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Research Paper

CASP8 rs3834129 (-652 6N insertion/deletion) Polymorphism and Colorectal Cancer Susceptibility: An Updated Meta-Analysis

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Abstract

CASP8 rs3834129 polymorphism (-652 6N insertion/deletion) is a genetic alteration which might affect the apoptosis pathway caspase enzyme. The impaired caspase enzyme would lead to the change of cancer risk. By now, the role of *CASP8* rs3834129 polymorphism has been widely investigated. However, the relationship of this genetic variant on colorectal cancer (CRC) susceptibility still remains inconsistent. Therefore, we further investigated the role of rs3834129 polymorphism on CRC risk. Eligible published studies were retrieved from EMBASE, PubMed, CNKI and WANFANG database updates to March 2018. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to assess the relationship strengths. In general, we successfully retrieved 13 studies (8 publications) involving 13058 cases and 14418 controls. The meta-analysis results demonstrated that rs3834129 polymorphism was associated with a decreased CRC risk in heterozygous model (ID vs. II: OR = 0.94, 95% CI = 0.88-0.99), but not the homozygous and allele models. Furthermore, significantly decreased risk was also found among Asian (ID vs. II: OR = 0.86, 95% CI = 0.76-0.98), and high quality score group (ID vs. II: OR = 0.90, 95% CI = 0.81-1.00) in the stratified analyses. Taken together, we showed that *CASP8* rs3834129 polymorphism influences CRC susceptibility in a weak impact manner. More case-control studies are warranted to validate such relationship.

Key words: colorectal cancer; *CASP8;* polymorphism; susceptibility; meta-analysis

Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the fourth most cause of cancer death in the world [1]. In China, CRC ranks the top five cancers both in new diagnoses and in the cancer-related cause death [2]. The definitive etiology of CRC remains unknown. Evidence suggests that CRC derives from combinations of genetic and environmental factors [3]. Previous epidemiological studies have elucidated that inherited susceptibility is a major component of CRC predisposition.

Apoptosis, also called as programmed cell death, is a pivotal mechanism to maintain the normal cellular growth [4]. Aberrant functions of apoptosis pathway are associated with the development of CRC [5, 6]. Caspases are the main regulative and executive enzymes in apoptosis pathway [7]. Among them, caspase 8 was one of the most important components of the caspases family proteins [5, 8]. Caspase 8 plays a critical role in mediating the extrinsic apoptosis pathway [9]. The human gene *CASP8* is located on chromosome 2q33-q34 with 11 exons. Several SNPs of *CASP8* gene have been identified to be associated with cancer risk [10-13]. Among them, *CASP8* rs3834129 polymorphism (-652 6N ins/del), a six

six-nucleotide insertion/deletion variant, leads to the decreased expression of *CASP8* mRNA [14].

CASP8 SNPs are reported to predispose to the susceptibility of several cancers, including neuroblastoma [12], bladder cancer [15], breast cancer [13]. Among them, *CASP8* rs3834129, namely -652 6N ins/del polymorphism, is one of the most investigated SNP. Extensively epidemiological studies have assessed the association between *CASP8* rs3834129 (-652 6N ins/del) polymorphism and CRC risk, yet with discrepant results. Therefore, we conducted this meta-analysis to provide a precise evaluation of the association of interest.

Materials and Methods

Publication search

We first searched the following key words: 'Caspase 8' or '*CASP8*' or 'rs3834129' and 'SNP' or 'polymorphism' or 'polymorphisms' or 'single nucleotide polymorphism' or 'variant' and 'colorectal cancer' or 'colorectal tumor' or 'colorectal carcinoma' or 'colorectal neoplasm' or 'CRC' in database of PubMed and EMBASE. We also searched the Chinese database CNKI and WANFANG to include more eligible studies. Further, additional studies were also manually extracted from the references of the above obtained publications. The latest search was done in March 2018 without any language restriction. The article will be considered as different studies if it contains more than two ethnicities. Among overlapping reports, only the largest one will be retained.

Eligibility criteria

The final including studies in this meta-analysis should fulfill all the following requirements: 1) unrelated case-control studies; 2) original epidemiological studies; 3) analyzing the relationship between *CASP8* rs3834129 polymorphism and CRC risk; 4) Sufficient genotype data were presented to obtain odds ratios (ORs) and 95% confidence intervals (CIs); 5) articles written in English or in Chinese.

Data extraction

We arranged two authors to identify all eligible studies independently. The following items were recorded from each study: first author's name, year of publication, Hardy-Weinberg equilibrium (HWE), quality score, country, ethnicity, source of controls, genotyping method, and genotype distributions of cases and controls. All the disagreed information was settle down after fully discussed by the two authors.

Statistical analysis

Fisher's exact test was applied to check whether the genotype frequency distribution of rs3834129 in controls was deviated from Hardy-Weinberg equilibrium. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated from multivariate logistic regression, and then used to estimate the associations between rs3834129 and CRC risk. Subgroup analyses were performed by ethnicity, source of control, and quality score. Between-study heterogeneity was determined by a chi-square-based *Q*-Test. The random-effects model (the DerSimonian and Laird method) would be performed in the presence of heterogeneity, whereas the fixed-effects model (the Mantel-Haenszel method) would be performed. Publication bias was assessed by visual inspection of funnel plots and the Egger's linear regression test. The asymmetric plot and P value less than 0.5 was considered as the existence of publication bias. In addition, sensitivity analysis was also applied to assess the robustness of the results. Quality assessment for each study was performed using the quality assessment criteria (Table 1). The meta-analysis was conducted using STATA version 11.0 (Stata Corporation, College Station, TX, USA). All p values were two-sided.

Table 1. Score of quality assessment

Criteria	Score
Representativeness of cases	
Selected from population cancer registry	2
Selected from hospital	1
No method of selection described	0
Representativeness of controls	
Population-based	3
Blood donors	2
Hospital-based	1
Not described	0
Ascertainment of cancer cases	
Histopathologic confirmation	2
Patient medical record	1
Not described	0
Control selection	
Controls matched with cases by age and sex	2
Controls matched with cases only by age or by sex	1
Not matched or not descried	0
Genotyping examination	
Genotyping done blindly and quality control	2
Only genotyping done blindly or quality control	1
Unblinded and without quality control	0
Total sample size for both cases and controls	
Larger than 1000	3
Larger than 500, but less than 1000	2
Larger than 200, but less than 500	1
Less than 200	0

Results

Study characteristics

Using the above mentioned database, we firstly identified 59 potentially relevant published records.

After literature screening and abstracts reading, we kept 7 publications in the analysis [16-22]. We also extracted 1 article from the references of the retrieval articles [23]. The general work flow of the selection process was graphically shown in Figure 1. In all, 13 studies (8 publications) with 13058 cases and 14418 controls were used in the pooled analysis (Table 2). Among them, 4 studies focused on Asians and 9 on Caucasians. 4 studies were hospital-based design, 9 were population-based design. 6 studies with a quality score >9, and 7 studies with a quality score ≤9.

Meta-analysis results

Overall meta-analysis information was shown in Table 3 and Figure 2. In the pooled analysis, statistically significant protection role of *CASP8* rs3834129 polymorphism in CRC was observed among heterozygous models (ID vs. II: OR=0.94, 95% CI=0.88-0.99). Statistically significant relationship was not observed in homozygous and allele model. When stratified by population, significant association between *CASP8* rs3834129 polymorphism and CRC risk was detected among African (ID vs. II: OR=0.86, 95% CI=0.76-0.98). Such association was not observed for the Caucasians. In terms of source of controls, we failed to detect any significant relationship in hospital-based group and in population-based group. Further subgroup analysis by quality score yielded a significant association for allele model (D vs. I: OR=0.90, 95% CI=0.81-1.00).

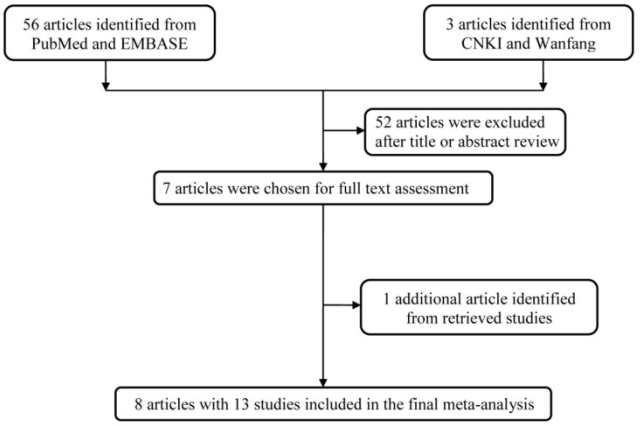

Figure 1. The work flow of the current process of handling selection.

Table 2. Characteristics of studies included in the current meta-analysis

Surname	Year	Country	Ethnicity	Control Source	Genotype method	Case				Control				MAF	HWE
						II	ID	DD	All	II	ID	DD	All		
Sun	2007	China	Asian	PB	PCR-RFLP	605	280	33	918	528	304	58	890	0.24	0.116
Pittman	2008	England	Caucasian	PB	AS-PCR	995	1897	987	3879	892	1872	897	3661	0.50	0.170
Liu	2010	China	Asian	PB	PCR-RFLP	233	116	21	370	528	278	32	838	0.20	0.538
Theodoropoulos	2011	Greece	Caucasian	HB	RFLP-PCR	103	201	98	402	120	254	106	480	0.49	0.194
Xiao	2013	China	Asian	HB	PCR-PAGE	187	107	11	305	212	115	15	342	0.21	0.905
Wu	2013	China	Asian	HB	PCR-SSCP	284	152	15	451	358	244	29	631	0.24	0.119
Pardini	2014	Spain	Caucasian	PB	Taqman	500	996	482	1978	425	802	420	1647	0.50	0.290
Pardini	2014	Italy	Caucasian	PB	Taqman	195	285	137	617	783	1230	538	2551	0.45	0.178
Pardini	2014	USA	Caucasian	PB	Taqman	237	514	259	1010	383	794	403	1580	0.51	0.835
Pardini	2014	England	Caucasian	PB	Taqman	410	825	341	1576	165	393	209	767	0.53	0.436
Pardini	2014	Czech	Caucasian	PB	Taqman	239	479	249	967	169	326	177	672	0.51	0.443
Pardini	2014	Netherlands	Caucasian	PB	Taqman	169	282	134	585	106	177	76	359	0.46	0.895
Diego Marques	2017	Brazil	Caucasian	HB	PCR	49	64	27	140	42	65	33	140	0.47	0.424

MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium; PB, population based; HB, hospital based; PCR-PAGE, polymerase chain reaction-polyacrylamide gel electrophoresis; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; AS-PCR, allele-specific polymerase chain reaction.

Table 3. Meta-analysis of the association between *CASP8* rs3834129 (-652 6N Ins/Del) polymorphism and CRC risk

Variables	No. of studies	Homozygous DD vs. II		Heterozygous ID vs. II		Allele D vs. I	
		OR (95% CI)	P ^{het}	OR (95% CI)	P ^{het}	OR (95% CI)	P ^{het}
All	13	0.92 (0.82-1.04)	0.022	**0.94 (0.88-0.99)**	0.602	0.95 (0.90-1.01)	0.015
Ethnicity							
Asian	4	0.78 (0.47-1.32)	0.028	**0.86 (0.76-0.98)**	0.734	0.88 (0.74-1.04)	0.046
Caucasian	9	0.96 (0.87-1.06)	0.166	0.96 (0.90-1.02)	0.408	0.98 (0.93-1.03)	0.191
Source of controls							
PB	9	0.93 (0.81-1.07)	0.007	0.94 (0.89-1.00)	0.566	0.96 (0.90-1.02)	0.009
HB	4	0.88 (0.67-1.16)	0.493	0.89 (0.75-1.04)	0.469	0.02 (0.81-1.05)	0.279
Quality score							
>9	6	0.82 (0.64-1.06)	0.004	0.91 (0.82-1.02)	0.174	**0.90 (0.81-1.00)**	0.006
≤9	7	1.00 (0.91-1.10)	0.925	0.95 (0.87-1.02)	0.891	1.00 (0.96-1.05)	0.942

Het, heterogeneity; HB, hospital based; PB, population based.

Heterogeneity and sensitivity analysis

We first used I^2 statistics and *Q* test to calculate between-study heterogeneity. Significant heterogeneity was detected among all three genetic models ($P<0.001$) in the pooled analysis. Therefore, we adopted the random-effect model to generate wider CIs. We also conducted sequential leave-one-out sensitivity analysis to assess the stability of the results. The results showed that no substantial changes in pooled results, after removing each study (Figure 3).

Publication bias

The Begg's funnel plots of the included studies showed no evidence of obvious asymmetry (Figure 4). Moreover, none-existence of publication bias among the studies was also approved by statistical evidence of Egger's test (data not shown).

Discussion

In this present meta-analysis, we comprehendsively assessed the relationship between *CASP8* rs3834129 polymorphism with CRC susceptibility. The obtained results suggested *CASP8* rs3834129 polymorphism may influence CRC risk in a low impact effect manner. To date, this meta-analysis represents the most powerful investigation in elucidating the role of *CASP8* rs3834129 polymorphism in CRC risk.

The role of *CASP8* rs3834129 polymorphism in CRC risk has attracted intensive attentions. The first case-control study with 4995 cases and 4972 controls was conducted by Sun et al. in 2007 [16]. They identified that -652 6N deletion allele would decrease the susceptibility of lung, colorectal, esophageal, breast, cervical and gastric cancer. Further biochemical assays illustrated that this variant might lead to decreased apoptotic reactivity of T lymphocytes upon cancer cells stimulation. A multi-centric study conducted by Pardini et al. indicated that rs3834129 was not associated with CRC risk in the full data set [21]. This study recruited 6,733 CRC cases and 7,576 controls by six different centers located in Spain, Italy, USA, England, Czech Republic and the Netherlands collaborating to the international consortium COGENT (Colorectal cancer GENeTics). Such null associations were also presented in a study conducted by Xiao MS et al. in Chinese population using 305 CRC patients and 342 healthy individuals [20].

To further clarify the role of *CASP8* rs3834129 polymorphism on the risk of CRC, we performed this meta-analysis. *CASP8* rs3834129 polymorphism was not associated with CRC risk, in some genetic models. This phenomenon may be due to the relatively small sample or the weak impact of single polymorphism in singe gene. Stratified analysis by ethnicity showed that significant association was observed between *CASP8* rs3834129 polymorphism and CRC risk among Asian, but not Caucasian. Allelic distributions of *CASP8* rs3834129 polymorphisms could vary geographically and ethnically.

When interpreting this meta-analysis, several limitations should be noted. First, we only used unadjusted estimates to assess the strength of association between *CASP8* rs3834129 polymorphism and CRC risk. We failed to conduct adjustment analysis as we could not obtain original data such as life habitat, environmental exposes, and gene-environment interactions, which restrains our further analysis for confounding factors. Second, language bias and selection bias could not be ruled out, as only published studies and papers written in English or Chinese were analyzed. Third, significant between-study heterogeneity was detected, which would impair the validity of conclusion. Fourth, in some subgroup analysis, the sample size was relatively small. Thus, the statistical power is to be impaired to estimate the real association. Last, nearly all the eligible

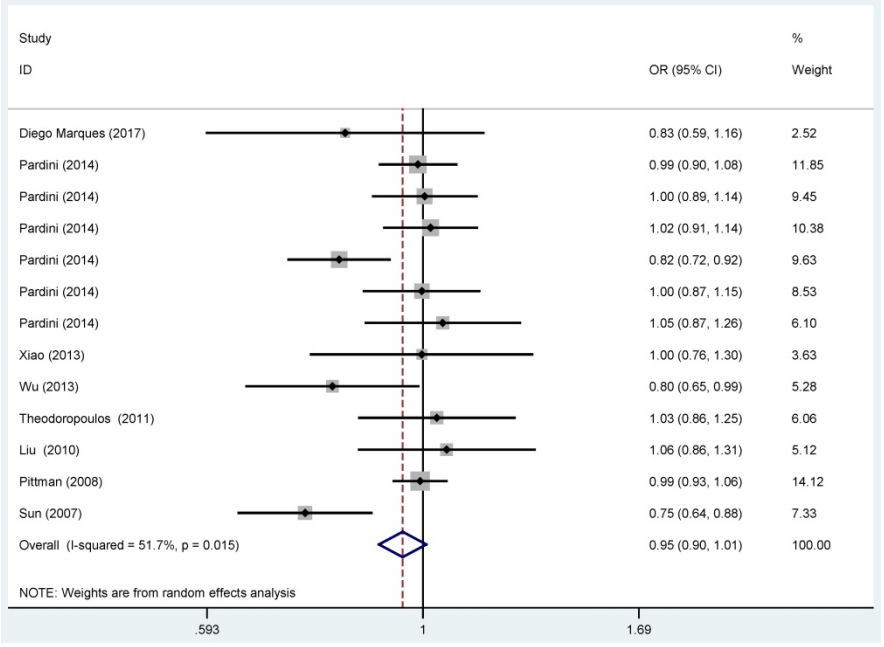

Figure 2. Forest plot for the CRC susceptibility associated with the *CASP8* rs3834129 polymorphism under allele comparison model. The horizontal lines represent the study-specific ORs and 95% CIs, respectively. The diamond represents the pooled results of OR and 95% CI.

case-control studies included were conducted in Asians and Caucasians. The studies of other ethnicities, such as Africans, were absence. Therefore, more studies from other ethnicities, especially Africans, are necessary to further confirm such conclusion, due to the geographical and genetic differences.

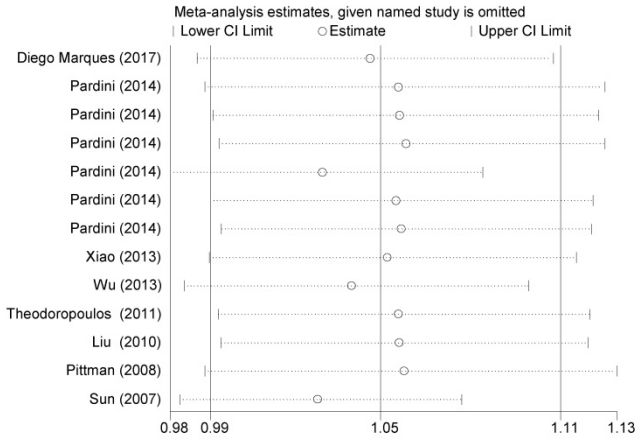

Figure 3. Sensitivity analysis of the summary OR coefficients on the association between *CASP8* rs3834129 polymorphism and CRC risk under allele comparison model.

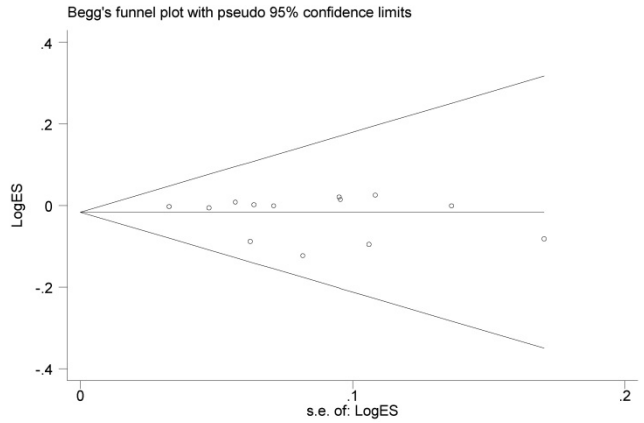

Figure 4. Funnel plot analysis to detect publication bias for *CASP8* rs3834129 polymorphism under allele comparison model. Each point represents a separate study for the indicated association.

Conclusion

In conclusion, the current meta-analysis provides strong evidence that *CASP8* rs3834129 polymorphism may not be strong enough to impact the risk of CRC, from the perspective of the formed case-control studies. Such relationship further helps to explain the etiology of CRC. Yet, further case-control studies with larger sample sizes, standardized unbiased design are warranted to confirm our findings.

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Competing Interests

The authors have declared that no competing interest exists.

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