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Original Article

Association between *ICOS* polymorphisms and risk of colorectal cancer: a case-control study involving 2,606 subjects

Jiakai Jiang[1*], Weifeng Tang[2*], Chao Liu[2*], Yafeng Wang[3], Sheng Zhang[1], Yu Chen[4,5,6]

[1]Department of General Surgery, Changzhou No. 3 People's Hospital, Changzhou, Jiangsu Province, China; [2]Department of Cardiothoracic Surgery, Affiliated People's Hospital of Jiangsu University, Zhenjiang, Jiangsu Province, China; [3]Department of Cardiology, The People's Hospital of Xishuangbanna Dai Autonomous Prefecture, Jinghong, Yunnan Province, China; [4]Cancer Bio-immunotherapy Center, [5]Department of Medical Oncology, Fujian Cancer Hospital & Fujian Medical University Cancer Hospital, Fuzhou, Fujian Province, China; [6]Fujian Provincial Key Laboratory of Translational Cancer Medicine, Fuzhou, Fujian Province, China. [*]Equal contributors.

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Abstract: Functional variants in *inducible T cell costimulator* (*ICOS*) gene are predicted to be associated with the susceptibility of colorectal cancer (CRC). In this study, we enrolled 2,606 participants (involving 1,003 CRC cases and 1,303 healthy controls) and conducted a case-control study to explore the potential relationship of *ICOS* rs4404254 T>C and rs10932029 T>C polymorphisms with the risk of CRC. A custom-by-design 48-Plex SNPscan Kit was used to obtain the genotypes of *ICOS* rs4404254 T>C and rs10932029 T>C variants. We found that *ICOS* rs10932029 T>C polymorphism was associated with risk of CRC in several subgroups (female subgroup: CC vs. TT: adjusted OR = 6.49, 95% CI 1.36-30.90, P = 0.019 and CC vs. CT/TT: adjusted OR = 6.38, 95% CI 1.34-30.32, P = 0.020; < 61 years subgroup: CC vs. TT: adjusted OR = 4.23, 95% CI 1.10-16.24, P = 0.036 and CC vs. CT/TT: adjusted OR = 4.20, 95% CI 1.10-16.09, P = 0.036; never smoking subgroup: CC vs. TT: adjusted OR = 2.82, 95% CI 1.04-7.64, P = 0.041 and CC vs. CT/TT: adjusted OR = 2.83, 95% CI 1.05-7.66, P = 0.041 and BMI ≥ 24 subgroup: CC vs. TT: adjusted OR = 6.81, 95% CI 1.39-33.30, P = 0.018 and CC vs. CT/TT: adjusted OR = 6.79, 95% CI 1.39-33.11, P = 0.018). In addition, we found that *ICOS* rs4404254 T>C polymorphism was associated with the susceptibility of CRC in never smoking subgroup (CC/TC vs. TT: adjusted OR = 1.23, 95% CI 1.01-1.51, P = 0.045). In summary, our findings suggest that *ICOS* rs10932029 T>C and *ICOS* rs4404254 T>C polymorphisms may be associated with the risk of CRC. In the future, a fine-mapping study with a functional evaluation is needed to explore the relationship between *ICOS* polymorphisms and the risk of CRC.

Keywords: *ICOS*, polymorphism, colorectal cancer, risk

Introduction

Colorectal cancer (CRC) remains a common public health problem, accounting for over 1.4 million newly diagnosed CRC patients in 2012 worldwide [1]. In China alone, there were approximately 376,300 CRC cases and 191,000 people died from CRC in 2015 [2]. The etiology of CRC remains unclear. Individuals with similar lifestyles, for example, only a small proportion of them might progress to CRC. Thus, the incidence of CRC may be influenced by various environmental factors, and an individual's genetic background. Recently, a number of studies have focused on the potential role of the immune system on the development of CRC (immune responses and individual's gene regulation or variants) [3-5]. Thus, investigation of host immune response-related polymorphisms could lead to novel insights into host immune responses in developing CRC.

It is reported that the CD28 family plays a vital role in human T-lymphocyte-dependent humoral immunity. Inducible T cell costimulator (ICOS), a member of the CD28 family, is expressed on activated T cells, and then forms homodimers to regulate cell signaling transduction, immune responses, and cell proliferation [6, 7]. Due to ICOS sharing homology with CD28, early

Table 1. Distribution of selected characteristics in CRC cases and controls

Variable	Cases (n = 1,003)		Controls (n = 1,303)		P^a
	n	%	n	%	
Age (years)	61.10 (± 12.17)		61.40 (± 9.61)		0.496
Age (years)					0.605
< 61	451	44.97	600	46.05	
≥ 61	552	55.03	703	53.95	
Sex					0.867
Male	620	61.81	801	61.47	
Female	383	38.19	502	38.53	
Smoking status					**0.002**
Never	744	74.18	1038	79.66	
Ever	259	25.82	265	20.34	
Alcohol use					**< 0.001**
Never	829	82.65	1,167	89.56	
Ever	174	17.35	136	10.44	
BMI (kg/m^2)					
< 24	670	66.80	688	52.80	**< 0.001**
≥ 24	333	33.20	615	47.20	
Site of tumor					
Colon cancer	431	42.97			
Rectum cancer	572	57.03			

[a]Two-sided X^2 test and student t test; Bold values are statistically significant ($P < 0.05$). BMI: body mass index.

research sought to characterize the potential role of ICOS in T-lymphocyte proliferation and activation. Interestingly, some previous studies suggested that ICOS-deficient T-cells might lead to a significant proliferation defect *in vitro* when compared to wild-type $CD4^+$ T-lymphocytes [8, 9]. A recent study indicated that expression of ICOS improved prognosis in CRC patients and the percentage of $ICOS^{(+)}CD4^{(+)}$ cells acting as Th1 cells [10]. Thus, ICOS may interact with its ligand (ICOSL) and influence the development of CRC. The *ICOS* gene is located on chromosome 2 (position: 203936748-203961577) in humans. The *ICOS* gene is polymorphic. There are more than 5,000 single-nucleotide polymorphisms (SNPs) in *ICOS* gene which have been identified (https://www.ncbi.nlm.nih.gov/snp/?term=ICOS), such as ICOS rs10932029 T>C, rs10932037 C>T, rs4675379 G>C, rs4404254 T>C and rs10183087 A>C polymorphisms, etc. Among these SNPs, *ICOS* rs4404254 T>C and rs10932029 T>C SNPs have been extensively investigated for their susceptibility to cancer [11-14]. But these previous studies reported inconsistent findings rather than conclusive evidence. The aim of this case-control study was to assess the association between *ICOS* polymorphisms and risk of CRC.

Materials and methods

Study subjects

The protocol of this case-control study was approved by the Medical Ethics Committee at Fujian Medical University and Jiangsu University and conformed to the Declaration of Helsinki. Prior to recruitment, each participant signed an informed consent. Enrollees from Fujian and Jiangsu province of China were accepted after seeking treatment at Fujian Medical University Union Hospital or Affiliated People's Hospital of Jiangsu University between October 2014 and August 2017. The diagnosis of CRC patients was made based on pathology. We enrolled a total of 1,003 patients with CRC (620 males, 383 females), with a mean age of 61.10 ± 12.17 years. And 1,303 healthy controls (801 males and 502 females) were also recruited, with a mean age of 61.40 ± 9.61 years. The controls were matched with CRC cases with respect to gender, age, geographic origin, and ethnic background. Demographics and risk factors were obtained by using a questionnaire. Overweight and obesity were defined as body mass index (BMI) ≥ 24 [15, 16].

DNA extraction and genotyping

Ethylenediamine tetraacetic acid (EDTA)-anticoagulated venous blood donated by all participants was harnessed to isolate genomic DNA by using a Promega DNA Blood Mini Kit (Promega, Madison, USA). A custom-by-design 48-Plex SNPscan Kit (Genesky Biotechnologies Inc., Shanghai, China) was used to obtain the genotypes of *ICOS* rs4404254 T>C and rs10932029 T>C SNPs as previously described [17]. For quality control, ninety-two DNA samples were randomly selected and analyzed by SNPscan Kit. The obtained genotypes were not changed.

Statistical analysis

The demographic and selected risk factors between CRC cases and controls were com-

Table 2. Primary information for *ICOS* polymorphisms

Genotyped SNPs	Chromosome	Chr Pos (NCBI Build 37)	Region	MAF[a] for Chinese in database	MAF in our controls (n = 782)	*P* value for HWE[b] test in our controls	Genotyping method	Genotyping value (%)
ICOS rs10932029 T>C	2	204801768	Intron1	0.084	0.100	0.538	SNPscan	98.87
ICOS rs4404254 T>C	2	204825286	3'UTR	0.131	0.168	0.295	SNPscan	98.87

[a]MAF: minor allele frequency; [b]HWE: Hardy-Weinberg equilibrium.

pared by using X^2 test or Fisher exact tests. An online goodness-of-fit Chi-square test software (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) was used to assess whether the genotype distributions of *ICOS* rs4404254 T>C and rs10932029 T>C polymorphisms were in Hardy-Weinberg equilibrium (HWE). Genotype frequencies of *ICOS* rs4404254 T>C and rs10932029 T>C polymorphisms in CRC cases and controls were also compared by X^2 test or Fisher exact tests. The potential association between *ICOS* rs4404254 T>C and rs10932029 T>C polymorphisms and CRC risk were estimated as odds ratios (ORs) with their 95% intervals (CIs). The interaction of gene and environment in the risk of CRC was determined by conditional logistic regression. *P* value < 0.05 (two-tailed) was considered as significant. All data analyses were carried out by using the SAS version 9.4 statistical software (SAS Institute, Cary, USA).

Results

Study characteristics

The demographic variables and selected risk factors are listed in **Table 1**. We found no difference between CRC cases and healthy controls considering sex (*P* = 0.867), age (*P* = 0.496), suggesting that the two groups were well matched. When regarding alcohol consumption, BMI and cigarette use, we found there were significant differences (*P* < 0.001, *P* < 0.001 and *P* = 0.002, respectively). The primary information of *ICOS* rs4404254 T>C and rs10932029 T>C SNPs is shown in **Table 2**. Genotype distributions of *ICOS* rs4404254 T>C and rs10932029 T>C in controls were in accordance with HWE (*P* = 0.295 and 0.538, respectively).

Association of ICOS rs4404254 T>C and rs10932029 T>C SNPs with CRC risk

Genotype distributions of *ICOS* rs4404254 T>C and rs10932029 T>C SNPs are listed in **Table 3**. The genotype frequencies of *ICOS* rs4404254 T>C polymorphism were 65.41% (TT), 31.02% (TC) and 3.57% (CC) in CRC cases and 69.62% (TT), 27.15% (TC) and 3.23% (CC) in controls. When the frequency of *ICOS* rs4404254 TT genotype was considered as reference, individuals carrying the *ICOS* rs4404254 TC genotype had a tendency of increased risk to CRC (crude OR = 1.18, 95% CI = 0.98-1.41 for TC vs. TT, *P* = 0.080). When compared with the frequency of *ICOS* rs4404254 TT genotype, individuals carrying the *ICOS* rs4404254 CC genotype were not associated with the risk of CRC (crude OR = 1.14, 95% CI = 0.72-1.80 for CC vs. TT, *P* = 0.578). When the frequency of *ICOS* rs4404254 TT genotype was used as reference, individuals carrying the *ICOS* rs4404254 TC/CC genotype significantly increased the susceptibility of CRC (crude OR = 1.21, 95% CI = 1.02-1.45 for TC/CC vs. TT, *P* = 0.033). When compared with the frequency of *ICOS* rs4404254 TC/TT genotype, individuals carrying the CC genotype were not associated with the risk of CRC (crude OR = 1.11, 95% CI = 0.70-1.75 for CC vs. TT/TC, *P* = 0.654). Adjustments for smoking, BMI, age, sex and drinking, a tendency of increased risk to CRC was also found (adjusted OR = 1.15, 95% CI = 0.96-1.39 for TC vs. TT, *P* = 0.141; adjusted OR = 1.07, 95% CI = 0.67-1.71 for CC vs. TT, *P* = 0.767, adjusted OR = 1.18, 95% CI = 0.98-1.41 for TC/CC vs. TT, *P* = 0.074 and adjusted OR = 1.05, 95% CI = 0.66-1.67 for CC vs. TT/TC, *P* = 0.831; **Table 3**).

The genotype frequencies of *ICOS* rs10932029 T>C SNP were 78.57% (TT), 19.90% (TC) and 1.53% (CC) in CRC cases and 81.62% (TT), 17.62% (TC) and 0.77% (CC) in controls. When the frequency of *ICOS* rs10932029 TT genotype was used as reference, individuals carrying the *ICOS* rs10932029 TC genotype were not associated with the risk of CRC (crude OR = 1.14, 95% CI = 0.92-1.41 for TC vs. TT, *P* = 0.218). When compared with the frequency of *ICOS* rs10932029 TT genotype, individuals carrying the *ICOS* rs10932029 CC genotype

Table 3. Logistic regression analyses of associations between *ICOS* polymorphisms and the risk of overall CRC

Genotype	Cases (n = 1,003)		Controls (n = 1,303)		Crude OR (95% CI)	*P*	Adjusted OR[a] (95% CI)	*P*[a]
	n	%	n	%				
rs10932029 T>C								
TT	770	78.57	1,061	81.62	1.00		1.00	
TC	195	19.90	229	17.62	1.14 (0.92-1.41)	0.218	1.11 (0.89-1.37)	0.354
CC	15	1.53	10	0.77	2.01 (0.90-4.50)	0.089	1.92 (0.85-4.36)	0.117
TC+CC	210	21.43	239	18.38	1.21 (0.98-1.49)	0.071	1.17 (0.95-1.45)	0.139
TT+TC	965	98.47	1,290	99.23	1.00		1.00	
CC	15	1.53	10	0.77	2.01 (0.90-4.48)	0.090	1.93 (0.85-4.37)	0.116
C allele	225	11.48	249	9.58				
rs4404254 T>C								
TT	641	65.41	905	69.62	1.00		1.00	
TC	304	31.02	353	27.15	1.18 (0.98-1.41)	0.080	1.15 (0.96-1.39)	0.141
CC	35	3.57	42	3.23	1.14 (0.72-1.80)	0.578	1.07 (0.67-1.71)	0.767
TC+CC	339	34.59	395	30.38	**1.21 (1.02-1.45)**	**0.033**	1.18 (0.98-1.41)	0.074
TT+TC	945	96.43	1,258	96.77	1.00		1.00	
CC	35	3.57	42	3.23	1.11 (0.70-1.75)	0.654	1.05 (0.66-1.67)	0.831
C allele	374	19.08	437	16.81				

[a]Adjusted for age, sex, BMI, smoking and drinking status; Bold values are statistically significant ($P < 0.05$).

had a tendency of increased risk to CRC (crude OR = 2.01, 95% CI = 0.90-4.50 for CC vs. TT, *P* = 0.089). When the frequency of *ICOS* rs10932029 TT genotype was used as reference, individuals carrying the *ICOS* rs109320-29 TC/CC genotype also had a tendency of increased risk to CRC (crude OR = 1.21, 95% CI = 0.98-1.49 for TC/CC vs. TT, *P* = 0.071). When *ICOS* rs10932029 TC/TT genotype was considered as reference, individuals carrying the CC genotype also had a tendency of increased susceptibility to CRC (crude OR = 2.01, 95% CI = 0.90-4.48 for CC vs. TT/TC, *P* = 0.090). However, after adjusting for smoking, BMI, age, sex, and drinking, the association between *ICOS* rs10932029 T>C polymorphism and risk of CRC was not found (adjusted OR = 1.11, 95% CI = 0.89-1.37 for TC vs. TT, *P* = 0.354; adjusted OR = 1.92, 95% CI = 0.85-4.36 for CC vs. TT, *P* = 0.117, adjusted OR = 1.17, 95% CI = 0.95-1.45 for TC/CC vs. TT, *P* = 0.139 and adjusted OR = 1.93, 95% CI = 0.85-4.37 for CC vs. TT/TC, *P* = 0.116; **Table 3**).

Association of ICOS rs4404254 T>C and rs10932029 T>C SNPs with CRC risk in a stratified analysis

To further assess the correlation of *ICOS* rs4404254 T>C and rs10932029 T>C SNPs with CRC susceptibility, a detailed stratified analysis was carried out by gender, age, BMI, smoking, and drinking. **Table 4** summarizes the genotype frequencies of *ICOS* rs10932029 T>C variants in different subgroups. After adjusting by gender, age, BMI, smoking, and drinking, we found that *ICOS* rs10932029 T>C polymorphism was associated with development of CRC in several subgroups [female subgroup: CC vs. TT: adjusted OR = 6.49, 95% CI 1.36-30.90, *P* = 0.019 and CC vs. CT/TT: adjusted OR = 6.38, 95% CI 1.34-30.32, *P* = 0.020; < 61 years subgroup: CC vs. TT: adjusted OR = 4.23, 95% CI 1.10-16.24, *P* = 0.036 and CC vs. CT/TT: adjusted OR = 4.20, 95% CI 1.10-16.09, *P* = 0.036; never smoking subgroup: CC vs. TT: adjusted OR = 2.82, 95% CI 1.04-7.64, *P* = 0.041 and CC vs. CT/TT: adjusted OR = 2.83, 95% CI 1.05-7.66, *P* = 0.041 and BMI ≥ 24 subgroup: CC vs. TT: adjusted OR = 6.81, 95% CI 1.39-33.30, *P* = 0.018 and CC vs. CT/TT: adjusted OR = 6.79, 95% CI 1.39-33.11, *P* = 0.018 (**Table 4**)].

Table 5 presents the genotype frequencies of *ICOS* rs4404254 T>C variants in different subgroups. Adjustment by conditional logistic regression with gender, age, BMI, smoking, and drinking, we found that *ICOS* rs4404254 T>C polymorphism was associated with develop-

Table 4. Stratified analyses between *ICOS* rs10932029 T>C polymorphism and CRC risk by sex, age, BMI, smoking status and alcohol consumption

Variable	*ICOS* rs10932029 T>C (case/control)[a]			Adjusted OR[b] (95% CI); *P*			
	TT	TC	CC	Additive model	Homozygote model	Dominant model	Recessive model
Sex							
Male	478/646	120/145	6/8	1.05 (0.80-1.38); *P*: 0.713	0.91 (0.31-2.69); *P*: 0.859	1.08 (0.82-1.41); *P*: 0.590	0.92 (0.31-2.72); *P*: 0.878
Female	292/415	75/84	9/2	1.20 (0.85-1.71); *P*: 0.303	6.49 (1.36-30.90); *P*: 0.019	1.35 (0.96-1.90); *P*: 0.087	6.38 (1.34-30.32); *P*: 0.020
Age							
< 61	342/482	92/113	9/3	1.12 (0.82-1.53); *P*: 0.489	4.23 (1.10-16.24); *P*: 0.036	1.22 (0.90-1.66); *P*: 0.209	4.20 (1.10-16.09); *P*: 0.036
≥ 61	428/579	103/116	6/7	1.13 (0.84-1.52); *P*: 0.423	1.10 (0.36-3.33); *P*: 0.872	1.16 (0.87-1.56); *P*: 0.308	1.10 (0.36-3.34); *P*: 0.869
Smoking status							
Never	575/849	141/180	12/6	1.10 (0.86-1.40); *P*: 0.472	2.82 (1.04-7.64); *P*: 0.041	1.18 (0.93-1.50); *P*: 0.183	2.83 (1.05-7.66); *P*: 0.041
Ever	195/212	54/49	3/4	1.15 (0.74-1.78); *P*: 0.542	0.74 (0.16-3.43); *P*: 0.695	1.16 (0.75-1.78); *P*: 0.509	0.74 (0.16-3.42); *P*: 0.695
Alcohol consumption							
Never	642/952	155/204	13/8	1.08 (0.86-1.37); *P*: 0.515	2.31 (0.94-5.67); *P*: 0.067	1.16 (0.92-1.45); *P*: 0.214	2.33 (0.95-5.69); *P*: 0.065
Ever	128/109	40/25	2/2	1.30 (0.74-2.29); *P*: 0.367	0.73 (0.10-5.47); *P*: 0.756	1.29 (0.74-2.25); *P*: 0.365	0.70 (0.09-5.28); *P*: 0.733
BMI (kg/m^2)							
< 24	514/549	134/129	8/8	1.07 (0.82-1.41); *P*: 0.608	1.01 (0.37-2.72); *P*: 0.993	1.09 (0.84-1.43); *P*: 0.506	1.01 (0.37-2.72); *P*: 0.987
≥ 24	256/512	61/100	7/2	1.17 (0.82-1.66); *P*: 0.398	6.81 (1.39-33.30); *P*: 0.018	1.32 (0.93-1.86); *P*: 0.118	6.79 (1.39-33.11); *P*: 0.018

[a]The genotyping was successful in 980 (97.71%) CRC cases and 1,300 (99.77%) controls for *ICOS* rs10932029 T>C; [b]Adjusted for age, sex, BMI, smoking status and alcohol consumption (besides stratified factors accordingly) in a logistic regression model.

Table 5. Stratified analyses between *ICOS* rs4404254 T>C polymorphism and CRC risk by sex, age, BMI, smoking status and alcohol consumption

Variable	*ICOS* rs4404254 T>C (case/control)[a]			Adjusted OR[b] (95% CI); *P*			
	TT	TC	CC	Additive model	Homozygote model	Dominant model	Recessive model
Sex							
Male	401/564	184/207	19/28	1.19 (0.93-1.51); *P*: 0.162	0.82 (0.45-1.51); *P*: 0.532	1.18 (0.94-1.49); *P*: 0.155	0.80 (0.44-1.47); *P*: 0.479
Female	240/341	120/146	16/14	1.11 (0.82-1.49); *P*: 0.509	1.62 (0.76-3.43); *P*: 0.210	1.18 (0.88-1.57); *P*: 0.267	1.59 (0.75-3.35); *P*: 0.223
Age							
< 61	277/414	148/166	18/18	1.24 (0.94-1.63); *P*: 0.128	1.38 (0.70-2.73); *P*: 0.358	1.28 (0.98-1.67); *P*: 0.067	1.31 (0.66-2.58); *P*: 0.438
≥ 61	364/491	156/187	17/24	1.07 (0.83-1.38); *P*: 0.621	0.87 (0.46-1.66); *P*: 0.671	1.08 (0.85-1.39); *P*: 0.522	0.88 (0.46-1.66); *P*: 0.685
Smoking status							
Never	465/718	237/287	26/30	1.19 (0.97-1.47); *P*: 0.104	1.26 (0.73-2.18); *P*: 0.399	1.23 (1.01-1.51); *P*: 0.045	1.22 (0.71-2.10); *P*: 0.470
Ever	176/187	67/66	9/12	1.00 (0.67-1.50); *P*: 0.994	0.69 (0.28-1.70); *P*: 0.415	0.99 (0.67-1.46); *P*: 0.960	0.71 (0.29-1.73); *P*: 0.447
Alcohol consumption							
Never	532/816	251/312	27/36	1.18 (0.96-1.44); *P*: 0.116	1.08 (0.65-1.81); *P*: 0.768	1.20 (0.99-1.46); *P*: 0.065	1.05 (0.63-1.76); *P*: 0.848
Ever	109/89	53/41	8/6	0.99 (0.60-1.64); *P*: 0.980	1.08 (0.36-3.26); *P*: 0.899	1.04 (0.64-1.68); *P*: 0.870	1.10 (0.37-3.30); *P*: 0.864
BMI (kg/m^2)							
< 24	423/465	208/198	25/23	1.13 (0.89-1.43); *P*: 0.327	1.14 (0.63-2.04); *P*: 0.665	1.16 (0.92-1.46); *P*: 0.207	1.12 (0.63-2.00); *P*: 0.706
≥ 24	218/440	96/155	10/19	1.19 (0.88-1.62); *P*: 0.253	0.99 (0.45-2.18); *P*: 0.977	1.22 (0.91-1.63); *P*: 0.195	0.96 (0.44-2.11); *P*: 0.927

[a]The genotyping was successful in 980 (97.71%) CRC cases and 1,300 (99.77%) controls for *ICOS* rs4404254 T>C; [b]Adjusted for age, sex, BMI, smoking status and alcohol consumption (besides stratified factors accordingly) in a logistic regression model.

ment of CRC in the never smoking subgroup: CC/TC vs. TT: adjusted OR = 1.23, 95% CI 1.01-1.51, P = 0.045 (**Table 5**).

Discussion

The etiology of CRC is very complicated, in which the individual's hereditary factor may play a vital role. Although some susceptibility genes suggest potent associations with the hereditary nonpolyposis CRC, many low-penetrant susceptibility factors involving SNPs predisposing to CRC remain to be elucidated. Recently, a pooled-analysis highlighted that variants of Cytotoxic T lymphocyte antigen-4 (CTLA-4) might influence the risk of CRC, which suggests the important role of costimulatory molecules in the development of CRC [18]. Additionally, ICOS and CTLA-4 are both located on chromosome 2q33. CTLA-4 blockade has been proven to be an active immunotherapeutic strategy in cancer. CTLA-4 blockade results in a higher frequency of $CD4^{+}ICOS^{hi}$ T cell and elevated IFN-gamma levels in both nonmalignant and malignant tissues, indicating that ICOS may interact with CTLA-4, and then plays a vital role in tumor immunity [19]. Several studies have focused on the relationship of *ICOS* rs10932029 T>C and rs4404254 T>C polymorphisms with susceptibility of cancer [11-14]. However, the results remain conflicting. In this study, we found that *ICOS* rs10932029 T>C polymorphism was associated with the development of CRC in female, < 61 years, never smoking, and BMI ≥ 24 subgroups. In addition, we found that *ICOS* rs4404254 T>C polymorphism was associated with the risk of CRC in the never smoking subgroup.

In *ICOS* rs10932029 T>C, we found that CC genotype in *ICOS* is relevant to an increased risk of CRC among female, < 61 years, never smoking, and BMI ≥ 24 patients. Xu et al. reported that *ICOS* rs10932029 was associated with the development of breast cancer (BC) in Chinese women, and the C allele may be a susceptibility factor in BC [13], suggesting that *ICOS* rs10932029 C allele may be related to a decrease activity of T cell. We also found that *ICOS* rs10932029 C allele was probably increased the risk of CRC, which was very similar to the previous study. *ICOS* rs10932029 T>C locates on intron region. Although this SNP is a non-coding polymorphism, it is proposed that a T→C substitution may influence expression of ICOS protein by altering gene splicing. *ICOS* rs10932029 T>C may accordingly confer susceptibility to CRC through these potential mechanisms, and this case-control study suggested that *ICOS* rs10932029 CC genotype and C allele could have a significant impact on colorectal carcinogenesis. Furthermore, we presumed that our findings could be explained by aberrant expression of ICOS in the presence of a C allele. *ICOS* rs4404254 T>C locates on 3'UTR region. In the current study, we found a potential association between *ICOS* rs4404254 T>C polymorphism and the development of CRC in the never smoking subgroup. A previous study indicated that *ICOS* rs4404254 CC genotype may increase the risk of cervical cancer [12], which is very analogous to our findings. However, Wu et al. reported that *ICOS* rs4404254 T>C might decrease the risk of CRC [11]. Clearly, these ambiguous results showed that the function of *ICOS* rs4404254 T>C polymorphism might be altered in different ethnicity or even influence by the environmental factors, which suggested larger case-control or cohort studies in different ethnicities with detailed information of risk factors and lifestyles were needed to extensively explore the potential association.

However, there are several limitations in this study. First, all participants were enrolled from two local hospitals, which suggesting the selection bias might have occurred. Second, a replicated investigation focusing on the correlation of *ICOS* rs10932029 T>C and rs4404254 T>C with the susceptibility of CRC was not performed. Third, for lack of the information of other environmental factors, possibly related to the etiology of CRC, we did not further evaluate the potential interaction of environmental factors with gene variants. Fourth, due to the limited participants in some subgroups, the power might be insufficient in these groups. Finally, we only selected two functional SNPs of *ICOS* gene and explored the association between these polymorphisms and risk of CRC. In the future, a fine-mapping study should be performed to extensively identify the potential association between *ICOS* SNPs and risk of CRC.

In summary, our findings suggest that *ICOS* rs10932029 T>C polymorphism may be associated with development of CRC in females, never smoking, < 61 years and BMI ≥ 24 sub-

groups. In addition, our findings highlight that *ICOS* rs4404254 T>C polymorphism is associated with the risk of CRC in the never smoking subgroup. In the future, a fine-mapping study with a functional evaluation is needed to further explore the potential relationship between *ICOS* polymorphisms and risk of CRC.

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Disclosure of conflict of interest

None.

Address correspondence to: Sheng Zhang, Department of General Surgery, Changzhou No. 3 People's Hospital, Changzhou 213000, Jiangsu Province, China. Tel: 0086-13601507172; E-mail: 136015-07172@163.com; Yu Chen, Department of Medical Oncology, Fujian Cancer Hospital & Fujian Medical University Cancer Hospital, Fuzhou 350000, Fujian Province, China. Tel: 0086-13859089836; E-mail: 13859089836@139.com

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