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Contribution of Caspase-8 Genotypes to Colorectal Cancer Risk in Taiwan

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Abstract. *Background/Aim: The aim of this study was to examine the role of caspase-8 rs3834129 polymorphism on colorectal cancer (CRC) risk in Taiwanese CRC patients and healthy controls. Materials and Methods: The caspase-8 rs3834129 (-652 6N insertion/deletion) polymorphic genotypes were analyzed in 362 patients with CRC and the same number of age- and gender-matched healthy subjects. The interaction of caspase-8 rs3834129 genotypes with personal behaviors and clinicopathological features were also examined. Results: The percentage of variants ID and DD for caspase-8 rs3834129 genotype were 37.6 and 5.8% in CRC group and 39.0 and 6.6% in the control group, respectively (p for trend=0.7987). The allelic frequency distribution analysis showed that caspase-8 rs3834129 D allele conferred a non-significant lower susceptibility for CRC compared with I allele (OR=0.92, 95%CI=0.74-1.20, p=0.5063). There was no obvious link between caspase-8 rs3834129 genotype and CRC risk among ever-smokers, non-smokers, non-alcohol drinkers or alcohol drinkers. No statistically significant correlation was observed between caspase-8 rs3834129 genotypic distribution and age, gender, tumor size, location or metastasis status. Conclusion: Overall, caspase-8 rs3834129 genotypes may not serve as predictors for CRC risk or prognosis.*

Colorectal cancer (CRC) is the third most prevalent type and the fourth most death-causing cancer worldwide (1, 2). In Taiwan, the incidence and mortality of CRC has been ranked as the first and third cancer among the common types of cancer for many years (3), and the extremely high incidence of CRC has been closely associated with the modern dietary habits of the Western world and the rapid decrease in the consumption of dietary fiber or grain-derived foods. However, the definitive mechanisms of CRC carcinogenesis remain largely unknown. Mounting evidence has suggested that CRC is the result of the interaction among individual genomic and environmental factors, which are waiting to be revealed in the era of precise medicine (4-6). This hypothesis is supported by epidemiological studies, which attributed indirect evidence for the involvement of specific lifestyle and environmental factors in the etiology of more than 85% of CRC cases, particularly meat consumption, cigarette smoking, and exposure to carcinogenic aromatic amines, such as arylamines and heterocyclic amines (7, 8). In addition, 15-20% of CRC cases have a history of familial cancer that have led molecular epidemiologists to search for genomic susceptibility factors that can serve as cancer predictors (9-11). In Taiwan, although several biomarkers for early detection of CRC have been revealed and published during the past decade (12-18), their phenotype and value in clinical practice is not yet known.

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Programmed cell death, also named apoptosis, is an essential mechanism to maintain proper cellular growth rate in a stable population (19). It has been reported that aberrant apoptotic pathway is closely associated with tumorigenesis in CRC (20, 21). Caspases are recognized as a family of regulative and executive proteins in the typical apoptosis processes (22). Among them, caspase-8 is one of the most important members of the caspase family of proteins playing an important role in apoptosis (20, 23), especially in mediating the extrinsic apoptotic pathway (24). The protein is encoded by the *caspase-8* (also known as *CASP8*) gene which has 11 exons and is located on chromosome 2q33 q34. Several *caspase-8* gene SNPs have been identified to be associated with the risk of various types of cancer (25-28). Among them, the *caspase-8* rs3834129 (-652, 6N ins/del) polymorphism, a six-nucleotide insertion (I)/deletion (D) variant, has been functionally shown to lead to the down-regulation of the mRNA of *caspase-8* (29). Caspase-8 SNPs are reported to be genomic markers for prediction of the personal risk for several types of cancer, including neuroblastoma (26), bladder cancer (30) and breast cancer (27). Among them, *caspase-8* rs3834129 is the most investigated SNP on *caspase-8*. Epidemiological studies have extensively examined the association between caspase-8 rs3834129 polymorphism and CRC risk, with discrepant results. Therefore, the current case–control study, aimed to investigate the distribution of genotypes of *caspase-8* rs3834129 polymorphism, evaluate its association with CRC risk in a Taiwanese population, and discuss concisely the differences and similarities among this and other studies.

Materials and Methods

Collected population. The investigated population was composed of 724 individuals, including 362 patients diagnosed with CRC and the same number of control subjects. The 362 patients diagnosed with CRC were recruited at the outpatient clinics of general surgery at the China Medical University Hospital by the surgical teams under the supervision of LB Jeng and MD Yang during the period between 2002 and 2008. The clinical characteristics for the investigated patients, including their histological details, were all identified and recorded by the well-trained surgeons (13, 18, 31). Well-matched for age (no different than 5 years), gender (most of them were the same) and some specific habits (such as smoking and alcohol drinking), 362 non-cancer healthy volunteers were selected as controls after initial random sampling from the Health Examination Cohort of the Hospital with the help of colleagues at the Department of Family Medicine. The exclusion criteria of the controls included previous malignancy, metastasized cancer from other or unknown origin and any familial or genetic diseases. All the 724 recruited participants completed a self-administered questionnaire and provided a 5-ml sample of peripheral blood for genotyping. This study has been approved by the Institutional Review Board of the China Medical University Hospital (IRB project identification coding number: DMR99-IRB-108) and written informed consents were obtained from all the participants with the help of Tissue-Bank of China Medical University Hospital. The selective demographic information for all the 724 participants in this study is summarized in Table I.

Table I. *Summary of selected data of the 362 patients with colorectal cancer and 362 matched non-cancer healthy controls.*

	Controls (n=362)	Cases (n=362)	
Character	n (%)	n (%)	*p*-Value[a]
Age (years)			
≤60	93 (25.7%)	95 (26.2%)	0.8654
>60	269 (74.3%)	267 (73.8%)	
Gender			
Male	209 (57.7%)	203 (56.1%)	0.6525
Female	153 (42.3%)	159 (43.9%)	
Tumor size (cm)			
<5		195 (53.9%)	
≥5		167 (46.1%)	
Location			
Colon		257 (71.0%)	
Rectum		105 (29.0%)	
Lymph node metastasis			
Negative		210 (58.0%)	
Positive		152 (42.0%)	

SD: Standard deviation; [a]based on Chi-square test without Yates' correction.

Genotyping conditions for caspase-8 rs3834129. Genomic DNA was extracted within 12 h after getting the blood from the peripheral blood leukocytes using the QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan, ROC), stored long-term at −80˚C, then diluted and aliquoted for *caspase-8* rs3834129 genotyping as a working stock at −20˚C as per our routine practice (12, 14). The primer pairs and the selection of restriction enzymes used for *caspase-8* rs3834129 genotyping are listed in Table II. The polymerase chain reaction (PCR) cycling conditions were: one cycle at 94˚C for 5 min; 35 cycles at 94˚C for 30 sec, 59˚C for 30 sec and 72˚C for 30 sec, and a final extension at 72˚C for 10 min. After PCR, the SNP-containing DNA amplicons were subjected to overnight digestion by restriction endonucleases. Following enzyme digestion, each sample was immediately subject to 3% agarose gel electrophoresis. All the genotypic processing was repeated by two well-trained researchers independently and blindly, and their results were 100% concordant to each other. In addition, the success rate of PCR-restrictive fragment length polymorphism (RFLP) was 100%, and the genotypes of 5% of the participants in both the control and patient groups were randomly selected and subject to PCR direct sequencing (Genomics BioSci & Tech Co. There was 100% concordance in the results obtained by direct sequencing and PCR-RFLP.

Statistical analysis. The Student's *t*-test was used for the comparison of the continuous variables such as age among the CRC cases and controls. The Pearson's Chi-square was used to compare the distribution of the *caspase-8* rs3834129 genotypes among the investigated subjects. The associations between *caspase-8* rs3834129 genotypes and CRC risk were estimated with the indexes of computing odds ratios (ORs) and their 95% confidence intervals

Table II. *Primers, and DNA products after PCR and after enzyme digestion for caspase-8 rs3834129.*

Polymorphic site	Primers	PCR product size	Restriction enzyme	Genotyping and adduct pattern after digestion
Caspase-8 rs3834129	Forward: 5'-ACTCTGCATGCCAGGAGCTA-3'	324 bp	*Pvu* II	D: 324 bp I: 176, 148 bp
	Reverse: 5'-CTGGGGAAGCCTCACTGTAT-3'			

D: Deletion; I: insertion.

Table III. *Distributions of caspase-8 rs3834129 genotypic frequencies among the patients with colorectal cancer and healthy controls.*

Genotype	Cases, n (%)	Controls, n (%)	Adjusted OR (95%CI)[a]	*p*-Value[b]
II	205 (56.6)	197 (54.4)	1.00 (Reference)	
ID	136 (37.6)	141 (39.0)	0.94 (0.71-1.23)	0.6270
DD	21 (5.8)	24 (6.6)	0.88 (0.49-1.46)	0.5818
ID+DD	157 (43.4)	165 (45.6)	0.90 (0.62-1.28)	0.5496
p_{trend}				0.7987

I: Insertion; D: deletion; OR: Odds ratio; CI: confidence interval; [a]Data adjusted for confounding factors: age, gender, smoking, alcohol and betel quid consumption; [b]Based on Chi-square test without Yates' correction. Significant *p*-values ($p<0.05$) are shown in bold.

Table IV. *Allelic frequencies for caspase-8 rs3834129 polymorphisms among the patients with colorectal cancer and healthy controls.*

Allele	Cases, n (%) (n=724)	Controls, n (%) (n=724)	Adjusted OR (95%CI)[a]	*p*-Value[b]
I	546 (75.4)	535 (73.9)	1.00 (Reference)	
D	178 (24.6)	189 (26.1)	0.92 (0.74-1.20)	0.5063

I: Insertion; D: deletion; OR: Odds ratio; CI: confidence interval. [a]Data adjusted for confounding factors: age, gender, smoking, alcohol and betel quid consumption. [b]Based on Chi-square test without Yates' correction. Significant *p*-values ($p<0.05$) are shown in bold.

(CIs) using logistic regression analysis. Similar to typical cancer genomic studies, any difference between the two groups at $p<0.05$ was identified as statistically significant.

Results

The recording and comparisons of the selected demographic characteristics, including age and gender for the 724 subjects, including 362 patients with CRC and 362 non-cancer healthy controls, are summarized Table I. In addition, the tumor size, location, and lymph node metastasis status of the 362 CRC patients are concisely summarized in Table I. Since the strategies of frequency matching were applied for age, gender and habits in recruiting the 362 non-cancer healthy individuals as controls, there was no difference in respect to the distributions of age and gender between the two groups ($p=0.8654$ and 0.6525, respectively) (Table I). Also, there was no difference in respect to the distributions of smoking and alcohol drinking status between the control and case groups (data not shown).

The distributions of the *caspase-8* rs3834129 genotypes among the 362 non-cancer healthy controls and the 362 patients with CRC are presented and analyzed in Table III. The genotypes of *caspase-8* rs3834129 were not differently distributed between the case and control groups (p for trend=0.7987) (Table III). In detail, the *caspase-8* rs3834129 heterozygous and homozygous variant ID and DD genotypes were not associated with altered CRC risk compared with the wild-type II genotype (adjusted OR=0.94 and 0.88, 95%CI=0.71-1.23 and 0.49-1.46, $p=0.6270$ and 0.5818, respectively). In addition, in the dominant model, there was no significant association between D allele carriers (ID+DD) of *caspase-8* rs3834129 and CRC risk compared with the II wild-type genotype (adjusted OR=0.90, 95%CI=0.62-1.28, $p=0.5496$).

Table V. *Odds ratios for association of caspase-8 rs3834129 genotype with colorectal cancer after stratification by smoking status.*

Genotype	Non-smokers, n		OR (95%CI)[a]	aOR (95%CI)[b]	*p*-Value[c]	Smokers, n		OR (95%CI)[a]	aOR (95%CI)[b]	*p*-Value[c]
	Controls	Cases				Controls	Cases			
II	149	153	1.00 (ref)	1.00 (ref)		48	52	1.00 (ref)	1.00 (ref)	
ID	109	101	0.90 (0.63-1.28)	0.93 (0.74-1.23)	0.5677	32	35	1.01 (0.54-1.88)	1.04 (0.67-1.79)	0.9758
DD	20	17	0.83 (0.42-1.64)	0.88 (0.65-1.58)	0.5881	4	4	0.92 (0.22-3.90)	0.98 (0.51-3.14)	1.0000
Total	278	271				84	91			
ptrend					0.7742					0.9929

[a]Multivariate logistic regression analysis; [b]multivariate logistic regression analysis after adjusting for age, gender and alcohol drinking status; [c]Chi-square without Yates' correction or Fisher's exact test (when n<5); insertion; D: deletion; CI: confidence interval; aOR: adjusted odds ratio. Significant *p*-values ($p<0.05$) are shown in bold.

Table VI. *Odds ratios for caspase-8 rs3834129 genotype and colorectal cancer after stratification by alcohol drinking status.*

Genotype	Non-drinkers, n		OR (95%CI)[a]	aOR (95%CI)[b]	*p*-Value[c]	Drinkers, n		OR (95%CI)[a]	aOR (95%CI)[b]	*p*-Value[c]
	Controls	Cases				Controls	Cases			
II	170	180	1.00 (ref)	1.00 (ref)		27	25	1.00 (ref)	1.00 (ref)	
ID	119	119	0.94 (0.68-1.31)	0.95 (0.65-1.33)	0.7338	22	17	0.83 (0.36-1.92)	0.91 (0.47-1.85)	0.6709
DD	22	19	0.82 (0.43-1.56)	0.89 (0.52-1.48)	0.5376	2	2	1.08 (0.14-8.26)	1.06 (0.38-6.27)	1.0000
Total	311	318				51	44			
ptrend					0.8076					0.9034

[a]Multivariate logistic regression analysis; [b]multivariate logistic regression analysis after adjusting for age, gender and smoking status; [c]Chi-square without Yates' correction or Fisher's exact test (when n<5); I: Insertion; D: deletion; CI: confidence interval; aOR: adjusted odds ratio. Significant *p*-values ($p<0.05$) are shown in bold.

In order to confirm the highlight findings in Table III, analysis of allelic frequency distributions for *caspase-8* rs3834129 was also conducted and the results are presented in Table IV. Supporting the conclusion that the genotype of *caspase-8* rs3834129 is not associated with CRC risk, the frequency of variant allele D was 24.6% in the case group, slightly but non-significantly lower than that of 26.1% in the control group (adjusted OR=0.92, 95%CI=0.74-1.20, $p=0.5063$) (Table IV).

Smoking and alcohol drinking habits are well-known risk factors for CRC in Taiwan. Thus, the interactions between the genotypes of *caspase-8* rs3834129 and personal cigarette smoking and alcohol drinking behaviors of Taiwanese were examined, and the results are presented in Tables V and VI. Firstly, among non-smokers, people with ID and DD genotypes at *caspase-8* rs3834129 were at 0.90- and 0.83-fold odds of having CRC (95%CI=0.63-1.28 and 0.42-1.64, $p=0.5677$ and 0.5881) (Table V, left panel). After adjusting for confounding factors including age, gender and alcohol drinking status, the statistical results still were maintained at a non-significant level for ID and DD genotypes (Table V, left panel). Similarly, a non-significant effect was found among the non-smokers (Table V, right panel). Secondly, among non-alcohol drinkers and alcohol drinkers, those with ID and DD genotypes at *caspase-8* rs3834129 did not have a significantly increased risk of having CRC (Table VI).

The correlations between genotypes of *caspase-8* rs3834129 and clinicopathological features of 362 patients with CRC were further stratified, analyzed and presented in Table VII. No statistically significant correlation was observed between *caspase-8* rs3834129 genotypic distributions and age, gender, tumor size, location or metastasis status (all $p>0.05$) (Table VII).

Discussion

Caspase-8 belongs to a family of cysteine proteases, which play critical roles in the regulation of apoptosis and cytokine processing (32). As originally identified, *caspase-8* plays a role as an initiator of both the extrinsic and the intrinsic apoptotic pathway after activation by Fas-FasL ligands (33, 34). Further, activated caspase-8 may team-up with initiator caspase-10, contributing to the formation of death inducing

Table VII. *Correlation between caspase-8 rs3834129 genotype and clinicopathological features of 362 patients with colorectal cancer.*

Characteristics	Cases, n	Genotype, n (%)			*p*-Value[a]
		II	ID	DD	
Age (years)					
≤60	95	52 (54.7)	37 (39.0)	6 (6.3)	
>60	267	153 (57.3)	99 (37.1)	15 (5.6)	0.9024
Gender					
Male	203	112 (55.2)	79 (38.9)	12 (5.9)	
Female	159	93 (58.5)	57 (35.8)	9 (5.7)	0.8162
Tumor size					
<5 cm	195	107 (54.9)	77 (39.5)	11 (5.6)	
≥5 cm	167	98 (58.7)	59 (35.3)	10 (6.0)	0.7177
Location					
Colon	257	142 (55.3)	99 (38.5)	16 (6.2)	
Rectum	105	63 (60.0)	37 (35.2)	5 (4.8)	0.6751
Lymph node metastasis					
Negative	210	115 (54.8)	82 (39.0)	13 (6.2)	
Positive	152	90 (59.2)	54 (35.5)	8 (5.3)	0.6942

I: Insertion; D: deletion; [a]Based on Chi-square test without Yates's correction.

signaling complex and subsequently the activation the downstream effector caspases to execute the overall apoptosis (35). Among *caspase-8* SNPs, rs3834129 is the most investigated. The first case-control study was conducted by Sun and his colleagues in 2007 to examine the contribution of *caspase-8* rs3834129 to several types of cancer (36). They found that the D allele at caspase-8 rs3834129 was associated with a decreased susceptibility to lung, colorectal, esophageal, breast, cervical and gastric cancer among 4995 cases and 4972 controls. Further studies have indicated that caspase-8 rs3834129 genotypes might be associated with a susceptibility to cancers such as melanoma (25), brain tumor (26), breast cancer (27) and kidney cancer (28). Sun and his colleagues have also reported that the deletion genotype at *caspase-8* rs3834129 destroys a stimulatory protein 1 binding element in the promoter regulatory region, which may cause decreased *CASP8* transcription and eventually reduced apoptosis of antitumor T lymphocytes (36).

There were several reports examining the contribution of *caspase-8* rs3834129 in CRC risk in other populations. A multicentric study that included 6,733 CRC cases and 7,576 controls recruited from Spain, Italy, USA, England, Czech Republic and the Netherlands has indicated that rs3834129 was not associated with CRC risk (37). This is consistent with the study conducted by Xiao MS *et al*. which included 305 CRC patients and 342 healthy Chinese controls (38). In order to clarify the role of caspase-8 rs3834129 polymorphism on the risk of CRC, Ying and his colleagues have performed a meta-analysis and concluded that *caspase-8* rs3834129 polymorphism was not associated with CRC risk (39). In agreement, the present study, indicated that ID or DD genotypes at *caspase-8* rs3834129 are not significantly associated with increased risk of CRC in this Taiwanese population (Table II). Therefore, the direct effect of *caspase-8* rs3834129 on carcinogenesis is still controversial and needs further investigation.

Long-term smoking habit has been reported as a risk factor for CRC (40), thus the interaction of *caspase-8* rs3834129 genotype and smoking on CRC risk was examined. Among both smokers and non-smokers, variant genotypes at *caspase-8* rs3834129 were not associated with altered risk of CRC in Taiwanese (Table V). Similarly, the variant genotypes at *caspase-8* rs3834129 were not associated with altered risk of CRC among non-alcohol drinkers or drinkers in Taiwanese (Table VI). In addition, no interaction was found between *caspase-8* rs3834129 genotype and age or gender on CRC risk. Regarding tumor size, location and lymph node metastasis, no determinant effect of *caspase-8* rs3834129 on CRC was found in the patients investigated (Table VII). These findings also need to be validated by further studies in additional and larger populations.

In conclusion, solid evidence was provided within a moderate and representative population showing that *caspase-8* rs3834129 was not associated with increased CRC risk in a Taiwanese population. Additionally, the stratified analyses indicated that smokers and alcohol drinkers do not bear additional risk for CRC. Moreover, *caspase-8* rs3834129 variant genotypes were not found to be predictors of poorer prognosis. Further validation in large population-based studies in different ethnicities are urgently encouraged and needed.

Conflicts of Interest

The Authors declare no conflict of interest in regard to this study.

Authors' Contributions

Research Design: Wu MH and Hung YW; Patient and Questionnaire Summarize: Yueh TC, Wang SC, Lai YL and Ke TW; Experiment Performance: Chao CC, Wang YC and Chang WS; Statistical Analysis: Hsu SW, Fu CK; Manuscript Writing: Tsai CW, Gong CL and Bau DT; Reviewing and Revising: Bau DT, Chang WS and Tsai CW

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