Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Association of *CASP8* rs3834129 and *CTGF* rs6918698 genotypes with susceptibility to colorectal cancer in a Mexican population

Anilú Margarita Saucedo-Sariñana,[1] Yuri Giovanna Vanessa Trujillo-Fernández[2], Clara Ibet Juarez-Vazquez[1], Miriam Yadira Godínez-Rodríguez[2], César de Jesús Tovar-Jácome[2], Patricio Barros-Nuñez[3], Tomas Daniel Pineda-Razo[4], María Eugenia Marín-Contreras[5], Mónica Alejandra Rosales-Reynoso[2*]

[1] Dirección Académica Aparatos y Sistemas I. Facultad de Medicina. Decanato Ciencias de la Salud, Universidad Autónoma de Guadalajara (UAG). Zapopan, Jalisco, México

[2] División de Medicina Molecular, Centro de Investigación Biomédica de Occidente, Instituto Mexicano del Seguro Social (IMSS), Guadalajara, Jalisco, México

[3] Doctorado en Genética Humana, Centro Universitario de Ciencias de la Salud (CUCS) Universidad de Guadalajara, Guadalajara, Jalisco, México

[4] Servicio de Oncología Médica, Hospital de Especialidades, Instituto Mexicano del Seguro Social (IMSS), Guadalajara, Jalisco, México

[5] Servicio de Gastroenterología, Hospital de Especialidades, Instituto Mexicano del Seguro Social (IMSS), Guadalajara, Jalisco, México

Article Info

Article history:

Received: October 24, 2023
Accepted: July 24, 2024
Published: November 30, 2024

Use your device to scan and read the article online

Abstract

Connective tissue growth factor (*CTGF*) and Caspase 8 (*CASP8*) have been implicated in cancer development and progression. Variants such as *CASP8* rs3834129 (-652 6N I/D) and *CTGF* rs6918698 (-945 C>G) have been associated with several cancers, although their association is still debated between populations. This study investigates the possible association between the *CASP8* rs3834129 and *CTGF* rs6918698 variants with colorectal cancer (CRC) in Mexican patients. Genomic DNA was extracted from 250 CRC patients and 250 control subjects. The identification of *CASP8* and *CTGF* variants was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methodology. The association was determined by the odds ratio (OR) analysis and *P* values were adjusted by the Bonferroni correction. Patients carrying the D/D genotype for the *CASP8* rs3834129 variant exhibited an increased susceptibility to CRC ($P = 0.012$). The D/D genotype was associated with older 50-year-old patients ($P = 0.006$). In addition, this same D/D genotype was associated with TNM II stage ($P = 0.013$) and rectal localization ($P = 0.023$). Additionally, patients carrying the G/G genotype for the *CTGF* rs6918698 variant showed a decreased susceptibility to CRC ($P = 0.009$), and in the sex stratification, this gene has protective role in males ($P = 0.008$). This same genotype was associated with decreased susceptibility to early TNM stages (I+II) ($P = 0.023$) and right-sided colon tumor localization ($P = 0.002$). There was no association between response to treatment and the variants analyzed. Our findings suggest that the *CASP8* rs3834129 and *CTGF* rs6918698 variants have a significant impact on the development of CRC.

Keywords: Colorectal cancer; *CASP8*, *CTGF*; Variants; Cancer susceptibility

1. Introduction

Worldwide, CRC is the third type of cancer with the highest incidence after breast cancer and lung cancer. According to Globocan 2022, there were 1,926,425 new cases reported for both sexes and 904,019 deaths [1]. In Mexico, CRC ranks as the third among the top five cancers with the highest number of affected patients. In addition, the statistics indicate 16,082 new cases of CRC, corresponding to an incidence rate of 10.9 per 100,000 inhabitants and mortality rate of 8.6 per 100,000 [2]. CRC is a heterogeneous disease initiated by a succession of genetic, epigenetic, and environmental events leading to the uncontrolled development of cells in the epithelial lining of the colon segments [3]. The development and progression of CRC involve dysregulated proliferation of epithelial cells, which is associated with a sequence of cumulative genetic and epigenetic alterations [4]. Evidence suggests that the prolonged survival of these genetically unstable colorectal epithelial cells may culminate in a final malignant transformation due to random events. This transformation is associated with the gradual inhibition of apoptosis, a mechanism in which *CASP8* plays a critical role [5].

CASP8 is a crucial regulator of apoptosis, serving as an

* Corresponding author.
E-mail address: mareynoso77@yahoo.com.mx; monica.rosales@imss.gob.mx (M. A. Rosales-Reynoso).
Doi: http://dx.doi.org/10.14715/cmb/2024.70.11.2

essential protective mechanism against hyperproliferation and tumorigenesis [4]. CASP8 is a protease that initiates apoptosis within cells via the Fas/FasL pathway [6]. The gene is located at chromosome 2q33.1 and comprises 15 exons and 14 introns [7]. The principal function is to prevent the development of tumors by controlling the hyperproliferation of cancer cells [6]. The altered expression or function of this gene affects the immune system's response and apoptosis. While CASP8 typically acts as a tumor suppressor by regulating cell death, it can paradoxically promote tumor formation and cancer progression when its function is compromised; additionally, alterations in this protein have been described to give rise to resistance to treatments in various cancers, including breast, cervical, lung, gastric, esophageal, renal, and CRC [5, 8]. Currently, significant research has focused on the rs3834129 (-652 6N I/D) (del AGT AAG) variant in the promoter region of this gene [9]. This variant has been reported to influence cancer risk. However, the association between rs3834129 and the risk of CRC remains unclear, with studies showing inconclusive results [8, 10, 11].

The connective tissue growth factor (CTGF), also known as CCN-2 (cysteine-rich 61/connective tissue growth factor/nephroblastoma), corresponds to the CCN family [12, 13]. The *CTGF* gene is located at 6q23.2 and contains 5 exons and 4 introns [13]. It encodes a member of the CCN protein group (extracellular matrix-associated proteins)[12]. *CTGF* participates in several cellular processes, including adhesion, cell proliferation, development, angiogenesis, chemotaxis, migration, tumorigenesis, and apoptosis [14–18]. Studies have indicated that CTGF is activated by basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) [15,17]. Transforming growth factor β (TGF-β) is one of the primary regulators of CTGF production since it influences on tumor development and progression [17, 19, 20]. In vitro studies have indicated that when antagonists block the functional effect of *CTGF*, endothelial cell migration and proliferation are reduced [14]. The alteration of *CTGF* expression and its association with cancer development have been studied in various types of cancer, including CRC, and it is considered a prognostic marker in several human cancers [17, 21–25]. A few variants in the *CTGF* gene have been analyzed concerning different pathologies; however, the rs6918698 variant has been associated with CRC [17] and Crohn's disease [14, 26, 27]. For this reason, this study aims to examine for the first time the distribution of alleles and genotypes of these two variants, *CASP8* (rs3834129) and *CTGF* (rs6918698), evaluating their possible association with the development and clinicopathological characteristics of CRC in Mexican patients.

2. Materials and methods

The study included 250 patients clinically diagnosed and histologically confirmed as having sporadic colorectal adenocarcinoma according to the Clinical Practice Guidelines on Colon and Rectal Cancer and the clinicopathological criteria of the Specialty Hospital of West National Medical Center in the IMSS in Guadalajara, Mexico. The control group included 250 healthy blood-donating individuals. In this study, CRC patients and unrelated healthy individuals, matched in age and sex with the case group, were recruited from 2018 to 2022 at the Specialty Hospital of West National Medical Center at IMSS in Guadalajara, Mexico. Tumor staging and grading were performed according to the Tumor-Node-Metastasis (TNM) classification. The study was approved by the Ethics Committee 1305 of the West Biomedical Research Center of the Mexican Institute of Social Security (IMSS) (R-2018-1305-001) and conducted under national and international ethical standards. The inclusion criteria for the CRC patients were patients diagnosed with sporadic CRC, according to the clinical and pathological criteria, and staged by TNM classification, without another type of cancer, with age and sex indistinct. The control group was composed of healthy individuals, none of whom were related, regardless of age and sex. Demographic, clinical, and anatomopathological data of the CRC patients and controls were obtained from hospital records.

2.1. Genotyping

Genomic DNA was extracted from peripheral blood lymphocytes using the salting out method [28]. The genotyping was performed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphisms (PCR-RFLPs) using the following primer pairs: for rs3834129 (*CASP8*): forward 5'-CTGCATGCCAGGAGCTAAGT-3' and reverse 5'-GCCATAGTAATTCTTGCTCTGC-3' [6], for rs6918698 (*CTGF*): forward 5'-GAGACCAAAGACGCGTGTGA-3' and reverse 5'-CTCCTAGGTGAACCCCCTTT-3'[29]. The PCR amplification of rs3834129 (*CASP8*) and rs6918698 (*CTGF*) was carried out with the following reaction mixture: 100 ng of DNA, 1 X PCR buffer (500 mM KCl, 100 mM Tris-HCl, and 0.1% Triton X-100), 2.0 mM $MgCl_2$, 150 µM dNTPs, 1 µM of each primer, 2 U Taq DNA Polymerase, and H_2O to adjust the volume of 10 µL. The primers for rs3834129 (*CASP8*) were: forward 5'-CTGCATGCCAGGAGCTAAGT -3' and reverse 5'-GCCATAGTAATTCTTGCTCTGC-3' [6]; for rs6918698 (*CTGF)*: forward 5'-GAGACCAAAGACGCGTGTGA-3' and reverse 5'-CTCCTAGGTGAACCCCCTTT-3' [29]. The PCR reaction was performed under the following conditions: initial denaturation at 95 °C for 5 min, 30 cycles of denaturation at 95 °C for 45 s, annealing at 59 °C (for rs3834129-*CASP8*) and 54 °C (for rs6918698-*CTGF*) for 45 s, extension at 72 °C for 45 s, and final extension at 72°C for 10 min.

For rs3834129 (*CASP8*), the PCR fragment had a length of 171 bp, and it was verified in polyacrylamide gels at 6%. The fragment was digested with the restriction enzyme *BfaI* according to the manufacturer's instructions and separated on 6% polyacrylamide gels. The *BfaI* endonuclease recognizes the C^TAG restriction site. The homozygous genotype wild type I/I contains a recognition site for the enzyme *BfaI*, so the digestion yields two DNA fragments of 146 and 31 bp. The homozygous polymorphic genotype D/D does not contain a recognition site for the enzyme *BfaI*, so the 171 bp amplicon remains unaltered after incubation with endonuclease. Lastly, the heterozygous genotype I/D produces three DNA fragments of 171, 146, and 31 bp.

The rs6918698 (*CTGF*) PCR fragment had a length of 244 bp (verified in polyacrylamide gels at 6%), it was digested with the restriction enzyme *MnlI* according to the manufacturer's instructions and was separated on 6% polyacrylamide gels.

The *MnlI* endonuclease recognizes the $CCTC(N)_7$^ restriction site. The homozygous polymorphic genotype

G/G contains a recognition site for the enzyme *MnlI*, so the digestion yields two DNA fragments of 120 and 93 bp. The homozygous genotype wild type C/C does not contain a recognition site for the enzyme *MnlI*, so the 244 bp amplicon remains unaltered after incubation with endonuclease. Lastly, the heterozygous genotype C/G produces three DNA fragments of 244, 120, and 93 bp. The quality control for these assays was evaluated by re-genotyping 10% of samples, randomly selected by an independent technician. Concordance between genotyping tests was 100%.

2.2. Statistical Analysis

The genotypic and allelic frequencies were determined by direct counting. In both variants, the Hardy-Weinberg equilibrium was calculated with the Chi-square test. Analysis of the association of demographic, clinical, and anatomopathological characteristics with the genotypes was performed with Odds ratio (OR) analysis with confidence intervals (CI) of 95% and Yates corrected Chi-square test in SPSS 25.0 and EpiInfo 7.2.3.1 software packages. The significance level considered in all analyses was $p < 0.05$. A Bonferroni correction test was applied to adjust the *P* values ($P < 0.025$).

3. Results

3.1. Characteristics of the individuals studied

Table 1 shows the demographic, clinical, and anatomopathological data of CRC patients and the control group. The group of CRC patients included 140 men and 110 females, and the mean age was 58.39 years. The control group included 120 men and 130 females, with a mean age of 58.03 years. Non-statistical significance was observed for sex and age between the groups; however, we found significant differences in tobacco and alcohol consumption ($P = 0.001$). In the description of the CRC patients, 69.6% were in clinical stages TNM III and IV; the most frequent tumor location was the rectum, and the liver was the most frequent site of metastasis. 43.6% of the patients had a complete pathological response.

3.2. Genotype frequencies of the *CASP8* and *CTGF* variants in CRC patients and control individuals

The variants rs3834129 (*CASP8*) and rs6918698 (*CTGF*) in the control group were in Hardy-Weinberg equilibrium (data not shown). For the variant rs3834129 (*CASP8*), the genotype wild type I/I was found in 22.4% of the CRC patients, while in the control group it was found in 28.8% of individuals; the I/D genotype was found in 40.8% of the CRC patients and 46% of the controls, and the D/D polymorphic genotype was found in 36.8% and 25.2% in CRC patients and controls, respectively. In the association analysis, we observed that patients carrying the D/D genotype have an increased susceptibility to developing colorectal cancer (OR = 1.87; 95% CI 1.16-

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

	CRC n=250 (100%)	Control n=250 (100%)	*P value*
Age mean (±S.D.)	58.39(±12.49)	58.03(±10.84)	0.731
>50 years	187 (74.8%)	200 (80%)	0.199
<50 years	63 (25.2%)	50 (20%)	
Sex			
Male	140 (56%)	120 (48%)	0.089
Female	110 (44%)	130 (52%)	
Tobacco	85 (34%)	34 (13.6%)	**0.001**
Alcohol	73 (29.2%)	29 (11.6%)	**0.001**
TNM stage			
I	6 (2.4%)	-	
II	70 (28%)	-	
III	94 (37.6%)	-	
IV	80 (32%)	-	
Tumor location			
Ascendent colon	50 (20%)	-	
Descendent colon	3 (1.2%)	-	
Sigmoid colon	62 (24.8%)	-	
Rectum	135 (54%)	-	
Pathological Response			
Complete response	109 (43.6%)	-	
Partial response	73 (29.2%)	-	
Non-response	68 (27.2%)	-	
Metastasis			
Liver	30 (37.5%)	-	
Lung	13 (16.2%)	-	
Liver and lung	9 (11.3%)	-	
Peritoneum	3 (3.7%)	-	
Lung and peritoneum	3 (3.7%)	-	
Ovary	2 (2.5%)	-	
Brain	1 (1.2%)	-	
Non-available	19 (23.9%)	-	

P-values were adjusted by the Bonferroni test (0.025); Bold text highlights statistically significant findings.

3.01; P = 0.012). Allele frequencies were also statistically significant (OR = 1.43; 95% CI 1.11–1.84; P = 0.005) (Table 2). For the variant rs6918698 (*CTGF*) the C/C homozygous genotype was observed in 37.6% of the CRC patients and 26.8% of the control group; the C/G heterozygous genotype was observed in 46% of the CRC patients and 49.6% of the control group, and the G/G polymorphic genotype was observed in 16.4% of the CRC patients and 23.6% of the controls. In the association analysis, we observed that patients carrying the G/G genotype have a decreased susceptibility to developing CRC (OR = 0.49; 95% CI 0.29–0.82; P = 0.009). This decreased susceptibility was observed under the dominant model of inheritance C/G+G/G (OR = 0.60; 95% CI 0.41-0.88, P = 0.012). Allele frequencies were also statistically significant (OR = 0.69; 95% CI 0.53-0.89, P = 0.005) (Table 2).

3.3. Association of the genotypes with demographical, clinical and anatomopathological features

In the analysis by age of diagnosis, for the variant *CASP8* rs3834129, patients over 50 years old and carrying the D/D genotype have an increased susceptibility to CRC (OR = 2.18; 95% CI 1.27–3.73, P = 0.006). We did not observe an association between the genotypes I/D or D/D in the *CASP8* rs3834129 variant with the characteristics as sex, tobacco, and alcohol consumption (Table 3). For the variant *CTGF* rs6918698, the genotype G/G in male patients was associated with decreased susceptibility to CRC (OR = 0.36; 95% CI 0.17–0.74, P = 0.008). We did not find an association of the *CTGF* rs6918698 variant with the variables age, tobacco, and alcohol consumption (P > 0.05) (Table 3).

In the TNM stage and tumor location analysis for the *CASP8* rs3834129 variant, we observed that the patients in TNM stage II and carriers of the D/D genotype showed an increased susceptibility (OR = 2.80; 95% CI 1.28-6.10; P = 0.013); in addition, we observed that the patients with tumor location in the rectum and carriers of the D/D genotype showed an increased susceptibility (OR = 2.03; 95% CI 1.13-3.62; P = 0.023) (Table 4). For the *CTGF* rs6918698 variant, we observed that the patient's carriers of C/G and G/G genotypes and with TNM stage I+II had a decreased susceptibility (OR = 0.47; 95% IC 0.26-0.84; P = 0.016) and (OR = 0.40; 95% CI 0.19-0.75; P = 0.023), respectively. In addition, we observed that the patient's carriers of the G/G genotype and tumor location in the colon showed a decreased susceptibility to developing CRC (OR = 0.38; 95% IC 0.19–0.75; P = 0.007). Interestingly, we observed that the patient's carriers of C/G and G/G genotypes and tumor location in the right colon showed the same decreased susceptibility (Table 4). Finally, in the analysis of response to treatment between patients, we do not observe any statistical significance (Table 5).

4. Discussion

It has been described that familial and hereditary predisposition accounts for ~20% of all colorectal cancers [30]; likewise, germline mutations in mismatch repair (MMR) genes, *APC*, *SMAD4*, *STK11/LKB1*, *MUTYH/MYH*, and *ALK3* account for 5% of cases [31]. Variation in genetic risk is probably manifested by combinations of frequent variants with lower penetrance. Although these common alleles may confer only minute differences in CRC risk, some people may be at significant risk through interaction with other alleles. In this study, we investigated the potential association of *CASP8* rs3834129 (-652 6N I/D) and *CTGF* rs6918698 (-945 C>G) variants with the susceptibility to developing colorectal cancer. The results of this study carried out in the Mexican population show an evident increase of CRC in people older than 50 years (74.8%), a finding that has been previously reported when considering the average age of diagnosis between 50 and 75 years of age [8, 10]. According to international guidelines, consumption of tobacco and alcohol are risk factors

Table 2. Genotypes and Allelic Frequencies of the *CASP8* (rs3834129) and *CTGF* (rs6918698) variants in the Study Subjects.

Genotype	CRC n=250(100%)	Control n=250(100%)	OR (C.I. 95%)	*P value*
***CASP8* rs3834129**				
I/I	56(22.4%)	72(28.8%)	1.00 (Reference)	-
I/D	102(40.8%)	115(46.0%)	1.14 (0.73-1.76)	0.635
D/D	92(36.8%)	63(25.2%)	**1.87 (1.16-3.01)**	**0.012**
I/D + D/D vs. I/I	194(77.6%)	178(71.2%)	1.40 (0.93-2.09)	0.124
Allele				
I	214(42.8%)	259(51.8%)	1.00 (Reference)	-
D	286(57.2%)	241(48.2%)	**1.43 (1.11-1.84)**	**0.005**
***CTGF* rs6918698**				
C/C	94(37.6%)	67(26.8%)	1.00 (Reference)	-
C/G	115(46.0%)	124(49.6%)	0.66 (0.44-0.98)	0.055
G/G	41(16.4%)	59(23.6%)	**0.49 (0.29-0.82)**	**0.009**
CG+GG	156(62.4%)	183(73.2%)	**0.60 (0.41-0.88)**	**0.012**
Allele				
C	303(60.6%)	258(51.6%)	1.00 (Reference)	-
G	197(39.4%)	242(48.4%)	**0.69 (0.53-0.89)**	**0.005**

P-values were adjusted by the Bonferroni test (0.025); Bold text highlights statistically significant findings.

Table 3. Association of *CASP8* (rs3834129) and *CTGF* (rs6918698) variants with demographic variables.

				***CASP8* rs3834129**		
	CRC/Control			**OR (95% C.I.); *P value***		
Variable	**I/I**	**I/D**	**D/D**	**I/D vs. I/I**	**D/D vs. I/I**	**I/D + D/D vs. I/I**
Age						
>50	45/60	83/83	72/44	1.33 (0.81-2.18);0.306	**2.18 (1.27-3.73);0.006**	1.62 (1.03-2.55);0.044
<50	11/12	19/32	20/19	0.64 (0.23-1.75);0.547	1.14 (0.40-3.22);1.000	0.83 (0.33-2.08);0.879
Sex						
Male	30/36	60/54	50/30	1.33 (0.72-2.44);0.439	2.00 (1.03-3.88);0.058	1.57 (0.89-2.75);0.149
female	26/36	42/61	42/33	0.95 (0.50-1.80);1.000	1.76 (0.89-3.47);0.142	1.23 (0.69-2.21);0.570
Tobacco	24/14	32/10	29/10	1.86 (0.70-4.91);0.304	1.69 (0.63-4.48);0.415	1.77 (0.77-4.08);0.250
Alcohol	16/12	29/10	28/7	2.17 (0.77-6.13);0.223	3.00 (0.98-9.16);0.091	2.51 (0.99-6.33);0.081
				***CTGF* rs6918698**		
	CRC/Control			**OR (95% C.I.); *P value***		
Variable	**C/C**	**C/G**	**G/G**	**C/G vs. C/C**	**G/G vs. C/C**	**C/G+G/G vs C/C**
Age						
>50	80/58	87/88	33/41	0.71 (0.45-1.12);0.180	0.58 (0.33-1.03);0.086	0.67(0.44-1.02);0.082
<50	14/9	28/36	8/18	0.50 (0.18-1.32);0.243	0.28 (0.08-0.93);0.067	0.42(0.16-1.09);0.118
Sex						
Male	48/26	70/61	22/33	0.62 (0.34-1.11);0.148	**0.36 (0.17-0.74);0.008**	0.53(0.30-0.92);0.034
female	46/41	45/63	19/26	0.63 (0.36-1.12);0.157	0.65 (0.31-1.34);0.328	0.64(0.37-1.08);0.129
Tobacco	25/14	46/11	14/9	2.34 (0.92-5.92);0.113	0.87 (0.30-2.52);1.000	1.68(0.73-3.84);0.308
Alcohol	20/8	44/9	9/12	1.95 (0.65-5.81);0.351	0.30 (0.09-0.98);0.085	1.00(0.38-2.64);1.000

P-values were adjusted by the Bonferroni test (0.025); Bold text highlights statistically significant findings.

Table 4. Association of *CASP8* (rs3834129) and *CTGF* (rs6918698) variants with clinicopathological variables.

				***CASP8* rs3834129**		
	CRC/Control			**OR (95% C.I.); *P value***		
Variable	**I/I**	**I/D**	**D/D**	**I/D vs. I/I**	**D/D vs. I/I**	**I/D + D/D vs. I/I**
TNM						
II	11/72	32/115	27/63	1.82 (0.86-3.83);0.157	**2.80 (1.28-6.10);0.013**	2.16 (1.07-4.36);0.040
III	24/72	36/115	34/63	0.93 (0.51-1.70);0.956	1.61 (0.86-3.01);0.171	1.17 (0.68-2.02);0.640
IV	16/72	34/115	30/63	1.33 (0.68-2.58);0.496	2.14 (1.06-4.29);0.045	1.61 (0.87-2.98);0.160
I+II	16/72	32/115	28/63	1.25 (0.64-2.44);0.622	2.00 (0.99-4.03);0.074	1.51 (0.81-2.80);0.236
III+IV	40/72	70/115	64/63	1.09 (0.67-1.78);0.807	1.82 (1.08-3.07);0.031	1.35 (0.86-2.11);0.221
Tumor location						
Colon	29/72	42/115	44/63	0.90 (0.51-1.58);0.840	1.73 (0.97-3.09);0.083	1.19 (0.72-1.98);0.558
Rectum	27/72	60/115	48/63	1.39 (0.80-2.39);0.287	**2.03 (1.13-3.62);0.023**	1.61 (0.97-2.67);0.077
Right colon	12/72	18/115	20/63	0.93 (0.42-2.06);1.000	1.90 (0.86-4.20);0.157	1.28 (0.63-2.59);0.604
Left colon	14/72	24/115	24/63	1.07 (0.52-2.20);0.992	1.95 (0.93-4.10);0.106	1.38 (0.72-2.67);0.410
				***CTGF* rs6918698**		
	CRC/Control			**OR (95% C.I.); *P value***		
Variable	**C/C**	**C/G**	**G/G**	**C/G vs. C/C**	**G/G vs. C/C**	**C/G+G/G vs C/C**
TNM						
II	30/67	28/124	12/59	0.50 (0.27-0.91);0.033	0.45 (0.21-0.96);0.058	0.48 (0.28-0.84);0.014
III	34/67	46/124	14/59	0.73 (0.42-1.24);0.310	0.46 (0.22-0.95);0.052	0.64 (0.38-1.07);0.116
IV	26/67	39/124	15/59	0.81 (0.45-1.44);0.573	0.65 (0.31-1.35);0.334	0.76 (0.44-1.31);0.398
I+II	34/67	30/124	12/59	**0.47 (0.26-0.84);0.016**	**0.40 (0.19-0.84);0.023**	**0.45 (0.26-0.76);0.004**
III+IV	60/67	85/124	29/59	0.76 (0.49-1.19);0.286	0.54 (0.31-0.96);0.051	0.69 (0.45-1.05);0.111
Tumor Location						
Colon	47/67	52/124	16/59	0.59 (0.36-0.97);0.054	**0.38 (0.19-0.75);0.007**	**0.52 (0.33-0.84);0.010**
Rectum	47/67	63/124	25/59	0.72 (0.44-1.17);0.232	0.60 (0.33-1.09);0.131	0.68 (0.43-1.07);0.126
Right colon	29/67	15/124	6/59	**0.27 (0.14-0.55);0.001**	**0.23 (0.09-0.60);0.002**	**0.26 (0.14-0.49);0.001**
Left colon	18/67	37/124	10/59	1.11 (0.58-2.09);0.871	0.63 (0.27-1.47);0.390	0.95 (0.51-1.76);1.000

P-value were adjusted by the Bonferroni test (0.025); Bold text highlights statistically significant findings.

Table 5. Association of *CASP8* (rs3834129) and *CTGF* (rs6918698) variants and response to treatment.

***CASP8* rs3834129**					
Genotype	**Complete Responders** n= 109(100%)	**Partial Responders** n=73 (100%)	***P value; OR (95%CI)***	**Non-Responders** n=68 (100%)	***P value; OR (95%CI)***
I/I	22 (20.18)	23 (31.51)	1.0 (reference)	11 (16.18)	1.0 (reference)
I/D	45 (41.29)	27 (36.99)	0.209; 0.57 (0.26-1.22)	30 (44.11)	0.658; 1.33 (0.56-3.14)
D/D	42 (38.53)	23 (31.50)	0.147; 0.52 (0.24-1.13)	27 (39.71)	0.728; 1.28 (0.53-3.07)
I/D + D/D	87 (79.82)	50 (68.49)	0.118; 0.54 (0.27-1.08)	57 (83.82)	0.640; 1.31 (0.59-2.90)
***CTGF* rs6918698**					
Genotype	**Complete Responders** n= 109(100%)	**Partial Responders** n=73 (100%)	***P value; OR (95%CI)***	**Non-Responders** n=68 (100%)	***P value; OR (95%CI)***
CC	43 (39.45)	23 (31.51)	1.0 (reference)	28 (41.18)	1.0 (reference)
CG	45 (41.28)	37 (50.68)	0.272; 1.53 (0.78-2.99)	33 (48.53)	0.850; 1.12 (0.58-2.16)
GG	21 (19.27)	13 (17.81)	0.909; 1.15 (0.49-2.72)	7 (10.29)	0.262; 0.51 (0.19-1.36)
CG+GG	66 (60.55)	50 (68.49)	0.349; 1.41 (0.75-2.64)	40 (58.82)	0.943; 0.93 (0.50-1.72)

that play an important role in colorectal carcinogenesis [32–35]. This finding was consistent with our results, showing that 34% of CRC patients consume tobacco and 29.2% consume alcohol (P = 0.001). Regarding the clinical stage, most studies show that due to the lack of early diagnosis, the majority are diagnosed in stages III and IV. As shown in this study, with 69.6 percent overall, most are in stage III with 37.6%, while in stage IV with 32% [35, 36].

The gene *CASP8* encodes the protein Caspase 8, a protein that participates in the extrinsic pathway of apoptosis. The rs3834129 is the most investigated promoter, and it is well documented that this variant abolishes a binding site for the transcription activator 1 (Sp1), causing a decreased activity of *CASP8* due to lower expression of the *CASP8* protein in lymphocytes, reducing apoptosis [37]. This type of variant influences immune status and can modify CRC susceptibility.

Regarding the *CASP8* rs3834129 variant, in this study, the risk of developing CRC was statistically evident in individuals carrying the D/D genotype. Similar findings were reported in a previous study performed in patients with cutaneous melanoma [5] and colorectal cancer [11]. We can observe that a statistically significant risk association was found in this study with the D/D genotype, as well as in the frequency of the D allele. These results are similar to those reported by Ying Y et al. (2018) in the Asian population, where an association with CRC was found [11]. However, in other populations, controversial results have been observed; in a Chinese population, this variant was associated with a decreased susceptibility to developing colorectal cancer as well as other types of cancer such as breast, lung, gastric, esophageal, and cervical cancers [38]. These controversial results may be due to variations between the analyzed populations.

Connective tissue growth factor (CTGF) is an important signal-regulating molecule that plays an essential role in the processes of cell adhesion, angiogenesis, proliferation, migration, and tissue repair. *CTGF* expression has previously been described to increase the growth of hepatocellular cancer as well as in the early stages of colon cancer; however, it appears to have a protective effect against metastasis in the late stages of colon cancer [39]. Normally, the presence of the C allele at position −945 is paramount to suppressing CTGF transcription via the Sp3 junction. As a result of this suppression, the production of CTGF would be reduced [40]. Sp1 frequently participates as a stronger activator than Sp3, and Sp3 also acts as a repressor at the CTGF promoter [17]. In vitro studies have shown that when antagonists block the functional effect of CTGF, endothelial cell proliferation and migration are decreased [41].

Regarding the rs6918698 (-945 C>G) variant, it has been little studied in cancer, and only one study has been associated with the risk of developing CRC [17]. In this study, a decreased risk of developing CRC was statistically evident in individuals carrying the G/G genotype. However, in 2015, Ahmad et al. in the Swiss population found that the G/G genotype is associated with an increased risk of developing colon cancer [17]. The results shown are contradictory, maybe due to variations between the analyzed populations. In India, a study analyzed the expression of *CTGF* in patients with CRC, demonstrated that patients in early TNM stage (I/II) had better survival and lower probability of recurrence compared to those in advanced stages (III/IV) and that the expression increased according to the stage [18], also in a CRC meta-analysis, indicates that high expression of *CTGF* decreases the risk of lymph node metastasis and that it has been observed in patients in early TNM stages [41]. Contrary to this, in prostate cancer it was observed that a high expression of *CTGF* favored cancer cells to progress to advanced stages and later metastasis to bone [42]. Therefore, the presence of this variant could be considered as a prognostic factor regarding the survival of patients with CRC.

Our results show that the presence of at least one polymorphic G allele in the genotype of the individuals confers protection against tumors located in the colon. However, there are currently no other studies that demonstrate this association.

5. Conclusion

In conclusion, the results of the present study indicate that the D/D genotype of rs3834129 (*CASP8*) and the G/G of rs6918698 (*CTGF)* are significantly associated with colorectal carcinogenesis. Functional studies, including larger sample sizes, should be performed to corroborate the results of this study.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

This study was supported by grants from the “Fondo de Investigación en Salud. Instituto Mexicano del Seguro Social” (FIS/IMSS/PROT/G18/1822) and Foundation IMSS (F-2018-1305-001).

Author contributions

Designed the study: Mónica Alejandra Rosales-Reynoso and Patricio Barros-Núñez.
Clinical advice and patient recruitment: César de Jesús Tovar-Jácomé, Clara Ibet Juárez-Vázquez, Tomas Daniel Pineda-Razo and María Eugenia Marín-Contreras.
Performed experiments, data analysis and results: Anilú Margarita Saucedo-Sariñana, Yuri Giovanna Vanessa Trujillo-Fernández and Miriam Yadira Godínez-Rodríguez.
Supervised, directed and managed the study; Mónica Alejandra Rosales-Reynoso and Patricio Barros-Núñez.
Approved of the version to be published: Anilú Margarita Saucedo-Sariñana, Yuri Giovanna Vanessa Trujillo-Fernández, Clara Ibet Juarez-Vazquez, Miriam Yadira Godínez-Rodríguez, César de Jesús Tovar-Jácome, Patricio Barros-Nuñez, Tomas Daniel Pineda-Razo, María Eugenia Marín-Contreras, Mónica Alejandra Rosales-Reynoso.

References

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, Jemal A. Global cancer statistics 2022: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin (2024) 4(3):229–263. https://doi: 10.3322/caac.21834.
2. Globocan. Mexico (2022). https://gco.iarc.who.int/media/globocan/factsheets/ populations/484-mexico-fact-sheet.pdf
3. Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Piñeros M, Znaor A, et al. Cancer statistics for the year 2020: An overview.

Int J Cancer (2021). https://doi.org/10.1002/ijc.33588.
4. Theodoropoulos GE, Gazouli M, Vaiopoulou A, Leandrou M, Nikouli S, Vassou E, et al. Polymorphisms of caspase 8 and caspase 9 gene and colorectal cancer susceptibility and prognosis. Int J Colorectal Dis (2011) 26:1113–8. https://doi.org/10.1007/s00384-011-1217-5.
5. Li C, Zhao H, Hu Z, Liu Z, Wang L-E, Gershenwald JE, et al. Genetic variants and haplotypes of the caspase-8 and caspase-10 genes contribute to susceptibility to cutaneous melanoma. Hum Mutat (2008) 29:1443–51. https://doi.org/10.1002/humu.20803.
6. de Martino M, Haitel A, Schatzl G, Klingler HC, Klatte T. The CASP8 -652 6N insertion/deletion promoter polymorphism is associated with renal cell carcinoma risk and metastasis. J Urol (2013) 190:717–22. https://doi.org/10.1016/j.juro.2013.01.008.
7. Yao X, Hao S, Yu P. Association Study of the Caspase Gene Family and Psoriasis Vulgaris Susceptibility in Northeastern China. Biomed Res Int (2019) 2019:2417612. https://doi.org/10.1155/2019/2417612.
8. Wu M-H, Hung Y-W, Gong C-L, Chao C-C, Yueh T-C, Wang S-C, et al. Contribution of Caspase-8 Genotypes to Colorectal Cancer Risk in Taiwan. Anticancer Res (2019) 39:2791–7. https://doi.org/10.21873/anticanres.13406.
9. Fulda S. Caspase-8 in cancer biology and therapy. Cancer Lett (2009) 281:128–33. https://doi.org/10.1016/j.canlet.2008.11.023.
10. Pardini B, Verderio P, Pizzamiglio S, Nici C, Maiorana MV, Naccarati A, et al. Association between CASP8 -652 6N del polymorphism (rs3834129) and colorectal cancer risk: results from a multi-centric study. PLoS One (2014) 9:e85538. https://doi.org/10.1371/journal.pone.0085538.
11. Ying Y, Xu J, Qi Y, Zhang M, Yang Y. CASP8 rs3834129 (-652 6N insertion/deletion) Polymorphism and Colorectal Cancer Susceptibility: An Updated Meta-Analysis. J Cancer (2018) 9:4166–71. https://doi.org/10.7150/jca.27110.
12. Perbal B. CCN proteins: A centralized communication network. J Cell Commun Signal (2013) 7:169–77. https://doi.org/10.1007/s12079-013-0193-7.
13. Shen Y-W, Zhou Y-D, Luan X, Zhang W-D. Blocking CTGF-Mediated Tumor-Stroma Interplay in Pancreatic Cancer. Trends Mol Med (2020) 26:1064–7. https://doi.org/10.1016/j.molmed.2020.08.005.
14. Brigstock DR. The connective tissue growth factor/cysteine-rich 61/nephroblastoma overexpressed (CCN) family. Endocr Rev (1999) 20:189–206. https://doi.org/10.1210/edrv.20.2.0360.
15. Shimo T, Nakanishi T, Nishida T, Asano M, Kanyama M, Kuboki T, et al. Connective tissue growth factor induces the proliferation, migration, and tube formation of vascular endothelial cells in vitro, and angiogenesis in vivo. J Biochem (1999) 126:137–45. https://doi.org/10.1093/oxfordjournals.jbchem.a022414.
16. Dhar A, Ray A. The CCN family proteins in carcinogenesis. Exp Oncol (2010) 32:2–9.
17. Ahmad A, Askari S, Befekadu R, Hahn-Strömberg V. Investigating the association between polymorphisms in connective tissue growth factor and susceptibility to colon carcinoma. Mol Med Rep (2015) 11:2493–503. https://doi.org/10.3892/mmr.2014.3083.
18. Bhat IP, Rather TB, Maqbool I, Rashid G, Akhtar K, Bhat GA, et al. Connective tissue growth factor expression hints at aggressive nature of colorectal cancer. World J Gastroenterol (2022) 28:547–69. https://doi.org/10.3748/wjg.v28.i5.547.
19. Chen YQ, Sloan-Lancaster J, Berg DT, Richardson MA, Grinnell B, Tseng-Crank J. Differential mechanisms of plasminogen activator inhibitor-1 gene activation by transforming growth factor-beta and tumor necrosis factor-alpha in endothelial cells. Thromb Haemost (2001) 86:1563–72.
20. Robinson PM, Smith TS, Patel D, Dave M, Lewin AS, Pi L, et al. Proteolytic processing of connective tissue growth factor in normal ocular tissues and during corneal wound healing. Invest Ophthalmol Vis Sci (2012) 53:8093–103. https://doi.org/10.1167/iovs.12-10419.
21. Koliopanos A, Friess H, di Mola FF, Tang W-H, Kubulus D, Brigstock D, et al. Connective tissue growth factor gene expression alters tumor progression in esophageal cancer. World J Surg (2002) 26:420–7. https://doi.org/10.1007/s00268-001-0242-x.
22. Chen P-P, Li W-J, Wang Y, Zhao S, Li D-Y, Feng L-Y, et al. Expression of Cyr61, CTGF, and WISP-1 correlates with clinical features of lung cancer. PLoS One (2007) 2:e534. https://doi.org/10.1371/journal.pone.0000534.
23. Liu L-Y, Han Y-C, Wu S-H, Lv Z-H. Expression of connective tissue growth factor in tumor tissues is an independent predictor of poor prognosis in patients with gastric cancer. World J Gastroenterol (2008) 14:2110–4. https://doi.org/10.3748/wjg.14.2110.
24. Zhou Z-Q, Cao W-H, Xie J-J, Lin J, Shen Z-Y, Zhang Q-Y, et al. Expression and prognostic significance of THBS1, Cyr61 and CTGF in esophageal squamous cell carcinoma. BMC Cancer (2009) 9:291. https://doi.org/10.1186/1471-2407-9-291.
25. Chien W, O'Kelly J, Lu D, Leiter A, Sohn J, Yin D, et al. Expression of connective tissue growth factor (CTGF/CCN2) in breast cancer cells is associated with increased migration and angiogenesis. Int J Oncol (2011) 38:1741–7. https://doi.org/10.3892/ijo.2011.985.
26. Burke JP, O'Connell RM, Lennon G, Doherty GA, Keegan D, O'Donoghue D, et al. The influence of CTGF single-nucleotide polymorphisms on outcomes in Crohn's disease. Ann Surg (2013) 258:764–7. https://doi.org/10.1097/SLA.0000000000000247.
27. Li J, Ma X, Chakravarti D, Shalapour S, DePinho RA. Genetic and biological hallmarks of colorectal cancer. Genes Dev (2021) 35:787–820. https://doi.org/10.1101/gad.348226.120.
28 Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res (1988) 16:1215. https://doi.org/10.1093/nar/16.3.1215.
29. Posch MG, Schmidt G, Steinhoff L, Perrot A, Drews T, Dandel M, et al. A promoter polymorphism -945C>G in the connective tissue growth factor in heart failure patients with mechanical circulatory support: a new marker for bridge to recovery? Eur J Cardio-Thoracic Surg Off J Eur Assoc Cardio-Thoracic Surg (2015) 47:e29-33. https://doi.org/10.1093/ejcts/ezu402.
30. Lichtenstein P, Holm N V, Verkasalo PK, Iliadou A, Kaprio J, Koskenvuo M, et al. Environmental and heritable factors in the causation of cancer--analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med (2000) 343:78–85. https://doi.org/10.1056/NEJM200007133430201.
31. Aaltonen L, Johns L, Järvinen H, Mecklin J-P, Houlston R. Explaining the familial colorectal cancer risk associated with mismatch repair (MMR)-deficient and MMR-stable tumors. Clin Cancer Res an Off J Am Assoc Cancer Res (2007) 13:356–61. https://doi.org/10.1158/1078-0432.
32. Tsong WH, Koh WP, Yuan JM, Wang R, Sun CL, Yu MC. Cigarettes and alcohol in relation to colorectal cancer: the Singapore Chinese Health Study. Br J Cancer (2007) 96:821–7. https://doi.org/10.1038/sj.bjc.6603623.
33. Argilés G, Tabernero J, Labianca R, Hochhauser D, Salazar R, Iveson T, et al. Localised colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol Off J Eur Soc Med Oncol (2020) 31:1291–305. https://doi.org/10.1016/j.annonc.2020.06.022.
34. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin (2020) 70:7–30. https://doi.org/10.3322/caac.21590.
35. Leal-Omar J, Zarate-Guzmán J, Peniche-Moguel P, Gil-Rojas N, Gutiérrez-Roa A, Briones-Fraga S, et al. Demografía del cáncer

colorrectal en los últimos 5 años de un hospital oncológico (2019) 31:363–9. https://doi.org/10.24875/END.M19000125.

36. Park S, Kim J, Cho Y, Ahn S, Kim G, Hwang D, et al. Promotion of tumorigenesis by miR-1260b-targeting CASP8: Potential diagnostic and prognostic marker for breast cancer. Cancer Sci (2022) 113:2097–108. https://doi.org/10.1111/cas.15345.
37. Yang M, Sun T, Wang L, Yu D, Zhang X, Miao X, et al. Functional variants in cell death pathway genes and risk of pancreatic cancer. Clin Cancer Res an Off J Am Assoc Cancer Res (2008) 14:3230–6. https://doi.org/10.1158/1078-0432.
38. Sun T, Gao Y, Tan W, Ma S, Shi Y, Yao J, et al. A six-nucleotide insertion-deletion polymorphism in the CASP8 promoter is associated with susceptibility to multiple cancers. Nat Genet (2007) 39:605–13. https://doi.org/10.1038/ng2030.
39. Ramazani Y, Knops N, Elmonem MA, Nguyen TQ, Arcolino FO, van den Heuvel L, et al. Connective tissue growth factor (CTGF) from basics to clinics. Matrix Biol (2018) 68–69:44–66. https://doi.org/10.1016/j.matbio.2018.03.007.
40. Fonseca C, Lindahl GE, Ponticos M, Sestini P, Renzoni EA, Holmes AM, et al. A polymorphism in the CTGF promoter region associated with systemic sclerosis. N Engl J Med (2007) 357:1210–20. https://doi.org/10.1056/NEJMoa067655.
41. Zhao F, Li C, Wu Y, Xia J, Zeng M, Li T, et al. Connective Tissue Growth Factor in Digestive System Cancers: A Review and Meta-Analysis. Biomed Res Int (2020) 2020:8489093. https://doi.org/10.1155/2020/8489093.
42. Kim B, Kim H, Jung S, Moon A, Noh D-Y, Lee ZH, et al. A CTGF-RUNX2-RANKL Axis in Breast and Prostate Cancer Cells Promotes Tumor Progression in Bone. J Bone Miner Res Off J Am Soc Bone Miner Res (2020) 35:155–66. https://doi.org/10.1002/jbmr.3869.