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ORIGINAL ARTICLE

Case Control Study

CYP1A1, *CYP2E1* and *EPHX1* polymorphisms in sporadic colorectal neoplasms

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Abstract

AIM

To investigate the contribution of polymorphisms in

the *CYP1A1*, *CYP2E1* and *EPHX1* genes on sporadic colorectal cancer (SCRC) risk.

METHODS

Six hundred forty-one individuals (227 patients with SCRC and 400 controls) were enrolled in the study. The variables analyzed were age, gender, tobacco and alcohol consumption, and clinical and histopathological tumor parameters. The *CYP1A1**2A, *CYP1A1**2C *CYP2E1**5B and *CYP2E1**6 polymorphisms were analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The *EPHX1* Tyr113His, *EPHX1* His139Arg and *CYP1A1**2C polymorphisms were detected by real-time PCR. Chi-squared test and binary logistic regression were used in the statistical analysis. Haplotype analysis was conducted using the Haploview program, version 2.05.

RESULTS

Age over 62 years was a risk factor for SCRC development (OR = 7.54, 95%CI: 4.94-11.50, $P < 0.01$). Male individuals were less susceptible to SCRC (OR = 0.55, 95%CI: 0.35-0.85, $P < 0.01$). The *CYP2E1*5B* polymorphism was associated with SCRC in the codominant (heterozygous genotype: OR = 2.66, 95%CI: 1.64-4.32, $P < 0.01$), dominant (OR = 2.82, 95%CI: 1.74-4.55, $P < 0.01$), overdominant (OR = 2.58, 95%CI: 1.59-4.19, $P < 0.01$), and log-additive models (OR = 2.84, 95%CI: 1.78-4.52, $P < 0.01$). The *CYP2E1*6* polymorphism was associated with an increased SCRC risk in codominant (heterozygous genotype: OR = 2.81, 95%CI: 1.84-4.28, $P < 0.01$; homozygous polymorphic: OR = 7.32, 95%CI: 1.85-28.96, $P < 0.01$), dominant (OR = 2.97, 95%CI: 1.97-4.50, $P < 0.01$), recessive (OR = 5.26, 95%CI: 1.35-20.50, $P = 0.016$), overdominant (OR = 2.64, 95%CI: 1.74-4.01, $P < 0.01$), and log-additive models (OR = 2.78, 95%CI: 1.91-4.06, $P < 0.01$). The haplotype formed by the minor alleles of the CYP2E1*5B (C) and CYP2E1*6 (A) polymorphisms was associated with SCRC ($P = 0.002$). However, the *CYP1A1**2A, *CYP1A1**2C, *EPHX1 Tyr113His* and *EPHX1 His139Arg* polymorphisms were not associated with SCRC.

CONCLUSION

In conclusion, the results demonstrated that *CYP2E1*5B* and *CYP2E1*6* minor alleles play a role in the development of SCRC.

Key words: Single-nucleotide polymorphisms; Colorectal neoplasms; Cytochrome P-450 CYP2E1; Cytochrome P-450 CYP1A1; Epoxide hydrolases 1

Core tip: Sporadic colorectal cancer (SCRC) includes malignancies that occur in the colon and rectum. This type of cancer is the third most common cancer worldwide. The main etiological factors are age over 50 years and tobacco and alcohol consumption. The elimination of environmental carcinogens contained in tobacco, as well as alcohol, requires metabolic activation mediated by xenobiotic-metabolizing enzymes (XMEs). The *CYP2E1*5B* and *CYP2E1*6* polymorphisms were associated with SCRC, as well as the *CYP2E1*5B* (*C*) and *CYP2E1*6* (*A*) haplotype (minor alleles). Polymorphisms in several genes encoding these XMEs may be involved in alterations in gene expression related to important processes of colorectal carcinogenesis such as inflammation and angiogenesis.

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INTRODUCTION

Sporadic colorectal cancer (SCRC) includes malignancies that occur in the large intestine (colon) and rectum. This type of cancer is the fifth most common cancer in Brazil. In 2016, an estimated 34280 new cases of SCRC will be diagnosed in Brazil, according to a survey conducted by the National Cancer Institute (INCA)[1]. This is the third most common cancer worldwide with an estimated 136100 new cases each year, mainly in developed regions. The overall mortality rate is estimated to be 694000 deaths, 8.5% of all cases. Fifty-two percent of these deaths occur in developing regions of the world[2]. The main etiological factors related to SCRC are age over 50 years[1] and tobacco[3] and alcohol consumption[4].

Tobacco and alcohol are environmental carcinogens responsible for the release of exogenous compounds, including reactive oxygenated intermediates (ROMs) represented by benzo[a]pyrene (BaP) N-nitrosamines, heterocyclic amines (HAs) and polycyclic aromatic hydrocarbons (PAHs). These compounds are metabolically activated in electrophilic forms before interaction with DNA, and they generate adducts and contribute to tumor initiation[5].

The elimination of these environmental carcinogens requires metabolic activation mediated by xenobiotic-metabolizing enzymes (XMEs), such as cytochrome P-450 (CYP) and epoxide hydrolase (EPHX1). Polymorphisms in several genes encoding these XMEs are responsible for metabolism errors, which can contribute to the development of several cancer types[5-7].

In the liver and intestine, Phase I oxidative enzymes convert the compounds to highly reactive metabolites by introducing one or more hydroxyl groups in the substrate, increasing its water solubility and converting it into a form that will be more easily expelled. These enzymes, including CYPs and EPHX1, are involved in cellular pathways required for the

carcinogenesis process, such as the metabolism of eicosanoids, the biosynthesis of cholesterol and bile acids, steroid synthesis, biogenic amine synthesis and degradation, vitamin D3 synthesis, hydroxylation of retinoic acid, and arachidonic acid metabolism[5,6,8].

Single-nucleotide polymorphisms (SNPs) in genes encoding XMEs can modify the enzyme expression or function and, consequently, alter the activation or detoxification of carcinogenic compounds. The balance between metabolic activation and detoxification can affect the risk of cancer once DNA adducts play an important role in the carcinogenic process[5,6].

SNPs in the *CYP1A1* and *CYP2E1* genes, which encode important XMEs, can lead to alterations of the function of these enzymes, resulting in the activation of carcinogens, which are involved in tumor initiation[5]. These polymorphisms have been associated with colorectal cancer development[9,10]. Among the polymorphisms, the main ones are *CYP1A1**2A (rs4646903), resulting in the substitution of thymine for cytosine (*T3801C*) in the poly (A) tail of the 3′ untranslated gene region[11,12]; *CYP1A1**2C (rs1048943), resulting from the transition of adenine to guanine (*A2455G*)[13,14]; *CYP2E1**5B (rs3813867), with the substitution of guanine for cytosine at the -1293 nucleotide position[12,15]; and *CYP2E1**6 (rs6413432), caused by the alteration of thymine to adenine at position 7632 of the gene[16,17].

EPHX1 Tyr113His (rs1051740) and *EPHX1 His139Arg* (rs2234922), functional polymorphisms of the *EPHX1* gene, have been well characterized[18]. These polymorphisms are associated with the susceptibility to SCRC[19,20]. The *EPHX1 Tyr113His* polymorphism, located at position 337 in exon 3 of the *EPHX1* gene, is characterized by a substitution of the amino acid histidine for tyrosine at position 113 of the protein. This change leads to a decrease of approximately 40-50% of the enzyme activity and stability *in vitro*. The polymorphism *EPHX1 His139Arg*, localized in exon 4 at position 416 of the *EPHX1* gene, results in the amino acid substitution of arginine to histidine at position 139 of the protein. These modifications increase the enzyme activity and stability by 25%[18,21].

In the present study, we investigated the association between the *CYP1A1**2A, *CYP1A1**2C, *CYP2E1**5B, *CYP2E1*6, EPHX1 Tyr113His* and *EPHX1 His139Arg* polymorphisms and SCRC risk, the interaction between these polymorphisms with tobacco and alcohol consumption, and the association of SCRC with sociodemographic factors.

MATERIALS AND METHODS

Approval and consent

After approval by the Ethics in Research Committee CEP/FAMERP, protocol No. 012/2012 (CAAE: 0237.0.140.00011), the individuals who agreed to participate in the study signed an informed consent form. Information about current and past occupations, tobacco and alcohol consumption, and family history of cancer or adenomatous polyps and lesions were collected using a standard interviewer-administered questionnaire. The ethnicity was not evaluated during this study because of the miscegenation of the studied population.

Study populations

Six hundred twenty-seven individuals (227 patients with sporadic colorectal cancer and 400 controls) were included in the study (Table 1). The recruitment of patients and controls, as well as the collection of peripheral blood and clinical and histopathological data, was performed between 2010 and 2013 at the Coloproctology Service of Hospital de Base/Sao Jose do Rio Preto Medical School, Sao Jose do Rio Preto, SP, Brazil. In the present study, it was not necessary for a follow-up of the individuals. The case group consisted of individuals with a clinical and histopathological diagnosis of SCRC. The exclusion criteria were patients with hereditary cancer and those previously treated with chemotherapy and/or radiotherapy. The control group consisted of healthy individuals, blood donors with no history of a cancer diagnosis and no family history of cancer in at least three previous generations and other diseases according to the criteria of the American Association of Blood Donors[22].

We considered smoker individuals as those patients who consumed >100 cigarettes in a lifetime. We considered alcohol drinkers as those patients who consumed > 1 drink per week (one drink was defined as approximately 44 mL of liquor or 118 mL of wine or 350 mL of beer)[23].

Tumors were TNM classified according to the following three criteria: the tumor extent (T), the presence of regional lymph node involvement (N) and the presence of distant metastasis (M)[24]. T1 and T2 tumors were classified as smaller tumors, and T3 and T4 tumors were classified as larger tumors. Lymph node involvement was classified according to its absence (N0) and presence (N1, N2, N3). Tumors were classified as non-aggressive (stage Ⅰ and Ⅱ) and aggressive (stage Ⅲ and Ⅳ) according to the clinical staging (TNM)[25]. Information about TNM was impossible in all cases. The analysis of these parameters was performed in a smaller group. Therefore, for the analysis of tumor extension, only 200 samples were analyzed. For the analysis of regional lymph node involvement, 198 samples were analyzed. For the evaluation of aggressiveness, 114 samples were included in the analysis.

Nucleic acid extraction

DNA extraction was performed from peripheral blood leukocytes according to the procedure by Miller and collaborators with modifications[26]. Quantification and the purity of DNA samples were determined by absorbance at a wavelength (λ) at 260 and 280 nm using the Picodrop Pico200™ spectrophotometer

Table 1 Sociodemographic data of patients with sporadic colorectal cancer and controls *n* (%)

Variables	Control (*n* = 400)	Case (*n* = 227)	OR[1]	95%CI	*P* value
Gender					
Female	125 (31.3)	106 (46.7)	1.00 (reference)		
Male	275 (69.7)	121 (53.3)	0.55	0.35-0.85	< 0.01[2]
Age (mean)					
< 62	350 (87.5)	105 (46.3)	1.00 (reference)		
⩾ 62	50 (12.5)	122 (53.7)	7.54	4.94-11.50	< 0.01[2]
Tobacco consumption					
Non-smokers	243 (60.8)	131 (57.7)	1.00 (reference)		
Smokers	157 (39.2)	96 (42.3)	1.12	0.73-1.70	0.60
Alcohol consumption					
Non-drinkers	218 (54.5)	127 (55.9)	1.00 (reference)		
Drinkers	182 (45.5)	100 (44.1)	1.44	0.93-2.24	0.10

[1]Odds ratio (OR) adjusted for age, gender, tobacco and alcohol consumption and polymorphisms in the dominant model; [2]Significant *P* values < 0.05.

Table 2 Description of the primers sequences and restriction enzymes for *CYP1A12A, *CYP2E1**5B and *CYP2E1**6 polymorphisms analysis**

Polymorphisms	Sequence of primers	Restriction Enzyme T/*t*
*CYP1A1**2A		MspI
Sense	5′-GA TGA AGA GGT GTA GCC GCT-3′	37 ℃/3 h
Antisense	5-TAG GAG TCT TGT CTC ATG CCT-3′	
*CYP2E1**5B		PstI
Sense	5′-CCA GTC GAG TCT ACA TTG TCA-3′	37 ℃/3 h
Antisense	5′-TTC ATT CTG TCT TCT AAC TGG-3′	
*CYP2E1**6		DraI
Sense	5′-TCG TCA GTT CCT GAA AGC AGG-3′	37 ℃/3 h
Antisense	5′-GAG CTC TGA TGC AAG TAT CGC-3′	

(Thermo Scientific).

Polymorphism genotyping

The genotyping of *CYP1A1**2A (rs4646903), *CYP2E1**5B (rs3813867) and *CYP2E1**6 (rs6413432) polymorphisms was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The primer sequences used for amplification and the enzymes used to identify polymorphic sites are shown in Table 2.

EPHX1 Tyr113His (rs1051740) and *EPHX1 His139Arg* (rs2234922) and *CYP1A1**2C (rs1048943) polymorphism genotyping was performed by real-time PCR. The reactions were established according to the manufacturer's protocol (Applied Biosystems) with specific primers and probes validated (TaqMan MGB-probes: Assay ID C__14938_30, C__11638783_30 and C_25624888_50, respectively). The reactions were performed using the Step One Plus™ Real-Time PCR System (Applied Biosystems).

Statistical analysis

Descriptive statistics included the mean values, standard deviation for continuous data and percentages for categorical data. The BioEstat software, version 5.0 was used to evaluate the Hardy-Weinberg equilibrium (HWE). The software Minitab, version 16.0, was used to perform the normality test (similar to the Shapiro-Wilk method) of the variable age, and a binary logistic regression model was used to evaluate the association between the variables and SCRC and also to evaluate the association of polymorphisms with clinical and histopathological parameters after the adjustment for age, gender, and tobacco and alcohol consumption.

The SNPStats software (available at: < http://bioinfo.iconcologia.net/SNPstats_web>) was used to perform binary logistic regression to evaluate the association of polymorphisms with SCRC risk in the log-additive model (major allele homozygotes *vs* heterozygotes + minor allele homozygotes with weight 2), the dominant model (major allele homozygotes *vs* heterozygotes + minor allele homozygotes), the recessive model (major allele homozygotes + heterozygotes *vs* minor allele homozygotes), the codominant model (heterozygotes *vs* major allele homozygotes and minor allele homozygotes *vs* major allele homozygotes), and the overdominant model (major allele homozygotes *vs* heterozygotes + minor allele homozygotes), after adjustment for age, gender and tobacco and alcohol consumption. The SNPStats program was also used to evaluate the potential interaction between the polymorphisms and tobacco or alcohol consumption, adjusted for the other variables on SCRC risk. The results are presented as odds ratios (ORs) and 95%CI. Linkage disequilibrium between the polymorphism and haplotype frequencies was determined using the Haploview program, version 2.05. Results with a *P* value < 0.05 were considered statistically significant. The statistical review of the study was performed by a biomedical statistician.

RESULTS

The normality test was performed for the variable age, which had a normal distribution ($P < 0.01$). Table 1 shows the sociodemographic data of the SCRC patients and controls. Age over 62 years (mean age of the case group; OR = 7.54, 95%CI: 4.94-11.50, $P < 0.01$)

Table 3 Alleles frequencies of *CYP1A12A, *CYP1A1**2C, *CYP2E1**5B, *CYP2E1**6, *EPHX1 Tyr113His* and *EPHX1 His139Arg* polymorphisms in the sample of this study**

Polymorphisms	Allele	Control *n*	Allele frequencies	Case *n*	Allele frequencies
*CYP1A1*2A*	T	617	0.77	383	0.84
	C	183	0.23	71	0.16
*CYP1A1*2C*	A	699	0.87	416	0.92
	G	101	0.13	38	0.08
*CYP2E1*5B*	G	751	0.94	381	0.84
	C	49	0.06	73	0.16
*CYP2E1*6*	T	710	0.89	345	0.76
	A	90	0.11	109	0.24
EPHX1 Tyr113His	T	586	0.73	340	0.75
	C	214	0.27	114	0.25
EPHX1 His139Arg	A	615	0.77	373	0.82
	G	185	0.23	81	0.18

and male gender (OR = 0.55, 95%CI: 0.35-0.85, $P < 0.01$) showed a statistically significant association with SCRC.

The allelic frequencies of the polymorphisms are shown in Table 3. The genotype frequencies are in HWE equilibrium in both groups for the *CYP2E1**5B, *CYP2E1**6, *EPHX1 Tyr113His* and *EPHX1 His139Arg* polymorphisms. For the *CYP1A1**2A and *CYP1A1**2C polymorphisms, only the case group is in HWE equilibrium (*CYP1A1*2A* case: $\chi^2 = 3.08$ and $P = 0.08$, control: $\chi^2 = 4.97$ and $P = 0.03$; *CYP1A1*2C* case: $\chi^2 = 3.40$ and $P = 0.06$; control: $\chi^2 = 8.59$ and $P = 0.003$). HWE analysis was performed in case-control studies to verify if the allele frequency is similar to the expected frequency throughout the generations and to allow the investigation of the association between an allele and pathological conditions.

The results of the association between the six polymorphisms with SCRC are shown in Table 4. *CYP2E1**5B and *CYP2E1**6 polymorphisms were associated with SCRC in all genotype models, except for the log-additive for *CYP2E1**5B because the minor allele was not represented in the control group. *CYP1A1**2A, *CYP1A1**2C, *EPHX1 Tyr113His* and *EPHX1 His139Arg* polymorphisms were not associated with SCRC.

In the present study, the interaction of the presence of polymorphisms and tobacco or alcohol consumption with the SCRC risk was not demonstrated (Table 5). We observed that heterozygous or homozygous polymorphic genotype carriers for the *CYP2E1**5B polymorphism showed an increased SCRC risk independent of tobacco consumption (non-smokers: OR = 2.69 and 95%CI: 1.41-5.10; smokers: OR = 2.68 and 95%CI: 1.33-5.41) or alcohol consumption (non-drinkers: OR = 3.07 and 95%CI: 1.63-5.80; drinkers: OR = 3.90 and 95%CI: 1.82-8.38). The same was observed for non-smokers (OR = 2.89; 95%CI: 1.7-4.93) or smokers (OR = 2.99, 95%CI: 1.58-5.64) and non-drinkers (OR = 3.1, 95%CI: 1.80-5.48) or drinkers (OR = 4.10, 95%CI: 2.18-7.72) carrying heterozygous or homozygous polymorphic genotypes for the *CYP2E1**6 polymorphism.

Regarding the clinical and histopathological parameters of SCRC, the most common variables were tumor extension T3 and T4 (61.63%), the absence of lymph node involvement (52.91%) and the rectum as the primary site (52.09%). The polymorphisms were not associated with clinical and histopathological parameters (data not shown).

Haplotype analyses were conducted to evaluate the combined effect of the polymorphisms on SCRC development. The *CYP1A1**2A and *CYP1A1**2C polymorphisms in our study were in strong linkage disequilibrium [logarithm of odds (LOD) = 39.44; Lewontin's D' (D') = 0.711]. The haplotype *CA* (minor alleles for both polymorphisms) was not associated with SCRC ($P > 0.05$).

The *CYP2E1**5B and *CYP2E1**6 polymorphisms were also in linkage disequilibrium [logarithm of odds (LOD) = 10.15; Lewontin's D' (D') = 0.39]. The haplotype formed by minor alleles (*CA*) of both polymorphisms presented a higher frequency in the case group ($P = 0.002$).

The *EPHX1 Tyr113His* and *EPHX1 His139Arg* polymorphisms were not in linkage disequilibrium in the population studied (logarithm of odds (LOD) = 0.17; Lewontin's D' (D') = 0.124).

DISCUSSION

The results of the present study showed that individuals aged 62 years and older are more susceptible to SCRC, corroborating the data reported by previous studies, which established that age is a risk factor for this disease[1,10,19,20]. We also observed that male subjects were less susceptible to SCRC, although the incidence of SCRC is similar between genders[1,21].

In the present study, the *CYP2E1**5B and *CYP2E1**6 polymorphisms were associated with increased SCRC risk. The *CYP2E1* haplotypes formed by both minor alleles (CA) were also associated with SCRC. The *CYP2E1**5B[10] and *CYP2E1**6[27] polymorphisms can enhance the transcription of the *CYP2E1* gene and increase the level of enzyme activity. CYP2E1 is involved in arachidonic acid metabolism, producing hydroxyeicosatetraenoic acids and epoxyeicosatrienoic acids, which have been implicated in inflammation and vascular endothelial growth factor-dependent angiogenesis[28-30]. Furthermore, CYP2E1 is involved in reactive oxygen species (ROS) production, which is related to angiogenesis induction and metastatic growth of tumor cells[31]. Therefore, the increase in enzyme activity as a result of the CYP2E1 polymorphism may contribute to an increased risk of cancer.

Studies have also shown an association between the polymorphic genotype of *CYP2E1**5B (*CC*)[10,27,32,33] and the polymorphic genotype of *CYP2E1**6 (*AA*)[10,34] and increased SCRC risk in Caucasians. However, in other studies, these polymorphisms were not

Table 4 Association of *CYP1A12A, *CYP1A1**2C, *CYP2E1**5B, *CYP2E1**6, *EPHX1 Tyr113His* and *EPHX1 His139Arg* polymorphisms with sporadic colorectal cancer**

Models	Genotype	Control, *n* (%)	Case, *n* (%)	OR[1] (95%CI)	*P* value	Genotype	Control, *n* (%)	Case, *n* (%)	OR[1] (95%CI)	*P* value
	*CYP1A1**2A					*CYP1A1**2C				
Codominant	T/T	246 (61.5)	165 (72.7)	1.00 (reference)		A/A	312 (78)	193 (85)	1.00 (reference)	
	T/C	125 (31.3)	53 (23.3)	0.76 (0.49-1.18)	0.27	A/G	75 (18.8)	30 (13.2)	0.70 (0.41-1.20)	0.13
	C/C	29 (7.2)	09 (4)	0.59 (0.25-1.39)		G/G	13 (3.2)	4 (1.8)	0.36 (0.10-1.31)	
Dominant	T/T	246 (61.5)	165 (72.7)	1.00 (reference)		A/A	312 (78)	193 (85)	1.00 (reference)	
	T/C-C/C	154 (38.5)	62 (27.3)	0.73 (0.49-1.10)	0.13	A/G-G/G	88 (22)	34 (15)	0.64 (0.38-1.06)	0.08
Recessive	T/T-T/C	371 (92.8)	218 (96)	1.00 (reference)		A/A-T/G	387 (96.8)	223 (98.2)	1.00 (reference)	
	C/C	29 (7.2)	09 (4)	0.64 (0.27-1.50)	0.29	G/G	13 (3.2)	4 (1.8)	0.38 (0.10-1.38)	0.12
Overdominant	T/T-C/C	275 (68.8)	174 (76.7)	1.00 (reference)		A/A-G/G	325 (81.2)	197 (86.8)	1.00 (reference)	
	T/C	125 (31.2)	53 (23.3)	0.80 (0.52-1.23)	0.30	A/G	75 (18.8)	30 (13.2)	0.72 (0.42-1.24)	0.23
Log-additive	T/T	246 (61.5)	165 (72.7)	1.00 (reference)		A/A	312 (78)	193 (85)	1.00 (reference)	
	T/C	125 (31.3)	53 (23.3)	0.77 (0.55-1.06)	0.11	A/G	75 (18.8)	30 (13.2)	0.66 (0.43-1.00)	0.05
	C/C	29 (7.2)	09 (4)			G/G	13 (3.2)	4 (1.8)		
	*CYP2E1**5B					*CYP2E1**6				
Codominant	G/G	351 (87.8)	157 (69.2)	1.00 (reference)		T/T	314 (78.5)	126 (55.5)	1.00 (reference)	
	G/C	49 (12.2)	67 (29.5)	2.66 (1.64-4.32)	< 0.01[2]	T/A	82 (20.5)	93 (41)	2.81 (1.84-4.28)	< 0.01[2]
	C/C	0	03 (1.3)	-		A/A	04 (1)	8 (3.5)	7.32 (1.85-28.96)	
Dominant	G/G	351 (87.8)	157 (69.2)	1.00 (reference)		T/T	314 (78.5)	126 (55.5)	1.00 (reference)	
	G/C-C/C	49 (12.2)	70 (30.8)	2.82 (1.74-4.55)	< 0.00[2]	T/A-A/A	86 (21.5)	101 (44.5)	2.97 (1.97-4.50)	< 0.01[2]
Recessive	G/G-G/C	400 (100)	224 (98.7)	1.00 (reference)		T/T-T/A	396 (99)	219 (96.5)	1.00 (reference)	
	C/C	0	03 (1.3)	-	-	A/A	4 (1)	8 (3.5)	5.26 (1.35-20.50)	0.016[2]
Overdominant	G/G-C/C	351 (87.8)	160 (70.5)	1.00 (reference)		T/T-A/A	318 (79.5)	134 (59)	1.00 (reference)	
	G/C	49 (12.2)	67 (29.5)	2.58 (1.59-4.19)	< 0.01[2]	T/A	82 (20.5)	93 (41)	2.64 (1.74-4.01)	< 0.01[2]
Log-additive	G/G	351 (87.8)	157 (69.2)	1.00 (reference)		T/T	314 (78.5)	126 (55.5)	1.00 (reference)	
	G/C	49 (12.2)	67 (29.5)	2.84 (1.78-4.52)	< 0.01[2]	T/A	82 (20.5)	93 (41)	2.78 (1.91-4.06)	< 0.01[2]
	C/C	0	03 (1.3)			A/A	4 (1)	8 (3.5)		
	EPHX1 Tyr113His					*EPHX1 His139Arg*				
Codominant	T/T	214 (53.5)	126 (55.5)	1.00 (reference)		A/A	235 (58.8)	153 (67.4)	1.00 (reference)	
	T/C	158 (39.5)	88 (38.8)	0.95 (0.63-1.41)	0.84	A/G	145 (36.2)	67 (29.5)	0.79 (0.52-1.20)	0.18
	C/C	28 (7)	13 (5.7)	0.80 (0.36-1.76)		G/G	20 (5)	7 (3.1)	0.42 (0.14-1.26)	
Dominant	T/T	214 (53.5)	126 (55.5)	1.00 (reference)		A/A	235 (58.8)	153 (67.4)	1.00 (reference)	
	T/C-C/C	186 (46.5)	101 (44.5)	0.92 (0.63-1.36)	0.68	A/G-G/G	165 (41.2)	74 (32.6)	0.74 (0.50-1.11)	0.15
Recessive	T/T-T/C	372 (93)	214 (94.3)	1.00 (reference)		A/A-A/G	380 (95)	220 (96.9)	1.00 (reference)	
	C/C	28 (7)	13 (5.7)	0.81 (0.37-1.77)	0.60	G/G	20 (5)	7 (3.1)	0.45 (0.15-1.35)	0.14
Overdominant	T/T-C/C	242 (60.5)	139 (61.2)	1.00 (reference)		A/A-G/G	255 (63.8)	160 (70.5)	1.00 (reference)	
	T/C	158 (39.5)	88 (38.8)	0.97 (0.65-1.44)	0.88	A/G	145 (36.2)	67 (29.5)	0.83 (0.55-1.25)	0.37
Log-additive	T/T	214 (53.5)	126 (55.5)	1.00 (reference)		A/A	235 (58.8)	153 (67.4)	1.00 (reference)	
	T/C	158 (39.5)	88 (38.8)	0.92 (0.67-1.25)	0.59	A/G	145 (36.2)	67 (29.5)	0.74 (0.52-1.04)	0.08
	C/C	28 (7)	13 (5.7)			G/G	20 (5)	7 (3.1)		

[1]Odds ratio (OR) adjusted for age, gender and tobacco and alcohol consumption and polymorphisms in the dominant model; [2]Significant *P* values < 0.05.

associated with SCRC[17,34-36].

The *CYP1A1**2A, *CYP1A1**2C, *EPHX1 Tyr113His* and *EPHX1 His139Arg* polymorphisms were not associated with SCRC risk in the present study. The literature has shown controversial results from the influence of these polymorphisms on SCRC development. Studies in Japanese[37] and Lebanese[35] populations, as well as a recent meta-analysis[38], did not find an association between the *CYP1A1**2A polymorphism and this tumor type.

On the other hand, a study conducted in Asia showed that the *CYP1A1**2A and *CYP1A1**2C polymorphisms increase the SCRC risk in this population[39]. The association of the *CYP1A1**2C with SCRC was also evidenced in a study conducted in Hungary[32] and was confirmed in two meta-analyses, especially in Asians and Caucasians[40,41]. Two other studies conducted in an Asian population, similar to our findings, did not observe the influence of the *CYP1A1**2C polymorphism on SCRC[37,42].

The genotype frequencies of *CYP1A1**2A and *CYP1A1**2C are in HWE equilibrium in only the case group. According to the literature, case-control studies with SNP analysis have shown HWE disequilibrium in patients or controls or in both groups[43].

Regarding the *Tyr113His* and *His139Arg* polymorphisms of the *EPHX1* gene, our results are consistent with another study from North America that did not find a significant association between these polymorphisms and SCRC[21]. Some studies have shown an association between SCRC and these polymorphisms[19,20,36]. A meta-analysis showed that there are differences between studies of different populations that explain the contradictory results. The authors have observed that the allele frequencies of *EPHX1* polymorphisms and their effects on cancer risk are different depending on the population studied. Different ethnic compositions, inclusion criteria, the

Table 5 Interaction between *CYP1A12A, *CYP1A1**2C, *CYP2E1**5B, *CYP2E1**6, *EPHX1 Tyr113His* and *EPHX1 His139Arg* polymorphisms and tobacco or alcohol consumption on the risk of SCRC**

	Tobacco consumption							Alcohol consumption						
	Non-smoker			Smoker			*P* value	Non-drinker			Drinker			*P* value
	Control	Case	OR[1] (95%CI)	Control	Case	OR[1] (95%CI)		Control	Case	OR[1] (95%CI)	Control	Case	OR[1] (95%CI)	
*CYP1A1**2A														
T/T	156	96	1	90	69	1.12 (0.69-1.83)	0.33	137	92	1	109	73	1.62 (0.97-2.70)	0.35
T/C-C/C	87	35	0.87 (0.51-1.49)	67	27	0.65 (0.35-1.21)		81	35	0.88 (0.50-1.52)	73	27	0.95 (0.50-1.81)	
*CYP1A1**2C														
A/A	190	107	1	122	86	1.08 (0.68-1.69)	0.21	165	105	1	147	88	1.42 (0.89-2.29)	0.91
A/G-G/G	53	24	0.81 (0.43-1.52)	35	10	0.44 (0.18-1.06)		53	22	0.65 (0.34-1.25)	35	12	0.87 (0.38-2.00)	
*CYP2E1**5B														
G/G	215	94	1	136	63	0.90 (0.56-1.44)	0.83	190	85	1	161	72	1.56 (0.96-2.53)	0.68
G/C-C/C	28	37	2.69 (1.41-5.10)	21	33	2.68 (1.33-5.41)		28	42	3.07 (1.63-5.80)	21	28	3.90 (1.82-8.38)	
*CYP2E1**6														
T/T	189	72	1	125	54	0.96 (0.57-1.63)	0.87	171	70	1	143	56	1.47 (0.85-2.55)	0.78
T/A-A/C	54	59	2.89 (1.70-4.93)	32	42	2.99 (1.58-5.64)		47	57	3.14 (1.80-5.48)	39	44	4.10 (2.18-7.72)	
EPHX1 Tyr113His														
T/T	129	75	1	85	51	0.89 (0.51-1.54)	0.60	122	75	1	92	51	1.30 (0.74-2.31)	0.57
T/C-C/C	114	56	0.84 (0.51-1.40)	72	45	0.92 (0.52-1.62)		96	52	0.83 (0.49-1.41)	90	49	1.36 (0.78-2.37)	
EPHX1 His139Arg														
A/A	141	89	1	94	64	0.87 (0.52-1.44)	0.38	132	83	1	103	70	1.66 (0.98-2.80)	0.34
A/G-G/G	102	42	0.64 (0.38-1.09)	63	32	0.79 (0.44-1.44)		86	44	0.89 (0.52-1.53)	79	30	1.00 (0.54-1.85)	

[1]Odds Ratio (OR) adjusted for age, gender and tobacco or alcohol consumption.

quality of original studies, selection bias and study sample size may contribute to the discrepancy. In this meta-analysis, the *EPHX1 Tyr113His* polymorphism was not associated with SCRC risk, and the *EPHX1 His139Arg* polymorphism was associated with decreased SCRC risk[44].

The present study did not show a potential interaction between the presence of the polymorphisms investigated and tobacco and alcohol consumption concerning SCRC risk. These results agree with the study that evaluated the interaction of these variables with the *CYP1A1**2A, *CYP1A1**2C, *EPHX1 Tyr113His* and *EPHX1 His139Arg* polymorphisms[45-47]. On the other hand, an interaction was observed between the *CYP1A1**2A, *CYP1A1**2C polymorphisms (heterozygous) and tobacco consumption concerning SCRC risk[39]. Huang and colleagues[17] found an elevated risk of SCRC in individuals who were *EPHX1 Tyr113His* and *EPHX1 His139Arg* polymorphisms carriers and smokers.

To our knowledge, no study evaluated the interaction between *CYP2E1**6 polymorphisms and tobacco and alcohol consumption concerning SCRC risk. Regarding *CYP2E1**5B, an interaction between the *CYP2E1**5B polymorphism and alcohol consumption concerning SCRC risk was described in the literature[33]. Interestingly, the observation in the present study about the increased SCRC risk in the presence of *CYP2E1**5B and *CYP2E1**6 polymorphisms, independent of tobacco and alcohol consumption, reinforces the influence of these polymorphisms in the etiology of SCRC.

The most representative primary site in this study was the rectum, corroborating a previous report regarding the higher occurrence of primary SCRC at this anatomical location[48]. The tumor extent and presence of lymph node involvement were not associated with the polymorphisms evaluated during this study. According to our knowledge, there are no studies evaluating the association between these clinical variables and *CYP2E1* and *EPHX1* polymorphisms in SCRC. The association between the *CYP1A1**2A polymorphism and clinical and histopathological data were investigated in lung cancer. However, no association was found[49].

The discrepancy between these studies may be the result of several variables, such as differences in gender, epidemiological factors and study design. Therefore,

Table 6 Comparison between the results of this study and the results of other studies presented in the discussion

Ref.	Country study	Sample size		Gender				Age		Tobacco consumption				Alcohol consumption				Polymorphisms		
		Case N	Control N	Female		Male		Mean (SD)		Non-smokers		Smokers		Non-drinkers		Drinkers		*CYP1A1*	*CYP2E1*	*EPHX1*
				Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control			
Huang *et al*[19], 2005	Bethesda, Maryland	772	777	237	241	535	536	-	-	-	-	-	-	-	-	-	-	-	-	Tyr113His[1], His139Arg[1]
van der Logt *et al*[36], 2006	Netherlands	371	415	159	247	212	168	42	64.0	-	-	-	-	-	-	-	-	-	*5B, *6	Tyr113His, His139Arg
Kiss *et al*[32], 2007	Hungary	500	500	278	278	222	222	64.1	63.8	-	-	-	-	-	-	-	-	-	*5B[1]	Tyr113His[1], His139Arg
Yeh *et al*[42], 2007	China	727	736	317	327	410	409	-	-	-	-	-	-	-	-	-	-	*2C[1]	-	-
Yoshida *et al*[39], 2007	Japan	66	121	26	48	36	73	67.3[1]	67.3	35	55	261	61	-	-	-	-	*2A, *2C	-	-
Morita *et al*[33], 2009	Japan	685	778	259	288	426	490	60.2	58.6	-	-	-	-	272	264	413	468	-	*5B	-
Hlavata *et al*[20], 2010	Czech	495	495	206	230	289	265	57.2[1]	55.5	243	195	220	169	-	-	-	-	-	-	Tyr113His, His139Arg
Nisa *et al*[37], 2010	Japan	685	778	259	288	426	490	-	-	299	326	386	452	-	-	-	-	*2A, *2C	-	-
Northwood *et al*[21], 2010	United Kingdom	317	296	911	122	226	174	62.5	62.0	-	-	-	-	-	-	-	-	*2C	-	Tyr113His, His139Arg
Darazy *et al*[35], 2011	Lebanon	57	70	-	-	-	-	60.3	62.8	-	-	-	-	-	-	-	-	*2A	*6	-
Jin *et al*[40], 2011	China	5336	6226	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*2C[1]	-	-
Sameer *et al*[10], 2011	India	86	160	37	72	49	88	52.0	52.0	31	75	55	85	-	-	-	-	-	*5B[1]	-
Liu *et al*[43], 2012	China	6395	7893	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Tyr113His, His139Arg[1]
Silva *et al*[34], 2012	Brazil	131	206	70	124	61	82	62.4	61.7	-	-	-	-	-	-	-	-	-	*5B[1]	-
Zheng *et al*[41], 2012	China	6673	8102	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*2A[1], *2C	-	-
Jiang *et al*[27], 2013	China	5137	6330	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*5B[1], *6	-
Qian *et al*[17], 2013	China	4592	5918	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*6	-
He *et al*[38], 2014	China	6975	8651	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*2A	-	-
This Study	Brazil	227	400	125	106	2751	121	62.0[1]	46.7	243	131	157	96	218	127	182	100	*2A, *2C	*5B[1], *6[1]	Tyr113His, His139Arg

[1]*P* value significant; the variable or polymorphism was associated with SCRC.

further studies are needed to better understand the factors involved in SCRC etiology. A summary of the comparison between our results and the literature data can be observed in Table 6.

In conclusion, our data demonstrate the influence of the *CYP2E1**5B and *CYP2E1**6 polymorphisms in SCRC development for the population studied. In addition, individuals aged 62 years and older are more susceptible to the SCRC. Male individuals are less susceptible. These results can contribute to the identification of biomarkers for SCRC and understanding of the mechanisms involved in colorectal carcinogenesis.

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COMMENTS

Background

Colorectal cancer is the third most common cancer worldwide and can be related to altered metabolism of carcinogens. Therefore, it is interesting to evaluate polymorphisms in genes related to this process, such as *Cytochrome P-450* (*CYP450*) and *Epoxide hydrolase 1* (*EPHX1*). Polymorphisms in the genes encoding CYP1A1, CYP2E1, and EPHX1 may alter the levels of gene transcription and enzyme activity. This alteration can lead to DNA damage and the deregulation of mechanisms involved in colorectal cancer.

Research frontiers

Polymorphisms in the genes encoding *CYP1A1*, *CYP2E1,* and *EPHX1* have been extensively studied in the susceptibility to diseases such as cancer. However, the literature presents conflicting results. Therefore, several studies are necessary to evaluate and confirm the real role among the factors that influence alterations in metabolic processes related during colorectal cancer.

Innovations and breakthroughs

For the first time, a study evaluated the haplotype formed by minor alleles of polymorphisms of the *CYP2E1* and *CYP1A1* genes in colorectal cancer development. The haplotype formed by minor alleles of polymorphisms *CYP2E1*5B* and *CYP2E1*6* was associated with increased colorectal cancer risk.

Applications

Data showed that carriers of polymorphisms *CYP2E1*5B* and *CYP2E1*6* constitute a risk group for sporadic colorectal cancer (SCRC). Thus, considering the high incidence of this cancer, it is important for the comprehension of the factors that lead to carcinogenesis for the development of preventive and therapeutic strategies for cancer management.

Terminology

CYP1A1: Cytochrome P-450 CYP1A1 (cytochrome P450 family 1 subfamily A member 1), gene located on chromosome 15 (NC_000015.10). CYP2E1: Cytochrome P-450 CYP2E1 (cytochrome P450 family 2 subfamily E member 1), gene located in chromosome 10 (NC_000010.11). *EPHX1*: Epoxide Hydrolases 1, gene located in chromosome 1 (NC_000001.11).

Peer-review

Fernandes *et al* have conducted a very good case control study examining the involvement of CYP1A1, CYP2E1, and EPHX1 polymorphisms in SCRC. They find age over 62, female gender, CYP2E1*5B and CYP2E1*6 polymorphisms associated with SCRC.

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