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Original Article

Genetic variants of *HOTAIR* (rs920778) and *miR-3117* (rs7512692) influence susceptibility to colorectal cancer in a Mexican population

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Abstract

Colorectal cancer (CRC) is the most prevalent type of gastrointestinal cancer. Genetic, epigenetic, and lifestyle factors have been implicated in the development of CRC. Non-coding RNAs such as HOX transcript antisense RNA (*HOTAIR*) and *miR-3117* have been associated with cell proliferation, progression, invasion, and metastasis, as well as poor survival in several cancer types. This study examines the potential association between the *HOTAIR* (rs920778 T>C) and *miR-3117* (rs7512692 C>T and rs4655646 G>A) variants and the clinicopathological features of CRC in Mexican patients. The study included genomic DNA of peripheral blood samples from 557 individuals (296 CRC patients and 261 controls). The variants were identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The association was calculated using the odds ratio (OR) test. *P*-values were adjusted using the Bonferroni test (0.016). Individuals carrying the T/C and C/C genotypes for the *HOTAIR* rs920778 variant exhibited a higher susceptibility to CRC (OR=1.73, 95% CI: 1.15-2.58, P=0.009 and OR=2.78, 95% CI: 1.74-4.45, P=0.001, respectively). Male patients older than 50 years and carrying the C/C genotype demonstrated an increased susceptibility to developing CRC (OR=2.77, 95% CI: 1.63–4.70, P=0.001). Additionally, C/C genotype carriers exhibited an association with the advanced TNM stage. Furthermore, for the rs7512692 variant of the *miR-3117* gene, patients carrying the C/T genotype exhibited increased susceptibility to developing CRC (OR=1.92, 95% CI: 1.35-2.74, P=0.001). Male patients over 50 years of age and carrying the C/T genotype demonstrated increased susceptibility for early TNM stages and tumor location in the colon. The results obtained suggest that the *HOTAIR* rs920778 and *miR-3117* rs7512692 variants play a significant role in colorectal cancer risk.

Keywords: Colorectal cancer; *HOTAIR*; *miR-3117*; Genotypes; Susceptibility

1. Introduction

Colorectal cancer (CRC) is a neoplasm caused by the uncontrolled development of cells located in the layers of the colon wall [1]. It is estimated that CRC is among the main types of cancer with the highest incidence and mortality worldwide, according to GLOBOCAN statistics. In Mexico, it is in third position both in terms of incidence and mortality [2]. CRC is a multifactorial disease, the pathogenesis of which is not fully understood. At present, several risk factors have been identified, including age, ethnicity, genetic mutations, epigenetic factors, dietary factors, smoking, and alcohol consumption [3]. Various studies have shown that its occurrence and development are closely related to the inactivation of tumor suppressor genes, oncogene activation, apoptosis imbalance, and cell proliferation [4]. Non-coding RNAs (ncRNAs) have recently gained attention because of their involvement in different biological processes. An increasing number of studies have demonstrated that mutations or abnormal expression of ncRNAs are closely associated with various

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diseases, including CRC [5].

HOTAIR is a long non-coding RNA (lncRNA), located in chromosome 12q13.13 that has been linked to cancer development, progression, and metastasis [6]. It has been observed that the overexpression of *HOTAIR* can contribute to the progression of multiple cancers, including ovarian, breast, pancreatic, hepatocellular, lung, gastric, and colorectal cancers [7]. In addition, there is growing evidence that *HOTAIR* expression levels may be useful as a prognostic and predictive cancer biomarker [6]. It has also been suggested that *HOTAIR* may function as a molecular decoy in tumors, where it may sequester RNA-binding proteins (RBPs) and several microRNAs (miRNAs) [6]. Peng et al. have proposed that *HOTAIR* may play a role in accelerating colorectal cancer development by downregulating miRNA-34a [8].

A considerable number of studies have suggested that variants in the *HOTAIR* gene may serve as potential genetic markers for cancer susceptibility [9]. Some studies have demonstrated that the rs920778 T>C variant is associated with increased susceptibility to developing cancer, including breast cancer [10], oral cancer [11], and colorectal cancer [6], [12]; however, the results remain controversial between populations.

miRNAs are short, non-coding sequences of approximately 20-24 nucleotides that play an important role in post-transcriptional regulation, which can affect the stability and translation of several mRNAs [12]. Recent studies have demonstrated that miRNAs can modulate up to 60% of protein-coding genes in the human genome [13]. Consequently, due to their pivotal role in gene regulation, miRNAs are implicated in numerous and significant processes that facilitate the maintenance and homeostasis of the tissues [14], through the regulation of proliferation [15], differentiation [16], apoptosis [17], and hematopoiesis [18]. Most miRNAs are situated in genomic regions that frequently undergo rearrangements as amplifications or deletions, indicating that abnormalities in miRNAs play a significant role in carcinogenesis [19].

miR-3117 is located at chromosome 1p31.3 and produces the *miR-3117*-3p, which has affinity for genes involved in the mitogen-activated protein kinase (MAPK) pathway [20]. Alterations in this pathway have been described in several types of cancer, including colorectal cancer [21]. The role of the rs7512692 and rs4655646 variants in miR-3117 gene regarding cancer has only been investigated in acute lymphoblastic leukemia [22] and breast cancer [23]. In CRC, these variants have not been analyzed.

This study aims to investigate, for the first time, the potential association of *HOTAIR* (rs920778) and the *miR-3117* (rs7512692 and rs4655646) variants with the development and clinicopathological characteristics of CRC in Mexican patients.

2. Materials and Methods

The study included 296 patients with sporadic colorectal adenocarcinoma who were clinically diagnosed and histologically confirmed between the years of 2019 and 2023. The patients were diagnosed according to the Clinical Practice Guidelines for colon and rectal cancer and the clinicopathological criteria of the Specialty Hospital of the West National Medical Center of the Mexican Institute of Social Security (IMSS) in Guadalajara, Mexico. Pathologic tumor staging and grading were done according to the tumor-node-metastasis (TNM) classification. The control group comprised 261 individuals who were not related and had undergone a negative colonoscopy for malignancy. Patients and controls were excluded if they were diagnosed with an autoimmune or inflammatory bowel disease or had a family history of any known hereditary cancer syndrome. The control group was age-matched to the patients group. Informed consent forms were signed by both cases and controls. The study was approved by the National Committee for Scientific Research of the Mexican Institute of Social Security (IMSS) (R-2019-785-171) and conducted in accordance with national and international ethical standards. All participants provided informed consent for participation in this study. A standard epidemiological questionnaire was used to collect personal data, including age, sex, drinking and smoking status, and familial history. Information on the clinical and pathological characteristics of the patients was obtained from their medical records.

2.1. Genotyping

Genomic DNA was extracted from peripheral blood using standard methods, as described by Miller et al. (1988). The variants rs920778 (C>T) in the *HOTAIR* gene and rs7512692 (C>T) and rs4655646 (G>A) in the *miR-3117* gene were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using the following primer pairs: For the *HOTAIR* rs920778 variant, the following primer pairs were used: rs920778-F: 5'- TTA CAG CTT AAA TGT CTG AAT GTT CC-3′ and rs920778-R: 5'- GCC TCT GGA TCT GAG AAA GAA A -3′ [31]. For the *miR-3117* gene, the following sequences were used: rs7512692-F: 5'-TGG CAG TTG CTG GTA CTC TT-3' and rs7512692-R: 5'- CTC AAG TCT CCT CCC CCA TC-3' and for the variant rs4655646-F: 5′ TGG CAT GTG AGG AAA GTT GGA-3′ and rs4655646-R: 5′ AGA TAT TGG GCC TCT ACC CGT-3' [30].

A polymerase chain reaction (PCR) reaction was conducted for the *HOTAIR* (rs920778) and *miR-3117* (rs7512692 and rs4655646) variants in a 10 µL volume. The reaction mixture contained 100 ng of DNA, 10X buffer (500 mM KCl, 100 mM Tris-HCl, and 0.1% Triton X-100), 2.0 mM MgCl2, 200 mM dNTPs, 1 pM of each primer, and 2 U Taq DNA polymerase (Thermo Fisher Scientific, USA). The amplification program consisted of an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 62 °C for (rs920778), 58 °C for (rs7512692), and 60 °C for (rs4655646) respectively for 45 s, and extension at 72 °C for 30 s, followed by final extension at 72 °C for 10 min. Five microliters of each PCR product were subjected to digestion with 5U of the following restriction enzymes: The *HOTAIR* rs920778 (*MspI*) and *miR-3117* rs7512692 (*Hpy1881II*) and rs4655646 (*TaqI*) (New England Biolabs, USA), and were digested according to the manufacturer's instructions and separated in a 6% polyacrylamide gel. Genotypes were identified according to the methods described by Kashani et al. (2021) and Sarabandi et al. (2021) [24, 25]. To ensure the quality of the genotyping processes, approximately 10% of the randomly selected samples were reprocessed, and the results were found to be 100% consistent.

2.2. Statistical Analysis

Genotype and allele frequencies were estimated by direct counting in both groups. The Chi-square test was employed to assess the Hardy-Weinberg equilibrium (HWE) and to identify differences in genotype and allele distributions in relation to the clinical features of patients and controls. Statistical analysis included odds ratio analysis and the Yates-corrected Chi-square test. The association of genotypes or alleles with CRC and stratification by demographic and clinicopathological characteristics was calculated by odds ratio (OR) and confidence intervals (CI) in an SPSS v25.0 software package (SPSS Inc., Chicago, IL, USA). All statistical analyses were conducted with a significance level of P <0.05. A Bonferroni correction test was applied to adjust the P values (P < 0.016).

3. Results

3.1. Characteristics of the subjects included in the study

Table 1 presents the clinicopathological characteristics of the subjects included in the study. The mean age observed was 58.59 (±12.31) for the CRC group and 59.56 (±12.50) years in the control group (*P*=0.360). Tobacco consumption exhibited significant differences (*P*=0.003) between these groups. The analysis revealed that age, sex, and alcohol consumption did not exhibit significant differences between the analyzed groups ($P > 0.05$).

For the rs920778 variant in the *HOTAIR* gene, a statistically significant association was observed in individuals over 50 years of age and carrying the T/C and C/C genotypes (OR = 1.88, 95% CI = 1.19-2.95, P = 0.008 and OR = 2.77, 95% CI = 1.63-4.70, P = 0.001, respectively). Males with the C/C genotype exhibited a significantly increased susceptibility to developing CRC (OR = 2.55, 95% CI = 1.36-4.80, P = 0.005). The analysis of disease development revealed that individuals carrying the C/C genotype exhibited a significantly increased susceptibility to developing CRC in advanced TNM stages (III+IV) (OR = 2.03, 95% CI = 1.26-4.80, P = 0.002) (Table 3).

A statistical significance was observed in patients over 50 years of age and carrying the C/T genotype for the *miR-3117* (rs7512692) variant (OR = 1.92, 95% CI = 1.28-2.87, P = 0.001). The C/T genotype was found to confer a significantly increased susceptibility to developing CRC (OR = 2.36, 95% CI = 1.38–4.04, P = 0.002). The consumption of tobacco was found to be statistically significant (OR = 2.64, 95% CI = 1.33-5.25, P = 0.008). The analysis of disease development revealed that individuals carrying the C/T genotype exhibited a significantly increased susceptibility to develop CRC in both early and advanced TNM

Table 1. Demographic and clinical features of the colorectal cancer patients and control subjects.

Characteristic	CRC Group n=296 (100%)	Control Group n=261 (100%)	*P-value*
Mean Age (years SD)	58.59 (±12.31) 20-92	59.56 (±12.50) 37-93	0.360
Age (in years)			
< 50	53 (18)	67 (26)	0.020
> 50	243 (82)	194 (74)	
Sex			
Male	169 (57)	138 (53)	0.318
Female	127 (43)	123 (47)	
Smoking status			
Yes	101 (34)	59 (23)	**0.003**
No	195 (66)	202 (77)	
Drinking status			
Yes	85 (29)	56 (21)	0.049
No	211 (71)	205 (79)	
Clinical stage TNM			
I	5 (2)		
II	101 (34)		
III	116 (39)		
IV	74 (25)		
Tumor location			
Colon	91 (31)		
Rectum	205 (69)		
Pathological Response			
Complete response	126 (43)		
Partial response	92 (31)		
Without response	78 (26)		

P-values were calculated by the Chi-square test. *P*-values were adjusted by the Bonferroni test (0.016).

Table 2. Distribution of genotypes and allelic frequencies of the *HOTAIR* rs920778, *miR-3117* rs7512692 and rs4655646 variants in CRC and control group.

Genotype	CRC Group n= 296 (100%)	Control Group n=261 (100%)	OR (95% CI)	*P value*
		***HOTAIR* (rs920778 T>C)**		
T/T	64 (21.6)	94 (36.0)	1.00 (Reference)	
C/T	139 (47.0)	118 (45.2)	1.73 (1.15 – 2.58)	**0.009**
C/C	93 (31.4)	49 (18.8)	2.78 (1.74 – 4.45)	**0.001**
C/T+T/T vs. C/C	232 (78.4)	167 (64.0)	2.04 (1.40 – 2.96)	**0.001**
Allele				
T	267 (45.1)	306 (58.6)	1.00 (Reference)	
C	325 (54.9)	216 (41.6)	1.72 (1.35 – 2.18)	**0.001**
		***miR-3117* (rs7512692 C>T)**		
C/C	95 (32.1)	118 (45.2)	1.00 (Reference)	
C/T	188 (63.5)	121 (46.4)	1.92 (1.35 – 2.74)	**0.001**
T/T	13 (4.4)	22 (8.4)	0.73 (0.35 – 1.53)	0.521
C/T+T/T vs. C/C	201 (67.9)	143 (54.8)	1.74 (1.23 – 2.46)	**0.002**
Allele				
C	378 (63.9)	357 (68.4)	1.00 (Reference)	
T	214 (36.1)	165 (31.6)	1.22 (0.95 – 1.57)	0.125
		***miR-3117* (rs4655646 G>A)**		
G/G	14 (4.7)	18 (6.9)	1.00 (Reference)	
G/A	110 (37.2)	85 (32.6)	1.66 (0.78- 3.53)	0.253
A/A	172 (58.1)	158 (60.5)	1.39 (0.67- 2.90)	0.471
G/A+A/A vs. G/G	282 (95.3)	243 (93.1)	1.49 (0.72 – 3.06)	0.360
Allele				
G	138 (23.3)	121(23.2)	1.00 (Reference)	
A	454 (76.7)	401(76.8)	0.99 (0.75 – 1.31)	1.000

P-value were adjusted by the Bonferroni test (0.016).

stages (I+II and III+IV) (OR = 2.37, 95% CI = 1.44-3.89, P = 0.001 and OR = 1.72, 95% CI = 1.16-2.56, P = 0.009), respectively. The analysis based on tumor location indicates that individuals with the C/T genotype are more susceptible to developing tumors in the colon (OR = 2.01; 95% CI = 1.21-3.36, P = 0.009) (Table 4). Regarding the *miR-3117* (rs4655646) variant, no statistical association was observed when comparing sex, age, alcohol, tobacco consumption, TNM stage, and tumor location among groups (Table 5). Four distinct haplotypes were identified within the *miR-3117* gene, yet none of these exhibited a statistically significant association (Table 2).

The results of the multiple logistic regression analysis, which included confounding variables, are presented in Table 6. Statistical significance was observed for tobacco consumption in the presence of the two variants associated with *HOTAIR* (rs920778) and miR3117 (rs7512692) (OR = 2.12; 95% CI = 1.44-3.12; P = 0.001 and OR = 1.81; 95% CI = 1.27-2.59; P = 0.001, respectively), suggesting that tobacco consumption increases the risk and susceptibility of developing CRC.

4. Discussion

Colorectal cancer is a complex disease that is influenced by numerous factors, including genetics, epigenetics, and environmental factors. Non-coding RNAs have recently emerged as a subject of interest due to their involvement in a variety of biological processes. An increasing number of studies have demonstrated that mutations or abnormal expression of ncRNAs are closely associated with CRC.

The present investigation aimed to examine the impact of genetic variants in *HOTAIR* (rs920778) and *miR-3117* (rs7512692 and rs4655646) on the likelihood of developing CRC in the Mexican population. In this study, involving 296 patients, a significant increase in CRC was observed in individuals over the age of 50 (82%), which is consistent with the established statistics [25]. Although the incidence of early-onset CRC in individuals under the age of 50 is increasing, the risk is much higher in those over the age of 50 [26]. Significant statistical differences

Table 3. Association of the *HOTAIR* rs920778 variant with demographic and clinical variables.

	***HOTAIR* (rs920778)**					
	CRC/Control			**OR (95% CI); *P* value**		
Variable	**TT**	**TC**	**CC**	**TC versus TT**	**CC versus TT**	**TC + CC versus TT**
Age (years)						
<50	10/20	25/35	18/12	1.42 (0.57 -3.57); 0.592	3.00 (1.04-8.60); 0.070	1.82 (0.77 -4.34); 0.243
>50	54/74	114/83	75/37	1.88 (1.19 -2.95); **0.008**	2.77 (1.63-4.70); **0.001**	2.15 (1.41 -3.28); **0.001**
Sex						
Male	39/48	76/64	54/26	1.46 (0.85 -2.50); 0.211	2.55 (1.36-4.80); **0.005**	1.77 (1.07 -2.93); 0.032
Female	25/46	63/54	39/23	2.14 (1.16 -3.94); 0.019	3.12 (1.53 -6.33); **0.002**	2.43 (1.37 -4.30); **0.003**
Smoke status						
Yes	23/21	43/28	35/10	1.40 (0.65 -2.99); 0.496	3.19 (1.27 -8.00); 0.021	1.87 (0.92 -3.80); 0.116
Drinking status						
Yes	18/22	40/21	27/13	2.32 (1.02 -5.26); 0.065	2.53 (1.02 -6.29); 0.071	2.40 (1.14 -5.08); 0.032
TNM stage						
I+II	23/64	54/118	29/49	1.27 (0.71 -2.26); 0.496	1.64 (0.84-3.19); 0.188	1.38 (0.80 -2.38); 0.300
III+IV	41/64	85/118	64/49	1.12 (0.69 -1.81); 0.722	2.03 (1.18-3.50); **0.013**	1.39 (0.88 -2.18); 0.182
Localization						
Colon	21/64	40/118	30/49	1.03 (0.56 -1.90); 1.000	1.84 (0.95 -3.64); 0.095	1.27 (0.72 -2.25); 0.478
Rectum	43/64	98/118	63/49	1.23 (0.77 -1.97); 0.444	1.91 (1.11 -3.27); 0.024	1.43 (0.92 -2.23); 0.136

P-values were adjusted by the Bonferroni test (0.016).

Table 4. Association of the *miR-3117* (rs7512692) variant with demographic and clinical variables.

	***miR-3117* (rs7512692)**					
	CRC/Control			**OR (95% CI); *P* value**		
Variable	**CC**	**CT**	**TT**	**CT versus CC**	**TT versus GG**	**CT + TT versus CC**
Age (years)						
<50	20/32	32/28	1/8	1.82 (0.85 -3.88); 0.166	0.19 (0.02 -1.66); 0.211	1.46 (0.70 -3.04); 0.399
>50	75/86	156/93	12/14	1.92 (1.28 -2.87); **0.001**	0.98 (0.42 -2.25); 1.000	1.80 (1.21 -2.66); **0.004**
Sex						
Male	58/62	102/66	9/10	1.65 (1.02 -2.65); 0.049	0.96 (0.36 -2.53); 1.000	1.56 (0.98 -2.47); 0.075
Female	37/56	86/55	4/12	2.36 (1.38 -4.04); **0.002**	0.50 (0.15 -1.68); 0.396	2.03 (1.20 -3.42); **0.010**
Smoke status						
Yes	33/30	67/23	1/6	2.64 (1.33 -5.25); **0.008**	0.15 (0.01 -1.33); 0.129	2.13 (1.1 -4.11); 0.035
Drinking status						
Yes	33/30	47/21	5/5	2.03 (0.99 -4.15); 0.074	0.90 (0.23 -3.45); 1.000	1.81 (0.91 -3.59); 0.121
TNM stage						
I+II	30/118	73/121	3/22	2.37 (1.44 -3.89); **0.001**	0.53 (0.15 -1.91); 0.485	2.09 (1.28 -3.4); **0.004**
III+IV	65/118	115/121	10/22	1.72 (1.16 -2.56); **0.009**	0.82 (0.36 -1.84); 0.789	1.58 (1.07 -2.33); 0.024
Localization						
Colon	29/118	60/121	2/22	2.01 (1.21 -3.36); **0.009**	0.36 (0.08 -1.66); 0.290	1.76 (1.06 -2.91); 0.035
Rectum	66/118	128/121	11/22	1.89 (1.27 -2.79); **0.001**	0.89 (0.40 -1.95); 0.934	1.73 (1.18 -2.54); **0.005**

P-value were adjusted by the Bonferroni test (0.016).

Table 5. Association of the *miR-3117* rs4655646 variant with demographic and clinical variables.

	***miR-3117* (rs4655646)**					
	CRC/Control			**OR (95% CI); *P* value**		
Variable	**GG**	**GA**	**AA**	**GA versus GG**	**AA versus GG**	**GA + AA versus GG**
Age (years)						
<50	3/5	25/21	25/42	1.98 (0.42 -9.29); 0.619	0.99 (0.21 -4.51); 1.000	1.32 (0.30 -5.80); **0.001**
>50	11/13	85/64	147/116	1.59 (0.66 -3.73); 0.421	1.49 (0.64 -3.46); 0.462	1.52 (0.66 -3.48); 0.427
Sex						
Male	7/11	66/42	96/85	2.46 (0.88 -6.87); 0.131	1.77 (0.65 -4.78); 0.368	2.00 (0.75 -5.31); 0.239
Female	7/7	44/43	76/73	1.02 (0.33 -3.16); 1.000	1.04 (0.34 -3.11); 1.000	1.03 (0.35 -3.04); 1.000
Smoke status						
Yes	6/4	39/22	56/33	1.18 (0.30 -4.64); 1.000	1.13 (0.29 -4.30); 1.000	1.15 (0.31 -4.25); 1.000
Drinking status						
Yes	2/8	32/18	51/30	7.11 (1.36 -37.16); 0.026	5.10 (1.02 -25.35); 0.066	5.72 (1.17 -27.94); 0.039
TNM stage						
I+II	4/18	37/85	65/158	1.95 (0.62 -6.18); 0.365	1.85 (0.60 -5.68); 0.399	1.88 (0.62 -5.71); 0.368
III+IV	10/18	73/85	107/158	1.54 (0.67 -3.55); 0.410	1.21 (0.54 -2.74); 0.782	1.33 (0.60 -2.95); 0.608
Localization						
Colon	5/18	35/85	51/158	1.76 (0.48 -6.48); 0.565	1.29 (0.36 -4.63); 0.928	1.45 (0.41 -5.10); 0.760
Rectum	9/18	75/85	121/158	1.76 (0.74 -4.16); 0.271	1.53 (0.66 -3.52); 0.421	1.61 (0.7 -3.67); 0.342

P-values were adjusted by the Bonferroni test (0.016).

Table 6. Logistic regression analysis for the *HOTAIR* and *miR-3117* variants analyzed with confounding variables.

Independent Variable	Regression coefficient	Standard error	Wald test	Degrees of freedom	*P* value	OR (95% IC)
Age **>50 vs. <50**	0.489	0.216	5.131	1	0.024	1.63 (1.06 -2.48)
Sex **Male vs. Female**	0.127	0.178	0.507	1	0.477	1.13 (0.80 -1.61)
Smoking status **Yes vs. No**	0.530	0.204	6.747	1	**0.009**	1.69 (1.13 -2.53)
Drinking status **Yes vs. No**	0.333	0.213	2.439	1	0.118	1.39 (0.91 -2.11)
miR-3117 rs7512692 **CT+TT**	0.597	0.182	10.699	1	**0.001**	1.81 (1.27 -2.59)
HOTAIR rs920778 TC+CC	0.754	0.197	14.677	1	**0.001**	2.12 (1.44-3.12)
Constant	-0.126	0.085	2.196	1	0.138	
Model	$X^2 = 42.215$ d.f.=6 P= **0.001**					

Bonferroni test was used to adjust the *P* value (0.016).

were observed between the groups in terms of tobacco consumption. These findings are consistent with those reported by Yang LP et al. in 2021, who observed a significant CRC increase in smoking patients, particularly those with left-sided tumors; a longer duration and higher rate of smoking were associated with the CRC risk, being up to 55% higher than in non-smokers [27]. In a different study, Huang et al. (2022) found that smoking is associated with the development of CRC and with a high risk of mortality (1.11 times higher in smokers than in non-smokers); such a risk was present in patients who smoked more than 12 cigarettes per day or for more than 30 years [28].

HOTAIR has been identified as a functional lncRNA that plays a role in the development of several types of cancer. It is located on chromosome 12, within the HOXC locus [29]. *HOTAIR* interacts with epigenetic regulators, including the polycomb repressive complex 2 (PRC2) and the lysine-specific demethylase 1A (LSD1) complex, to regulate the epigenetic silencing of various genes associated with cancer, such as the HOXD gene [28], [30]. It has been reported that RNA interference-mediated knockdown of *HOTAIR* causes altered gene expression of the *HOTAIR* target genes and suppresses the invasion of gastrointestinal stromal tumor cells [6]. This study reported that *HOTAIR* functions as a "miRNA sponge," which silences some miRNAs with tumor suppressor functions and thereby induces upregulation of oncogenic genes [6]. Recently, *HOTAIR* SNPs have also been widely studied; however, there are insufficient studies to establish the link between *HOTAIR* variants and CRC. The most extensively studied SNP in the *HOTAIR* gene is rs920778, which has been associated with cancer by *HOTAIR* upregulation [6], [10]. In this study, an analysis of genotypic and allelic frequencies for the *HOTAIR* rs920778 T>C variant revealed significant differences. The T/C and C/C genotypes were found to be significantly associated with an increased risk of CRC, thereby confirming the relation as a risk factor for CRC susceptibility. Furthermore, males over the age of 50 exhibited an association with advanced TNM stages (III and IV).

Although these findings are reported for the first time among Mexican patients with CRC, such an association between colorectal cancer and the T/C and C/C genotypes had already been reported in the Saudi population [12]; on the other hand, differences were observed regarding the susceptibility and the clinicopathological characteristics such as TNM stage, tumor location, and mortality in the Korean population [6]. Several investigations have demonstrated that miRNAs are involved in regulating more than 30% of the human genome [31], [32]; moreover, it is postulated that miRNAs may be involved in numerous biological pathways, including proliferation and metastasis.

Neerincx et al. (2015) examined miRNA expression profiles in paired non-cancerous, primary, and metastatic colorectal cancer tissues and found that *miR-3117* exhibited specific expression in advanced colorectal cancer tissues [32], [33]. Nevertheless, the role of *miR-3117* in tumor progression remains to be elucidated. The studies conducted on the SNPs rs7512692 and rs4655646 of the *miR-3117* gene have been relatively limited in scope and primarily focused on analyzing the risk associated with these variants in breast cancer [23].

The analysis of genotypic and allelic frequencies for the *miR-3117* rs7512692 and rs4655646 variants revealed significant differences only for the rs7512692 variant. Individuals with the C/T genotype of the rs7512692 variant exhibited a significantly elevated risk of developing CRC. This association was observed in females over the age of 50 with early TNM stages (I and II) and tumor location in the colon. Furthermore, a significant difference was observed in patients who used tobacco (Table 6). Haplotype analysis did not reveal statistically significant differences among the two SNPs analyzed in the *miR-3117* gene. These findings are consistent with those reported in breast cancer [23]. Another set of SNPs analyzed in *miR-3117* (rs12402181 and rs62571442), showed no association with acute lymphoblastic leukemia [33].

Previous studies have demonstrated that miRNA-3117 plays a role in the development of CRC and exhibits higher expression in metastatic CRC [32]. Although the expression of miRNA-3117 was not analyzed in this study, it is reasonable to hypothesize that the polymorphic alleles could influence the precise identification of its target mRNA sequences. The loss of binding could result in an increase in the expression of its target genes. In silico analysis revealed that the target genes of *miR-3117* regulate the mRNA of the MAPK signaling pathway [34]. The genes predicted to be targeted by *miR-3117* are in the initial stages of the cascade (*CACNG1*, *CACNG8, PDGFR, GRB2, SOS1*, and *RAS*), which could result in the deregulation of subsequent stages. Aberrant expression of this pathway is a major and highly prevalent oncogenic event in many human cancers [34].

In conclusion, the failure of *miR-3117* to recognize its targets due to the change of the rs7512692 C allele to the A allele in the seed region may contribute to colorectal carcinogenesis by leading to an aberrant activation of the RAS-MAPK pathway. The multivariable analysis demonstrated, for the first time, that tobacco consumption is a risk factor for CRC in carriers of the T/C or C/C genotypes of the rs920778 *HOTAIR* variant and the C/T or T/T genotypes of the rs7512692 *miR-3117* variant. Lifestyle factors that may be modified, such as tobacco consumption, have been associated with an increased risk of CRC in studies conducted in Western populations [35].

Further studies with larger sample sizes, functional genomics, and analysis in other populations are necessary to validate the genetic effects of *HOTAIR* and *miR-3117* polymorphisms in CRC. One limitation of this study was the absence of follow-up data on the patients. Further studies are required to investigate the association between other SNPs in the *HOTAIR* and *miR-3117* genes and the risk of CRC.

5. Conclusion

This study represents the first to identify the *HOTAIR* rs920778 and *miR-3117* rs7512692 variants as potential markers for CRC risk. Furthermore, these polymorphic variants were found to be associated with age, sex, TNM stages, and tumor localization.

Conflict of Interest

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate
The study was approved by the National Committee for Scientific Research of the Mexican Institute of Social Security (IMSS) (R-2019-785-171) and conducted in accordance with national and international ethical standards.

Availability of data and material
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author Contributions
All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Yuri Giovanna Vanessa Trujillo-Fernández], [Dalia Elizabeth Rodríguez-Torres], [Cesar de Jesús Tovar-Jacome], [Patricio Barros-Núñez], [Miriam Yadira Godínez-Rodríguez], [Perla Janeth Pérez-Bojórquez], [Luis Alberto Flores-Martínez], [Tomás Daniel Pineda-Razo], [María Eugenia Marín-Contreras], [Aldo Antonio Alcaraz-Wong], [Ignacio Mariscal-Ramírez] and [Mónica Alejandra Rosales-Reynoso]. The first draft of the manuscript was written by [Mónica Alejandra Rosales-Reynoso and Patricio Barros-Núñez] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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