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A literature-based systematic HuGE review and meta-analysis show that CASP gene family polymorphisms are associated with risk of lung cancer

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ABSTRACT. The caspase (CASP) gene family is known to be involved in apoptosis, cytokine maturation, cell growth, and differentiation. A large number of single nucleotide polymorphisms (SNPs) in the CASP gene family have been increasingly recognized as important regulators in the development of lung cancer. However, this specific association is still controversial. In this Human Genome Epidemiology review and meta-analysis, we summarized the available evidence associating lung cancer with the CASP gene family. Seven studies, which included 1155 lung cancer cases and 1120 healthy controls, met the inclusion criteria and were included in our meta-analysis. In seven studies, 19 different SNPs have been studied in seven CASP genes, including CASP-1, -2, -5, -7, -8, -9, and -10. Meta-analysis results showed positive associations between heterozygote (A/G) of rs507879 in the CASP-5 gene, the T allele of rs12415607 in the CASP-7 gene, and the T allele and T carrier (C/T+T/T) of rs4645981 in the CASP-9 gene with lung cancer susceptibility [odds ratio (OR) = 1.83, 95% confidence interval (95%CI)

= 1.07-3.12, P = 0.03; OR = 1.18, 95%CI = 1.02-1.37, P = 0.03; 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, we found that homozygote (G/G) of rs2227310 in the CASP-7 gene, del allele, heterozygote (ins/del), and del carrier (ins/del + del/del) of rs3834129 in CASP-8 could be protective factors for lung cancer (OR = 0.17, 95%CI = 0.14-0.21, P = 0.0003; OR = 0.83, 95%CI = 0.72-0.97, P = 0.02; OR = 0.74, 95%CI = 0.64-0.85, P < 0.0001; OR = 0.81, 95%CI = 0.71-0.93, P = 0.002; respectively). In conclusion, based on this meta-analysis, we suggest that SNPs in CASP-5, -7, -8, and -9 are associated with susceptibility to lung cancer.

Key words: Caspase; Gene polymorphisms; Lung cancer; Meta-analysis

INTRODUCTION

Lung cancer (LC), characterized by uncontrolled cell growth in tissues of the lung (D'Amico et al., 2010), is one of the most common malignant tumors, representing a significant threat to human health (Han et al., 2011). According to the statistics collected by WHO, every year around 1.10 million people die from LC (Long et al., 2008). LC is the leading cause of cancer death in the United States, and has a 5-year relative survival rate of only 16% (Stewart, 2010). There are two main pathological types of LC, namely small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC) (Bandi et al., 2009). NSCLC is the most common form of LC, where about 85% of cancers are classified as NSCLC, while SCLC occurs in approximately 13-15% of patients (Wood et al., 2012). Those who suffer from LC may be afflicted with shortness of breath, coughing (including coughing up blood), and weight loss (Zhao et al., 2010). LC is caused by both genetic and environmental factors and their interactions, and susceptibility differences in the form of rare high-penetrance genes and genetic polymorphism (Vaissière et al., 2009).

Caspases (CASP), with 14 known members, comprise a family characterized by highly conserved intracellular aspartate-specific cysteine proteases (Van De Water et al., 2004; Mittal et al., 2011). Two types are recognized in the CASP family: initiator caspases and effector caspases. CASP-2, -8, -9, -10 are initiator caspases and CASP-3, -6, -7 are executioner caspases (Yu et al., 2009). CASP-4 and -5 are not currently classified as initiator or effector, because they are inflammatory enzymes that, in concert with CASP-1, are involved in T-cell maturation (Van De Water et al., 2004). CASP-14 is not involved in apoptosis or inflammation, but instead is involved in skin cell development (Yu et al., 2009). Studies have shown that the CASP gene family plays an important role in executing cell apoptosis (Du et al., 2005). CASP causes cell death by nuclear membrane breakdown, DNA fragmentation, and chromatin condensation, and the formation of apoptotic genetic polymorphisms for genes controlling the cell cycle or apoptosis has been found to increase the risk for a number of human malignancies (Theodoropoulos et al., 2011). Single nucleotide polymorphisms (SNPs) are the most common form of human genetic variation and may lead to an individual's susceptibility to cancer. Recently, a large number of SNPs in the CASP apoptotic pathway have been increasingly rec-

ognized. Several studies have demonstrated that some variants in apoptosis pathway genes are associated with the susceptibility to various human cancers, especially LC (MacPherson et al., 2004; Zhang et al., 2005; Kesarwani et al., 2011). However, this specific association of CASP gene polymorphisms with LC susceptibility remains controversial. Therefore, we performed a Human Genome Epidemiology (HuGE) review and meta-analysis by including the most recent and relevant articles to identify statistical evidence of the associations between CASP gene family polymorphisms and risk of LC that have been investigated.

MATERIAL AND METHODS

Literature search

PubMed, Cochrane Library, Embase, Web of Science, Springerlink, CNKI, and CBM databases were searched (last search was updated on April 27, 2012) extensively to identify relevant studies, using the following queries: ["caspase" or "CASP" or "CASPASE" (Mesh)] and ["SNPs" or "SNP" or "polymorphism, genetic" (Mesh)] and ["lung cancer" or "lung tumor" or "lung neoplasms" (Mesh)]. The references in the eligible studies or textbooks were also checked.

Inclusion and exclusion criteria

The included studies had to meet the following criteria: i) the type of study was a case-control study; ii) the study focused on associations between CASP gene family polymorphisms and LC susceptibility; iii) all patients had a diagnosis of LC confirmed by pathological examination of the surgical specimen; iv) the frequencies of alleles or genotypes in case and control groups could be extracted; v) the publication was in English or Chinese. Studies were excluded when they were: i) not case-control studies about CASP gene family polymorphisms and LC susceptibility; ii) case reports, letters, reviews, and editorial articles; iii) studies that were based on incomplete data; useless or overlapping data were reported; iv) duplicate data were contained in the studies.

Data extraction

Using a standardized form, data from the studies published were extracted independently by two reviewers (Z.Y.Z. and Y.X.) to populate the necessary information. The following information extracted from each of the articles included: 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 a discussion with a third reviewer (R.W.).

Quality assessment of the studies included

Two reviewers (X.Y.J. and X.T.) independently assessed the quality of papers according to modified STROBE quality score systems (Son et al., 2006; Zhang et al., 2011). Forty

assessment items related to quality appraisal 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 the SNPs of CASP gene family from the relevant studies were determined by the allele counting method. 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 the STATA Version 12.0 software (Stata Corp., College Station, TX, USA). Between-study variations and heterogeneities were estimated using the Cochran Q-statistic (Higgins and Thompson, 2002; Zintzaras and Ionnidis, 2005) ($P \leq 0.05$ was considered to be a manifestation of statistically significant heterogeneity). We also quantified the effect of heterogeneity by using the I^2 test. I^2 ranges between 0 and 100% and represents the proportion of inter-study variability that can be attributed to heterogeneity rather than chance. I^2 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 meta-analysis, or else the fixed-effect model was used (Viechtbauer, 2007). To establish the effect of heterogeneity on conclusions of meta-analyses, subgroup analysis was performed. We tested whether genotype frequencies of controls were in HWE using the χ^2 test. Subgroup analysis based on nationality was used to explore and to explain the diversity among the results of different studies. Sensitivity analysis was mainly 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), where statistical significance was considered when $P < 0.05$. All the P values were two-sided. To ensure the reliability and the accuracy of the results, two reviewers (Z.Y.Z. and Y.X.) independently populated the data in the statistics software programs and got the same results.

RESULTS

Characteristics of the studies included

The search strategy retrieved 94 potentially relevant studies. According to the inclusion criteria, 7 studies (Park et al., 2006; Son et al., 2006; Lou et al., 2007; Sun et al., 2007; Lee et al., 2009; Ulybina et al., 2009; Hart et al., 2011) were included and 87 were excluded in this meta-analysis. The flow chart of study selection is shown in Figure 1. These 7 case-control studies selected included a total of 1155 LC cases and 1120 healthy controls. All studies were case-control studies, which evaluated the association of CASP gene family polymorphisms and LC susceptibility. The publication year of the studies included ranged from 2006 to 2010. All patients fulfilled the diagnosis criteria of LC confirmed by pathological examination of the surgical specimen. The source of controls was based on a healthy population. The HWE test was performed on the genotype distribution of the controls in all the studies included, and all of them were found to be in HWE ($P > 0.05$). Seven CASP genes with 19 SNPs were addressed, including CASP-1 (rs501192), CASP-2 (rs4647297), CASP-5 (rs507879, rs523104),

CASP-7 (rs12415607, rs1593766, rs2227310, rs10787498), CASP-8 (rs3834129, rs3769818, rs1045485), CASP-9 (rs4645978, rs4645980, rs4645981, rs4645982, rs1052571, rs1052576, rs2308950), and CASP-10 (rs13006529). All quality scores of the studies included were higher than 20 (moderate to high quality). The baseline characteristics and methodological quality of all the studies included are summarized in Table 1. The genotype distribution and risk allele frequency are summarized in Table 2.

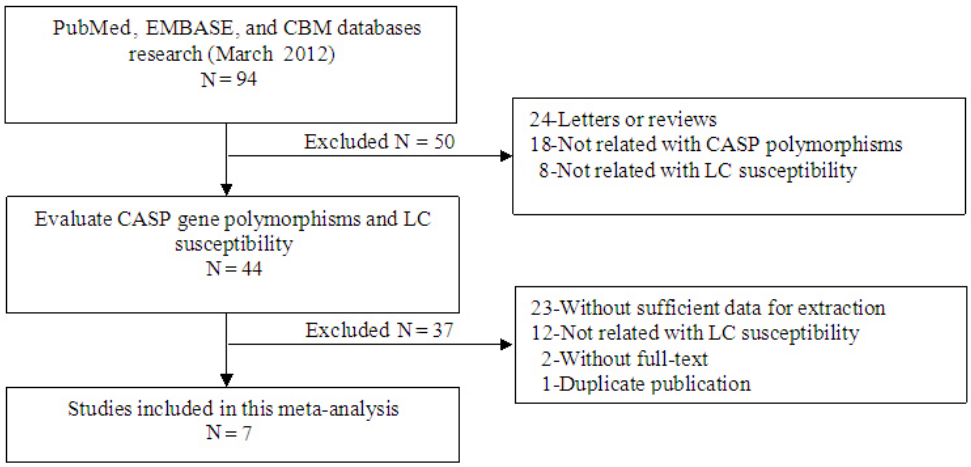

Figure 1. Flow chart shows study selection procedure. CASP = caspase; LC = lung cancer.

Association between CASP gene family polymorphisms and LC risk

A summary of the meta-analysis findings of the association between CASP gene family polymorphisms and LC susceptibility is provided in Table 3. The meta-analysis results showed that the heterozygote (A/G) of rs507879 in the CASP-5 gene was positively associated with LC risk (OR = 1.83, 95%CI = 1.07-3.12, P = 0.03). In addition, the T allele of rs12415607 in the CASP-7 gene also showed a significantly positive association with LC susceptibility (OR = 1.18, 95%CI = 1.02-1.37, P = 0.03). However, the homozygote (G/G) of rs2227310 in the CASP-7 gene showed a negative relation to LC susceptibility (OR = 0.17, 95%CI = 0.14-0.21, P = 0.0003). Similarly, the del allele, heterozygote (ins/del), and del carrier (ins/del + del/del) of rs3834129 in CASP-8 also showed a negative association with LC risk (OR = 0.83, 95%CI = 0.72-0.97, P = 0.02; OR = 0.74, 95%CI = 0.64-0.85, P < 0.0001; OR = 0.81, 95%CI = 0.71-0.93, P = 0.002, respectively). Only the T allele and T carrier (C/T+T/T) of rs4645981 in the CASP-9 gene showed a positive association with LC risk (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). On the other hand, there was no significant associations found between CASP-1 (rs501192), CASP-2 (rs4647297), CASP-5 (rs523104), CASP-7 (rs1593766, rs2227310), CASP-8 (rs3769818, rs1045485), CASP-9 (rs4645978, rs4645980, rs4645982, rs1052571, rs1052576, rs2308950), and CASP-10 (rs13006529) (all P > 0.05) and LC risk. Sensitivity analysis was performed by sequential omission of each study. The significance of pooled OR in all individual analyses and subgroup analyses was not influenced excessively by omitting any single study.

Table 1. Characteristics of individual studies included in meta-analysis.

First author (year)	Country	Ethnicity	Case number		Sample	Detection	Gene	Chromosome	SNP	Quality scores
			LC	Control						
Park et al. (2006)	Korea	Asian	432	432	Blood	PCR-RFLP	CASP-9	1	rs4645978 (A/G) rs4645980 (T/G) rs4645981 (C/T) rs4645982 (del/ins)	23
Son et al. (2006)	Korea	Asian	432	432	Blood	PCR-RFLP	CASP-8	2	rs3834129 (ins/del) rs3769818 (G/A)	22
Lou et al. (2007)	China	Asian	81	100	Blood	PCR-RFLP	CASP-9	1	rs1052571 (C/T) rs1052576 (A/G)	24
Sun et al. (2007)	China	Asian	1155	1120	Blood	PCR-RFLP	CASP-8	2	rs3834129 (ins/del)	25
Lee et al. (2009)	Korea	Asian	720	720	-	-	CASP-7	10	rs12415607 (G/T) rs1593766 (A/G) rs2227310 (C/G) rs10787498 (G/T)	23
Ulybina et al. (2009)	Russia	Caucasian	111	110	Blood	AS-PCR	CASP-2	7	rs4647297 (C/G)	27
							CASP-5	11	rs507879 (A/G)	
							CASP-7	10	rs2227310 (C/G)	
							CASP-9	1	rs1052576 (A/G) rs2308950 (A/G) rs1052576 (A/G)	
							CASP-10	2	rs13006529 (A/T)	
			647	833			CASP-5	11	rs523104 (G/C)	
			647	833			CASP-8	2	rs1045485 (G/C)	
Hart et al. (2011)	Norway	Caucasian	442	440	Blood/Tissue	TaqMan	CASP-8	2	rs3834129 (ins/del)	29
							CASP-1	11	rs501192 (G/A)	

LC = lung cancer; SNP = single nucleotide polymorphisms; PCR = polymerase chain reaction; RFLP = restriction fragment length polymorphism; AS = allele specific.

Table 2. Frequency distribution of single nucleotide polymorphisms (SNPs) in the studies included.

First author	Year	Gene	SNPs	LC group						Control group				HWE test	
				Total	1	2	1/1	1/2	2/2	Total	1	2	1/1	1/2	2/2	χ^2	P
Park et al.	2006	CASP-9	rs4645978 (A/G)	432	521	343	148	225	59	432	491	373	138	215	79	0.088	0.766
			rs4645980 (T/G)	432	497	367	143	211	78	432	510	354	152	206	74	0.087	0.769
			rs4645981 (C/T)	432	671	193	261	149	22	432	719	145	298	123	11	0.162	0.688
			rs4645982 (del/ins)	432	529	335	159	211	62	432	523	341	161	201	70	0.297	0.586
Son et al.	2006	CASP-8	rs3834129 (ins/del)	432	210	654	25	160	247	432	205	659	22	161	249	0.380	0.537
			rs3769818 (G/A)	432	628	236	226	176	30	432	618	246	226	166	40	1.384	0.239
Lou et al.	2007	CASP-9	rs1052571 (C/T)	81	134	28	55	24	2	100	162	38	66	30	4	0.064	0.800
			rs1052576 (A/G)	81	79	83	18	43	20	100	132	68	45	42	13	0.412	0.521
Sun et al.	2007	CASP-8	rs3834129 (ins/del)	1149	1860	438	756	348	45	1111	1687	535	640	407	64	0.004	0.947
Lee et al.	2009	CASP-7	rs12415607 (G/T)	720	856	584	260	336	124	720	913	527	293	327	100	0.328	0.567
			rs1593766 (A/G)	720	1279	161	565	149	6	720	1295	145	582	131	7	0.015	0.902
			rs2227310 (C/G)	720	813	627	246	321	153	720	878	562	272	334	114	0.460	0.497
			rs10787498 (G/T)	720	1150	290	467	216	37	720	1141	299	455	231	34	0.449	0.503
Ulybina et al.	2009	CASP-2	rs4647297 (C/G)	111	213	9	102	9	0	110	211	9	101	9	0	0.200	0.655
		CASP-5	rs507879 (A/G)	111	119	103	29	61	21	110	112	108	34	44	32	2.388	0.122
			rs523104 (G/C)	647	772	522	240	292	115	833	1037	629	328	381	124	0.601	0.438
		CASP-7	rs2227310 (C/G)	111	164	58	57	50	4	110	158	62	57	44	9	0.015	0.901
		CASP-9	rs1052571 (C/T)	111	137	85	40	57	14	110	128	92	38	52	20	0.090	0.765
			rs2308950 (A/G)	111	219	3	108	3	0	110	212	8	102	8	0	0.157	0.692
			rs1052576 (A/G)	111	134	88	39	56	16	110	127	93	37	53	20	0.018	0.893
		CASP-10	rs13006529 (A/T)	111	118	104	33	52	26	110	129	91	43	43	24	1.146	0.284
		CASP-8	rs1045485 (G/C)	647	1128	166	489	150	8	833	1474	192	652	170	11	0.000	0.983
Hart et al.	2011	CASP-8	rs3834129 (ins/del)	436	460	412	125	210	101	433	421	445	106	209	118	0.498	0.481
		CASP-1	rs501192 (G/A)	436	706	166	286	134	16	435	705	165	282	141	12	1.294	0.255

LC = lung cancer; HWE = Hardy-Weinberg equilibrium; all $P < 0.05$ was considered to be statistically significant.

Table 3. Meta-analysis of the association between CASP gene polymorphisms and lung cancer (LC).

Polymorphisms		LC (cases n/N)	Controls (n/N)	OR (95%CI)	P	Effect model
CASP-1						
Rs501192	A allele	166/872	165/870	1.00 (0.79-1.28)	0.97	Fixed
	G/A + A/A	150/436	153/435	0.97 (0.73-1.28)	0.81	Fixed
	A/A	16/436	12/435	1.34 (0.63-2.87)	0.45	Fixed
	G/A	134/436	141/435	0.93 (0.70-1.23)	0.59	Fixed
CASP-2						
Rs4647297	G allele	9/222	9/220	0.99 (0.39-2.54)	0.98	Fixed
	C/G + G/G	9/111	9/110	0.99 (0.38-2.60)	0.98	Fixed
	G/G	-	-	-	-	-
	C/G	9/111	9/110	0.99 (0.38-2.60)	0.98	Fixed
CASP-5						
Rs507879	G allele	103/222	108/220	0.90 (0.62-1.30)	0.57	Fixed
	A/G + G/G	82/111	76/110	1.26 (0.70-2.27)	0.43	Fixed
	G/G	21/111	32/110	0.57 (0.30-1.07)	0.08	Fixed
	A/G	61/111	44/110	1.83 (1.07-3.12)	0.03	Fixed
Rs523104	C allele	522/1294	629/1666	1.11 (0.96-1.29)	0.15	Fixed
	G/C + C/C	407/647	505/833	1.10 (0.89-1.36)	0.37	Fixed
	C/C	115/647	124/833	1.24 (0.94-1.63)	0.13	Fixed
	G/C	292/647	381/833	0.98 (0.79-1.20)	0.82	Fixed
CASP-7						
Rs12415607	T allele	584/1440	527/1440	1.18 (1.02-1.37)	0.03	Fixed
	G/T + T/T	460/720	427/720	1.21 (0.98-1.50)	0.07	Fixed
	T/T	124/720	100/720	1.29 (0.97-1.72)	0.08	Fixed
	G/T	336/720	327/720	1.05 (0.85-1.29)	0.63	Fixed
Rs1593766	G allele	161/1440	145/1440	1.12 (0.89-1.43)	0.33	Fixed
	A/G + G/G	155/720	138/720	1.16 (0.89-1.50)	0.27	Fixed
	G/G	6/720	7/720	0.86 (0.29-2.56)	0.78	Fixed
	A/G	149/720	131/720	1.17 (0.90-1.52)	0.23	Fixed
Rs2227310	G allele	685/1662	624/1660	1.12 (0.87-1.43)	0.39	Fixed
	C/G + G/G	528/831	501/830	1.15 (0.94-1.40)	0.18	Fixed
	G/G	157/831	457/830	0.17 (0.14-0.21)	0.0003	Random
	C/G	371/831	492/830	0.75 (0.30-1.85)	0.53	Random
Rs10787498	T allele	290/1440	299/1440	0.96 (0.80-1.15)	0.68	Fixed
	G/T + T/T	253/720	265/720	0.93 (0.75-1.15)	0.51	Fixed
	T/T	37/720	34/720	1.09 (0.68-1.76)	0.72	Fixed
	G/T	216/720	231/720	0.91 (0.73-1.13)	0.39	Fixed
CASP-8						
Rs3834129	del allele	1504/4034	1639/1639	0.83 (0.72-0.97)	0.02	Random
	ins/del + del/del	1111/2017	1208/1976	0.74 (0.64-0.85)	<0.0001	Fixed
	del/del	393/2017	431/1976	0.84 (0.71-1.01)	0.06	Fixed
	ins/del	819/2017	895/1976	0.81 (0.71-0.93)	0.002	Fixed
Rs3769818	A allele	236/864	246/864	0.94 (0.77-1.17)	0.59	Fixed
	G/A + A/A	206/432	206/432	1.00 (0.77-1.31)	0.11	Fixed
	A/A	30/432	40/432	0.73 (0.45-1.20)	0.21	Fixed
	G/A	176/432	166/432	1.10 (0.84-1.45)	0.49	Fixed
Rs1045485	C allele	166/1294	192/1666	1.13 (0.91-1.41)	0.28	Fixed
	G/C + C/C	158/647	181/833	1.16 (0.91-1.48)	0.22	Fixed
	C/C	8/647	11/833	0.94 (0.37-2.34)	0.89	Fixed
	G/C	150/647	170/833	1.18 (0.92-1.51)	0.20	Fixed

Continued on next page

Table 3. Continued.

Polymorphisms		LC cases n/N	Controls n/N	OR (95%CI)	P	Effect model
CASP-9						
Rs4645978	G allele	343/864	373/864	0.87 (0.72-1.05)	0.14	Fixed
	A/G + G/G	284/432	294/432	0.90 (0.68-1.20)	0.47	Fixed
	G/G	59/432	79/432	0.71 (0.49-1.02)	0.06	Fixed
	A/G	225/432	215/432	1.10 (0.84-1.43)	0.50	Fixed
Rs4645980	G allele	367/864	354/864	1.06 (0.88-1.29)	0.53	Fixed
	T/G + G/G	289/432	280/432	1.10 (0.83-1.45)	0.52	Fixed
	G/G	78/432	74/432	1.07 (0.75-1.51)	0.72	Fixed
	T/G	211/432	206/432	1.05 (0.80-1.37)	0.73	Fixed
Rs4645981	T allele	193/864	145/864	1.43 (1.12-1.81)	0.004	Fixed
	C/T+T/T	171/432	134/432	1.46 (1.10-1.93)	0.009	Fixed
	T/T	22/432	11/432	2.05 (0.98-4.29)	0.06	Fixed
	C/T	149/432	123/432	1.32 (0.99-1.76)	0.06	Fixed
Rs4645982	ins allele	335/864	341/864	0.97 (0.80-1.18)	0.77	Fixed
	del/ins + ins/ins	273/432	271/432	1.02 (0.77-1.34)	0.89	Fixed
	ins/ins	62/432	70/432	0.87 (0.60-1.26)	0.45	Fixed
	del/ins	211/432	201/432	1.10 (0.84-1.43)	0.50	Fixed
Rs1052571	T allele	113/384	130/420	0.87 (0.64-1.19)	0.39	Fixed
	C/T + T/T	97/192	106/210	0.93 (0.61-1.40)	0.72	Fixed
	T/T	16/192	24/210	0.64 (0.33-1.27)	0.20	Fixed
	C/T	81/192	82/210	1.09 (0.73-1.64)	0.66	Fixed
Rs1052576	G allele	171/384	161/420	1.34 (0.60-3.01)	0.47	Random
	A/G + G/G	135/192	128/210	1.61(0.54-4.83)	0.39	Random
	G/G	36/192	33/210	1.28 (0.45-3.62)	0.65	Random
	A/G	99/192	95/210	1.28 (0.87-1.90)	0.21	Fixed
Rs2308950	G allele	3/222	8/220	0.36 (0.10-1.39)	0.14	Fixed
	A/G + G/G	3/111	8/110	0.35 (0.09-1.37)	0.13	Fixed
	G/G	-	-	-	-	-
	A/G	3/111	8/110	0.35 (0.09-1.37)	0.13	Fixed
CASP-10						
Rs13006529	T allele	104/222	91/220	1.25 (0.86-1.82)	0.25	Fixed
	A/T + T/T	78/111	67/110	1.52 (0.87-2.65)	0.14	Fixed
	T/T	26/111	24/110	1.10 (0.58-2.06)	0.78	Fixed
	A/T	52/111	43/110	1.37 (0.80-2.34)	0.24	Fixed

OR = odds ratio; 95%CI = confidence interval.

The positive associations between CASP gene family polymorphisms and LC susceptibility are shown in Figure 2.

Publication bias

Publication bias of the studies was assessed based on rs3834129 in the CASP-8 gene 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. The funnel plot of the studies included appeared to be symmetrical (Figure 3). The Egger test also showed that there was no statistical significance for all evaluations of publication bias (P = 0.061).

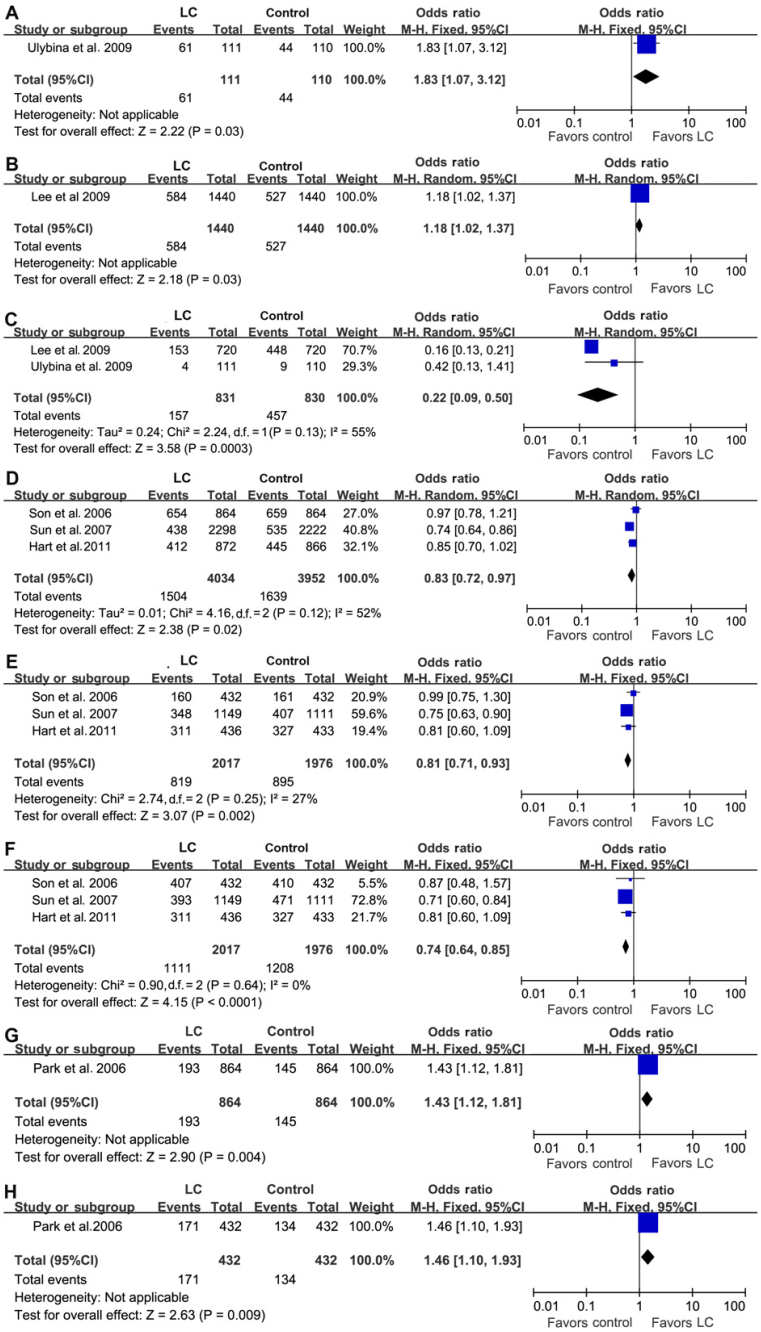

Figure 2. Positive associations between CASP gene family polymorphisms and lung cancer susceptibility. **A.** Heterozygote of rs507879; **B.** T allele of rs12415607; **C.** homozygote of rs2227310; **D.** del allele of rs3834129; **E.** heterozygote of rs3834129; **F.** del carrier of rs3834129; **G.** T allele of rs4645981; **H.** T carrier of rs4645981. LC = lung cancer; M.-H. = Mantel-Haenszel; 95%CI = 95% confidence interval.

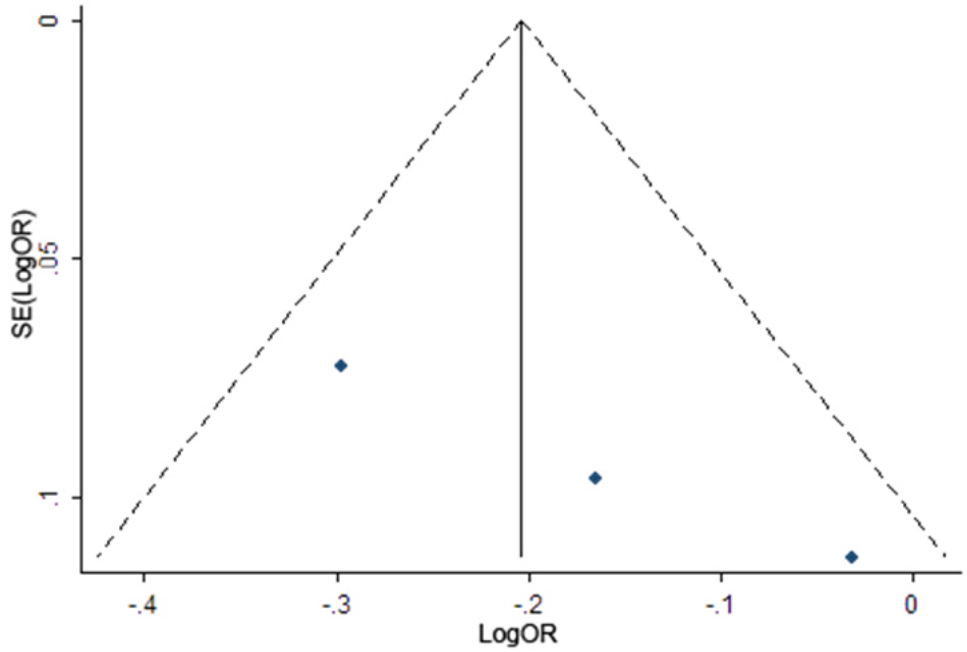

Figure 3. Begger's funnel plot of publication bias based on the association between rs3834129 in the CASP-8 gene and susceptibility to lung cancer.

DISCUSSION

LC ranks as the most malignant tumors around the world (D'Amico et al., 2010). It is estimated that 1.10 million people die from LC each year (Han et al., 2011). The most common form of LC is NSCLC, and the other is SCLC (Bandi et al., 2009). Smoking is the leading cause of lung cancer (Thorgeirsson et al., 2008). Besides that, some studies have found that genetic factors play an important role in the pathology of LC (El-Zein et al., 2012). It has been demonstrated that caspases are closely related to genetic susceptibility to cancers. The somatic mutations in CASP genes repress apoptosis leading to unwanted cell proliferation and anomalous cell survival (Srivastava et al., 2010). Caspases are a big family of highly conserved intracellular aspartate-specific cysteine proteases (Mittal et al., 2011). Polymorphism in CASP-9 gene has been shown to be associated with LC (Kesarwani et al., 2011). However, the clear connection between other genes in the caspase family and LC risk is not yet known.

In this meta-analysis, we quantitatively assessed the association between SNPs in CASP genes and LC risk. Finally, 7 case-control studies were included with a total of 1155 LC cases and 1120 healthy controls. We examined 7 CASP genes with 19 SNPs, including CASP-1, -2, -5, -7, -8, -9, and -10. The main meta-analysis results showed a significant association between CASP-5, -7, -8, and -9 and susceptibility to LC. There was a positive association between the heterozygote (A/G) of rs507879 in the CASP-5 gene and LC risk, which indicated that the heterozygote (A/G) of rs507879 may be a potential risk factor for LC. Moreover, the T allele of rs12415607 in the CASP-7 gene and T allele and T carrier (C/T+T/T) of rs4645981

in the CASP-9 gene also may increase the risk of LC. Interestingly, we found that the homozygote (G/G) of rs2227310 in the CASP-7 gene, del allele, heterozygote (ins/del), and del carrier (ins/del + del/del) of rs3834129 in CASP-8 may decrease the risk of LC, suggesting that rs2227310 in the CASP-7 gene and rs3834129 in CASP-8 are protective factors against LC. In contrast, we found no significant associations between CASP-1 (rs501192), CASP-2 (rs4647297), CASP-5 (rs523104), CASP-7 (rs1593766, rs2227310), CASP-8 (rs3769818, rs1045485), CASP-9 (rs4645978, rs4645980, rs4645982, rs1052571, rs1052576, rs2308950), and CASP-10 (rs13006529) (all $P > 0.05$) and LC risk.

Limitations in our meta-analysis should be addressed. First, because only the studies published were included in the meta-analysis, the relevant research articles are not many, and the sample size of this study was not large. Second, 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 among all studies. In addition, although all cases and controls of each study were well defined with similar inclusion criteria, there might have been potential factors that were not taken into account and that may have influenced our results. Most important of all, our meta-analysis was based on unadjusted OR estimates because not all papers presented adjusted ORs or when they did, the ORs were not adjusted by the same potential confounders, such as ethnicity, gender, geographic distribution, etc. Given these considerations, additional investigation in these areas is needed, and our conclusions should be interpreted cautiously.

In conclusion, this meta-analysis of 7 case-control studies demonstrated that SNPs in CASP-5, -7, -8, and -9 are associated with susceptibility to LC. The heterozygote (A/G) of rs507879, T allele of rs12415607, and T allele and T carrier (C/T+T/T) of rs4645981 may be potential risk factors for LC, while the homozygote (G/G) of rs2227310 in the CASP-7 gene and del allele, heterozygote (ins/del), and del carrier (ins/del + del/del) of rs3834129 in CASP-8 may decrease the risk of LC. Since few studies are available in this field and current evidence remains limited, it should be emphasized that there is a need to conduct large studies with an adequate methodological quality, properly controlling confounders to obtain valid results.

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