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Original Research

TCF7L2 and *CCND1* polymorphisms and its association with colorectal cancer in Mexican patients

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Abstract: Accumulative evidence suggests that alterations due to mutations or genetic polymorphisms in the *TCF7L2* and *CCND1* genes, which are components of the Wnt signaling pathway, contributes to carcinogenesis. The present study was designated to clarify whether common single nucleotide polymorphisms (SNPs) of the transcription factor *7-* like 2 (*TCF7L2*) and cyclin D1 (*CCND1)* genes are associated with colorectal cancer risk in Mexican patients. A case-control study including 197 colorectal cancer patients and 100 healthy subjects was conducted in a Mexican population. Identification of polymorphisms was made by the polymerase chain reaction-restriction fragment length polymorphism methodology. The association was calculated by the odds ratio (OR) test. The results demonstrate that patients with the T/T genotype for the rs12255372 polymorphism of the *TCF7L2* gene present an increased colorectal cancer risk (OR=2.64, *P*=0.0236). Also, the risk analysis for Tumor-Nodule-Metastasis (TNM) stage and tumor location showed association with this polymorphism under the over-dominant model of inheritance (OR=1.75, *P*=0.0440). A similar relation was observed for the genotype T/T of the rs7903146 polymorphism and the rectal location of cancer (OR=7.57, *P*=0.0403). For the rs603965 polymorphism of the *CCND1* gene, we observed a protection effect for the colon cancer location under the dominant model (OR=0.49, *P*=0.0477). These results reveal a significant role of the analyzed polymorphisms in the *TCF7L2* and *CCND1* genes on the susceptibility or protection for developing colorectal cancer in the Mexican population.

Key words: Colorectal cancer, genetic polymorphisms, *TCF7L2, CCND1,* Mexican population.

Introduction

Worldwide, colorectal cancer (CRC) is the second most common type of cancer in women and the third in men (1, 2). The multistep carcinogenic mechanism, typical in the adenoma-carcinoma sequence, probably is also occurring in colorectal carcinogenesis (3, 4); however, currently is accepted that the pathogenesis of CRC involves multifactorial interactions of environmental factors and genetic susceptibility (5).

Since 1988, it had been proposed that CRC result of a series of mutational events, with the majority of tumors initially carrying an Adenomatous Polyposis Coli *(APC)* mutation and followed by mutations in the *p53* and K-*ras* genes (1, 6). It is possible that colorectal tumors result of the conjunction of inherited susceptibility, epigenetic changes, diet, lifestyle, and environmental exposure factors (7).

The Wnt/β-catenin pathway modulates cell proliferation, cell polarity, and cell differentiation during embryonic development and also plays a key role in homeostasis of mature tissues. This pathway is related to colon cancer since the recognition that abnormalities of chromosome 5q are early events in the tumorigenic process for sporadic and hereditary familial adenomatous polyposis (FAP) (5, 8). Close to 90% of sporadic colon cancers show mutations in the Wnt pathway, most of them inhibiting the APC function (5). The Wnt/β-catenin signaling depends on the recruitment of β-catenin into the nucleus. The gene of the transcription factor 7-like 2 (*TCF7L2)* is located on chromosome 10q and encodes for a protein known as T-cell factor 4 (TCF-4), which forms a complex with the lymphoid factor 1 (LEF) (9). Interaction of LEF/TCF with the β-catenin protein constitutes a transcription complex that regulates expression of target genes such as *CCND1*, *PPARD* and *c-Myc*, which participate on the colorectal carcinogenesis (10, 11). The bipartite transcription factor β-catenin/TCF is the major effector of the Wnt/β-catenin signaling (6).

Recently, several studies have investigated the association between the *TCF7L2* gene variants: rs7903146 (C>T) and rs12255372 (G>T), and several types of cancer, including breast (12-15), prostate (16-19), colorectal (20-24), lung (20), gastric (25) and ovarian (26); however, the results and conclusions are even controversial.

The *CCND1* gene is a main regulatory protein in the cell cycle control, principally in the transition from

G1 to S phase, which is regulated by cyclin-dependent kinases (27, 28). The overexpression of the *CCND1* gene, located on chromosome 11q13 [29], have been described in a wide variety of tumors, including colon (30-32), breast (33, 34), head and neck (35), laryngeal (36) and lung (37-39).

The change G to A in the splice donor region of exon 4 in the *CCND1* gene, located at nucleotide 870 (codon 242) (rs603965), is implicated in the splicing of the cyclin D1 transcript (40). The polymorphic allele A preferentially transcribes a truncated transcript "b" and encodes a protein with a longer half-life (41). The allele A in the homozygous state (A/A genotype) is associated with an increased risk of colorectal cancer (CRC) (42) and adenomas, mostly in younger patients and in patients with family history of this illness (43). However, the role of the rs603965 SNP in CRC risk remains controversial due to the results obtained from studies in different populations.

This is the first study assessing the potential risk of the rs7903146 and rs12255372 SNPs in the *TCF7L2* gene and the rs603965 SNP in the *CCND1* gene for the developing CRC in Mexican patients. Certain biological risk factors as age, gender, tobacco smoking and alcohol consumption are also analyzed together with these polymorphisms.

Materials and Methods

Ethical considerations

The present study was approved by the local institutional review board (CIBO, IMSS-R-2012-1305-10), and the participants signed an informed consent before the blood sample donation. This study was carried according to the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Participants

The selection criteria for all patients were: unrelated Mexican mestizos (at least two generations) over 18 years old. The CRC patients group involved 197 DNA samples obtained from individuals diagnosed with sporadic colorectal cancer (93 females and 104 males), according to clinicopathological criteria at the Hospital de Especialidades from Centro Médico Nacional de Occidente of the Instituto Mexicano del Seguro Social (IMSS) in Guadalajara, Jalisco between the years 2012-2015. The control group included 100 healthy volunteers (38 females and 62 males between 29 and 59 years old). These volunteers were not age-matched with the patient group. The exclusion criteria for the control group were no previous history of cancer and no chemotherapy for the patients. Data from all patients were obtained by clinical reviews and medical records. Also, we used a standard epidemiologic questionnaire to collect personal data, including age, sex, family history, drinking and smoking status.

DNA isolation and genotyping

Genomic DNA was extracted from peripheral blood using standard methods (44). The genomic regions encompassing the SNPs were amplified by a polymerase chain reaction previous to a restriction fragment length polymorphism assay (PCR-RFLP). Prim-

ers for rs12255372 (G>T) and rs7903146 (C>T) in the *TCF7L2* gene were those described by Bodhini *et al.* 2007 (45). The genotyping assay was made using the restriction enzyme *FokI* (New England Biolabs, USA) at 37°C for 16 hours for rs12255372 polymorphism. This enzyme cleaves the amplified product (118 bp), where the G allele creates a restriction site and gives two fragments with 85 and 33 bp (Figure 1). For the rs7903146, the genotyping was made using the restriction enzyme *RsaI* (New England Biolabs, USA), at 37°C for 16 hours; this enzyme cleavage the amplified product (107 bp) where the C allele creates a restriction site and gives two fragments of 82 and 25 bp (Figure 2). For the rs603965 (G>A) in the *CCND1* gene, primers were those described by Catarino RJ, *et al.* (46). The genotyping assay was made using the restriction enzyme *ScrfI* (New England Biolabs, USA) at 37°C for 16 hours. This enzyme digests the amplified product (167 bp) into two fragments (142 and 25 bp) when the G allele is present at the nucleotide 870 of the *CCND1* gene (Figure 3). Enzyme digestion products were visualized on 6% polyacrylamide gels stained with silver nitrate.

For the quality control of these assays, some randomly selected DNA samples were re-genotyped by a different technician. The observed concordance between genotyping assays was 100%.

Statistical methods

Allele and genotype frequencies were estimated by direct counting in both groups. Hardy-Weinberg equilibrium (HWE) was assessed by the chi-square test, and logistic regression was used to analyze the interaction
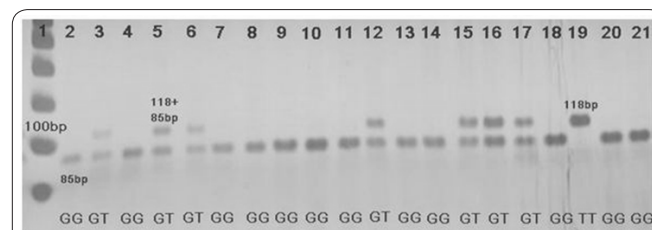
Figure 1. Polyacrylamide gel at 6% dyed with AgNO$_3$. The gel corresponds to the enzymatic digestion procedure with the *FokI* enzyme to identify the genotypes for the rs12255372 polymorphism in the *TCF7L2* gene. Line 1: 50 bp molecular marker. Lines 2, 4, 7, 8-11,13, 14, 18, 20, 21: G/G genotype (wild homozygous) with a size of 85 bp. Lines 3, 5, 6, 12, 15-17: G/T genotype (heterozygous) with a size of 118+85 bp. Line 19: T/T genotype (polymorphic homozygous) with a size of 118 bp.
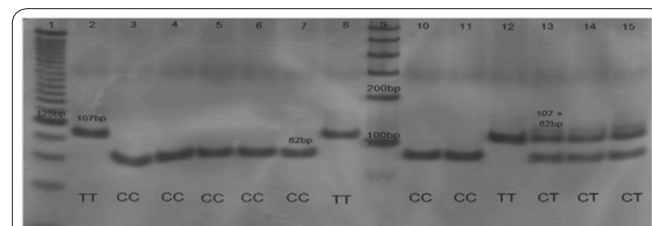
Figure 2. Polyacrylamide gel at 6% dyed with AgNO$_3$. The gel corresponds to the enzymatic digestion procedure with the *RsaI* enzyme to identify the genotypes for the rs7903146 polymorphism in the *TCF7L2* gene. Line 1: Molecular Marker of 25 bp. Line 9: Molecular Marker of 100 bp. Lines 3-7, 10, 11: C/C genotype (wild homozygous) with a size of 82 bp. Lines 13-15: C/T genotype (heterozygous) with a size of 107+82 bp. Lines 2, 8, 12: T/T genotype (polymorphic homozygous) with a size of 107 bp.
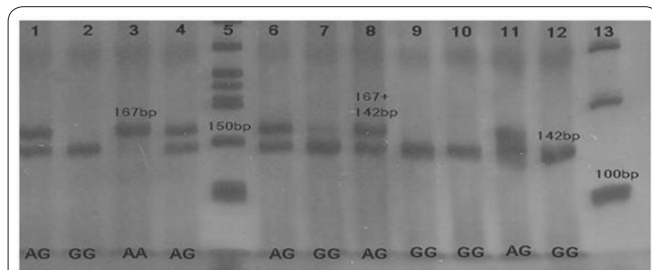
Figure 3. Polyacrylamide gel at 6% dyed with AgNO$_3$. The gel corresponds to the enzymatic digestion procedure with the *ScrfI* enzyme to identify the genotypes for the rs603965 polymorphism in the *CCND1* gene. Line 5: 50 bp molecular marker. Line 13: Molecular Marker of 100 bp. Lines 2, 7, 10, 12: G/G genotype (wild homozygous) with a size of 142 bp. Lines 1, 4, 6, 8, 11: G/A genotype (heterozygous) with a size of 167+142 bp. Line 3: A/A genotype (polymorphic homozygous) with a size of 167 bp.

between genotypes, genetics model of inheritance and the biological risk factors. To measure the association of CRC with the presence of alleles or genotypes and with the stratified analysis by TNM stage, the odds ratio (OR) and corresponding 95% confidence intervals (CI) were calculated using SPSS v17.0 software package (SPSS Inc., Chicago, IL, USA). For all the statistic analysis, p<0.05 was considered to be significant.

Results

Socio-demographic data analysis

Socio-demographic information was obtained from individuals of the CRC and control groups (healthy subjects) through interviews or medical records. A comparison of some biological risk factors for colorectal cancer such as age, gender, and tobacco and alcohol consumption, was made between affected individuals and a control group (Table 1). At the time that the blood samples were drawn, the average age of the CRC patients and controls were 59.03 and 36.88 years, respectively. Except by the age, there were no significant differences

Table 1. Biological risk factors and clinical features in CRC patients and controls.

Risk factors and clinical features	Patients n=197	Controls n=100	*p-value*
Age	59.03	36.88	**<0.0001**
Gender			
Female	93	38	0.1311
Male	104	62	
Tobacco smoking			
Yes	65	33	0.6008
No	111	67	
Alcohol consumption			
Yes	54	26	0.4113
No	121	74	
Cancer Family History			
Yes	30	---	
No	125	---	
Tumor location			
Colon	80	---	
Rectum	19	---	
TNM Stage			
Stage I	1	---	
Stage II	30	---	
Stage III	59	---	
Stage IV	86	---	

P values calculated by Chi-square test. *p<0.05 for statistical significance.*

between patients and the control group (gender, smoking and drinking status).

Distribution of genotypes and allele frequencies and risk analysis by genetic models

Table 2 shows the genotype and allele frequencies of the selected polymorphisms for the *TCF7L2* and *CCND1* genes. For the control group, the Hardy-Weinberg was calculated, and the three SNPs analyzed were in equilibrium (data not shown). There was no significant difference between patients with CRC and the control group for the rs7903146 and rs603965 poly-

Table 2. Genotype distribution and allele frequencies for the *TCF7L2* and *CCND1* genetic polymorphisms in the patients and control groups.

Genotype	Frequencies Patients group n= 197 (%)	Control group n= 100 (%)	OR [95% CI]	*P*-value
***TCF7L2* (rs7903146)**				
C/C	91 (46.2)	53 (53.0)	1.00 [Reference]	
C/T	93 (47.2)	44 (44.0)	1.23 [0.73- 2.08]	0.4834
T/T	13 (6.6)	3 (3.0)	2.52 [0.63-11.73]	0.2459
T/T+C/T *vs.* C/C	106 (53.8)	47 (47.0)	1.31 [0.79-2.19]	0.3239
C/C+C/T *vs.* T/T	184 (93.4)	97 (97.0)	2.28 [0.59-10.36]	0.3047
T/T+C/C *vs.* C/T	104 (52.8)	56 (56.0)	1.14 [0.68-1.90]	0.6884
Allele				
C	0.69 (275)	0.75 (150)	1.00 [Reference]	
T	0.31 (26)	0.25 (6)	1.29 [0.88-1.90]	0.2110
***TCF7L2* (rs12255372)**				
G/G	104 (52.8)	65 (65.0)	1.00 [Reference]	
G/T	89 (45.2)	30 (30.0)	1.63 [0.80-3.29]	0.1964
T/T	4 (2.0)	5 (5.0)	**2.64 [1.12-6.24]**	**0.0236**
T/T+G/T *vs.* G/G	93 (47.2)	35 (35.0)	**1.64 [1.00-2.70]**	**0.0487**
G/G+G/T *vs.* T/T	193 (98.0)	95 (95.0)	0.39 [0.10-1.49]	0.1558
T/T+G/G *vs.* G/T	108 (54.8)	70 (70.0)	**1.91 [1.14-1.79]**	**0.0128**
Allele				
G	0.75 (297)	0.81 (160)	1.00 [Reference]	
T	0.25 (8)	0.19 (10)	1.47 [0.96-2.23]	0.0797
***CCND1* (603965)**				
G/G	54 (27.4)	23 (23.0)	1.00 [Reference]	
G/A	97 (49.2)	56 (56.0)	0.74 [0.39-1.38]	0.3858
A/A	46 (23.4)	21 (21.0)	0.93 [0.43-2.02]	0.9919
A/A+G/A vs. G/G	143 (72.6)	77 (77.0)	0.79 [0.45-1.39]	0.4966
G/G+G/A vs. A/A	151 (76.6)	79 (79.0)	1.15 [0.64-2.05]	0.7557
G/G+A/A vs. G/A	100 (50.8)	44 (44.0)	0.76 [0.47-1.24]	0.3275
Allele				
G	0.52 (205)	0.51 (102)	1.00 [Reference]	
A	0.48 (92)	0.49 (42)	0.95 [0.68-1.34]	0.8623

P values calculated by Chi-square test.

morphisms. However, the distribution of genotypes between patients and controls was significantly different for the rs12255372 polymorphism of the *TCF7L2* gene. The risk analysis for the rs12255372 polymorphism showed an OR of 2.64 (95% CI=1.12-6.24, *P*=0.0236) under a co-dominant pattern of allelic interaction (individuals with T/T genotype). Our results showed a marginally significant association with the dominant model of inherited with an OR of 1.64 (95% CI=1.00-2.70, *P*=0.0487), and a quite significant association with the over-dominant model: OR of 1.91 (95% CI=1.14-1.79, *P*=0.0128). We did not observe statistical differences in the allele distribution: OR of 1.47 (95% CI=0.96-2.23, *P*=0.0797).

Logistic regression analysis

Considering that biological and environmental variables as age, gender, and smoking and drinking may affect the development of CRC, we further performed a logistic regression analysis, including the biological risk factors and its interaction with the selected polymorphisms, according to the different genetics models (codominant, dominant, recessive and over-dominant). None significant association (*P*<0.05) was found in this comparison among the CRC risk, the biological and environmental factors, and the selected polymorphisms of the *TCF7L2* and *CCND1* genes (Data not shown).

Risk analysis for TNM staging and tumor location

Stratified analyzes for each SNP by TNM stage and tumor location are shown in Tables 3 and 4, respectively. We do not found evidence for association of the rs7903146 and rs603965 polymorphisms with the cancer TNM stages; however, a statistically significant association between TNM stages III+IV was observed under an over-dominant model of allelic interaction for the rs12255372 polymorphism (OR=1.75; 95% CI=1.02-3.02, *P*=0.0440).

Our stratified analysis by tumor location revealed an increased risk of colon and rectal cancer in the presence of the genotype T/T of the rs7903146 polymorphism (OR=4.87; 95% CI=1.19-19.80, *P*=0.0234, and OR=7.57; 95% CI=1.27-45.07, *P*=0.0403) respectively. Additionally, this polymorphism showed association with the colon cancer location, under a dominant model (OR=1.98; 95%CI=1.08-3.61, *P*=0.0347), and an increased risk of 6.46 with the rectal tumor site under a recessive model (95% CI=1.19-35.05, *P*=0.0449). For the rs12255372 polymorphism, the association was observed with the colon tumor location under an over-dominant model of allelic interaction (OR=1.90; 95% CI=1.03-3.52, *P*=0.0438). Finally, for the rs603965 polymorphism of the *CCND1* gene, we observed an interesting inverse association with the colon tumor location under a dominant model (OR=0.49; 95% CI=0.26-0.95, *P*=0.0477).

Discussion

Colorectal cancer is a common and afflictive disease caused by genetic mutations and environmental factors. Lifestyle and dietary factors largely influence this neoplasia; biological risk factors such as age, gender, tobacco smoking and alcohol consumption have been implicated in the development and progression of this disease. To our knowledge, this is the first report analyz-

Table 3. Genotypic distribution of polymorphisms of the *TCF7L2* and *CCND1* genes by TNM stage between controls and the colorectal cancer group.

SNP	Genotype	Controls n=100 (%)	Stage I+II CCR n=31 (%)	Stage III+IV CCR n=144 (%)	Stage I+II CCR vs. Controls OR (95% CI)	*P-value*	Stage III+IV CCR vs. Controls OR (95% CI)	*P-value*
	C/C	53 (53)	10 (32.3)	68 (47.2)	1 (Reference)		1 (Reference)	
	C/T	44 (44)	20 (64.5)	64 (44.4)	2.40 (1.20-5.68)	0.0593	1.71 (0.99-2.96)	0.0568
	T/T	3 (3)	1 (32.2)	12 (8.3)	1.76 (0.16-18.74)	0.5208	0.67 (0.17-2.61)	0.7336
rs7903146								
	Dom	47 (47)	21 (67.7)	76 (52.8)	2.36 (1.01-5.53)	0.0632	1.56 (0.92-2.65)	0.1118
	Rec	97 (97)	30 (96.8)	132 (91.7)	1.07 (0.10-10.74)	1.0000	0.55 (0.14-2.10)	0.4944
	Over-Dom	56 (56)	11 (35.5)	80 (55.6)	2.31 (1.00-5.33)	0.0636	1.01 (0.60-1.70)	1.0000
		n=100 (%)	n=31 (%)	n=142 (%)				
	G/G	65 (65)	17 (54.8)	77 (54.2)	1 (Reference)		1 (Reference)	
rs12255372	G/T	30 (30)	14 (45.2)	61 (42.9)	1.78 (0.77-4.08)	0.1958	1.71 (0.99-2.96)	0.0568
	T/T	5 (5)	0 (0)	4 (2.8)	-	0.5781	0.67 (0.17-2.61)	0.7336
	Dom	35 (35)	14 (45.2)	65 (45.8)	1.52 (0.67-3.46)	0.3957	1.56 (0.92-2.65)	0.1118
	Rec	95 (95)	31 (100)	138 (97.2)	-	0.5915	0.55 (0.14-2.10)	0.4944
	Over-Dom	70 (70)	17 (54.8)	81 (57.0)	1.92 (0.84-4.39)	0.1320	**1.75 (1.02-3.02)**	***0.0440**
		n=100 (%)	n=31 (%)	n=145 (%)				
	G/G	23 (23)	6 (19.4)	44 (30.3)	1 (Reference)		1 (Reference)	
	G/A	56 (56)	15 (48.4)	70 (48.3)	1.02 (0.35-2.97)	1.0000	0.65 (0.35-1.20)	0.2187
	A/A	21 (21)	10 (32.2)	31 (21.4)	1.82 (0.56-5.89)	0.3873	0.77 (0.36-1.63)	0.5673
rs603965								
	Dom	77 (77)	25 (80.6)	101 (69.7)	1.24 (0.45-3.40)	0.8065	0.68 (0.38-1.23)	0.2438
	Rec	79 (79)	21 (67.7)	114 (78.6)	1.79 (0.73-4.37)	0.2289	1.02 (0.54-1.90)	1.0000
	Over-Dom	44 (44)	16 (51.6)	75 (51.7)	0.73 (0.32-1.65)	0.5375	0.73 (0.43-1.22)	0.2447

P values calculated by Chi-square test. * Significant *P* values. Dom: Dominant model. Rec: Recessive model. Over-Dom: Over-dominant model.

Table 4. Comparison of *TCF7L2* (rs7903146 and rs12255372) and *CCND1* (rs603965) genotypic distribution by tumor location.

SNP	Genotype	Controls n=100 (%)	Colon cancer n=80 (%)	Rectal cancer n=18 (%)	Colon cancer vs. controls OR (95% CI)	P value	Rectal cancer vs. controls OR (95% CI)	P value
	C/C	53 (53)	29 (36.3)	7 (38.8)	1		1	
	C/T	44 (44)	43 (53.8)	8 (44.4)	1.78 (0.96-3.31)	0.0866	1.37 (0.46-4.09)	0.5901
	T/T	3 (3)	8 (10)	3 (16.6)	4.87 (1.19-19.80)	*0.0234	7.57 (1.27-45.07)	*0.0403
rs7903146								
	Dom	47 (47)	51 (63.6)	11 (61.1)	1.98 (1.08-3.61)	*0.0347	1.77 (0.63-4.94)	0.3131
	Rec	97 (97)	72 (90)	15 (83.3)	3.59 (0.92-14.01)	0.0639	6.46 (1.19-35.05)	*0.0449
	Over-Dom	56 (56)	37 (46.3)	10 (55.5)	1.39 (0.77-2.53)	0.2926	1.10 (0.37-2.79)	1.0000
		n=100 (%)	n=79 (%)	n=19 (%)				
	G/G	65 (65)	44 (55)	10 (52.6)	1		1	
	G/T	30 (30)	35 (45)	8 (45.1)	1.77 (0.95-3.28)	0.0851	1.73 (0.62-4.83)	0.2919
rs12255372	T/T	5 (5)	0 (0)	1 (5.3)	-	0.1545	1.30 (0.16-12.30)	1.0000
	Dom	35 (35)	35 (45)	9 (47.3)	1.51 (0.83-2.77)	0.2194	1.67 (0.62-4.49)	0.3133
	Rec	95 (95)	79 (100)	18 (94.7)	-	0.0667	1.05 (0.11-9.57)	1.0000
	Over-Dom	70 (70)	44 (55)	11 (57.8)	1.90 (1.03-3.52)	*0.0438	1.69 (0.62-4.64)	0.2983
		n=100 (%)	n=80 (%)	n=19 (%)				
	G/G	23 (23)	30 (37.5)	8 (42.1)	1		1	
	G/A	56 (56)	39 (48.7)	10 (52.3)	0.53 (0.27-1.05)	0.0860	0.51 (0.17-1.46)	0.2640
	A/A	21 (21)	11 (13.8)	1 (5.3)	0.40 (0.16-0.99)	0.0724	0.13 (0.01-1.18)	0.0639
rs603965								
	Dom	77 (77)	50 (62.5)	11 (57.8)	0.49 (0.26-0.95)	*0.0477	0.41 (0.14-1.14)	0.0935
	Rec	79 (79)	69 (86.2)	18 (94.7)	0.59 (0.27-1.33)	0.2420	0.19 (0.02-1.56)	0.1184
	Over-Dom	44 (44)	41 (51.2)	9 (49.3)	0.74 (0.41-1.34)	0.3690	0.87 (0.32-2.33)	0.8061

P values calculated by Chi-square test. * Significant *P* values. Dom: Dominant model, Rec: Recessive model, Over-Dom: Over-dominant model.

ing the gene polymorphisms of *TCF7L2* (rs12255372 and rs7903146) and *CCND1* (rs603965), about tumor location, TNM staging and biological risk factors in Mexican patients with CRC.

In this study, comparison of biological risk factors for CRC such as age, gender, smoking and alcohol consumption, achieved between affected individuals and a control group, did not show significant differences except by the age. As it was mentioned before, the control group was not age-matched with the patients group, and certainly CRC is a disease more frequently occurring in older people (50 years and over); therefore, it is expected that the age average between the control and CRC groups will be differents.

The *TCF7L2* gene was firstly reported associated with type-2 diabetes (T2D) by Grant et al., (47) and it has been confirmed as one of the strongest T2D susceptibility genes. On the other hand, the *TCF7L2* gene may develop cancer because the TCF4 protein is involved in the Wnt/β-catenin signaling pathway, as a transcription factor that induces expression of target genes such as *CCND1* and *c-Myc,* both oncogenes involved in cellular proliferation, evasion of apoptosis, and tissue invasion and metastasis (25). Recent studies demonstrate the association of some SNPs in the *TCF7L2* gene with various types of cancer (12-26). Among these SNPs, rs7903146 (intron 3) and rs12255372 (intron 4), have been described affecting the mRNA structural stability and/or the alternative splicing (17, 18, 25).

Here we found an association between CRC risk and the rs12255372 polymorphism of the *TCF7L2* gene, demonstrated by the co-dominant, dominant and overdominant statistical models (OR=2.64, *P*=0.0236, OR=1.64, *P*=0.0236 and OR=1.91, *P*=0.0128, respectively). Such results differ to the observations reported

in 2008 by Hazra et al. (26), according to which, a decreased cancer risk was associated with this polymorphism in a Health Professionals Follow-up Study; or by Paez et al. (48) who did not find an relation between this SNP and risk or recurrence for CRC. On the other hand, we found a statistically significant association for the TNM stages III+IV under the over-dominant pattern of allelic interaction (OR=1.75, *P*=0.0440). With this same over-dominant pattern of allelic interaction, a statistically significant association with the colon cancer location was also evident OR=1.90 (*P*=0.0438).

Such relationship between the rs12255372 polymorphism of the *TCF7L2* gene and colorectal cancer have not been reported before. Previous reports have analyzed the polymorphic genotype T/T regarding other types of cancer; thus, Agalliu et al. in 2008 (16) found that the genotype T/T had an elevated risk for more aggressive prostate cancer; however, Naidu et al., in 2012 (14) do not found an association of this genotype with breast cancer. The results here obtained demonstrate clearly that individuals with T/T genotype are exposed to an increased risk for CRC.

Regarding to the rs7903146 polymorphism of the *TCF7L2* gene, our stratified analyze for tumor location revealed an increased risk of colon cancer in individuals with the T/T genotype (OR=4.87, *P*=0.0234); this relationship was also observed in a dominant pattern of allelic interaction (OR=1.98, *P*=0.0347). Similar results were found for the rectal location of cancer in individuals with the T/T genotype (OR=7.57, *P*=0.0403), and also under a recessive model of inheritance (OR=6.46 *P*=0.0449). These observations are consistent with the results of a case-control study conducted by Tsilids et al., (22) in a population of EU (Washington DC). Other two studies performed by Folsom et al., (20) and Slattery et

al. (23), reported that the rs7903146 polymorphism was associated with an increased risk of CRC in EU population. Naidu et al., 2012 (14) found that the genotype T/T of the rs7903146 polymorphism may elevate the risk of breast cancer and increase the metastatic potential of the tumor. Although these polymorphisms in the *TCF7L2* gene have not been analyzed with cancer in our population, there are several studies demonstrating its association with other diseases such diabetes gestacional (49), obesity (50), and type 2 diabetes (51-53).

The *CCND1* or *Cyclin D1* gene plays a substantial role in the progression of the cell cycle, cell migration, injury response, DNA-repairing and chromosome stability (54-58). Cyclin D1 protein is known regulating the G1 to S phase transition during cell division. Increased Cyclin D1 protein activity leads to a premature transition from the G1 to S phase, carrying with the altered DNA progression and subsequent accumulation of genetic errors, and enhancing abnormal cell proliferation (58). The rs603965 SNP (G870A) in the *Cyclin D1* gene is widely studied due to the role that plays in cell cycle progression and its frequent association with several types of cancer, among them CRC. G870A is located in exon 4 and its presence produces two transcripts known as the D1a and D1b isoforms. Although the function of the D1b variant has not been described, it has a longer half live compared to the D1a variant; therefore, it is speculated that the D1b variant promotes the cell cycle progression (54-58).

In 2003, Grieu et al. (59) performed a case-control study in Australian population analyzing the association of G870A with breast and/or colorectal cancer and they reported no association with CRC. Likewise, by logistic regression analysis, Liu et al. (60) in 2010 observed no association between this SNP and CRC risk in a Chinese population. Contrarily, there exist some reports proposing different degrees of association between this polymorphism and CRC risk (61-71).

Our findings show that the presence of the G870A polymorphism is not associated with CRC risk in Mexican mestizo population; moreover, under a dominant model of inheritance, individuals carrying this polymorphism show a statistically significant decreased risk for presenting cancer in the colon (OR=0.49, *P*=0.0477). This result suggests an interesting protection factor for the G/A+ A/A individuals. Sofer-Levy (64) and Pérez-Morales (65) have reported that the overexpression of cyclin D1 induce apoptosis; in that sense, the CCND1 rs603965 genotype may be functionally protective, as observed in our study (64, 65). Interesting, Perez-Morales et al., 2013 found similar results in Mexican patients with lung cancer (65).

These controversial differences can be explained by the genetic component itself, where the same polymorphism have a different impact on the risk and progression of the same disease in different populations.

Mexican mestizos are mainly an admixture of Europeans and Amerindians and constitute the mayor component of the current Mexican populations (66, 67); therefore, the particular genetic background of our Mexican population can explain the marked differences in CRC risk observed between our and other populations.

This is the first study assessing together these three polymorphisms: two in the *TCF7L2* gene and other in the *CCND1* gene, and their possible implication in the susceptibility to CRC, compared with tumor location, TNM staging and biological risk factors in Mexican patients. The results show that the rs12255372 and rs7903146 polymorphisms in the *TCF7L2* gene are related to CRC risk in our population, indicating an important role in CRC genetic susceptibility. On the other hand, the rs603965 SNP (G870A) in the *Cyclin D1* gene suggests an interesting protection factor for the G/A+ A/A individuals. Additional studies with larger samples, more diverse populations, and functional analysis of these polymorphisms are necessary to confirm and extend our findings.

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