

# CASP-9 gene functional polymorphisms and cancer risk: a large-scale association study plus meta-analysis

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Genet. Mol. Res. 12 (3): 3070-3078 (2013) Received April 14, 2012 Accepted November 22, 2012 Published February 28, 2013 DOI http://dx.doi.org/10.4238/2013.February.28.22

ABSTRACT. We investigated the association between CASP-9 polymorphisms and susceptibility to neoplasm. Fourteen studies with a total of 2733 neoplasm cases and 3352 healthy controls were included. Meta-analysis showed that the rs4645981\*T allele and the rs4645981\*T allele carrier were positively associated with neoplasm susceptibility [odds ratio (OR) = 1.43, 95% confidence interval (95%CI) = 1.12-1.81, P = 0.004; OR = 1.46, 95%CI = 1.10-1.93, P = 0.009, respectively]. However, the rs1052576\*A allele, rs1052576\*A carrier, rs2308941\*T allele, and rs2308941\*T carrier might decrease the risk of cancer (OR = 0.72, 95%CI= 0.58-0.89, P = 0.003; OR = 0.76, 95%CI = 0.63-0.92, P = 0.004; OR = 0.20, 95%CI = 0.09-0.45, P < 0.0001; OR = 0.21, 95%CI = 0.06-0.75, P = 0.02, respectively). There was no significant association between rs1263, rs1052571, rs2308950, rs4645978, rs4645980, rs4645982, and rs4646018 and cancer risk (all P > 0.05). In conclusion, this meta-analysis suggests that CASP-9 gene polymorphisms are involved in the pathogenesis of various cancers. The rs4645981\*T allele and the rs4645981\*T allele carrier might increase the risk of cancer, but the rs1052576\*A allele, rs1052576\*A carrier, rs2308941\*T allele, and rs2308941\*T carrier might be protective.

Key words: CASP-9; Gene polymorphisms; Cancer; Meta-analysis

Genetics and Molecular Research 12 (3): 3070-3078 (2013)

# **INTRODUCTION**

Caspase-9 is a cysteine protease encoded by the CASP-9 gene (Thornberry, 1997; Shiozaki et al., 2003) located on chromosome 1p36.1 (Bian et al., 2004). A member of the caspase gene family, caspase-9, plays an important role in the mitochondrial death pathway. In this pathway, cell death signals lead to mitochondrial release of cytochrome c, which binds and facilitates formation of the heptameric apoptosome that recruits and activates caspase-9. During apoptosis, the extrinsic and intrinsic pathways in humans use the caspase enzyme cascade; the extrinsic pathway utilizes caspase-8 and -10, while the intrinsic pathway employs caspase-9 (Kesarwani et al., 2011). Complex recruitment of caspase-9 leads to activation through formation of the apoptosome (Gil et al., 2002). Many studies have identified significant associations between the caspase gene cluster and cancer susceptibility, particularly for CASP-7, -8, and -10 (Oliveira et al., 2004; De Vecchi et al., 2009; Liu et al., 2010). However, Catchpoole and Lock (2001) found that CASP-9 is also strongly associated with neuroblastoma. Liamarkopoulos et al. (2011) confirmed that CASP-9 genetic polymorphisms influence the risk of gastric cancer. Moreover, Choi et al. (2012) demonstrated CASP-9 involvement in the pathogenesis of lung cancer. Seo et al. (2011) suggested that ovarian cancer susceptibility is correlated with CASP-9. However, these studies did not characterize the precise relationship between CASP-9 and cancer risk, and uncertainty remains. We performed this study to describe the overall associations between CASP-9 and cancer risk.

## **MATERIAL AND METHODS**

#### Literature search

PubMed, Cochrane library, Embase, Web of Science, Springerlink, CNKI, and CBM databases were searched (last search was updated on May 10, 2012) extensively to identify relevant studies. The search terms included ["Caspase-9" or "ICE-Like Apoptotic Protease 6" or "Procaspase-9" or "Caspase 9" (Mesh)] and ["SNPs" or "SNP" or "polymorphism, genetic" (Mesh)] and ["cancer" or "tumor" or "Neoplasms" (Mesh)]. References in eligible studies or textbooks were also reviewed.

#### Inclusion and exclusion criteria

The studies included had to meet the following criteria: case-control study; focused on associations between CASP-9 polymorphisms and cancer susceptibility; all patients with the diagnosis of cancer confirmed by pathological examination of surgical specimens; the frequencies of alleles or genotypes in case and control groups could be determined; and the publication was in English or Chinese. Studies were excluded when they were not case-control studies about CASP-9 gene polymorphisms and cancer susceptibility; if they were based on incomplete data; or if useless or overlapping data were reported.

#### **Data extraction**

Using a standardized form, data from the studies published were extracted indepen-

Genetics and Molecular Research 12 (3): 3070-3078 (2013)

#### Z.Y. Zhang et al.

dently by 2 reviewers (Z.Y.Z. and Y.X.). The following information was extracted from each article: first author, year of publication, country, language, ethnicity, study design, diagnostic criteria, source of cases and controls, number of cases and controls, mean age, sample, pathological types, detection methods, polymorphism genotype frequency, and evidence of Hardy-Weinberg equilibrium (HWE) in controls. In case of conflicting evaluations, an agreement was reached following discussion with a third reviewer (R.W.).

#### Quality assessment

Two reviewers (X.Y.J. and X.T.) independently assessed the quality of the papers according to modified STROBE quality score systems (Son et al., 2006; Zhang et al., 2011). Forty quality appraisal assessment items were used in this meta-analysis, with scores ranging from 0 to 40. Scores of 0-20, 20-30, and 30-40 were defined as low, moderate, and high quality, respectively. Disagreement was resolved by discussion with a third reviewer (R.W.).

#### Statistical analysis

Allele or genotype frequencies of CASP-9 SNPs were determined by allele counting. The odds ratio (OR) and 95% confidence interval (95%CI) were calculated using Review Manager version 5.1.6 (provided by the Cochrane Collaboration, available at: http://ims. cochrane.org/revman/download) and STATA version 12.0 (Stata Corp., College Station, TX, USA). Between-study variation and heterogeneity were estimated using the Cochrane Q-test (Higgins and Thompson, 2002; Zintzaras and Ioannidis, 2005) ( $P \le 0.05$  indicated statistically significant heterogeneity). We also quantified the effect of heterogeneity by using the  $I^2$  test. I<sup>2</sup> ranges between 0 and 100% and represents the proportion of inter-study variability that can be attributed to heterogeneity rather than chance. I<sup>2</sup> values of 25, 50, and 75% were defined as low, moderate, and high estimates, respectively. When a significant Q-test (P < 0.10) or  $I^2$ > 50% indicated heterogeneity across studies, the random-effect model was used for metaanalysis, or the fixed-effect model was used. To establish the effect of heterogeneity on the meta-analysis conclusions, subgroup analysis was performed. We determined whether the genotype frequencies of the controls were in HWE using the  $\chi^2$  test. Subgroup analysis based on nationality was used to explore and explain diversity among the results of different studies. Sensitivity analysis was performed by sequential omission of individual studies. Publication bias was investigated by Begger's funnel plot, and funnel plot asymmetry was assessed by the Egger linear regression test (Peters et al., 2006); statistical significance was indicated by the Egger test (P < 0.05). All P values were two-sided. To ensure reliability and accuracy, two reviewers (Z.Y.Z. and Y.X.) independently populated the data in the statistical software programs and obtained the same results.

#### RESULTS

## Characteristics of the studies included

Fourteen studies (Park et al., 2006; Fang et al., 2007; Lan et al., 2007; Lou et al., 2007; He et al., 2008; Hosgood et al., 2008; Ye et al., 2008; Gangwar et al., 2009; Ulybina et al., 2009;

Genetics and Molecular Research 12 (3): 3070-3078 (2013)

Wu, 2009; Kesarwani et al., 2011; Ni et al., 2011; Theodoropoulos et al., 2010, 2011) were included and 80 were excluded, based on the inclusion and exclusion criteria. A flow chart representing study selection is shown in Figure 1. The total numbers of cancer cases and healthy controls were 2733 and 3352 in 14 case-control studies, which evaluated the relationship between CASP-9 gene polymorphisms and susceptibility to cancer. Publication year ranged from 2005 to 2011. All patients fulfilled the diagnosis criteria of malignant neoplasm confirmed by pathological examination of the surgical specimen. The source of controls was based on a healthy population. Ten SNPs in CASP-9 were addressed, including rs1263, rs1052571, rs1052576, rs2308941, rs2308950, rs4645978, rs4645980, rs4645981, rs4645982, and rs4646018. HWE tests were performed on the genotype distribution of controls in all the studies included. We found that 4 studies mainly from Asian populations were in non-HWE (P < 0.05), while all others were in HWE (P > 0.05). All quality scores were >20 (moderate to high quality). The characteristics and methodological quality of the studies included are summarized in Table 1.



Figure 1. Flow chart shows the study selection procedure.

# Association between CASP-9 gene polymorphisms and cancer risk

A summary of the meta-analysis of the association between CASP-9 gene polymorphisms and cancer susceptibility is provided in Table 2. The meta-analysis showed that rs4645981, including the rs4645981\*T allele and the rs4645981\*T allele carrier, was positively associated with cancer susceptibility (OR = 1.43, 95%CI = 1.12-1.81, P = 0.004; OR = 1.46, 95%CI = 1.10-1.93, P = 0.009, respectively). However, there were negative associations between rs1052576, rs2308941, and cancer susceptibility. The rs1052576\*A allele, rs1052576\*A carrier, rs2308941\*T allele, and rs2308941\*T carrier might decrease the risk of cancer (OR = 0.72, 95%CI = 0.58-0.89, P = 0.003; OR = 0.76, 95%CI = 0.63-0.92, P = 0.004; OR = 0.20, 95%CI = 0.09-0.45, P < 0.0001; OR = 0.21, 95%CI = 0.06-0.75, P = 0.02, respectively). In addition, there were no significant associations for rs1263, rs1052571, rs2308950, rs4645978, rs4645980, rs4645982, and rs4646018 (all P > 0.05). The significance of pooled OR in all individual and subgroup analyses was not excessively influenced by omitting any single study or the non-HWE studies. The positive associations between CASP-9 gene polymorphisms and cancer susceptibility are shown in Figure 2.

Genetics and Molecular Research 12 (3): 3070-3078 (2013)

Table 1. Characteristics	of individual	studies includ	led in the r	neta-analy:	sis.				
Reference	Country	Ethnicity	Num	lber	Detection	Disease	SNP		HWE
			Case	Control				Ь	Test
Park et al., 2006	Korea	Asian	432	432	PCR-RFLP	Lung cancer	rs4645978 (A/G)	0.77	HWE
							rs4645980 (T/G)	0.77	HWE
							rs4645981 (C/1) rs4645982 (del/ins)	0.09	HWE
Fang et al., 2007	China	Asian	70	100	PCR-RFLP	Gastric cancer	rs1052576 (G/A)	0.52	HWE
Lan et al., 2007	USA	Caucasian	461	535	DNA sequencing	Lymphoma	rs1052576 (G/A)	0.67	HWE
						Lymphoma	rs1052576 (G/A)	0.67	HWE
						Lymphoma	rs1052576 (G/A)	0.67	HWE
Lou et al., 2007	China	Asian	81	100	PCR-RFLP	Lung cancer	rs1052571 (C/T)	0.80	HWE
							rs1052576 (G/A)	0.52	HWE
He et al., 2008	China	Asian	170	100	PCR-RFLP	Colon cancer	rs1052576 (G/A)	0.52	HWE
Hosgood et al., 2008	USA	Caucasian	128	516	DNA sequencing	Multiple myeloma	rs1052576 (G/A)	0.76	HWE
Ye et al., 2008	China	Asian	33	33	DNA microarray	Variant cancer	rs2308941 (C/T)	<0.05	non-HWE
Gangwar et al., 2009	India	Asian	212	250	PCR-RFLP	Bladder cancer	rs4645978 (A/G)	<0.05	non-HWE
							rs4645982 (del/ins)	0.60	HWE
Ulybina et al., 2009	Russia	Caucasian	111	110	AS-PCR	Lung cancer	rs1052571 (C/T)	0.77	HWE
							rs2308950 (A/G)	0.69	HWE
							rs1052576 (G/A)	0.89	HWE
Wu, 2009	China	Asian	100	60	PCR-RFLP	Liver cancer	rs1052576 (G/A)	0.84	HWE
Theodoropoulos et al., 2010	Greece	Caucasian	80	160	PCR-RFLP	Pancreatic cancer	rs1263 (A/G)	0.08	HWE
Kesarwani et al., 2011	India	Asian	175	198	PCR-RFLP	Prostate cancer	rs4645978 (A/G)	<0.05	non-HWE
							rs4645982 (del/ins)	<0.05	non-HWE
Ni et al., 2011	China	Asian	278	278	PCR-RFLP	Gastric cancer	rs4646018 (G/A)	0.52	HWE
Theodoropoulos et al., 2011	Greece	Caucasian	402	480	PCR-RFLP	Colorectal cancer	rs1263 (A/G)	0.93	HWE
SNP = single nucleotide polymorphism; AS = allel	polymorphis e specific.	sms; HWE =	Hardy-We	inberg equ	uilibrium; PCR = p	olymerase chain reac	ction; RFLP = restri	ction frag	ment length

Z.Y. Zhang et al.

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Table 2 Mata analysis of the association between CASP 9 gene polymorphisms and cancer risk

SNPs		Cases (n/N)	Controls (n/N)	OR (95%CI)	Р	Heterogeneity test	Effect model
Rs1263	G allele	436/964	646/1280	1.44 (0.26-7.84)	0.67	$P < 0.00001, I^2 = 98\%$	Random
	G carrier	324/482	488/640	1.82 (0.12-26.97)	0.66	$P < 0.00001, I^2 = 95\%$	Random
Rs1052571	T allele	113/384	130/420	0.87 (0.64-1.19)	0.39	$P = 0.93, I^2 = 0\%$	Fixed
	T carrier	97/192	106/210	0.93 (0.61-1.40)	0.72	$P = 0.96, I^2 = 0\%$	Fixed
Rs1052576	A allele	1047/2226	1601/3022	0.72 (0.58-0.89)	0.003	$P = 0.009, I^2 = 65\%$	Random
	A carrier	806/1113	1160/1511	0.76 (0.63-0.92)	0.004	$P = 0.41, I^2 = 3\%$	Fixed
Rs2308941	T allele	33/66	55/66	0.20 (0.09-0.45)	< 0.0001	-	Fixed
	T carrier	20/33	29/33	0.21 (0.06-0.75)	0.02	-	Fixed
Rs2308950	G allele	3/222	8/220	0.36 (0.10-1.39)	0.14	-	Fixed
	G carrier	3/111	8/110	0.35 (0.09-1.37)	0.13	-	Fixed
Rs4645978	G allele	642/1634	771/1760	0.83 (0.72-0.95)	0.008	$P = 0.64, I^2 = 0\%$	Fixed
	G carrier	506/817	583/880	0.82 (0.67-1.00)	0.05	$P = 0.27, I^2 = 24\%$	Fixed
Rs4645980	G allele	367/864	354/864	1.06 (0.88-1.29)	0.53	-	Fixed
	G carrier	289/432	280/432	1.10 (0.83-1.45)	0.52	-	Fixed
Rs4645981	T allele	193/864	145/864	1.43 (1.12-1.81)	0.004	-	Fixed
	T carrier	171/432	134/432	1.46 (1.10-1.93)	0.009	-	Fixed
Rs4645982	T allele	666/1628	768/1760	0.90 (0.79-1.04)	0.14	$P = 0.57, I^2 = 0\%$	Fixed
	T carrier	541/814	609/880	0.90 (0.73-1.10)	0.29	$P = 0.38$ , $I^2 = 0\%$	Fixed
Rs4646018	A allele	233/556	251/556	0.88 (0.69-1.11)	0.28	-	Fixed
	A carrier	189/278	197/278	0.87 (0.61-1.25)	0.46	-	Fixed

 $\overline{OR} = \text{odds ratio}; 95\%CI = 95\%$  confidence interval.

Rs4645981*T allele							
01 J	Case		Contr	ol		Odds Ratio	Odds Ratio
	Events	10(a)	Events	100	too of	M+H, Fixed, 95%CI	M-H, Fixed, 95%CI
Park et al. 2006	1/1	432	134	432	100.0%	1.46 [1.10, 1.93]	_
Total (95%CI)		432		432	100.0%	1 46 [1 10 1 03]	•
Total events	171		134				-
Heterogeneity: Not anni	licable		104				
Test for overall effect: 2	= 2.63 /P	= 0.009	0				0.01 0.1 1 10 100
		0.000	×				Favors control Favors case
Rs4645981*T carrier							
	Case		Contr	ol		Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95%Cl	M-H, Fixed, 95%Cl
01 Park et al. 2006	171	432	134	432	100.0%	1.46 [1.10, 1.93]	-
Total (95%CI)		432		432	100.0%	1.46 [1.10, 1.95]	•
I otal events	1/1		134				
Heterogeneity: Not app	ICable	- 0.000	a.				0.01 0.1 1 10 100
rest for overall effect: 2	= 2.03 (P	= 0.008	9				Favors control Favors case
1002070 A allele	Case		Contr	ol		Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95%CI	M-H, Random, 95%CI
Fang et al. 2007	69	140	132	200	11.8%	0.50 [0.32, 0.78]	
Lan et al. 2007	413	910	526	1060	20.3%	0.84 [0.71, 1.01]	-
Lou et al. 2007	79	162	132	200	12.3%	0.49 [0.32, 0.75]	-
He et al. 2008	177	340	132	200	14.1%	0.56 [0.39, 0.80]	-
Hosgood et al. 2008	105	252	510	1022	16.9%	0.72 [0.54, 0.95]	-
Ulybina et al. 2009	134	222	127	220	13.6%	1.12 [0.76, 1.63]	I
Wu et al. 2009	70	200	42	120	11.0%	1.00 [0.62, 1.61]	T
Total (95%CI)		2226		3022	100.0%	0 72 10 58 0 891	•
Total evente	1047	LEEU	1601	OULL	1001070	011 1 [0100] 0100]	
Heterogeneity: Tau <sup>2</sup> = 0.1	05: Chi <sup>2</sup> = 1	6.96. df	= 6 (P = 1	0.009):	l <sup>2</sup> = 65%		1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
Test for overall effect: Z	= 2.98 (P =	0.003)					0.01 0.1 1 10 100
		-					Favors control Favors case
DetOEOE76tA service							
Rs1052576 A carrier	Case		Contr	al		Odde Patio	Odda Patio
Study or Subgroup	Case Events	Total	Contre	Iol Total	Weight	Odds Ratio M-H Eixed 95%CI	Odds Ratio
Study or Subgroup	Case Events	Total	Events 87	ol Total	Weight	Odds Ratio M-H. Fixed, 95%CI	Odds Ratio M-H. Fixed, 95%Cl
Study or Subgroup Fang et al. 2007	Case Events 54 318	<u>Total</u> 70 455	Contro Events 87 393	ol <u>Total</u> 100 530	Weight 6.6% 44.1%	Odds Ratio M-H, Fixed, 95%Cl 0.50 [0.23, 1.13] 0.81 [0.61, 1.07]	Odds Ratio M-H. Fixed. 95%Cl
Study or Subgroup Fang et al. 2007 Lan et al. 2007 Lou et al. 2007	Case Events 54 318 61	Total 70 455 81	Contr Events 87 393 87	ol <u>Total</u> 100 530 100	Weight 6.6% 44.1% 7.8%	Odds Ratio <u>M-H. Fixed. 95%CI</u> 0.50 [0.23, 1.13] 0.81 [0.61, 1.07] 0.46 [0.21, 0.99]	Odds Ratio M-H. Fixed. 95%Cl
Fang et al. 2007 Lan et al. 2007 Lou et al. 2007 He et al. 2008	Case Events 54 318 61 136	<u>Total</u> 70 455 81 170	Contr Events 87 393 87 87	ol <u>Total</u> 100 530 100 100	Weight 6.6% 44.1% 7.8% 8.8%	Odds Ratio M-H. Fixed, 95%CI 0.50 [0.23, 1.13] 0.81 [0.61, 1.07] 0.46 [0.21, 0.99] 0.60 [0.30, 1.20]	Odds Ratio M-H. Fixed, 95%Cl
Fang et al. 2007 Lan et al. 2007 Lou et al. 2007 He et al. 2008 Hosgood et al. 2008	Case Events 54 318 61 136 85	Total 70 455 81 170 126	Contro Events 87 393 87 87 381	ol Total 100 530 100 100 511	Weight 6.6% 44.1% 7.8% 8.8% 19.8%	Odds Ratio M-H, Fixed, 95%CI 0.50 [0.23, 1.13] 0.81 [0.61, 1.07] 0.46 [0.21, 0.99] 0.60 [0.30, 1.20] 0.71 [0.46, 1.08]	Odds Ratio M-H. Fixed, 95%Cl
Rs 1032576 A Carter Study or Subgroup Fang et al. 2007 Lan et al. 2007 Lou et al. 2007 He et al. 2008 Hosgood et al. 2008 Ulybina et al. 2009	Case Events 54 318 61 136 85 95	Total 70 455 81 170 126 111	Contre Events 87 393 87 87 381 90	ol Total 100 530 100 100 511 110	Weight 6.6% 44.1% 7.8% 8.8% 19.8% 5.3%	Odds Ratio M-H. Fixed, 95%Cl 0.50 [0.23, 1.13] 0.81 [0.61, 1.07] 0.46 [0.21, 0.99] 0.60 [0.30, 1.20] 0.71 [0.46, 1.08] 1.32 [0.64, 2.70]	Odds Ratio
Rs 1032576 A Carrier Study or Subgroup Fang et al. 2007 Lou et al. 2007 He et al. 2008 Hosgood et al. 2008 Ulybina et al. 2009 Wu et al. 2009	Case Events 54 318 61 136 85 95 57	Total 70 455 81 170 126 111 100	Contre Events 87 393 87 87 87 381 90 35	ol Total 100 530 100 100 511 110 60	Weight 6.6% 44.1% 7.8% 8.8% 19.8% 5.3% 7.6%	Odds Ratio <u>M-H. Fixed, 95%C1</u> 0.50 [0.23, 1.13] 0.81 [0.61, 1.07] 0.46 [0.21, 0.99] 0.60 [0.30, 1.20] 0.71 [0.46, 1.08] 1.32 [0.64, 2.70] 0.95 [0.50, 1.81]	Odds Ratio MH. Fixed. 35%Cl
Rs 1032576 A Carrier Study or Subgroup Fang et al. 2007 Lan et al. 2007 Loc et al. 2007 He et al. 2008 Hosgood et al. 2008 Ulybina et al. 2009 Uu et al. 2009	Case Events 54 318 61 136 85 95 57	Total 70 455 81 170 126 111 100	Contro Events. 87 393 87 87 381 90 35	ol Total 100 530 100 100 511 110 60	Weight 6.6% 44.1% 7.8% 8.8% 19.8% 5.3% 7.6%	Odds Ratio M-H. Fixed, 95%Cl 0.50 [0.23, 1.13] 0.81 [0.61, 1.07] 0.46 [0.21, 0.99] 0.60 [0.30, 1.20] 0.71 [0.46, 1.08] 1.32 [0.64, 2.70] 0.95 [0.50, 1.81]	Odds Ratio MH. Fixed. 95%Cl
Rs 1092070 A Carrier Study or Subgroup Fang et al. 2007 Lou et al. 2007 Lou et al. 2008 Hosgood et al. 2008 Ulybina et al. 2009 Wu et al. 2009 Total (95%CI)	Case Events 54 318 61 136 85 95 57	Total 70 455 81 170 126 111 100 1113	Contro Events. 87 393 87 87 381 90 35	ol Total 100 530 100 511 110 60 1511	Weight 6.6% 44.1% 7.8% 8.8% 19.8% 5.3% 7.6% 100.0%	Odds Ratio M-H. Fixed. 95%CI 0.50 [0.23, 1.13] 0.48 [0.61, 1.07] 0.46 [0.21, 0.99] 0.60 [0.30, 1.20] 0.71 [0.46, 1.08] 1.32 [0.64, 2.70] 0.95 [0.50, 1.81] 0.76 [0.63, 0.92]	Odds Ratio MH-Fixed 393kGl
Rs 1052576 A Carrier Study or Subgroup Fang et al. 2007 Lon et al. 2007 Lou et al. 2007 Ho et al. 2008 Hospood et al. 2008 Ulybina et al. 2009 Wu et al. 2009 Total (95%CI) Total events	Case Events 54 318 61 136 85 95 57 806	Total 70 455 81 170 126 111 100 1113	Contro Events 87 393 87 87 381 90 35 1160	ol Total 100 530 100 511 110 60 1511	Weight 6.6% 44.1% 7.8% 8.8% 19.8% 5.3% 7.6% 100.0%	Odds Ratio M-H. Fixed, 95%CI 0.50 [0.23, 1.13] 0.41 [0.61, 1.07] 0.46 [0.21, 0.99] 0.60 [0.30, 1.20] 0.71 [0.46, 1.08] 1.32 [0.64, 2.70] 0.55 [0.50, 1.81] 0.76 [0.63, 0.92]	Odds Ratio MH-Fixed 35%CI
Is 102237.0 A callier Study or Subgroup Fang et al. 2007 Lon et al. 2007 Lon et al. 2007 Ho et al. 2008 Hosgood et al. 2008 Wu et al. 2009 Total (95%CT) Total events Heterogeneity: Ch <sup>a</sup> et al.	Case Events 54 318 61 136 85 95 57 57 806 16, df = 6 (	Total 70 455 81 170 126 111 100 1113 P = 0.41	Contri- Events 87 393 87 87 381 90 35 1160 (); I <sup>2</sup> = 3%	ol Total 100 530 100 511 110 60 1511	Weight 6.6% 44.1% 7.8% 8.8% 19.8% 5.3% 7.6%	Odds Ratio M-H. Fixed, 95%C1 0.50 [0.23, 1.13] 0.81 [0.61, 1.07] 0.46 [0.21, 0.99] 0.60 [0.30, 1.20] 0.71 [0.46, 1.08] 1.32 [0.64, 2.70] 0.35 [0.50, 1.81] 0.76 [0.63, 0.92]	Odds Ratio MH-Excel 35%Cl
Is 1022/75 A Callier Study or Subgroup. Fang et al. 2007 Lou et al. 2007 He et al. 2007 He et al. 2008 Ubybina et al. 2009 Wu et al. 2009 Total (95%CI) Total (95%CI) Total (95%CI) Total events Heterogeneity: ChIP = 6. Test for overall effect: Z	Case Events 54 318 61 136 85 95 57 806 16, df = 6 ( = 2.86 (P	Total 70 455 81 170 126 111 100 1113 P = 0.41 = 0.004)	Contro Events 87 393 87 87 381 90 90 35 1160 1); I <sup>2</sup> = 3%	ol Total 100 530 100 511 110 60 1511	Weight 6.6% 44.1% 7.8% 8.8% 19.8% 5.3% 7.6% 100.0%	Odds Ratio M-H. Fixed, 95%CI. 0.50 (0.23, 1.13) 0.48 (0.61, 1.07) 0.46 (0.21, 0.99) 0.60 (0.30, 1.20) 0.71 (0.46, 1.08) 1.32 (0.64, 2.70] 0.95 (0.50, 1.81] 0.76 [0.63, 0.92]	Odds Ratio MH-Fixed, 95(C)
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**Figure 2.** Association of the rs4645981\*T allele and the rs4645981\*T carrier with susceptibility to neoplasm. The squares and horizontal lines correspond to the study-specific odds ratio (OR) and 95% confidence interval (95%CI). The diamond represents the summary OR and 95%CI. M.-H. = Mantel-Haenszel estimator.

Genetics and Molecular Research 12 (3): 3070-3078 (2013)

Z.Y. Zhang et al.

#### **Publication bias**

Publication bias of the literatures was accessed by Begger's funnel plot and the Egger linear regression test. The Egger linear regression test was used to measure the asymmetry of the funnel plot. All graphical funnel plots appeared to be symmetrical (Figure 3). The Egger test also showed no statistically significant publication bias (all P > 0.05). Findings of the Egger publication bias test are shown in Table 3.



Figure 3. Begger's funnel plot of publication bias for the association between rs1052576, rs4645978, rs4645982, and susceptibility to lung cancer.

Table 3. Evaluation of publication bias by the Egger linear regression test.								
SNPs	Coefficient	SE	t	Р	95%CI			
rs4645978 (A/G)	-1.416	2.584	-0.550	0.681	(-34.248-31.416)			
rs4645982 (del/ins) rs1052576 (G/A)	-3.228 -1.633	1.284 1.838	-2.510 -0.890	0.241 0.415	(-19.537-13.081) (-6.359-3.092)			

SE = standard error; 95%CI = 95% confidence interval.

## DISCUSSION

Caspase-9 may cause aberrant apoptosis inhibition, and the relevance of this process has been demonstrated in a number of cancer types (Kelly et al., 2010). Caspase-9 is a member of the Caspase family of cysteine proteases that have been implicated in apoptosis and cytokine processing (Bian et al., 2004). As a central initiator caspase, caspase-9 can be triggered in a response to stimuli that damage mitochondria directly or by signals originating in other parts of the cell (Potokar et al., 2003). Although many studies have evaluated the association between CASP-9 gene polymorphisms and cancer risk, the results remain controversial.

In this meta-analysis, we examined 10 SNPs in the CASP-9 gene, including rs1263, rs1052571, rs1052576, rs2308941, rs2308950, rs4645978, rs4645980, rs4645981, rs4645982, and rs4646018. Our study showed that the rs4645981\*T allele and the rs4645981\*T allele carrier had significant associations with cancer risk after adjustment for multiple testing. However, the rs1052576\*A allele, rs1052576\*A carrier, rs2308941\*T allele, and rs2308941\*T carrier might decrease the risk of cancer. There was no association between rs1263, rs1052571, rs2308950, rs4645978, rs4645980, rs4645982, and rs4646018 and cancer risk (all P > 0.05). Although a recent collaborative study found an association for other SNPs of the CASP-9

Genetics and Molecular Research 12 (3): 3070-3078 (2013)

gene and their haplotypes, there has been no pooled analysis of rs1263, rs1052571, rs2308950, rs4645978, rs4645980, rs4645982, and rs4646018 and cancer risk. Some studies have shown that ethnicity may influence cancer susceptibility through variations in genetic background and environmental exposure leading to various gene-gene and gene-environmental interactions. Sensitivity analysis was performed by omitting any single study and non-HWE studies; no influence was found.

The many limitations of our meta-analysis should be addressed. First, the relevant research articles are few and the sample size of this meta-analysis was not large. In addition, some relevant studies could not be included in our analysis due to incomplete raw data. Third, we were not able to address the sources of heterogeneity in all studies. Fourth, although all cases and controls were well defined with similar inclusion criteria, there may be factors not taken into account that may have influenced our results. Most important, our meta-analysis was based on unadjusted OR estimates because not all publications presented adjusted OR; when they did, the OR were not adjusted by the same potential confounders, such as ethnicity, gender, geographic distribution, etc. Given these results, additional investigation in these areas is needed, and our conclusions should be interpreted cautiously.

In conclusion, this meta-analysis of 14 case-control studies demonstrated that CASP-9 gene polymorphisms are involved in the pathogenesis of various cancer. The rs4645981\*T allele and the rs4645981\*T allele carrier might increase the risk of cancer, but the rs1052576\*A allele, rs1052576\*A carrier, rs2308941\*T allele, and rs2308941\*T carrier might be protective. As few studies are available in this field and evidence remains limited, we emphasize the necessity to conduct large studies with adequate methodological quality and proper control of confounding factors in order to obtain valid results.

## **ACKNOWLEDGMENTS**

We would like to thank Liang Yuan (Department of Oncology, Liaoning Cancer Hospital and Institute) for her valuable contribution and kind revision of the manuscript.

#### REFERENCES

- Bian X, Giordano TD, Lin HJ, Solomon G, et al. (2004). Chemotherapy-induced apoptosis of S-type neuroblastoma cells requires caspase-9 and is augmented by CD95/Fas stimulation. J. Biol. Chem. 279: 4663-4669.
- Catchpoole DR and Lock RB (2001). The potential tumour suppressor role for caspase-9 (CASP9) in the childhood malignancy, neuroblastoma. *Eur. J. Cancer* 37: 2217-2221.
- Choi JY, Kim JG, Lee YJ, Chae YS, et al. (2012). Prognostic impact of polymorphisms in the CASPASE genes on survival of patients with colorectal cancer. *Cancer Res. Treat.* 44: 32-36.
- De Vecchi G, Verderio P, Pizzamiglio S, Manoukian S, et al. (2009). Evidences for association of the CASP8 -652 6N del promoter polymorphism with age at diagnosis in familial breast cancer cases. *Breast Cancer Res. Treat.* 113: 607-608.
- Fang CQ, Liu SL, Lou Y and Li JH (2007). Expression of the caspase 9 gene and its polymorphism distribution in gastric cancer. World Chin. J. Digestol. 15: 3190-3193.
- Gangwar R, Mandhani A and Mittal RD (2009). Caspase 9 and caspase 8 gene polymorphisms and susceptibility to bladder cancer in north Indian population. *Ann. Surg. Oncol.* 16: 2028-2034.
- Gil J, Garcia MA and Esteban M (2002). Caspase 9 activation by the dsRNA-dependent protein kinase, PKR: molecular mechanism and relevance. FEBS Lett. 529: 249-255.
- He XM, Wang LL, Fang CQ, Liu SL, et al. (2008). Expression of CASP9 gene and its polymorphism distribution in colon cancer. *Shijie Huaren Xiaohua Zazhi* 16: 2371-2375.

Genetics and Molecular Research 12 (3): 3070-3078 (2013)

Higgins JP and Thompson SG (2002). Quantifying heterogeneity in a meta-analysis. *Stat. Med.* 21: 1539-1558.

- Hosgood HD, III, Baris D, Zhang Y, Zhu Y, et al. (2008). Caspase polymorphisms and genetic susceptibility to multiple myeloma. *Hematol. Oncol.* 26: 148-151.
- Kelly JL, Novak AJ, Fredericksen ZS, Liebow M, et al. (2010). Germline variation in apoptosis pathway genes and risk of non-Hodgkin's lymphoma. *Cancer Epidemiol. Biomarkers Prev.* 19: 2847-2858.
- Kesarwani P, Mandal RK, Maheshwari R and Mittal RD (2011). Influence of caspases 8 and 9 gene promoter polymorphism on prostate cancer susceptibility and early development of hormone refractory prostate cancer. BJU Int. 107: 471-476.
- Lan Q, Zheng T, Chanock S, Zhang Y, et al. (2007). Genetic variants in caspase genes and susceptibility to non-Hodgkin lymphoma. *Carcinogenesis* 28: 823-827.
- Liamarkopoulos E, Gazouli M, Aravantinos G, Tzanakis N, et al. (2011). Caspase 8 and caspase 9 gene polymorphisms and susceptibility to gastric cancer. *Gastric. Cancer* 14: 317-321.
- Liu CY, Wu MC, Chen F, Ter-Minassian M, et al. (2010). A large-scale genetic association study of esophageal adenocarcinoma risk. *Carcinogenesis* 31: 1259-1263.
- Lou Y, Fang CQ and Li JH (2007). A study on the expression of CASP9 gene and its polymorphism distribution in nonsmall cell lung cancer. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 24: 59-62.
- Ni Q, Jiang X, Jin MJ, Liu B, et al. (2011). Association of CASP3 and CASP9 polymorphisms with genetic susceptibility to stomach cancer. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 28: 318-322.
- Oliveira C, Ferreira P, Nabais S, Campos L, et al. (2004). E-cadherin (CDH1) and p53 rather than SMAD4 and caspase-10 germline mutations contribute to genetic predisposition in Portuguese gastric cancer patients. *Eur. J. Cancer* 40: 1897-1903.
- Park JY, Park JM, Jang JS, Choi JE, et al. (2006). Caspase 9 promoter polymorphisms and risk of primary lung cancer. *Hum. Mol. Genet.* 15: 1963-1971.
- Peters JL, Sutton AJ, Jones DR, Abrams KR, et al. (2006). Comparison of two methods to detect publication bias in metaanalysis. JAMA 295: 676-680.
- Potokar M, Milisav I, Kreft M, Stenovec M, et al. (2003). Apoptosis triggered redistribution of caspase-9 from cytoplasm to mitochondria. *FEBS Lett.* 544: 153-159.
- Seo HW, Rengaraj D, Choi JW, Park KJ, et al. (2011). The expression profile of apoptosis-related genes in the chicken as a human epithelial ovarian cancer model. *Oncol. Rep.* 25: 49-56.
- Shiozaki EN, Chai J, Rigotti DJ, Riedl SJ, et al. (2003). Mechanism of XIAP-mediated inhibition of caspase-9. *Mol. Cell* 11: 519-527.
- Son JW, Kang HK, Chae MH, Choi JE, et al. (2006). Polymorphisms in the caspase-8 gene and the risk of lung cancer. *Cancer Genet. Cytogenet.* 169: 121-127.
- Theodoropoulos GE, Michalopoulos NV, Panoussopoulos SG, Taka S, et al. (2010). Effects of caspase-9 and survivin gene polymorphisms in pancreatic cancer risk and tumor characteristics. *Pancreas* 39: 976-980.

Theodoropoulos GE, Gazouli M, Vaiopoulou A, Leandrou M, et al. (2011). Polymorphisms of caspase 8 and caspase 9 gene and colorectal cancer susceptibility and prognosis. *Int. J. Colorectal Dis.* 26: 1113-1118.

Thornberry NA (1997). The caspase family of cysteine proteases. Br. Med. Bull. 53: 478-490.

- Ulybina YM, Kuligina ES, Mitiushkina NV, Rozanov ME, et al. (2009). Coding polymorphisms in Casp5, Casp8 and DR4 genes may play a role in predisposition to lung cancer. *Cancer Lett.* 278: 183-191.
- Wu H (2009). Correlation Between DNA Repair Gene XRCC1 Single Nucleotide Polymorphism and Susceptibility to Hepatocellular Carcinoma in Fusui County of Guangxi. Master's thesis, Guangxi Medical University.
- Ye CQ, Duan JT, Xue XP, Luo YY, et al. (2008). A study on the polymorphism distribution of caspase 9 gene with the susceptibility of cancer. *Tianjin Med. J.* 36: 420-422.
- Zhang L, Liu JL, Zhang YJ and Wang H (2011). Association between HLA-B\*27 polymorphisms and ankylosing spondylitis in Han populations: a meta-analysis. *Clin. Exp. Rheumatol.* 29: 285-292.
- Zintzaras E and Ioannidis JP (2005). Heterogeneity testing in meta-analysis of genome searches. *Genet. Epidemiol.* 28: 123-137.

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