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Correlation between the rs7101 and rs1063169 polymorphisms in the *FOS* noncoding region and susceptibility to and prognosis of colorectal cancer

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Abstract

Background: The *FOS* gene is located on human chromosome 14q21–31 and encodes the nuclear oncoprotein c-Fos. This study analyzed the correlation between the *FOS* noncoding region rs7101 and rs1063169 polymorphisms and colorectal cancer susceptibility and prognosis.

Methods: We analyzed the *FOS* genotypes in 432 colorectal cancer patients and 315 healthy subjects by PCR/Sanger sequencing. Survival was analyzed by Kaplan–Meier and Cox regression analysis. Western blot was used to detect the expression of c-Fos protein in cancer tissues and adjacent tissues in colorectal cancer patients with different genotypes.

Results: The presence of a T allele at rs7101 and a T allele at rs1063169 in *FOS* carried a higher risk of colorectal cancer [adjusted odds ratio (OR) = 1.237, 95% confidence interval (95% CI) = 1.131–1.346, $P \leq .001$ and adjusted OR = 1.218, 95% CI = 1.111–1.327, $P \leq .001$, respectively]. c-Fos protein levels were significantly higher in variant cancer tissues than in normal mucosa tissues ($P < .05$), and c-Fos proteins levels were also higher in homozygous variant cancer tissues than in heterozygous variant cancer tissues. The 3-year survival rate of patients with wild-type *FOS* was higher than that of patients with variant *FOS* ($P < .05$).

Conclusion: The rs7101 and rs1063169 polymorphisms in the noncoding region of *FOS* are associated with the risk of developing colorectal cancer and the progression of colorectal cancer, which may be because the mutation enhances the expression of c-Fos protein to promote the incidence and development of colorectal cancer.

Abbreviations: BMI = body mass index, ELISA = enzyme-linked immunosorbent assay, MAF = minor allele frequency, MDR = mutifactor dimensionality reduction, SNPs = single nucleotide polymorphisms.

Keywords: colorectal cancer, Fos, prognosis, single nucleotide polymorphism, survival

1. Introduction

Colorectal cancer is one of the most common malignant tumors, and it is the third most common cancer worldwide, after lung and breast cancer.[1] According to the most recent statistics, the annual incidence of colorectal cancer worldwide is nearly 1.2 million, and the number of deaths is as high as 600,000.[2] In recent years, the incidence of colorectal cancer in China has increased annually,[3] which seriously affects patients' health and quality of life. Because the early symptoms of colorectal cancer are difficult to detect, most patients are diagnosed in advanced stages. By this point, cancer cells may have already infiltrated the vasculature and metastasized, and treatment can be difficult. Therefore, early detection and early treatment have significant clinical significance for prolonging the survival of patients with colorectal cancer.

Colorectal cancer is caused by a combination of multiple factors, including genetic factors, environmental factors, lifestyle, and dietary habits.[4] Single nucleotide polymorphisms (SNPs) are DNA sequence polymorphisms at the genome level resulting from a single nucleotide variation, and are the most common mutations in human genetic variation. SNPs can reflect the individual's phenotype and response to drugs and environmental factors, and susceptibility to disease. In recent years, SNPs have taken center stage in the field of cancer research. Many studies have shown that SNPs are associated with cancer risk.[5–7] A correlation between SNPs and the risk of colorectal cancer has been confirmed.[8] *FOS* is a nuclear proto-oncogene and encodes c-Fos, which is involved in the regulation of cell proliferation and apoptosis. High c-Fos expression can promote the formation of colorectal cancer.[9] However, the relationship between *FOS* SNPs and the risk and prognosis of colorectal cancer has rarely been reported.

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HC and LJ contributed equally to this work.

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All data generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Using the Ensembl database, we selected 2 SNP loci with minor allele frequency (MAF) > 0.05 in the noncoding region of *FOS*, that is, rs7101 and rs1063169. The former is located in the 5 'UTR and the latter is in the intron. We analyzed the effects of these 2 SNPs on the susceptibility to colorectal cancer and survival to guide the judgment of clinical prognosis.

2. Material and methods

2.1. Subjects

Four hundred thirty-two patients with colorectal cancer treated in the Yidu Central Hospital of Weifang from February 2012 to August 2014 were randomly enrolled as a case group, and the data for gender, age, height, weight, smoking, alcohol consumption, clinical pathological stage, and degree of histological differentiation were collected. In addition, 315 patients with a healthy physical examination in the same period were recruited as the control group, using the basic data of patients in the case group as a guide. This study was approved by the Medical Ethics Committee of Yidu Central Hospital of Weifang. All subjects signed an informed consent for the collection of 8 mL venous blood. Patients in the case group also signed the informed consent for the subsequent protein expression analysis and genotyping of *c-Fos* rs7101 and rs1063169 of the surgical excised tissue specimens.

2.2. Genotyping

Eight milliliters of fasting venous blood was extracted from each subject, and 6 mL of whole blood was centrifuged at 3000 rpm for 20 minutes after rest for 30 minutes and stored at -80°C. Two milliliters of whole blood was treated with EDTA after anticoagulation to extract genomic DNA using a QIAamp DNA Blood Mini Kit (51104; QIAGEN, Hilden Geschäftsführer, Germany). Primers were designed to amplify the rs7101 and rs1063169 loci of *FOS*. The primer sequences for the rs7101 locus were Forward primer: 5'-CAGTGACCGTGCTCCTACC-3', Reverse primer: 5'-CAAAGCCGGGCGAGGG-3', Tm: 60°C. The primer sequences for the rs1063169 locus were Forward primer: 5'-TCTTTGTTCTCTTGCTGAGGATCT-3', Reverse primer: 5 '-GCACCCCACTGTGAAACCA-3', Tm: 60°C. The PCR reaction mix included 5 μL 10 × Buffer (with Mg^{2+}) (Invitrogen Corporation, Carlsbad, CA), 2 μL 2.5 mM dNTPs, 1 μL 10 μM Forward primer, 1 μL 10 μM Reverse primer, 10 ng template gDNA, 0.5 μL Taq polymerase, and ddH_2O to 50 μL. The PCR reaction conditions were as follows: 95°C, 5 minutes (94°C, 30 seconds; 60°C, 30 seconds; 72°C, 30 seconds), 30 cycles; 72°C, 5 minutes. After PCR, the target band was purified using a QIAquick Gel Extraction Kit (cat No. 28704; QIAGEN), and the sequence of the target band was analyzed using Sanger sequencing.

2.3. Protein extraction and expression analysis

Cancer tissue and adjacent normal tissueous tissue were selected from 16 patients with colorectal cancer, including 8 patients with colon cancer and 8 with rectal cancer. There were 6 CC genotypes, 5 CT genotypes, and 5 TT genotypes at rs7101 locus, 6 GG genotype, 5 GT genotype, and 5 TT genotype at rs1063169 locus. The protein was extracted using a T-PER tissue protein extraction kit (Thermo Scientific, Waltham, America), strictly following the kit instructions. Using β-actin as an internal reference protein, the c-Fos protein expression of 1 subject of each rs7101 locus CC, CT, TT genotype and 1 subject of each rs1063169 locus GG, GT, TT genotype was detected by Western blot (c-fos: Catalog # AF7254-SP, β-actin: Catalog # MAB8929-SP; R&D Systems, Minneapolis, MN) (Fig. 1). Concentrations of c-Fos were measured in the serum using an enzyme-linked immunosorbent assay (ELISA) kit (Catalog # CSB-E09261 h; Cusabio Biotech Co. Ltd, Newark, NJ).

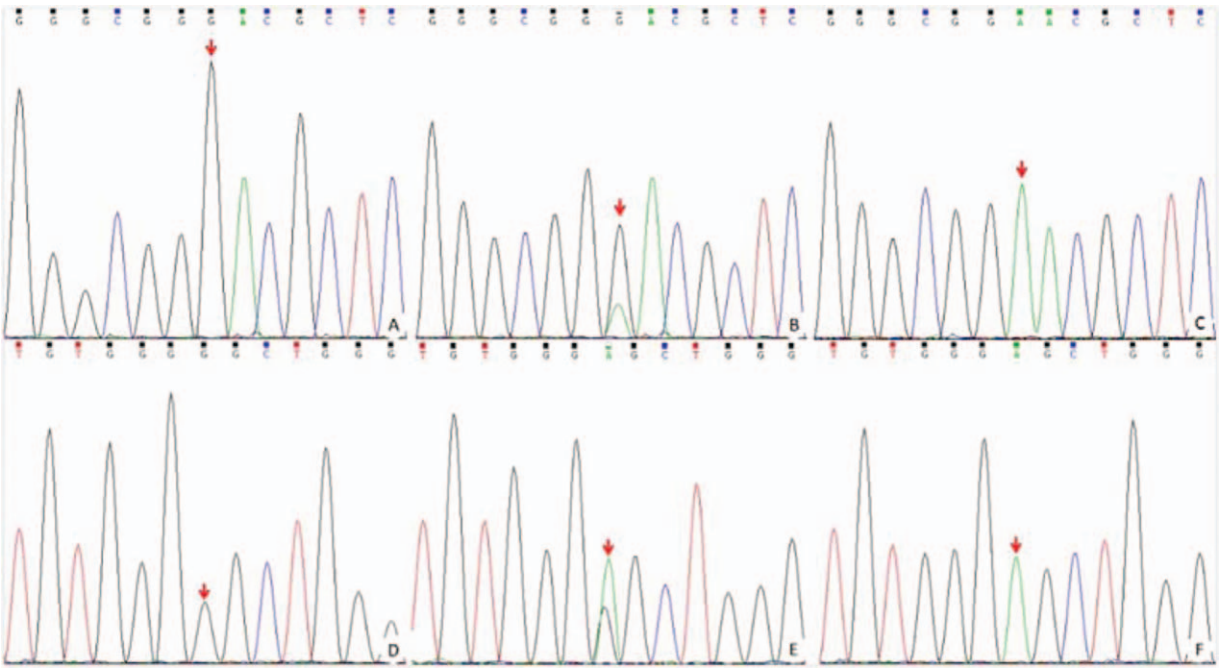

Figure 1. Expression of c-Fos protein was detected by western blot analysis in cancerous and adjacent normal tissues of different polymorphic loci. (A) Serum c-fos levels in different genotypes of rs7101 locus; (B) serum c-fos levels in different genotypes of rs1063169 locus.

Table 1

General clinical data of case group and healthy control group.

Parameters	Case group (n = 432)	Control group (n = 315)	P
Gender			.746
Male	267 (61.8%)	191 (60.6%)	
Female	165 (38.2%)	124 (39.4%)	
Age, y			.660
≤60	273 (63.2%)	204 (64.8%)	
>60	159 (36.8%)	111 (35.2%)	
Smoking			.711
Yes	151 (35.0%)	106 (33.7%)	
No	281 (65.0%)	209 (66.3%)	
Drinking			.720
Yes	121 (28.0%)	92 (29.2%)	
No	311 (72.0%)	223 (70.8%)	
BMI, kg/m^2			.240
<18.5	29 (6.7%)	22 (7.0%)	
18.5~24	246 (56.9%)	183 (58.1%)	
≥24	157 (36.3%)	110 (34.9%)	
Histological differentiation			
High	91 (21.1%)		
Medium	284 (65.7%)		
Low	57 (13.2%)		
Distant metastasis			
Yes	62 (14.4%)		
No	370 (85.6%)		
Lymph node metastasis			
Yes	217 (50.2%)		
No	215 (49.8%)		
Tumor location			
Colon	208 (48.1%)		
Rectum	224 (51.9%)		
TNM staging			
I	70 (16.2%)		
II	139 (32.2%)		
III	161 (37.3%)		
IV	62 (14.4%)		

TNM = tumor node metastasis.

2.4. Follow-up

Using the day of the patient's diagnosis as the first day of follow-up, the patient's state and the specific time of death were collected using telephone follow-up and home visits. The last recorded follow-up date was September 1, 2017.

2.5. Statistical analysis

SPSS v20.0 (SPSS Inc., Chicago) was used for statistical analysis in this study. Whether the *FOS* rs7101 and rs1063169 SNPs were in accordance with Hardy–Weinberg equilibrium was tested using the χ^2 test. The differences in demographic characteristics between the case group and the control group were analyzed using the χ^2 test. A multivariate logistic regression model was used to analyze the association of *FOS* polymorphisms with the risk of colorectal cancer, and it was adjusted for gender, age, smoking, alcohol intake, and BMI (body mass index). The effect of gene–environment interaction on the risk of colorectal cancer was analyzed by mutifactor dimensionality reduction (MDR). One-way analysis of variance (ANOVA) was used to compare the differences in the expression levels of c-Fos protein among different genotypes. We performed multivariate Cox regression analysis to assess factors such as age, gender, smoking, alcohol consumption, BMI, and the effects of *c-Fos* gene rs7101 and rs1063169 SNP on 3-year survival in patients with colorectal cancer. Survival analysis was performed using Kaplan–Meier curves, and log-Rank test was used to compare survival differences. All statistical analyses were 2-tailed, and $P < .05$ indicated that the difference was statistically significant.

3. Results

3.1. General clinical characteristics of the case group and the healthy control group

Four hundred thirty-two patients with colorectal cancer were enrolled in the case group, and 315 healthy subjects were enrolled in the control group. The general characteristics of the 2 groups are summarized in Table 1. The χ^2 test was used to compare and analyze the characteristics of the case group and the control group. The results showed that there was no statistically significant difference between the gender ratio, age, smoking, alcohol consumption, and BMI of the case group and the control group ($P > .05$). There were 208 cases (48.1%) of colon cancer and 224 cases (51.9%) of rectal cancer. Tissue differentiation, metastasis, and TNM staging are summarized in Table 1.

3.2. The relationship between the rs7101 and rs1063169 polymorphisms and susceptibility to colorectal cancer

The genotypes of the rs7101 and rs1063169 loci and allele frequencies of *FOS* in the case group and the control group are summarized in Table 2. The genotype and allele frequencies of the rs7101 and rs1063169 loci in the 2 groups all conformed to the Hardy–Weinberg balance ($P > .05$). The relationship between genotypes of rs7101 and rs1063169 and colorectal cancer was analyzed by logistic regression analysis, with gender, age, smoking status, alcohol consumption status, and BMI as the confounding factors. The results showed that rs7101 locus T allele carriers and rs1063169 locus T allele carriers had a higher risk of developing colorectal cancer (adjusted OR = 1.237, 95% CI = 1.131–1.346, $P \leq .001$ and adjusted OR = 1.218, 95% CI = 1.111–1.327, $P \leq .001$, respectively).

3.3. Interaction between c-fos gene rs7101, rs1063169 locus SNP, and environmental factors

The interaction of c-fos gene *rs7101* and rs1063169 SNP and age, sex, smoking, and alcohol consumption was analyzed by MDR. The results showed that rs7101 had the strongest interaction with age, followed by rs1063169 and alcohol consumption. The interaction between sex and smoking has a positive interaction effect on colorectal cancer risk (Fig. 2). The linkage disequilibrium analysis of *c-Fos* rs7101 and rs1063169 showed $D = 0$, $r^2 = 0.25$.

3.4. Association between c-Fos rs7101 and rs1063169 gene polymorphisms and staging of colorectal cancer

The frequency of *c-Fos* rs7101 T genotype in stage III/IV patients was significantly higher than that in stage I/II patients (adjusted OR = 1.230, 95% CI = 1.075–1.398, $P = .003$). CT and TT genotypes were risk factors for progression of colorectal cancer (adjusted OR = 1.394, 95% CI = 1.125–1.715, $P = .002$; adjusted OR = 1.329, 95% CI = 1.002–1.700, $P = .049$). The T allele at rs1063169 was a risk factor for the progression of colorectal cancer (adjusted OR = 1.372, 95% CI = 1.203–1.553, $P < .001$),

Table 2

The genotype and allele frequencies of rs7101 and rs1063169 in the case group and control group.

SNPs	Case group (n = 432)	Control group (n = 315)	*P*	Crude OR (95% CI)	*P*	Adjusted OR (95% CI)*
rs7101						
genotype						
CC	204 (47.2%)	194 (61.6%)		1		1
CT	153 (35.4%)	91 (28.9%)	.005	1.599 (1.140~2.244)	.006	1.223 (1.059~1.403)
TT	75 (17.4%)	30 (9.5%)	≤.001	2.377 (1.455~3.898)	≤.001	1.394 (1.170~1.606)
Alleles						
C	561 (64.9%)	479 (76.0%)		1		1
T	303 (35.1%)	151 (24.0%)	≤.001	1.713 (1.352~2.172)	≤.001	1.237 (1.131~1.346)
rs1063169						
genotype						
GG	217 (50.2%)	201 (63.8%)		1		1
GT	150 (34.7%)	86 (27.3%)	.004	1.616 (1.149~2.272)	.005	1.224 (1.062~1.399)
TT	65 (15.0%)	28 (8.9%)	.002	2.150 (1.293~3.589)	.004	1.346 (1.116~1.563)
Alleles						
G	584 (67.6%)	488 (77.5%)		1		1
T	280 (32.4%)	142 (22.5%)	≤.001	1.648 (1.294~2.099)	≤.001	1.218 (1.111~1.327)

*Adjusted according to factors such as gender, age, smoking, alcohol consumption, BMI.

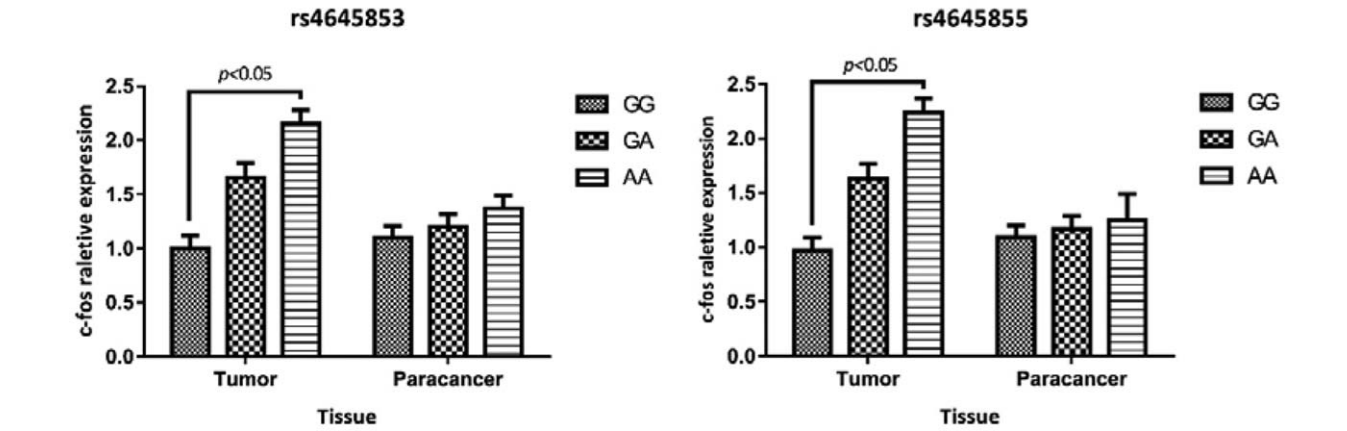

Figure 2. MDR analysis of the interaction between *c-Fos* gene *rs7101*, rs1063169 SNP, age, sex, smoking, alcohol consumption, and other factors. (A) Circle graph, "n%" indicates the size of the interaction, and a larger value indicates a stronger interaction. (B) Diagram of dendogram, red indicates strong interaction, and orange indicates weak interaction between them.

and TT genotype frequency was higher in patients with stage III/IV (adjusted OR = 1.722, 95% CI = 1.365–2.052, $P < .001$) (Table 3).

3.5. Effect of clinical parameters on the correlation between the rs7101 and rs1063169 polymorphisms and susceptibility to colorectal cancer

The relationship of the rs7101 and rs1063169 genotypes and colorectal cancer was analyzed by subgroup analysis (BMI, gender, age, smoking, and alcohol consumption). The results showed that the risk of colorectal cancer was significantly increased among the following subgroups harboring the rs7101 T allele: men, those aged >60 years, nonsmokers, nondrinkers, and individuals with a BMI ≥24 kg/m^2 ($P < .05$) (Table 4). This risk of colorectal cancer was significantly increased among the following subgroups harboring the rs1063169 T allele: women, those aged ≤60 years, smokers, those with alcohol use, and individuals with a BMI ≥24 kg/m^2 ($P < .05$) (Table 5).

3.6. Analysis of c-Fos protein expression

In order to further analyze the expression of c-Fos protein in each genotype, 16 cases of colorectal cancer were randomly selected, including 8 colon cancer and 8 rectal cancer cases, and Western blotting of cancerous tissue and corresponding normal mucosal tissue lysates was performed; meanwhile, the expression of c-Fos protein in serum was detected. (Figs. 1 and 3). Expression of c-Fos protein in colorectal cancer tissues and serum was significantly correlated with the rs7101 and rs1063169 genotypes, and the c-Fos protein expression was significantly higher in variants (CT +TT and GT+TT) than in wildtypes ($P < .05$). Moreover, the expression of c-Fos protein in patients with homozygous mutations (rs7101 TT and rs1063169 TT) was higher than that

Table 3

Association between *c-Fos rs2515621* and *rs10402762* gene polymorphisms and staging of colorectal cancer.

SNPs	I/II (209)	III/IV (223)	*P*	Crude OR (95% CI)	*P*	Adjusted OR (95% CI)*
rs7101						
genotype						
CC	116 (55.50%)	88 (39.46%)	1.00 (reference)			
CT	61 (29.19%)	92 (41.26%)	.001	1.988 (1.270–3.116)	.002	1.394 (1.125–1.715)
TT	32 (15.31%)	43 (19.28%)	.035	1.771 (1.003–3.133)	.049	1.329 (1.002–1.700)
Alleles						
C	293 (70.10%)	268 (60.09%)	1.00 (reference)			
T	125 (29.90%)	178 (39.91%)	.002	1.557 (1.162–2.086)	.003	1.230 (1.075–1.398)
rs1063169						
genotype						
GG	122 (58.37%)	95 (42.60%)	1.00 (reference)			
GT	71 (33.97%)	79 (35.43%)	.094	1.429 (0.921–2.219)	.116	1.203 (0.957–1.499)
TT	16 (7.66%)	49 (21.97%)	<.001	3.933 (2.023–7.722)	<.001	1.722 (1.365–2.052)
Alleles						
G	315 (75.36%)	269 (60.31%)	1.00 (reference)			
T	103 (24.64%)	177 (39.69%)	<.001	2.012 (1.486–2.725)	<.001	1.372 (1.203–1.553)

*Adjusted according to factors such as gender, age, smoking, alcohol consumption, BMI.

Table 4

Genotypes and allele frequencies of c-fos gene *rs7101* loci in different clinical parameter groups.

Parameters	Case group/ control group (432/315)	CC	CT	TT	C allele	T allele	Adjusted OR (95% CI)*	*P*
Gender								
Male	267/191	129/143	75/40	63/8	338/326	201/56	1.536 (1.388~1.679)	≤.001
Female	165/124	75/51	78/51	12/22	228/153	102/95	1.156 (0.986~1.369)	.077
Age, y								
≤60	273/204	183/154	72/38	18/12	438/346	108/62	1.137 (0.984~1.289)	.081
>60	159/111	21/40	81/53	57/18	123/133	195/89	1.429 (1.228~1.662)	≤.001
Smoking								
Yes	151/106	86/66	20/14	45/26	192/146	110/66	1.100 (0.938~1.275)	.250
No	281/209	118/128	133/77	30/4	369/333	193/85	1.321 (1.181~1.462)	≤.001
Drinking								
Yes	121/92	45/41	42/36	34/15	132/118	110/66	1.184 (0.994~1.398)	.059
No	311/223	159/153	111/55	41/15	429/361	193/85	1.278 (1.147~1.409)	≤.001
BMI, kg/m^2								
<18.5	29/22	15/12	9/7	5/3	39/31	19/13	1.066 (0.695~1.510)	.896
18.5~24	246/183	132/111	98/65	16/7	362/287	130/79	1.115 (0.972~1.262)	.121
≥24	157/110	57/71	46/19	54/20	160/161	154/59	1.451 (1.258~1.656)	≤.001

*Adjusted according to factors such as gender, age, smoking, alcohol consumption, BMI (Except for factors in this group).

Table 5

Genotype and allele frequencies of c-fos gene *rs1063169* loci in different clinical parameter groups.

Parameters	Case group/ control group (432/315)	GG	GT	TT	G allele	T allele	Adjusted OR (95% CI)*	*P*
Gender								
Male	267/191	134/110	101/58	32/23	369/278	165/104	1.075 (0.949~1.207)	.258
Female	165/124	83/91	49/28	33/5	215/210	115/38	1.486 (1.290~1.674)	≤.001
Age, y								
≤60	273/204	175/161	65/32	33/11	415/354	131/54	1.312 (1.158~1.460)	≤.001
>60	159/111	42/40	85/54	32/17	169/134	149/88	1.127 (0.972~1.301)	.115
Smoking								
Yes	151/106	75/88	60/12	16/6	210/188	92/24	1.503 (1.299~1.684)	≤.001
No	281/209	142/113	90/74	49/22	374/300	188/118	1.107 (0.983~1.238)	.094
Drinking								
Yes	121/92	41/61	42/25	38/6	124/147	118/37	1.664 (1.416~1.919)	≤.001
No	311/223	163/133	111/66	37/24	437/332	185/114	1.089 (0.970~1.212)	.152
BMI, kg/m^2								
<18.5	29/22	12/12	11/6	6/4	35/30	23/14	1.154 (0.777~1.618)	.544
18.5~24	246/183	109/87	102/80	35/16	320/254	172/112	1.086 (0.957~1.224)	.205
≥24	157/110	83/95	40/5	34/10	206/195	108/25	1.581 (1.382~1.757)	≤.001

*Adjusted according to factors such as gender, age, smoking, alcohol consumption, BMI (Except for factors in this group).

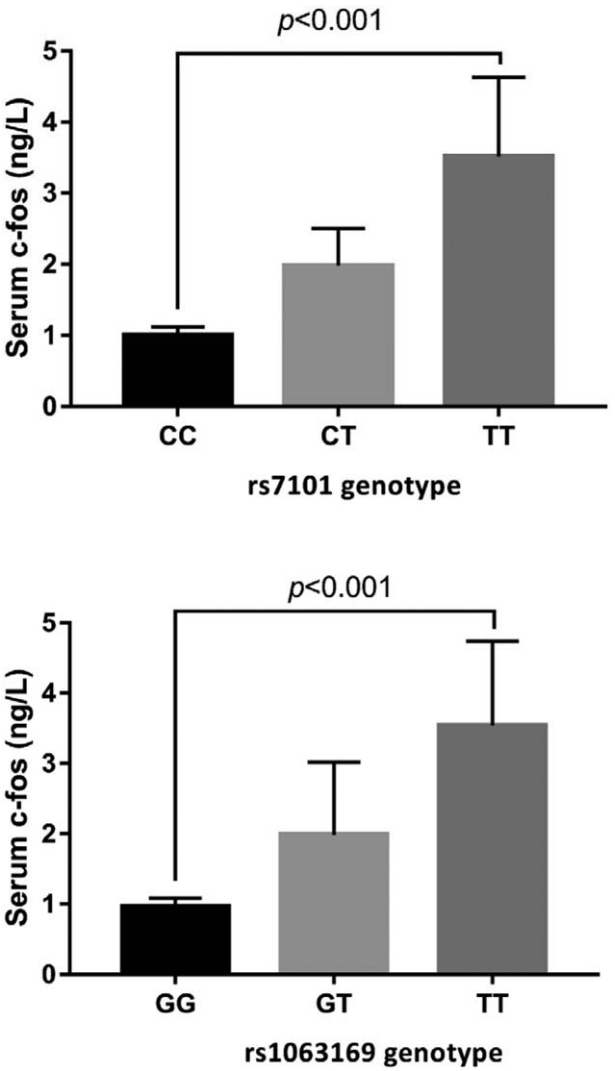

Figure 3. c-Fos protein levels in serum of subjects with different *FOS* rs7101 and rs1063169 genotypes.

in tumors with heterozygous mutations (rs7101 CT and rs1063169 GT) (Fig. 3).

3.7. Association of rs7101 and rs1063169 polymorphisms with survival of colorectal cancer patients

Multivariate Cox regression analysis showed that gender, age, smoking, alcohol consumption, BMI were not associated with 3-year survival in patients with colorectal cancer, and both rs7101 TT and rs1063169 TT were risk factors for 3-year survival in patients with colorectal cancer [hazard ratio (HR) = 2.38, 95% CI: 1.46–3.90, $P < .001$; HR = 2.15, 95% CI: 1.29–3.59, $P < .001$] (Table 6). The univariate survival function analysis showed that the 3-year survival of patients with the rs7101 CC genotype was 87.5%, and the median overall survival was 32.4 months. The 3-year survival of CT genotype patients was 69.8%, and the median overall survival was 26.5 months. The 3-year survival of TT genotype patients was 54.2%, and the median overall survival was 21.4 months. One-way ANOVA showed that the 3-year survival rates of patients harboring different rs7101 variants were significantly different ($P < .001$). The 3-year survival rate of wild-type homozygous patients was higher than that of heterozygous patients, and the 3-year survival rate of heterozygous patients was higher than that of variant homozygous patients.

Table 6

Mutivariate Cox regression analysis of the influence of general factors on the 3-year survival of patients with colorectal cancer.

Variants	HR (95% CI)	*P*
Gender	0.95 (0.54–1.42)	.74
Age	1.02 (0.97–1.09)	.41
Smoking	1.05 (0.97–1.34)	.35
Alcohol consumption	1.17 (0.99–1.35)	.24
BMI	1.05 (0.98–1.09)	.09
rs7101 TT	2.38 (1.46~3.90)	<.001
rs1063169 TT	2.15 (1.29~3.59)	<.001

Men were assigned "1" and women were assigned "2"; age >60 was assigned "1" and age <60 was assigned "2"; smoking was assigned "1" and nonsmoking was assigned "2"; drinking was assigned "1" and nondrinking was assigned "2"; BMI <24 kg/m^2 was assigned "1" and BMI >24 kg/m^2 was assigned "2."

The 3-year survival of patients with the rs1063169 GG genotype was 87.1%, and the median overall survival was 31.8 months. The 3-year survival of GT genotype patients was 68.7%, and the median overall survival was 26.1 months. The 3-year survival rate of TT genotype patients was 54.9%, and the median overall survival was 23.4 months. One-way ANOVA showed that the 3-year survival rates of patients harboring different rs1063169 variants were significantly different ($P < .001$). The 3-year survival rate of wild-type homozygous patients was higher than that of the heterozygous patients, but the 3-year survival rate of heterozygous patients and variant homozygous patients were not significantly different ($P > .05$) (Fig. 4).

4. Discussion

FOS, an oncogene located on human chromosome 14q21–31, encodes the nuclear protein c-Fos. It is a member of the immediate-early gene family. The c-Fos protein alone has no physiological function and must bind c-Jun to form a heterodimer (AP-1) with transcriptional activation activity.[10–12] AP-1 is closely related to the proliferation and differentiation of cells and plays an important role in the transformation and reversal of tumors.[13] The expression of c-Fos and c-Jun is low in normal tissues and high in many malignant tumors and cancerous processes.[14–16] At present, there are few reports on *FOS* polymorphisms or their relationship to susceptibility to and prognosis of colorectal cancer. In this study, only the rs7101 and rs1063169 polymorphisms were selected for analysis. These 2 SNP sites are in the noncoding region, which may participate in the regulation of *FOS*. Boyajyan et al[17] found that the rs7101 SNP is a risk factor for schizophrenia, and the rs1063169 SNP is a protective factor for schizophrenia. The expression of c-Fos protein in patients with schizophrenia is decreased compared with that in normal tissues. Similarly, in the study by Boyajjyan et al,[18] the T allele of the rs1063169 locus was found to reduce the risk of schizophrenia. This study found that the rs7101 and rs1063169 SNPs are risk factors for colorectal cancer, which is inconsistent with the findings of these previous studies. The authors believe that the *FOS* rs1063169 polymorphism may differ by ethnicity. The c-Fos protein expression level of the

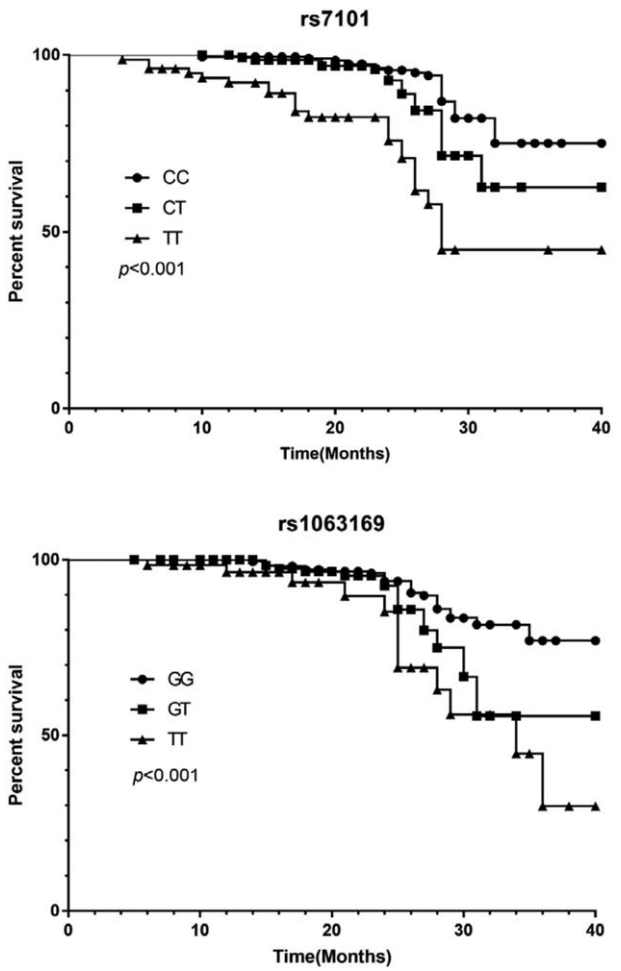

Figure 4. Survival of colorectal cancer by *FOS* rs7101 and rs1063169 genotype. The *P* value indicated the difference between the survival of the SNP locus in different genotypes. (A) Survival curve of different genotype colorectal cancer patients at rs7101 locus; (B) Survival curve of different genotype colorectal cancer patients at rs1063169 locus.

rs1063169 T allele in the Armenian population is lower than that of the G allele, but the reverse may be true in the Chinese population.

Tumorigenesis is often caused by a combination of genetic and environmental factors.[19] Therefore, there is great clinical significance in studying the interaction between genes and the environment on the risk of colorectal cancer. The risk factors for colorectal cancer in this study are gender, age, smoking, and alcohol consumption.[20] The results showed that in the male, older than 60, no smoking, no alcohol consumption, and BMI ≥24 subgroups, the risk of colorectal cancer with the rs7101 T allele was increased 1.536 times, 1.429 times, 1.321 times, 1.278 times, and 1.451 times, respectively, while the risk of colorectal cancer with the rs1063169 T allele was increased in the female, younger than 60, smoking, alcohol consumption, and BMI ≥24 subgroups 1.486 times, 1.312 times, 1.503 times, 1.664 times, and 1.581 times, respectively. The results of the study indicate that environment is also important in the process of genetic factors affecting the development of colorectal cancer. In the prevention of colorectal cancer, attention must be paid to the influence of internal factors (genes) and external factors (environments) on the disease. MDR methods are commonly used to analyze gene–gene and gene–environment interactions.[21] We used MDR to analyze the interaction between c-fos gene *rs7101* and rs1063169 SNPs and age, sex, smoking, and drinking. The results showed that the interaction between rs7101 locus and age was the strongest.

In order to further analyze the intrinsic mechanism of the influence of *FOS* polymorphisms on the susceptibility of colorectal cancer, the present study analyzed the expression of c-Fos protein in cancer tissues and adjacent normal tissueous tissues of patients with different genotypes. Because it was difficult to obtain tissue samples from rectal cancer patients, only 16 patients with colorectal cancer were selected for analysis in this study (8 patients with colon cancer and 8 with rectal cancer) to exclude the influence of different cancer tissue types. The results showed that the expression of c-Fos protein in colorectal cancer tissues was significantly related to the rs7101 and rs1063169 locus genotypes, and the expression level of c-Fos protein increased significantly in variant cancer tissue, compared with adjacent normal tissues. We believe that the rs7101 and rs1063169 mutations affect the regulation of c-Fos protein expression. Variant c-Fos protein expression is lower than wild-type protein expression. *FOS* is homologous to an oncogene in the FBJ and FBR mouse osteosarcoma viruses. Under normal conditions, c-Fos protein is in a low expression state. In recent years, it has been reported that abnormal expression of c-Fos in mammalian epithelial cells results in loss of epithelial cell polarity and the transformation between epithelial cells and fibroblastoid cells.[22,23] Some studies have also shown that the expression of c-Fos protein in cervical cancer is significantly increased.[24] Combined with the results of this study, this suggests that high expression of c-Fos may be a marker of tumorigenesis. As a nuclear proto-oncogene, abnormally high expression of c-Fos can lead to cell differentiation and tumor formation.

In addition, we performed a follow-up of the case group for 3 years, which showed that wild-type patients with c-fos gene *rs7101* and *rs1063169* have better prognosis than variant patients. The results also showed that the prognostic survival of colorectal cancer patients was correlated with rs7101 and rs1063169 SNPs of *c-Fos*. Although there are few studies on the correlation between the expression of c-Fos protein and the prognosis and survival of colorectal cancer, and at present, there are no cases reported in the TCGA database on polymorphisms of the *c-Fos* rs7101 and rs1063169 loci in colorectal cancer patients, the expression of c-Fos protein is related to the prognosis of colorectal cancer. For example, Jin et al[25] showed that loss of c-fos expression was associated with more advanced stage, lymph node metastasis, lymphatic invasion, and shorter survival, suggesting loss of c-fos expression in gastric cancer cells during progression, and this loss was associated with poor prognosis. Loss of c-fos expression has tumor suppressor activity in gastric cancer, suggesting that c-fos may have pro-apoptotic function.

There are several shortcomings in this study. Due to the limitation of objective conditions, no large-scale screening for SNPs was performed in this study. There are numerous SNPs in *FOS*, and there may be other SNPs related to the risk of disease and survival in colorectal cancer. In the TCGA database, only 19 cases of *c-Fos* mutations were reported, and only 5 SNPs were included. A large number of SNPs remain to be discovered. Therefore, it is necessary to use bioinformatics to screen onset risk-related SNPs. Furthermore, the limited source of tissue

samples in this study may have an impact on the objectivity of the analysis results.

5. Conclusion

The rs7101 and rs1063169 polymorphisms in noncoding regions of *FOS* are associated with the risk of colorectal cancer onset. The survival rate of colorectal cancer patients harboring these variants was significantly lower than that of patients with wild-type *FOS*, which may be due to the higher expression of c-Fos protein in patients with rs7101 and rs1063169 mutations, which promotes the occurrence and development of colorectal cancer, but its specific mechanism needs further study. In addition, screening for other *FOS* SNPs and analyzing their association with risk of colorectal cancer and colorectal cancer prognosis is a very clinically valuable research question. In order to better prevent and treat colorectal cancer and reduce its incidence, it is necessary to discover and study more SNP loci associated with the pathogenesis of colorectal cancer.

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