Rowe JD, Nieves E, Listowski I. Subunit diversity and tissue distribution of human glutathione S-transferases: interpretations based on electrospray ionization-MS and peptide sequence-specific antisera. Biochem J 1997; 325 (Pt 2): 481-486 [PMID: 9230131]

14 Economopoulos KP, Sargentinas TN. GSTM1, GSTT1, GSTP1, GSTA1 and colorectal cancer risk: a comprehensive meta-analysis. Eur J Cancer 2014; 50: 1617-1631 [PMID: 23207535 DOI: 10.1016/j.ejca.2013.02.006]

15 Oguztuzun S, Abu-Hijleh A, Coban T, Bulbul D, Kilic M, Iscan M, Iscan M. GST isoenzymes in matched normal and neoplastic breast tissue. Neoplasma 2011; 58: 304-310 [PMID: 21520986]

16 Hezova R, Bienertova-Vasku J, Sachlova M, Brezkova V, Vasku A, Svoboda M, Radová L, Kiss I, Vyzula R, Slaby O. Identification of genetic polymorphisms at the glutathione S-transferase Pi locus and association with susceptibility to bladder, testicular and prostate cancer. Carcinogenesis 1997; 18: 641-644 [PMID: 911193]

17 Fernandez GM, Russo A, Proenca MA, Gazoza NF, Rodgers GH, Biselli-Chicote PM, Silva AE, Netinho JG, Pavarrano EC, Golonovi-Bertolom EM. CYP1A1, CYP2E1 and EPHX1 polymorphisms in sporadic colorectal neoplasms. J World Gastroenterol 2016; 22: 9974-9983 [PMID: 28018104 DOI: 10.3748/wjg.v22.i45.9974]

18 Cong N, Liu L, Xie Y, Shao W, Song J. Association between glutathione S-transferase PI locus and association with susceptibility to bladder, testicular and prostate cancer. J Korean Med Sci 2014; 29: 1488-1492 [PMID: 25408570 DOI: 10.3346/jkms.2014.29.11.1488]

19 Kassab A, Msolly A, Lakhdar R, Gharbi O, Miled A. Polymorphisms of glutathione S-transferases M1, T1, P1 and susceptibility to colorectal cancer in a sample of the Tunisian population. Med Oncol 2014; 31: 760 [PMID: 24254297 DOI: 10.1007/s12032-013-0760-z]

20 Vlajkova T, Miteva L, Gulubova M, Staniolova S. Ile105Val GSTP1 polymorphism and susceptibility to colorectal carcinoma in Bulgarian population. Int J Colorectal Dis 2007; 12: 1209-1215 [PMID: 17404745 DOI: 10.1007/s00384-007-0305-z]

21 Osazuwa-Peters N, Massa ST, Christopher KM, Walker RJ, Varvares MA. Race and sex disparities in long-term survival of oral and oropharyngeal cancer in the United States. J Cancer Res Clin Oncol 2016; 142: 521-528 [PMID: 26507889 DOI: 10.1007/s00342-015-2061-8]

22 Turatti F, Rossi M, Pelucchi C, Levi F, La Vecchia C. Fruit and vegetables and cancer risk: a review of southern European studies. Br J Nutr 2015; 113 Suppl 2: S102-S110 [PMID: 26148912 DOI: 10.1017/s0007114515000148]

23 Hendifar A, Yang D, Lenz F, Lurje G, Pohl A, Lenz C, Ning Y, Zhang W, Lenz HJ. Gender disparities in metastatic colorectal cancer survival. Clin Cancer Res 2009; 15: 6391-6397 [PMID: 19789331 DOI: 10.1158/1078-0432.CCR-09-0877]

24 Iida Y, Kawan K, Tsuno N, Ishihara S, Yamaguchi H, Sunami E, Kitayama J, Watanabe T. Proximal shift of colorectal cancer along with aging. Clin Colorectal Cancer 2014; 13: 213-218 [PMID: 25245544 DOI: 10.1016/j.cccr.2014.06.005]

25 Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM, Ockene J, Writing Group for the Women’s Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women’s Health Initiative randomized controlled trial. JAMA 2002; 288: 321-333 [PMID: 11217397]

26 Calle EE, Miracle-McMahill HL, Thun MJ, Heath CW Jr. Estrogen replacement therapy and risk of fatal colon cancer in a prospective cohort of postmenopausal women. J Natl Cancer Inst 1995; 87: 517-523 [PMID: 7707438]

27 Chan JA, Meyerhardt JA, Chan AT, Giovannucci EL, Colditz GA, Fuchs CS. Hormone replacement therapy and survival after colorectal cancer diagnosis. J Clin Oncol 2006; 24: 5680-5686 [PMID: 17197103 DOI: 10.1200/JCO.2006.06.0580]

28 Mandelson MT, Miglioretti DL, Newcomb PA, Harrison R, Potter JD. Identification of genetic polymorphisms at the glutathione S-transferase Pi locus and association with susceptibility to bladder, testicular and prostate cancer. Cancer Res 2003; 63: 979-984 [PMID: 14750537]

29 Slattery ML, Anderson K, Samowitz W, Edwards SL, Curtin K, Caan B, Potter JD. Hormone replacement therapy and improved survival among postmenopausal women diagnosed with colon cancer (USA).
Cancer Causes Control 1999; 10: 467-473 [PMID: 10530618]

37 Yan L, Spitzenagel EL, Bosland MC. Soy consumption and colorectal cancer risk in humans: a meta-analysis. Cancer Epidemiol Biomarkers Prev 2010; 19: 148-158 [PMID: 20056634 DOI: 10.1158/1055-9965.EPI-09-0856]

38 Koh WP, Nelson HH, Yuan JM, Van den Berg D, Jin A, Wang R, Yu MC. Glutathione S-transferase (GST) gene polymorphisms, cigarette smoking and colorectal cancer risk among Chinese in Singapore. Carcinogenesis 2011; 32: 1507-1511 [PMID: 21803734 DOI: 10.1093/carcin/bgr175]

39 Rossi M, Jahanzaib Anwar M, Usman A, Keshavarzian A, Bishehsari F. Colorectal Cancer and Alcohol Consumption-Populations to Molecules. Cancers (Basel) 2018; 10: [PMID: 29385712 DOI: 10.3390/cancers10020038]

40 Cai S, Li Y, Ding Y, Chen K, Jin M. Alcohol drinking and the risk of colorectal cancer death: a meta-analysis. Eur J Cancer Prev 2014; 23: 532-539 [PMID: 25170915 DOI: 10.1097/CEJ.0000000000000076]

41 Wang J, Jiang J, Zhao Y, Gajalakshmi V, Kuriki K, Suzuki S, Nagaya T, Nakamura S, Akasaka S, Ishikawa H, Tokudome S. Genetic polymorphisms of glutathione S-transferase genes and susceptibility to colorectal cancer: a case-control study in an Indian population. Cancer Epidemiol 2011; 35: 66-72 [PMID: 20688591 DOI: 10.1016/j.canep.2010.07.003]

42 Khazab MN. The GSTP1 Ile105Val polymorphism is not associated with susceptibility to colorectal cancer. Asian Pac J Cancer Prev 2012; 13: 2949-2953 [PMID: 22938488]

43 Gonzales FJ, Coughtrie M, Tukey RH. Metabolismo dos fármacos. In: As bases Farmacológicas da Terapêutica de Goodman & Gilman. 12th ed. Rio de Janeiro: AMGH, 2012:135-136

44 Liu X, Tan N, Liao H, Pan G, Xu Q, Zhu R, Zou L, He S, Zhu H. High GSTP1 inhibits cell proliferation by reducing Akt phosphorylation and is associated with a better prognosis in hepatocellular carcinoma. Oncotarget 2017; 9: 8957-8971 [PMID: 29507666 DOI: 10.18632/oncotarget.23420]

45 Gurioli G, Martignano F, Salvi S, Costantino M, Gunelli R, Casadio V. GSTP1 methylation in a liquid biopsy biomarker? Clin Chem Lab Med 2018; 56: 702-717 [PMID: 29305565 DOI: 10.1515/celmn-2017-0703]

46 Saadat I, Saadat M. Glutathione S-transferase M1 and T1 null genotypes and the risk of gastric and colorectal cancers. Cancer Lett 2001; 169: 21-26 [PMID: 11410321 DOI: 10.1016/S0304-3835(01)00550-X]

47 Nissar S, Sameer AS, Rasool R, Chowdri NA, Rashid F. Evaluation of deletion polymorphisms of glutathione S-transferase genes and colorectal cancer risk in ethnic Kashmiri population: A case-control study. Indian J Cancer 2016; 53: 524-528 [PMID: 28485343 DOI: 10.4103/ijjc.IJC_17_17]

48 Nisa H, Kono S, Yin G, Toyomura K, Nagano J, Mibu R, Tanaka M, Kakeyi Y, Maehara Y, Okamura T, Ikejiri K, Futami K, Maekawa T, Yasunumi Y, Takenaka K, Ichimiyi H, Terasaka R. Cigarette smoking, genetic polymorphisms and colorectal cancer risk: the Fukuoka Colorectal Cancer Study. BMC Cancer 2010; 10: 274 [PMID: 20554171 DOI: 10.1186/1471-2407-10-274]

49 Piao JM, Shin MH, Kweon SS, Kim HK, Choi JS, Bae WK, Shim HJ, Kim HR, Park YK, Choi YD, Kim SH. Glutathione-S-transferase (GSTM1, GSTT1) and the risk of gastrointestinal cancer in a Korean population. World J Gastroenterol 2009; 15: 5716-5721 [PMID: 19960570 DOI: 10.3748/wjg.15.5716]

50 Zhu X, Wang Z, He J, Wang W, Xue W, Wang Y, Zheng L, Zhu ML. Associations between CYPIA1 rs1048943 A & G and rs4646903 T & G; C genetic variations and colorectal cancer risk: proof from 26 case-control studies. Oncotarget 2016; 7: 51365-51374 [PMID: 27384991 DOI: 10.18632/oncotarget.10331]

51 Hunter DJ, Riboli E, Haiman CA, Albanes D, Altshuler D, Chanock SJ, Haynes RB, Henderson BE, Kaaks R, Stram DO, Thomas G, Thun MJ, Blanché H, Buring JE, Burtt NP, Calle EE, Cann H, Canzian F, Chen YC, Colditz GA, Cox DG, Dungan AM, Feigelson HS, Friedman ML, Gaziano JM, Giovannucci E, Hankinson SE, Hirschorn JN, Hoover RN, Key T, Kolonel LN, Kraft P, Le Marchand L, Liu S, Ma J, Melnick S, Pharoah P, Pike MC, Rodriguez C, Setiawan VW, Stampfer MJ, Trapido E, Travis R, Virtamo J, Wacholder S, Willett WC; National Cancer Institute Breast and Prostate Cancer Cohort Consortium. A candidate gene approach to searching for low-penetrance breast and prostate cancer genes. Nat Rev Cancer 2005; 5: 977-985 [PMID: 16341085 DOI: 10.1038/nrc1754]

52 Mitsudomi T, Hamajima N, Ogawa M, Takahashi T. Prognostic significance of p53 alterations in patients with non-small cell lung cancer: a meta-analysis. Clin Cancer Res 2000; 6: 4055-4063 [PMID: 11051256]

53 Holley SL, Rajagopal R, Hoban PR, Deakin M, Fawole AS, Elder JB, Elder J, Smith V, Strange RC, Fryer AA. Polymorphisms in the glutathione S-transferase mu cluster are associated with tumour progression and patient outcome in colorectal cancer. Int J Oncol 2006; 28: 231-236 [PMID: 16328000]

54 Wu Y, Fan Y, Xue B, Luo L, Shen J, Zhang S, Jiang Y, Yin Z. Human glutathione S-transferase P1-1 interacts with TRAF2 and regulates TRAF2-ASK1 signals. Oncogene. 2006; 25: 5787-5800 [PMID: 16636664 DOI: 10.1038/sj.onc.1209576]

55 Adler V, Yin Z, Fuchs SY, Benezra M, Rosario L, Tew KD, Pincus MR, Sardana M, Henderson CJ, Wolf CR, Davis RJ, Ronai Z. Regulation of JNK signaling by GSTp. EMBO J 1999; 18: 1321-1334 [PMID: 10664598 DOI: 10.1093/emboj/18.5.1321]

56 de la Chapelle A. Genetic predisposition to colorectal cancer. Nat Rev Cancer 2004; 4: 769-780 [PMID: 15510158 DOI: 10.1038/nrc1453]
