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Association of *CASP9*, *CASP10* gene polymorphisms and tea drinking with colorectal cancer risk in the Han Chinese population*

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Abstract: The initiators caspase-9 (*CASP9*) and caspase-10 (*CASP10*) are two key controllers of apoptosis and play important roles in carcinogenesis. This study aims to explore the association between *CASPs* gene polymorphisms and colorectal cancer (CRC) susceptibility in a population-based study. A two-stage designed population-based case-control study was carried out, including a testing set with 300 cases and 296 controls and a validation set with 206 cases and 845 controls. A total of eight tag selected single nucleotide polymorphisms (SNPs) in *CASP9* and *CASP10* were chosen based on HapMap and the National Center of Biotechnology Information (NCBI) datasets and genotyped by restriction fragment length polymorphism (RFLP) assay. Multivariate logistic regression models were applied to evaluate the association of SNPs with CRC risk. In the first stage, from eight tag SNPs, three polymorphisms rs4646077 (odds ratio (OR)$_{AA+AG}$: 0.654, 95% confidence interval (CI): 0.406–1.055; P=0.082), rs4233532 (OR$_{CC}$: 1.667, 95% CI: 0.967–2.876; OR$_{CT}$: 1.435, 95% CI: 0.998–2.063; P=0.077), and rs2881930 (OR$_{CC}$: 0.263, 95% CI: 0.095–0.728, P=0.036) showed possible association with CRC risk. However, none of the three SNPs, rs4646077 (OR$_{AA+AG}$: 1.233, 95% CI: 0.903–1.683), rs4233532 (OR$_{CC}$: 0.892, 95% CI: 0.640–1.243; OR$_{CT}$: 1.134, 95% CI: 0.897–1.433), and rs2881930 (OR$_{CC}$: 1.096, 95% CI: 0.620–1.938; OR$_{CT}$: 1.009, 95% CI: 0.801–1.271), remained significant with CRC risk in the validation set, even after stratification for different tumor locations (colon or rectum). In addition, never tea drinking was associated with a significantly increased risk of CRC in testing set together with validation set (OR: 1.755, 95% CI: 1.319–2.334). Our results found that polymorphisms of *CASP9* and *CASP10* genes may not contribute to CRC risk in Chinese population and thereby the large-scale case-control studies might be in consideration. In addition, tea drinking was a protective factor for CRC.

Key words: *CASP9*, *CASP10*, Colorectal cancer, Single nucleotide polymorphisms, Susceptibility to cancer, Tea drinking
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1 Introduction

In the past few decades, the incidence of colorectal cancer (CRC) has increased drastically in many Asian countries, especially in China (Sung *et al*., 2005; Yee *et al*., 2010). The Chinese National Office for Cancer Prevention and Control revealed that the large increase in the number of CRC cases (19.1% in men and 17.7% in women) was observed between 2000 and 2005 (Yang *et al*., 2005). Environmental factors are thought to be related with risk of CRC, including lifestyle habits such as smoking, alcohol consumption, and tea consumption (Yang *et al*., 2007; Huxley *et al*., 2009). However, as a chronic complex disease, the genetic makeup of an individual might also contribute to CRC susceptibility (Zhang *et al*., 2009).

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Apoptosis is the process of programmed cell death (PCD), which mainly acts to develop and maintain tissue homeostasis in multicellular organisms (Grutter, 2000; Kumar, 2007). Dysregulation of apoptosis has been found to increase the risk of many human disorders, including human malignancies (Hajra and Liu, 2004). Caspases (CASPs) are a family of cysteine-dependent aspartate-specific proteases (Nicholson and Thornberry, 1997; Budihardjo *et al*., 1999) that play essential roles in apoptosis. The two distinct but converging pathways for *CASP* activation in human, the extrinsic or receptor-mediated pathway and the intrinsic or mitochondrial pathway, both own an independent group of initiator caspases (*CASP8* and *CASP10* for the extrinsic pathway, *CASP9* for the intrinsic pathway). The initiator *CASPs* (Degterev *et al*., 2003; Wang *et al*., 2005) transmit death signals and further activate a cascade of effector *CASPs* (*CASP3*, *CASP6*, and *CASP7*), which result in final cell death by the process of proteolysis.

The genetic variants of *CASP* genes and cancer risk have been studied, covering various *CASP* genes and multiple cancers; however, the results have been inconsistent (Chen *et al*., 2008; Han *et al*., 2008; Ter-Minassian *et al*., 2008; Lan *et al*., 2009; Srivastava *et al*., 2010). Nevertheless, the association between *CASP* polymorphisms and CRC risk has been reported in several studies. Liu B. *et al*. (2010) observed a marginally significant association of a 6-bp insertion/deletion polymorphism (−652 6N ins/del) of the *CASP8* gene and an increased risk of rectum cancer (odds ratio $(OR)_{del/del}$: 1.92, 95% confidence interval (CI): 0.97–3.79). Sun *et al*. (2007) identified the same variant, but with the opposite results of decreasing risk of colorectal cancer ($OR_{del/del}$: 0.50, 95% CI: 0.31–0.79; $OR_{ins/del}$: 0.80, 95% CI: 0.65–0.99). Haiman *et al*. (2008) and Pittman *et al*. (2008) performed case-control studies seeking to replicate Sun's finding. However, they did not find any statistically significant results. To our knowledge, no study has reported on the association of *CASP9*, *CASP10* polymorphisms and risk of CRC thus far.

Due to complexity of finding low-penetrance risk allele in molecular epidemiological research, expansion of sample size and adjustment of all possible confounding factors are an effective way to promote the efficiency (Ulybina *et al*., 2009). Nevertheless, it is difficult to collect big sample and identify all possible confounding factors. Therefore, alternative strategies and approaches of searching for polymorphic candidate SNPs are needed. In the present study, we have adopted a two-stage molecular epidemiological study, whereby DNA samples were divided into two parts according to the time when they collected. For the preliminary sorting, all the candidate SNPs were genotyped in the testing set, only those demonstrating possible association with CRC risk were further genotyped in the validation set. We hypothesized that *CASP9* and *CASP10* polymorphisms might affect the susceptibility of CRC. To test our hypothesis, eight tag SNPs in the two mentioned initiator *CASP* genes were selected and genotyped in a population-based case-control study.

2 Materials and methods

2.1 Study population

Recruitment of this population-based case-control study subjects had been previously reported (Fan *et al*., 2007; Zhang *et al*., 2009). The study protocol was approved by the Institutional Review Board of Medical School of Zhejiang University, China. Briefly, this study included 506 histologically confirmed CRC patients from 2002 to 2008 and 1141 controls without history of cancer randomly selected from the same population at the same time. In this study, a two-stage approach was utilized to compare single nucleotide polymorphism (SNP) frequencies (Thomas *et al*., 2004; Wang *et al*., 2006). The DNA samples were separated into two parts. Subjects recruited between 2006 and 2008, containing 300 cases and 296 controls, were considered the testing set, while subjects recruited between 2002 and 2005, containing 206 cases as well as 845 controls, were categorized as the validation set. All study subjects were the Han Chinese residents.

After receiving written informed consent from each participant, a face-to-face interview was performed by well-trained investigators utilizing a structured questionnaire, consisting of demographic characteristics (age, sex, occupation, education level, etc.), lifestyle habits (smoking, alcohol drinking, tea drinking, etc.), family history of cancer, as well as the reproductive history of the female participants.

Lifestyle factors were determined as follows: subjects who had smoked at least once a day for more than one year were considered smokers; those who consumed alcohol or tea once a day for at least three months were defined as alcohol drinkers or tea drinkers, respectively. In addition, a 2-ml venous blood specimen was collected from each participant using a vacuum tube containing ethylene diamine tetraacetic acid (EDTA) and subsequently stored at −60 °C freezer until use.

2.2 DNA isolation, SNP selection, and genotyping

The genomic DNA was isolated from peripheral blood samples using modified salting-out extraction (Nasiri *et al*., 2005), and the concentration as well as purity of DNA was measured by BioPhotometer (Eppendorf, Hamburg, Germany).

Selection of tag SNPs in *CASP9* and *CASP10* was based on the following criteria: at first, based on the National Center of Biotechnology Information (NCBI) SNP database and HapMap the Han Chinese population database, minor allele frequency (MAF) of SNPs more than 5% were picked up. Then, among selected SNPs, Haploview software was used for selecting tag SNP by implementing Paul de Bakker's Tagger tag SNP selection algorithm. Finally, a total of eight genetic polymorphisms were picked up (Table 1).

The genotypes for *CASP9* and *CASP10* were detected by restriction fragment length polymorphism (RFLP) assay. The details of this approach has been described earlier (Liu C.Y. *et al*., 2010; Wu *et al*., 2011). Information regarding sequences of the polymerase chain reaction (PCR) primer, restriction enzymes, PCR condition, as well as size of target PCR products is presented in Table 1. Additionally, 10% of the random samples were genotyped repeatedly for quality control and concordance rate was 100%.

2.3 Statistical analysis

The distribution of socio-demographic variables between the case and control was analyzed by the Pearson χ^2 test for categorical variables and Student's *t*-test for continuous variables. The Hardy-Weinberg equilibrium in control subjects was assessed by a goodness-of-fit χ^2 test with one degree of freedom for all SNPs.

The associations of eight tag SNPs with CRC risk were assessed by multivariate unconditional logistic regression models, adjusted by potential confounding covariates, including sex (binary), age (continuous), body mass index (BMI) (binary), smoking (binary), alcohol drinking (binary), tea drinking (binary), and family history of cancer (binary). The ORs and

Table 1 Basic information of SNPs

Gene/SNP	Location	SNP	Primer	Enzyme	PCR product* (bp)	T_m (°C)
CASP9						
rs4646018	Intron	A/G	L: 5′-TGTCGGGTATTGTTCTATGT-3′ R: 5′-CCCTAATGATGCACCTCTAA-3′	*Hae*III	469 (114+355)	56.8
rs4646077	Intron	A/G	L: 5′-TCCTTGCTGTGCTGTTTCAC-3′ R: 5′-ACTGGTCATTGGCATTCTCC-3′	*Xba*I	243 (93+150)	57.7
rs4233532	Intron	C/T	L: 5′-TGAGGCACGATTCCTATTCC-3′ R: 5′-TCTGTCTTTTGCCCCTCTGT-3′	*Bsh1236*I	189 (110+79)	58.0
rs4233533	Intron	C/T	L: 5′-CCGGTCCCTCTTTCTCCTAT-3′ R: 5′-CTTGGAAGCCCTGCTACTGA-3′	*Tru1*I	211 (139+72)	57.1
CASP10						
rs7576306	Intron	A/G	L: 5′-AACCATCTGCCTACCCTCCT-3′ R: 5′-TGAGGGCAAGGACTCTGTCT-3′	*Msp*I	198 (119+79)	56.0
rs12613347	Intron	C/T	L: 5′-TAGTTTGACAAGCGCTCCAG-3′ R: 5′-GACCTTTCTCTCGTCCCTCA-3′	*Fok*I	184 (130+54)	58.0
rs2881930	Intron	C/T	L: 5′-GCATGATTAAGCAATGTGTGG-3′ R: 5′-CTCGGCAACATGGTGAGAT-3′	*Bcl*I	285 (127+158)	55.6
rs3900115	Exon cds-synon	A/G	L: 5′-GGGTCCAAGATGTGGAGAAC-3′ R: 5′-GTCGCTCCACTTCCTCTTTG-3′	*Bts*CI	218 (82+136)	56.0

* After enzyme digestion; T_m: melting temperature

95% CIs were calculated to assess the significance of potential at-risk genotypes. Furthermore, the potential gene-environment interactions were evaluated by stratified analysis. The likelihood ratio test comparing a model with and without the interaction terms was conducted to assess multiplicative interaction (Kleinbaum, 2002). $P \leq 0.1$ was determined for statistical significance of the preliminary sorting, and the other statistical significance was assumed at $P \leq 0.05$. Every test was a two-side test. All of the statistical analyses were performed with statistical analysis system (SAS) software Version 9.1.3 (SAS Institute, Cary, NC).

3 Results

3.1 Characteristics of the study population

The distribution of demographics of the cases and controls in the testing set (with 300 cases and 296 controls) and validation set (with 206 cases and 845 controls) are presented in Table 2. Our data showed that the distribution of age, sex, BMI, marital status, education level, family history of cancer, smoking status, alcohol drinking as well as tea drinking in both sets between the cases and controls was similar, with no significant difference. However, in the validation set, the control group owned a higher percentage of

Table 2 Distribution of demographic characteristics among patients with CRC and control groups [a]

Characteristics	Years 2006–2008 (testing set)			Years 2002–2005 (validation set)		
	Case (*n*=300)	Control (*n*=296)	*P*	Case (*n*=206)	Control (*n*=845)	*P*
Age (year)	62.11±12.23	61.7158±11.10	0.098	60.51±10.08	60.31±10.88	0.805
Sex						
Male	159 (53.00%)	156 (52.70%)	0.942	104 (50.49%)	393 (46.51%)	0.305
Female	141 (47.00%)	140 (47.30%)		102 (49.51%)	452 (53.49%)	
BMI						
<24	239 (76.67%)	219 (73.99%)	0.100	160 (77.67%)	630 (74.56%)	0.354
>24	61 (20.33%)	77 (26.01%)		46 (22.33%)	215 (25.44%)	
Occupation[b]						
Farmers	269 (90.57%)	268 (90.54%)	0.989	144 (69.90%)	667 (78.93%)	0.006
Non-farmers	28 (9.43%)	28 (9.46%)		62 (30.10%)	178 (21.07%)	
Education[c]						
Illiterate	152 (50.84%)	136 (45.95%)	0.371	99 (48.29%)	449 (53.14%)	0.378
Primary school	107 (35.79%)	110 (37.16%)		64 (31.22%)	261 (30.89%)	
Junior school	34 (11.37%)	38 (12.84%)		28 (13.66%)	96 (11.36%)	
High school and above	6 (2.01%)	12 (4.05%)		14 (6.83%)	39 (4.61%)	
Marriage						
Unmarried	10 (3.33%)	7 (2.36%)	0.478	22 (10.68%)	80 (9.47%)	0.598
Married	290 (96.67%)	289 (97.64%)		184 (89.32%)	765 (90.53%)	
Smoking						
No	195 (65.00%)	184 (62.16%)	0.472	123 (59.71%)	527 (62.37%)	0.481
Yes	105 (35.00%)	112 (37.84%)		83 (40.29%)	318 (37.63%)	
Alcohol drinking						
No	218 (72.67%)	219 (73.99%)	0.716	146 (70.87%)	601 (71.12%)	0.943
Yes	82 (27.33%)	77 (26.01%)		60 (29.13%)	244 (28.88%)	
Tea drinking						
No	181 (60.33%)	162 (54.73%)	0.166	104 (50.49%)	416 (49.23%)	0.747
Yes	119 (39.67%)	134 (45.27%)		102 (49.51%)	429 (50.77%)	
Family history of cancer						
No	229 (76.33%)	244 (82.43%)	0.066	146 (70.86%)	645 (76.33%)	0.104
Yes	71 (23.67%)	52 (17.57%)		60 (29.13%)	200 (23.67%)	

[a] Data are expressed as mean±standard deviation (SD) for age and number (percentage) for others; [b] Data were missed for three cases; [c] Data were missed for one case. BMI: body mass index

farmers (cases vs. controls: 69.90% vs. 78.93%, P=0.006).

3.2 Association of lifestyle-related factors with CRC risk

When analyzed the adjusted association of lifestyle habits (smoking, alcohol drinking, and tea drinking) with CRC risk, we found that compared with the tea drinking group, the never tea drinking group showed a significantly increased risk of CRC in the testing set (OR: 1.603; 95% CI: 1.026–2.505) and overall subjects (testing set and validation set, OR: 1.755, 95% CI: 1.319–2.334). In validation set, only a marginally significant association was found (OR: 1.295; 95% CI: 0.858–1.955). No significant associations were revealed between smoking or alcohol drinking and CRC risk in any set.

3.3 Association of *CASP* gene with CRC risk

At the preliminary stage of the study, we used the tagging SNP approach for SNP sorting and selected nine tag SNPs in *CASP9* and *CASP10* genes for our study. However, we failed in analyzing one polymorphism (rs4661636) in spite of optimization primers and PCR conditions repeatedly, and the results were barely satisfactory. Subsequently, in the testing set, successful genotyping assays were developed for eight tag SNPs. The associations between the *CASP9* and *CASP10* gene polymorphisms with CRC risk are summarized in Table 3. All tag SNPs in the control group met to Hardy-Weinberg equilibrium. Of the eight tested SNPs, three of them demonstrated $P\leq0.1$ and showed a significant or marginally significant association with CRC risk. Based on the results of the preliminary screening, rs4646077 (OR_{AA+AG}: 0.654, 95% CI: 0.406–1.055; P=0.082), rs4233532 (OR_{CC}: 1.667, 95% CI: 0.967–2.876; OR_{CT}: 1.435, 95% CI: 0.998–2.063; P=0.077), and rs2881930 (OR_{CC}: 0.263, 95% CI: 0.095–0.728; P=0.036) were considered to significantly increase risk of CRC and were therefore subjected to the validation set as candidate SNPs for extend analysis.

The validation set consisted of 206 cases and 845 controls. We did not consider this validation set alone but jointly analyzed the data from both the two sets as recommended by Skol *et al*. (2006), which might increase power to detect genetic associations. However, for all the three SNPs, no significant statistical evidence of association with CRC risk was found, even after subgroup analysis for different tumor locations (Table 4).

Furthermore, the wild-type G allele of rs4646077, the variant C allele of rs4233532, and the wild-type T allele of rs2881930 were considered as risk alleles. Both cases and controls were classified based on the individual number of risk alleles, and divided into three groups (number of risk alleles ≤1, =2, and ≥3). No significant results were observed even after a stratified analysis for tumor location (Table 5).

3.4 Gene-environment interaction

Conducting likelihood ratio test was used to explore the multiplicative interaction between *CASP9* and *CASP10* polymorphisms and tea drinking (Table 6). For rs4646077, the significantly increased risk associated with GG or AA+AG genotype was apparent in the group of never tea drinking, with ORs of 1.832 (95% CI: 1.347–2.492) and 1.862 (95% CI: 1.168–2.969), respectively, compared with tea drinkers with the GG genotype. For rs4233532, never tea drinkers having CC+CT genotype significantly increased CRC risk, with an OR of 1.788 (95% CI: 1.245–2.568) compared with tea drinkers carrying the TT genotype. Additionally, for rs2881930, in the never tea drinking group, individuals carrying TT or CC+CT genotype were found to show an increased risk, the adjusted odds ratio being 1.695 (95% CI: 1.217–2.361) and 1.784 (95% CI: 1.246–2.555), respectively. Nevertheless, no significant multiplicative interaction was observed (P for interaction is 0.1559, 0.1255, and 0.7129, for rs4646077, rs4233532, and rs2881930, respectively). The interactions between *CASP* genotype and smoking or alcohol drinking on CRC risk were not statistically significant.

4 Discussion

In the present study, we investigated the association of *CASP9* and *CASP10* polymorphisms with CRC susceptibility in a two stage designed population-based case-control study in the Han Chinese population. However, no significant effects of eight tag SNPs involved in apoptosis genes on CRC predisposition were detected in our study. However, we found that tea drinking might be involved in reducing CRC risk.

Table 3 Adjusted association of *CASP* polymorphisms with CRC risk in testing set

Genotype	n*		Adjusted OR (95% CI)[a]	HWE P-value	P
	Case (n=300)	Control (n=296)			
CASP9					
rs4646018[b]					
GG	98 (32.67%)	92 (31.19%)	1.00	0.252	0.731
AG	145 (48.33%)	154 (52.20%)	0.881 (0.606–1.281)		
AA	57 (19.00%)	49 (16.61%)	1.019 (0.626–1.658)		
rs4646077[c]					
GG	262 (88.22%)	243 (82.94%)	1.00	0.824	0.082
AA+AG	35 (11.78%)	50 (17.06%)	0.654 (0.406–1.055)		
rs4233532[d]					
TT	90 (30.20%)	115 (38.98%)	1.00	0.123	0.077
TC	163 (55.37%)	148 (50.17%)	1.435 (0.998–2.063)		
CC	43 (14.43%)	32 (10.85%)	1.667 (0.967–2.876)		
rs4233533[e]					
TT	77 (25.93%)	80 (27.03%)	1.00	0.584	0.888
CT	150 (50.51%)	154 (52.03%)	0.995 (0.671–1.474)		
CC	70 (23.57%)	62 (20.95%)	1.100 (0.685–1.766)		
CASP10					
rs7576306[f]					
AA	185 (64.24%)	171 (58.56%)	1.00	0.784	0.314
AG	95 (32.99%)	106 (36.30%)	0.879 (0.616–1.253)		
GG	8 (2.78%)	15 (5.14%)	0.521 (0.213–1.274)		
rs12613347[g]					
CC	112 (39.72%)	105 (36.84%)	1.00	0.442	0.613
CT	139 (49.29%)	141 (49.47%)	0.908 (0.633–1.318)		
TT	31 (10.99%)	39 (13.68%)	0.762 (0.440–1.318)		
rs2881930[h]					
TT	186 (62.42%)	174 (59.18%)	1.00	0.550	0.036
CT	107 (35.91%)	102 (34.69%)	1.040 (0.733–1.467)		
CC	5 (1.68%)	18 (6.12%)	0.263 (0.095–0.728)		
rs3900115					
AA	172 (57.33%)	168 (56.76%)	1.00	0.816	0.208
AG	118 (39.33%)	109 (36.82%)	1.103 (0.783–1.554)		
GG	10 (3.33%)	19 (6.42%)	0.527 (0.236–1.177)		

* Data are expressed as number (percentage); [a] ORs were adjusted by sex, age, alcohol drinking, tea drinking, smoking, BMI, and family cancer history; [b] Genotyping failed in 1 control; [c] Genotyping failed in 3 cases and 3 controls; [d] Genotyping failed in 4 cases and 1 control; [e] Genotyping failed in 3 cases; [f] Genotyping failed in 12 cases and 4 controls; [g] Genotyping failed in 18 cases and 11 controls; [h] Genotyping failed in 2 cases and 2 controls. HWE: Hardy-Weinberg equilibrium

Many studies (Kuzuhara *et al*., 2007; Hakim *et al*., 2008; Kawai *et al*., 2008) have demonstrated that tea drinking was a protective factor for CRC risk, consistent with our results. Our data suggested that no statistical significance for interaction between *CASP9*, *CASP10* and tea drinking was detected, which was also consistent with previous studies. Zhang *et al*. (2005) found that never tea consumption increased risk of the non-cardiac gastric cancer with thymidylate synthase 3'-untranslated region (TS3'-UTR) 6-bp/6-bp genotype (OR: 3.19, 95% CI: 1.54–6.60). Wu *et al*. (2011) reported that never tea drinkers carrying xeroderma pigmentosum complementation group C (XPC) polymorphisms had a higher risk of CRC than tea drinkers. No significant interaction of TS3'-UTR or XPC polymorphism with tea drinking was found. Consequently, it was the positive effect of tea drinking itself mainly contributing to the association between

Table 4 Association of selected SNPs with risk of CRC in joint analyses

Genotype	Control (*n*=1 141)		All the cases (*n*=506)			Colon cancer (*n*=240)			Rectal cancer (*n*=266)		
	*n**	HWE *P*	*n**	Adjusted OR (95% CI)[a]	*P*	*n**	Adjusted OR (95% CI)[a]	*P*	*n**	Adjusted OR (95% CI)[a]	*P*
CASP9											
rs4646077[b]											
GG	925 (87.10%)	0.304	407 (84.79%)	1.00	0.188	196 (85.59%)	1.00	0.487	210 (84.06%)	1.00	0.199
AG+AA	137 (12.90%)		73 (15.21%)	1.233 (0.903–1.683)		33 (14.41%)	1.158 (0.766–1.751)		40 (15.94%)	1.291 (0.874–1.907)	
rs4233532[c]											
TT	414 (36.41%)	0.755	174 (34.52%)	1.00	0.275	83 (34.73%)	1.00	0.518	91 (34.34%)	1.00	0.463
CC	183 (16.09%)		70 (13.89%)	0.892 (0.640–1.243)		33 (13.81%)	0.899 (0.578–1.399)		37 (13.96%)	0.905 (0.589–1.390)	
CT	540 (47.49%)		260 (51.59%)	1.134 (0.897–1.433)		123 (51.46%)	1.127 (0.828–1.535)		137 (51.70%)	1.141 (0.845–1.541)	
CASP10											
rs2881930[d]											
TT	737 (64.71%)	0.484	323 (64.47%)	1.00	0.951	159 (67.09%)	1.00	0.811	164 (62.12%)	1.00	0.589
CC	39 (3.42%)		19 (3.79%)	1.096 (0.620–1.938)		7 (2.95%)	0.825 (0.361–1.889)		12 (4.55%)	1.377 (0.699–2.712)	
CT	363 (31.87%)		159 (31.74%)	1.009 (0.801–1.271)		71 (29.96%)	0.923 (0.678–1.256)		88 (33.33%)	1.092 (0.814–1.465)	

* Data are expressed as number (percentage); [a] ORs were adjusted for age, sex, alcohol drinking, tea drinking, smoking, BMI, and family cancer history; [b] Data were missed for 26 cases and 79 controls due to DNA exhaustion; [c] Genotyping failed in 2 cases and 4 controls; [d] Genotyping failed in 5 cases and 2 controls

Table 5 Combined effect of *CASP* polymorphisms and colorectal cancer risk

Number of risk alleles[a]	Controls (*n*=1 141)	All the cases (*n*=506)			Colon cancer (*n*=240)			Rectal cancer (*n*=266)		
	*n**	*n**	Adjusted OR (95% CI)[b]	*P*	*n**	Adjusted OR (95% CI)[b]	*P*	*n**	Adjusted OR(95% CI)[b]	*P*
≤1	610 (57.65%)	267 (56.45%)	1.00		142 (63.11%)	1.00		125 (50.40%)	1.00	
2	311 (29.40%)	143 (30.23%)	1.088 (0.848–1.396)	0.507	53 (23.56%)	0.760 (0.537–1.075)	0.121	90 (36.29%)	1.479 (0.983–2.019)	0.139
≥3	137 (12.95%)	63 (13.32%)	1.045 (0.747–1.462)	0.799	30 (13.33%)	0.965 (0.623–1.496)	0.874	33 (13.31%)	1.175 (0.764–1.806)	0.497

* Data are expressed as number (percentage); [a] Variant A allele of rs4646077, the variant C allele of rs4233532, and the variant C allele of rs2881930 were categorized as risk allele; [b] ORs were adjusted for age, sex, alcohol drinking, tea drinking, smoking, BMI, and family cancer history

CASP9, *CASP10* genotypes and CRC risk among never tea drinkers compared with tea drinkers.

Many epidemiological studies have focused on the association between *CASP* polymorphisms and risk of varied cancers, yet with inconsistent results. To our knowledge, only five articles related to *CASP* polymorphisms and CRC risk have been reported, with all the attention focused on *CASP8*. Sun *et al*. (2007) identified that a 6-bp deletion (−652 6N del) variant in the *CASP8* gene was associated with a decreased risk of multiple cancers, including CRC in a Chinese population. Haiman *et al*. (2008) and Pittman *et al*. (2008) conducted case-control studies in a Caucasian population with a relatively large sample size; however, no statistical significant results were observed in CRC ($OR_{del/del}$: 1.08, 95% CI: 0.90–1.30; $OR_{ins/del}$: 0.99, 95% CI: 0.86–1.14; and $OR_{del/del}$: 0.99, 95% CI: 0.87–1.12; $OR_{ins/del}$: 0.91, 95% CI: 0.81–1.01). In addition, our research group tested the same polymorphism in *CASP8*, and

Table 6 Interaction between *CASP* polymorphism and tea drinking

Tea drinking	Genotype	Control (*n*=1 141)	All cases (*n*=506)			Colon cancer (*n*=240)			Rectal cancer (*n*=266)		
		*n**	*n**	Adjusted OR (95% CI)[a]	*P*	*n**	Adjusted OR (95% CI)[a]	*P*	*n**	Adjusted OR (95% CI)[a]	*P*
	rs4646077[b]										
Yes	GG	467 (43.90%)	181 (37.71%)	1.00		93 (40.61%)	1.00		88 (35.06%)	1.00	
	AA+AG	56 (5.27%)	33 (6.88%)	1.599 (1.002–2.553)	0.048	15 (6.55%)	1.360 (0.735–2.515)	0.328	18 (7.17%)	1.853 (1.032–3.328)	0.039
No	GG	458 (43.10%)	226 (47.08%)	1.832 (1.347–2.492)	<0.001	103 (44.98%)	1.408 (0.937–2.115)	0.100	123 (49.00%)	2.302 (1.559–3.400)	<0.0001
	AA+AG	81 (7.63%)	40 (8.33%)	1.862 (1.168–2.969)	0.009	18 (7.86%)	1.439 (0.774–2.676)	0.250	22 (8.77%)	2.319 (1.297–4.146)	0.005
	rs4233532[c]										
Yes	TT	207 (18.21%)	87 (17.26%)	1.00		45 (18.82%)	1.00		42 (15.85%)	1.00	
	CC+CT	355 (31.22%)	132 (26.19%)	0.892 (0.645–1.233)	0.489	64 (26.78%)	0.846 (0.556–1.288)	0.435	68 (25.66%)	0.940 (0.612–1.443)	0.778
No	TT	207 (18.21%)	87 (17.26%)	1.414 (0.945–2.116)	0.092	38 (15.90%)	1.803 (0.633–1.852)	0.772	49 (18.49%)	1.784 (1.069–2.978)	0.027
	CC+CT	368 (32.36%)	198 (39.29%)	1.788 (1.245–2.568)	0.002	92 (38.50%)	1.447 (0.903–2.319)	0.125	106 (40.00%)	2.161 (1.357–3.441)	0.001
	rs2881930[d]										
Yes	TT	368 (32.31%)	147 (29.34%)	1.00		78 (32.91%)	1.00		69 (26.14%)	1.00	
	CC+CT	194 (17.03%)	73 (14.58%)	0.968 (0.693–1.350)	0.847	31 (13.08%)	0.770 (0.489–1.210)	0.257	42 (15.90%)	1.194 (0.779–1.830)	0.417
No	TT	369 (32.40%)	176 (35.13%)	1.695 (1.217–2.361)	0.002	81 (34.18%)	1.295 (0.838–2.003)	0.244	95 (35.99%)	2.164 (1.416–3.307)	0.000
	CC+CT	208 (18.26%)	105 (20.96%)	1.784 (1.246–2.555)	0.002	47 (19.83%)	1.354 (0.841–2.182)	0.213	58 (21.97%)	2.284 (1.450–3.600)	0.000

* Data are expressed as number (percentage); [a] ORs were adjusted for age, sex, alcohol drinking, smoking, BMI, and family cancer history; [b] Data were missed for 26 cases and 79 controls due to DNA exhaustion; [c] Genotyping failed in 2 cases and 4 controls; [d] Genotyping failed in 5 cases and 2 controls

also did not find any significant association in a Chinese population (Liu C.Y. *et al*., 2010).

It is plausible that *CASP9* and *CASP10*, as the initiator *CASPs*, might confer cancer susceptibility based on the results of previous studies. Lan *et al*. (2006; 2009) and Hosgood *et al*. (2008; 2009) carried out a series of case-control studies looking at the association between *CASP* polymorphisms and the risk of blood system neoplasms, and reported that rs1052576 of *CASP9* significantly decreased risk in both multiple myeloma (OR_{AG}: 0.8, 95% CI: 0.5–1.3; OR_{AA}: 0.5, 95% CI: 0.3–0.9) (Hosgood *et al*., 2008) and non-Hodgkin lymphoma (OR_{AG+AA}: 0.6, 95% CI: 0.4–1.0) (Lan *et al*., 2006), while *CASP10* rs11674246 showed a marginally protective effect in chronic lymphocytic leukemia (OR_{TT}: 0.74, 95% CI: 0.54–1.00) (Enjuanes *et al*., 2008). Liu C.Y. *et al*. (2010) suggested that the genotype rs4661636 in *CASP9* increased risk of esophageal adenocarcinoma (OR_{TT+CT}: 3.52, 95% CI: 2.12–5.87). Li *et al*. (2008) and Frank *et al*. (2006) evaluated *CASP10* rs13006529 polymorphism on the risk of cutaneous melanoma and familial breast cancer, with neither of the results reaching statistical significance. In the breast cancer, rs13010627 of *CASP10* was found to be associated with a decreased risk (OR_{AA+AG}: 0.62, 95% CI: 0.43–0.88) (Frank *et al*., 2006); however, this conclusion failed to remain significant in a subsequent large-scale pooled analysis (OR_{AA}: 0.94, 95% CI: 0.72–1.22; OR_{AG}: 1.03, 95% CI: 0.98–1.09) (Gaudet *et al*., 2009). Oh *et al*. (2010) showed that *CASP10* polymorphism increased the risk of CRC. Two *CASP10* mutations were observed in 43 colon cancers (2/43, 4.3%) harboring additional *CASP3*,

CASP7, and *CASP8* mutations. In spite of low incidence, the data indicated that a *CASP10* mutation might arouse the pathological changes of some colon carcinomas with other *CASP* gene mutations. Moreover, rs1052576, rs11674246, and rs13006529 detected above were in linkage disequilibrium with rs4646018 (r^2=0.857 for rs1052576) and rs7576306 (r^2=1.0 for rs11674246, r^2=0.932 for rs13006529), which were genotyped in our study; however, we failed to find an association between any polymorphisms of the two SNPs in *CASP9* and *CASP10* with CRC risk.

There were some limitations in our population-based case-control study. First, this study had insufficient power to detect very weak associations of *CASP* polymorphisms on CRC risk. Assuming the risk allele frequency ranged from 0.2 to 0.4, the power to detect an OR of 1.2 at a two-sided α=0.05 was less than 40% (from 28.8% to 39.2%). Second, because of the exhaustion of DNA samples, 26 cases (5.1%) and 79 (6.9%) controls were missed for rs4646077 in the joint analyses of both the testing set and validation set. However, these missing data displayed balanced distribution of demographic factors except tea drinking (tea drinkers 26.92% in cases vs. 50.63% in controls, P=0.03) (Table 7), thus the negative influence on our result due to missing data may be reduced. Moreover, our research was not able to cover all the tag SNPs of *CASP9* and *CASP10* because one polymorphism (rs4661636) failed to be genotyped using current experimental technologies.

Table 7 Distribution of demographic characteristics of 105 missing data of rs4646077 [a]

Characteristics	Missing data of rs4646077		
	Case (n=26)	Control (n=79)	P
Age (year)	56.69±10.48	58.61±10.37	0.421
Sex			
Male	7 (26.92%)	34 (43.04%)	0.144
Female	19 (73.08%)	45 (56.96%)	
BMI			
<24	21 (80.77%)	63 (79.75%)	0.910
≥24	5 (19.23%)	16(20.25%)	
Occupation			
Farmers	7 (26.92%)	66 (83.54%)	0.238
Non-farmers	19 (73.08%)	19 (73.08%)	
Education			
Illiterate	14 (53.85%)	38 (48.10%)	0.955
Primary school	8 (30.77%)	29 (36.71%)	
Junior school	3 (11.54%)	9 (11.39%)	
High school and above	1 (3.85%)	3 (3.80%)	
Marriage			
Unmarried	1 (3.85%)	6 (7.59%)	0.678
Married	25 (96.15%)	73 (92.41%)	
Smoking			
No	21 (80.77%)	49 (62.03%)	0.100
Yes	5 (19.23%)	30 (37.97%)	
Alcohol drinking			
No	23 (88.46%)	56 (70.89%)	0.070
Yes	3 (11.54%)	23 (29.11%)	
Tea drinking			
No	19 (73.08%)	39 (49.37%)	0.030
Yes	7 (26.92%)	40 (50.63%)	
Family history of cancer			
No	18 (69.23%)	6 (87.34%)	0.068
Yes	8 (30.77%)	10 (12.66%)	

[a] Data are expressed as mean±standard deviation (SD) for age, and number (percentage) for others

5 Conclusions

In conclusion, this study utilized a two-stage design for systematic analysis of association of tag SNPs in apoptotic genes *CASP9* and *CASP10* with CRC risk. Three candidate at-risk loci (rs4646077, rs2881930, and rs4233532) were identified in the testing set. However, in the joint analyses of both the testing set and validation set, these candidate genotypes showed no statistical significance. These findings should be confirmed in large-scale case-control studies.

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