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The relationship of CASP 8 polymorphism and cancer susceptibility: a meta-analysis

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Abstract

Caspase-8 (CASP8), member of the caspase cysteine protease family, plays an important role in cancer development. CASP8 D302H (rs1045485) (D, Aspartate; H, Histidine) and CASP8 -652 6N del (rs3834129) polymorphisms have been reported to be associated with Cancer susceptibility. However, there are many controversies on this issue. Therefore we performed this meta-analysis with 32 publications, which include 25800 case and 31964 control subjects for CASP8 -652 6N del polymorphism, and 36883 cases and 41089 controls for D302H polymorphism. The results demonstrated that the -652 6N del frequency showed significant difference between case and control group (del versus ins: OR=0.92; 95% CI: 0.90–0.95, p<0.00001). Homozygous, dominant and recessive genotypes were significantly associated with cancer risks. For D302H polymorphism, data indicated the association of allele C with decreased cancer risk (Overall, C versus G: OR=0.93; 95% CI: 0.86–0.99, p=0.03). All genetic models also indicated the significant association with cancer risk especially in Asian population. Further subgroup analysis indicated that CASP8 -652 6N del polymorphism was associated with breast cancer, lung and gastrointestinal cancer susceptibility. CASP8 D302H was found to be only associated with breast cancer risk. Therefore, these two CASP8 variations could be regarded as potential biomarkers for cancer risk.

Key words: CASP8, polymorphism, cancer, meta-analysis.

Introduction

Apoptosis which is also called programmed cell death, as a physiological process to protect the cells or tissue from being damaged by removing abnormal cells, is critical for successful tissue development and maintenance of normal tissue homeostasis. Aberrant regulation of apoptosis will lead to a large variety of disorders like autoimmune disease, degenerative disorder and cancer (1-3). In most cases, apoptosis is restrictively regulated in a well-conserved pathway, by which the cell death signals can be transmitted downward by a cascade of caspases activation. Caspases belong to a large family of cysteine proteases that can serve as apoptosis executioner. Caspases are located in the cytoplasm in inactivated form and then be activated by cleavage of specific aspartic acid residues substrate either by the same or other caspases. Although recently caspase-independent pathway was found out to be another way of apoptosis regulation, most of apoptosis are still be triggered and executed by caspases in order to keep the maintenance of cellular homeostasis (4).

Caspase 8 (CASP8), is an important member of the caspase cysteine protease family encoded by CASP8 gene. Its activation requires being cleaved by protealytic process from a 55kDa precursor into smaller active subunit (~20kDa) (5). Once caspase 8 is activated, it can function through substrate cleavage in either cytoplasm or nucleus, thus causing characteristic morphological as well as physical changes of apoptosis. Caspase 8 is chiefly involves in death receptor apoptosis pathway, also called extrinsic pathway; by the cleaveage of its downstream molecule, caspase 3 or 7 (6). Its deregulation or deactivity will lead to abnormal cancer progression as a result of disordered apoptosis.

According to NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP), CASP8 gene has 72 variations, among which the D302H (D, aspartate to H, histidine, G/C; rs1045485) of exon 10 and the promoter six-nucleotide deletion/insertion variation (–652 6N del; rs3834129) have drawn extensive attention. The reason is that previous results indicated that they might have some relationship with the function of CASP8.

For example, the nonsynonymous aspartate to histidine mutation at residue 302 locating on the surface of caspase 8 protein is hypothesized to influence the function of apoptosis regulation of CASP8 by influencing its autoprocessing or interactions with antiapoptotic molecules, such as the fas-associated protein with death domain-like apoptosis regulator (CFLAR) (7). The –652 6N del allele in CASP8 promoter region has been found to destruct the binding site for transcriptional activator Sp1, thus highly associated with decreased caspase 8 RNA expression levels (8). These all indicated us that these variants may contribute to the function of caspase 8 in regulating apoptosis, and furthermore, have potential role in cancer progression regulation.

However, there are still controversies on the association of caspase 8 with cancer susceptibility, especially for different cancer types. Studies by Shepherd (9),

Table 1. Characterization of CASP8 -652 6N del and D302H polymorphism in each study in this meta-analysis.

Study	Year	Country	Ethnicity	Cancer Type	Polymorphisms	Case			Control			HWE test
						AA	AB	BB	AA	AB	BB	
Shephard (9)	2009	UK	Caucasian	Breast Cancer	−652 6N del	738	1491	659	616	1316	675	Yes
Hashemi (11)	2012	Iran	Caucasian	Breast Cancer	−652 6N del	113	107	16	79	91	33	Yes
		UK	Caucasian	Breast Cancer	−652 6N del	631	224	22	665	188	25	Yes
Vecchi (18)	2009	Italy	Caucasian	Breast Cancer	−652 6N del	162	301	117	106	206	94	No
Cybulsky (13)	2008	Poland	Caucasian	Breast Cancer	−652 6N del	178	314	126	274	499	192	Yes
Haiman (12)	2008	African American	Caucasian	Breast Cancer	−652 6N del	86	211	117	100	222	125	Yes
		Latino	Caucasian	Breast Cancer	−652 6N del	177	185	47	169	194	52	Yes
		European American	Caucasian	Breast Cancer	−652 6N del	158	244	117	151	286	115	Yes
		Native Hawaiian	Caucasian	Breast Cancer	−652 6N del	52	64	23	97	130	60	Yes
		Japanese American	Caucasian	Breast Cancer	−652 6N del	502	189	23	596	223	26	Yes
		Filipino American	Caucasian	Breast Cancer	−652 6N del	187	88	12	196	76	15	No
		Chinese American,	Caucasian	Breast Cancer	−652 6N del	502	189	23	596	223	26	Yes
Sun (8)	2007	Chinese	Asian	Breast Cancer	−652 6N del	699	371	49	513	419	72	Yes
Sun (8)	2007	Chinese	Asian	Lung Cancer	−652 6N del	756	348	45	640	407	64	Yes
Lee (27)	2010	Korean	Asian	Lung Cancer	−652 6N del	440	240	40	422	257	41	Yes
Son (30)	2006	Korea	Asian	Lung Cancer	−652 6N del	247	160	25	249	161	22	Yes
Hart (28)	2011	Norway	Caucasian	Lung Cancer	−652 6N del	125	210	101	106	209	118	Yes
Haiman (12)	2008	African American	Caucasian	Colorectal Cancer	−652 6N del	49	109	59	217	522	309	Yes
		Japanese American	Caucasian	Colorectal Cancer	−652 6N del	257	108	11	828	302	35	Yes
		Native Hawaiian	Caucasian	Colorectal Cancer	−652 6N del	18	21	17	111	158	77	Yes
		Latino	Caucasian	Colorectal Cancer	−652 6N del	90	109	44	414	448	126	Yes
		European American	Caucasian	Colorectal Cancer	−652 6N del	161	265	136	346	681	308	Yes
Sun (8)	2007	Chinese	Asian	Colorectal Cancer	−652 6N del	605	280	33	528	304	58	Yes
Liu (31)	2010	Chinese	Asian	Colorectal Cancer	−652 6N del	311	160	25	528	278	32	Yes
Pittman (32)	2008	UK	Caucasian	Colorectal Cancer	−652 6N del	995	1897	987	892	1872	897	Yes
George (33)	2011	Greece	Caucasian	Colorectal Cancer	−652 6N del	103	201	98	120	254	106	Yes
Xiao (14)	2013	Chinese	Asian	Colorectal Cancer	−652 6N del	187	107	11	212	115	15	Yes
Wu (25)	2013	Chinese	Asian	Colorectal Cancer	−652 6N del	284	152	15	358	244	29	Yes
Sun (8)	2007	Chinese	Asian	Esophagus Cancer	−652 6N del	652	328	38	543	338	56	Yes
Umar (19)	2011	India	Caucasian	Esophagus Cancer	−652 6N del	139	103	17	138	93	28	No
Sun (8)	2007	Chinese	Asian	Gastric Cancer	−652 6N del	262	142	16	233	152	25	Yes
Liamarkopoulos(34)	2011	Greece	Caucasian	Gastric Cancer	−652 6N del	35	42	11	120	254	106	Yes
Yang (35)	2008	Chinese	Asian	Pancreatic cancer	−652 6N del	268	111	18	521	323	63	Yes
Srivastava(36)	2010	India	Caucasian	Gallbladder cancer	−652 6N del	147	69	12	122	84	24	Yes
George (37)	2010	India	Caucasian	Prostate cancer	−652 6N del	84	69	12	116	83	6	No
Haiman (12)	2008	African American	Caucasian	Prostate cancer	−652 6N del	175	437	240	127	308	181	Yes
		Japanese American	Caucasian	Prostate cancer	−652 6N del	497	194	16	502	187	20	Yes
		Native Hawaiian	Caucasian	Prostate cancer	−652 6N del	35	59	16	27	58	26	Yes
		Latino	Caucasian	Prostate cancer	−652 6N del	246	275	96	257	269	81	Yes
		European American	Caucasian	Prostate cancer	−652 6N del	121	244	78	108	210	104	No
Cybulsky (13)	2008	Poland	Caucasian	Prostate cancer	−652 6N del	139	236	110	274	499	192	Yes
Kesarwani (38)	2011	India	Caucasian	Prostate cancer	−652 6N del	86	72	12	109	83	6	No
Gangwar (39)	2009	Iran	Caucasian	Bladder cancer	−652 6N del	121	84	7	133	101	16	Yes
Wang (40)	2009	Chinese	Asian	Bladder cancer	−652 6N del	12	115	238	25	138	205	Yes

Xiao (41)	2011	Chinese	Asian	non-Hodgkin lymphoma	−652 6N del	92	40	7	152	78	10	Yes
Lv (42)	2010	Chinese	Asian	Lymphoid tumor	−652 6N del	48	46	6	344	179	21	Yes
Sun (8)	2007	Chinese	Asian	Cervical Cancer	−652 6N del	199	102	13	314	211	42	Yes
Chatterjee(22)	2011	African	Caucasian	Cervical Cancer	−652 6N del	18	63	25	43	129	85	No
		Mixed	Caucasian	Cervical Cancer	−652 6N del	84	188	67	265	510	189	No
Ma (23)	2011	Chinese	Asian	Ovarian Cancer	−652 6N del	128	87	3	138	122	25	No
Li (43)	2008	USA	Caucasian	Melanoma	−652 6N del	243	385	177	207	440	188	Yes
Li (24)	2010	USA	Caucasian	Head and neck carcinoma	−652 6N del	311	456	256	257	542	253	No
Wang (44)	2011	Chinese	Asian	Papillary thyroid Carcinoma	−652 6N del	65	45	8	106	92	15	Yes
Zhu (45)	2010	Chinese	Asian	Renal Cancer	−652 6N del	226	119	8	205	139	21	Yes
Shephard (9)	2009	UK	Caucasian	Breast Cancer	D302H	1728	498	36	534	1764	2226	Yes
Cox (10)	2007	European	Caucasian	Breast Cancer	D302H	12744	3440	239	12881	3900	328	Yes
Frank (46)	2006	German	Caucasian	Breast Cancer	D302H	377	101	8	109	385	478	Yes
Macpherson (7)	2004	UK	Caucasian	Breast Cancer	D302H	2186	579	37	616	2223	2765	Yes
Sigurdson (47)	2007	US	Caucasian	Breast Cancer	D302H	660	185	7	192	667	845	Yes
Pittman (32)	2008	UK	Caucasian	Colonrectal Cancer	D302H	59	894	2890	61	867	2703	Yes
Ramus (48)	2008	USA	Caucasian	Ovarian Cancer	D302H	2871	868	59	5005	1504	128	Yes
Li (43)	2008	USA	Caucasian	Melanoma	D302H	629	168	8	615	207	13	Yes
Li (24)	2010	USA	Caucasian	Head and neck Carcinoma	D302H	745	261	17	783	252	17	Yes
Bethke (49)	2009	Europe	Caucasian	Meningioma	D302H	476	121	10	490	110	7	Yes
Rajaraman (29)	2007	USA	Caucasian	Meningioma	D302H	117	38	5	426	118	6	Yes
		USA	Caucasian	Glioma	D302H	284	95	3	426	118	6	Yes
		USA	Caucasian	Acoustic neroma	D302H	50	20	3	426	118	6	Yes
Enjuanes (50)	2008	Spain	Caucasian	Chronic Lymphatic lymphoma	D302H	533	138	8	485	217	14	Yes
Lan (51)	2007	USA	Caucasian	non-Hodgkin lymphoma	D302H	1500	419	24	1388	393	24	Yes
Srivastava (36)	2010	India	Caucasian	Gallbladder Cancer	D302H	204	22	1	23	205	226	Yes
George (37)	2010	India	Caucasian	Prostate Cancer	D302H	111	48	6	54	117	159	Yes

For −652 6N del polymorphism, AA symbolizes ins/ins genotype; AB symbolizes ins/del genotype; BB symbolizes del/del genotype.

For D302H polymorphism, AA symbolizes DD genotype; AB symbolizes DH genotype; BB symbolizes HH genotype.

Cox (10) and Hashami (11) indicated the association of CASP 8 -652 6N del and D302H polymorphism with cancer risk. On the contrary, Haiman (12), Cybulski (13) and Xiao (14) demonstrated the negative association of these two variants with breast cancer, colon cancer and prostate cancer susceptibility. So we performed this meta-analysis based on most recent and relevant studies, aiming to summarize previous reports, and get an overall and objective understanding of the relationship between variant D302H, -652 6N del and multiple cancer risks that have been investigated till now.

Materials and methods

Identification of eligible studies

Relevant literatures published before 31 October 2014 in English by using the electronic MEDLINE, EMBASE and Chinese WANFANG (http://www.wanfangdata.com.cn/) database with the following keywords 'CASP8' or 'caspase 8', 'cancer', 'carcinoma', 'tumor' or 'tumour', 'neoplasm' and 'polymorphism' or 'variant'. References of the retrieved articles were also screened for original case-control studies. We included all the case-control and cohort studies that investigated the association between CASP8 polymorphisms and cancer risk with genotypic data for at least one of two polymorphisms, CASP8 D302H and CASP8 -652 6N del. Investigations in subjects with family cancer risks or cancer-prone disposition were also excluded. Additionally, when the case-control study was included by more than one article using the same case series, we selected the study that included the largest number of individuals. When the case-control study in one single publication was done in different ethnic groups, we regarded it as different case-control studies. If deviation from Hardy-Weinberg Equilibrium (HWE) was found of the control group, the publication was abandoned from this analysis.

Data extraction

The following information was extracted from each article: first author, year of publication, country where study was conducted, ethnicity of subjects, cancer types, and distribution of alleles and genotypes in the case and control groups.

Statistics

Crude odds ratios (ORs) with 95% confidence intervals (CIs) for alleles and genotypes were used to assess the strength of association between the CASP8 polymorphism and the risk of different types of cancer. Pooled ORs were calculated for the allele comparison, additive genetic model, dominant genetic model and recessive genetic model, respectively. The heterogeneity assumption was assessed using the Cochran's λ^2-based Q statistic test. Heterogeneity was not considered to be significant if P>0.10. The pooled OR estimate of each study was calculated using the fixed effects model if heterogeneity test was P<0.10, otherwise random effect model was employed to evaluate the significance. Stratification analyses were done by cancer types (if a cancer type was investigated in less than three individual studies, it was categorized into the "other cancer" group) and ethnicities.

Publication bias was tested using the funnel plot. All statistical tests were acquired with Review Manager (Cochrane Collaboration website Version 5.1). P<0.05 was considered to be statistically significant.

Results

53 publications about the association study of CASP8 D302H and CASP8 -652 6N del polymorphism with cancer were extracted from online Medline, EMBASE and WANFANG database. Of these, 28 publications involved the relationship of CASP8 -652 6N del with cancer including 44 case-control studies; 15 publications investigate the possible role of CASP8 D302H variant in cancer susceptibility including 17 case-control studies; 4 publications contain information about both variants in cancer. We excluded 10 studies that are found to be deviated from Hardy-weinberg equilibrium. Altogether, 32 publications which include 61 studies were identified to meet the criteria of inclusion (Table 1). There are 16 breast cancer studies, 12 colonrectum cancer studies, 6 prostate cancer studies and 4 lung cancer studies; all other 23 cancer types as gastric cancer, pancreatic cancer, brain tumor, etc, were categorized as «other cancer». Cancers were confirmed histologically or pathologically in all the studies.

Overall, all studies included in this analysis meet the criteria of Hardy-weinberg quilibrium. 10 studies excluded on CASP8 -652 6N del polymorphism were identified to deviate from the HWE although they have ever been referred by other meta-analysis (15-17). They are studies by De Vecchi (18), Meenakshi Umar (19), Ginu P. George (20), Pravin Kesarwani (21), Koushik Chatterjee (22), Xiangdong Ma(23), Haiman (12) and Chunying Li (24).

Publication bias was tested by funnel plot by Revman 5.1, all analysis showed no bias according to the funnel plot shown in Figure 1 & 2.

CASP8 -652 6N del

Altogether all studies in this analysis have included 25800 case and 31964 control subjects. The minor al-

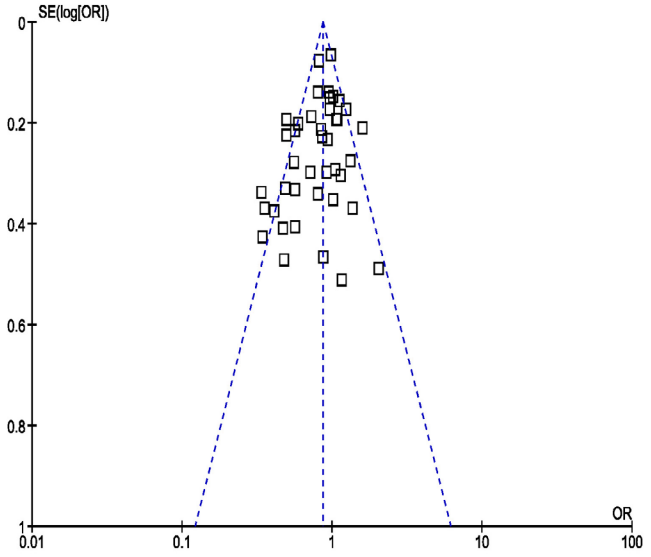

Figure 1. Funnel plot of the association between overall cancer risk and CASP8 -652 6N del polymorphism under fixed model (minor allele homozygotes vs common allele homozygotes).

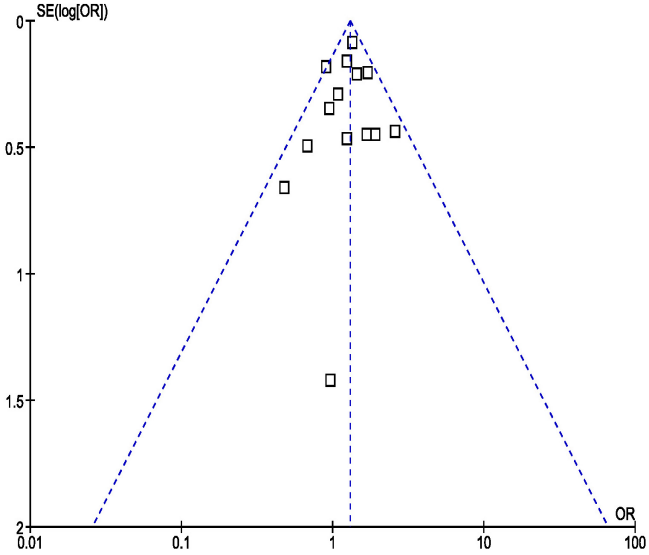

Figure 2. Funnel plot of the association between overall cancer risk and CASP8 D302H polymorphism under fixed model (minor allele homozygotes vs common allele homozygotes).

leles (-652 6N del) frequency showed significant difference between case and control groups (Overall, allele comparison, del versus ins: OR=0.92; 95% CI: 0.90–0.95, p<0.00001). Homozygous (Fig 3), dominant and recessive genotypes were significantly different between case and control group, and proves to be a protective factor for cancer susceptibility. In the stratified analysis, lung cancer and gastrointestinal cancer showed significant association with the polymorphism under homozygous and dominant model. For breast cancer, association only exists under recessive model. Dominant genotype showed significant difference between case and control group in colonrectum cancer. However, no significant association was observed for other cancer types. Additionally, significant association was seen in Asian people under all genetic models, but in Caucasian population only homozygous model showed significant result (Table 2).

CASP8 D302H

36883 cases and 41089 controls have been investi-

Study or Subgroup	Case Events	Case Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI
Cybulsky breast	126	304	192	466	3.6%	1.01 [0.75, 1.35]
Cybulsky Prostate	110	249	192	466	3.4%	1.13 [0.83, 1.54]
Gangwar Bladder	7	128	16	149	1.0%	0.48 [0.19, 1.21]
George Colonrectum	98	201	106	226	3.0%	1.08 [0.74, 1.58]
Haiman breast AA	117	203	125	225	3.0%	1.09 [0.74, 1.60]
Haiman breast CA	9	154	23	235	1.2%	0.57 [0.26, 1.27]
Haiman breast EA	117	275	115	266	3.2%	0.97 [0.69, 1.37]
Haiman breast JA	23	525	26	622	1.9%	1.05 [0.59, 1.86]
Haiman breast Latino	47	224	52	221	2.6%	0.86 [0.55, 1.35]
Haiman breast NH	23	75	60	157	1.9%	0.72 [0.40, 1.29]
Haiman Colonrectum AA	59	108	309	526	2.7%	0.85 [0.56, 1.28]
Haiman Colonrectum EA	136	297	308	654	3.7%	0.95 [0.72, 1.25]
Haiman Colonrectum JA	11	268	35	863	1.5%	1.01 [0.51, 2.02]
Haiman Colonrectum Latino	44	134	126	540	2.8%	1.61 [1.06, 2.43]
Haiman Colonrectum NH	17	35	77	188	1.4%	1.36 [0.66, 2.81]
Haiman Prostate AA	240	415	181	308	3.5%	0.96 [0.71, 1.30]
Haiman Prostate JA	16	513	20	522	1.6%	0.81 [0.41, 1.58]
Haiman Prostate Latino	96	342	81	338	3.2%	1.24 [0.88, 1.75]
Haiman Prostate NH	16	51	26	53	1.2%	0.47 [0.21, 1.06]
Hart Lung	101	226	118	224	3.0%	0.73 [0.50, 1.05]
Hashemi breast Iran	16	129	33	112	1.6%	0.34 [0.17, 0.66]
Hashemi breast UK	22	653	25	690	1.9%	0.93 [0.52, 1.66]
Lee Lung	40	480	41	463	2.5%	0.94 [0.59, 1.48]
Li Melanoma	177	420	188	395	3.7%	0.80 [0.61, 1.06]
Liamarkopoulos Gastric	11	46	106	226	1.4%	0.36 [0.17, 0.74]
Liu Colonrectum	25	336	32	560	2.1%	1.33 [0.77, 2.28]
Lv lymphoid	6	54	21	365	0.9%	2.05 [0.79, 5.33]
Pittman Colonrectum	987	1982	897	1789	4.7%	0.99 [0.87, 1.12]
Shephard breast	659	1397	675	1291	4.5%	0.81 [0.70, 0.95]
Son Lung Korean	25	272	22	271	1.8%	1.15 [0.63, 2.09]
Srivastava Gallbladder	12	159	24	146	1.4%	0.41 [0.20, 0.86]
Sun breast	49	748	72	585	3.0%	0.50 [0.34, 0.73]
Sun Cervix	13	212	42	356	1.7%	0.49 [0.26, 0.93]
Sun Colonrectum	33	638	58	586	2.6%	0.50 [0.32, 0.77]
Sun Esophagus	38	690	56	599	2.7%	0.57 [0.37, 0.87]
Sun Gastric	16	278	25	258	1.7%	0.57 [0.30, 1.09]
Sun Lung	45	801	64	704	2.9%	0.60 [0.40, 0.88]
Wang Bladder	238	250	205	230	1.5%	2.42 [1.19, 4.94]
Wang Thyroid	8	73	15	121	1.0%	0.87 [0.35, 2.16]
Wu Colorectal	15	299	29	387	1.7%	0.65 [0.34, 1.24]
Xiao Colorectal	11	198	15	227	1.2%	0.83 [0.37, 1.85]
Xiao Lymphoma	7	99	10	162	0.9%	1.16 [0.43, 3.14]
Yang Pancreatic	18	286	63	584	2.1%	0.56 [0.32, 0.96]
Zhu Renal	8	234	21	226	1.2%	0.35 [0.15, 0.80]
Total (95% CI)		**15461**		**18582**	**100.0%**	**0.83 [0.75, 0.92]**
Total events	3892		4927			

Heterogeneity: Tau² = 0.05; Chi² = 99.75, df = 43 (P < 0.00001); I² = 57%
Test for overall effect: Z = 3.50 (P = 0.0005)

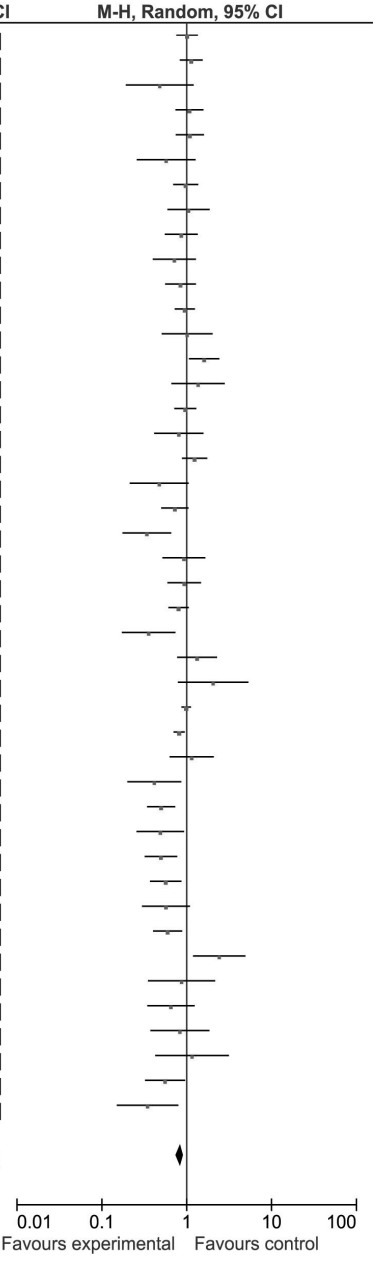

Figure 3. Forest plot of the association between overall cancer risk and CASP8 polymorphism CASP8 -652 6N del under fixed model (minor allele homozygotes vs common allele homozygotes).

Table 2. Association of CASP8 -652 6N del polymorphism and cancer susceptibility in subgroups.

Variables	Study included	Sample size Case-control	Homozygote (del/del vs. ins/ins)			Dominant (del/del + ins/del vs. ins/ins)			Recessive (del/del vs. ins/del + ins/ins)		
			OR (95% CI)	P	Phet [b]	OR (95% CI)	P	Phet [b]	OR (95% CI)	P	Phet [b]
Total	44	25800-31964	0.83 (0.75, 0.92)	P=0.0005	P<0.00001	0.89 (0.84, 0.95)	P=0.0002	P<0.00001	0.89 (0.82, 0.98)	P=0.01	P<0.00001
Cancer types											
Breast cancer	11	8183-8555	0.90 (0.65, 1.25)	P=0.53	P<0.00001	0.92 (0.80, 1.05)	P=0.23	P=0.0005	0.84 (0.71, 0.99)	P=0.03	P=0.02
Colonrectum Cancer	11	7905-11724	0.97 (0.82, 1.15)	P=0.72	P=0.04	0.92 (0.86, 0.99)	P=0.02	P=0.18 [a]	1.03 (0.89, 1.19)	P=0.06	P=0.71
Lung cancer	4	2737-2696	0.77 (0.62, 0.95)	P=0.02	P=0.25 [a]	0.82 (0.73, 0.91)	P=0.0004	P=0.10 [a]	0.83 (0.68, 1.01)	P=0.06	P=0.41 [a]
Gastrointestinal	16	10056-14688	0.81 (0.67, 0.98)	P=0.03	P=0.0002	0.85 (0.77, 0.94)	P=0.001	P=0.006	0.89 (0.76, 1.04)	P=0.15	P=0.001
Other cancer	13	4824-6025	0.90 (0.71, 1.14)	P=0.39	P=0.004	0.94 (0.82, 1.08)	P=0.41	P=0.003	0.96 (0.80, 1.16)	P=0.68	P=0.11
Ethnicity											
Asian	17	8461-10154	0.75 (0.60, 0.94)	P=0.01	P=0.001	0.83 (0.74, 0.92)	P=0.0006	P=0.0002	0.79 (0.64, 0.98)	P=0.03	P=0.0009
Caucasian	27	17339-21810	0.90 (0.81, 1.00)	P=0.04	P=0.005	0.94 (0.88, 1.00)	P=0.06	P=0.05	0.95 (0.87, 1.04)	P=0.26	P=0.002

[a] Fixed effects model. Other data were analyzed by random effects model. [b] Pvalue of Q-test for heterogeneity.

Table 3. Association of CASP8 D302H del polymorphism and cancer susceptibility in subgroups.

Variables	Study included	Sample size Case-control	Homozygote (HH vs. DD)			Dominant (HH vs. DH+DD)			Recessive (DH+HH vs. DD)		
			OR (95% CI)	P	Phet [d]	OR (95% CI)	P	Phet [d]	OR (95% CI)	P	Phet [d]
Total	17	36883-41089	0.79 (0.70, 0.88)	P=0.05	P=0.07	0.91 (0.84, 0.98)	P=0.02	P=0.01 [c]	0.91 (0.88, 0.94)	P<0.00001	P<0.0001 [c]
Cancer types											
Breast cancer	5	22825-24456	0.70 (0.61, 0.81)	P<0.00001	P=0.57	0.72 (0.62, 0.83)	P<0.00001	P=0.56	0.87 (0.84, 0.91)	P<0.00001	P=0.81
Other cancer	12	14058-16633	1.01 (0.79, 1.30)	P=0.93	P=0.19	1.01 (0.93, 1.11)	P=0.76	P=0.29	0.98 (0.93, 1.05)	P=0.61	P=0.0002
Ethnicity											
Caucasian	17	36883-41089	0.79 (0.70, 0.88)	P=0.05	P=0.07	0.91 (0.84, 0.98)	P=0.02	P=0.01 [c]	0.91 (0.88, 0.94)	P<0.0001	P<0.00001 [c]

[c] Fixed effects model. Other data were analyzed by random effects model. [d] P value Q-test for heterogeneity.

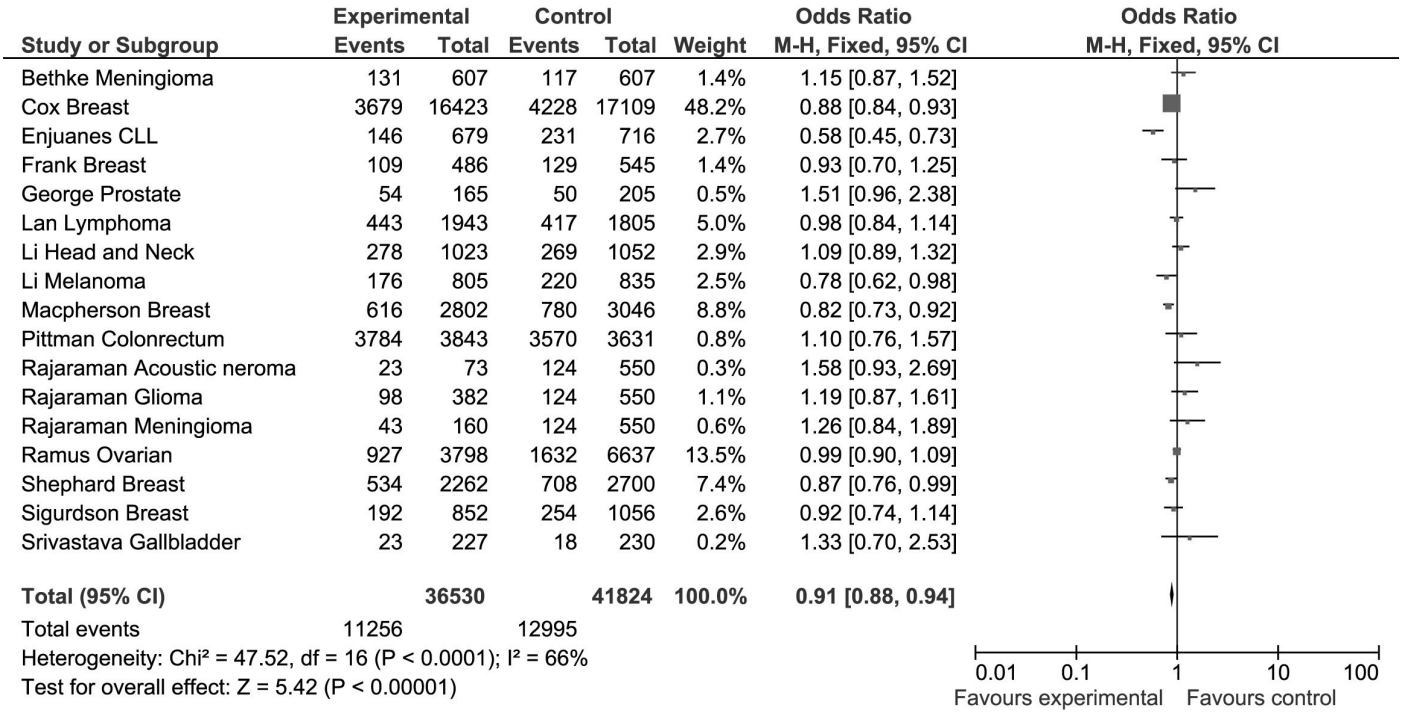

Figure 4. Forest plot of the association between overall cancer risk and CASP8 polymorphism CASP8 D302H under fixed model (minor allele homozygotes vs common allele homozygotes).

gated in eligible studies. All subjects were from caucasian population. The minor alleles (D302H) frequency showed significant difference between case and control group (Overall, allele comparison, C versus G: OR=0.93; 95% CI: 0.86–0.99, p=0.03). Overall, pooled data indicated the association of minor allele C or H with decreased cancer risk (homozygote comparison, C/C versus G/G: OR=0.79; 95% CI: 0.70–0.88; dominant comparison, C/C versus G/G +C/G: OR= 0.91; 95% CI: 0.84–0.98; recessive comparison, C/G+ C/C versus G/G: OR= 0.91; 95% CI: 0.88–0.94) (Fig 4, Table 3).

Subgroup analysis also showed that allele G or D is the protective factor in breast cancer susceptibility under all genetic models (homozygote comparison, C/C versus G/G: OR=0.70; 95% CI: 0.61–0.81; dominant comparison, C/C versus G/G +C/G: OR= 0.72; 95% CI: 0.62–0.83; recessive comparison, C/G+ C/C versus G/G: OR= 0.87; 95% CI: 0.84–0.91). This association was not observed in other cancer type groups (Table 3).

Discussion

Our meta-analysis summarized the association of two CASP8 gene polymorphism with cancer susceptibility, which has included 25800 cases and 31964 controls for -652 6N del polymorphism, and 36883 cases and 41089 controls for D302H in total. Results indicated that the minor alleles of CASP8 -652 6N del and D302H polymorphism were both associated with cancer risks, as a protective factor.

CASP8 functions as an upstream apoptosis signal regulator mainly in extracellular apoptotic signaling pathways (6). The D302H variation was hypothesized to affect CASP8 function by interfering its autoprocessing and interaction with anti-apoptotic proteins, which might be the cause of its association with cancer risk. Whereas CASP8 -652 6N del polymorphism, which was reported to decrease the CASP8 expression, theoretically leading to cancer development by apoptosis attenuation, was proved to be a protective factor for cancer in this analysis (8). Other scientific studies may provide explanation for this contradiction. Data has shown that T lymphocyte bearing CASP8 -652 6N del polymorphism shows decreased apoptosis, which relatively strengthen the surveillance power of T lymphocytes towards cancer cells (8). Since the definite role of CASP8 in apoptosis pathway has not been thoroughly elucidated till now, more work needs to be done to confirm the association of CASP8 -652 6N del polymorphism with cancer development.

Subgroup analysis demonstrated CASP8 -652 6N del polymorphism showed significant correlation with lung and gastrointestinal cancer susceptibility, which has not been reported in other similar studies by Yin, Fan and Sergentanis (15-17). Recently the association of lung cancer risk and CASP8-652 6N del polymorphism was confirmed by Zhang in a relatively smaller group meta-analysis (17). This indicates that the association can be extended into other cellular context. Certainly further analysis and more case-control studies needs to be done to validate whether this association could be general in all cancer types.

Our analysis did not find association of CASP8 -652 6N del polymorphism with colonrectum cancer susceptibility in any of the genetic models. However, three analysis has addressed this association. Yin (16) reported that CASP8 -652 6N del polymorphism is related to colonrectum cancer susceptibility under dominant model, and Zhang proposed that colonrectum cancer risk reduction is associated with CASP8 -652 6N del variation under recessive model (17). In Wu's study (25), CASP8 -652 6N del/ins polymorphism may be a prognositic marker of colon cancer. Similar controversy was also found in the association of breast cancer with CASP8 -652 6N del variation. The significance was seen under dominant model in our analysis, whereas no association was found in Zhang's study; different gene-

tic model showed significance association in Yin and Sergentanis' study (16,26). Carefully analysis on their studies found that the reason for different conclusion might be: Firstly, Our study has included more case-control studies in colonrectum and breast cancer types. So our analysis should have more statistical power to draw the conclusion. Secondly, no statistical results can be found in Yin and Sergentanis's study on the OR value. Thirdly, as a high heterogeneous disorder, different cancer types or even the same cancer type in different population could have different genetic context, so the association will certainly vary due to the complexity of different genetic background.

There's no much contradiction about the association of CASP8 D302H polymorphism with cancer risks among different studies. All studies were conducted in Caucasian population including our newly included studies (9, 11, 27-29). Therefore we cannot draw a conclusion about the association of this variant with cancer susceptibility in other ethnic population till now.

In total, this analysis indicates two variants, CASP8 -652 6N del and CASP8 D302H, are significantly associated with cancer susceptibility, especially with some specific cancer types. On account of the potential functions of CASP8 in apoptosis pathway as well as some other biological processes, more profound study should be carry out to further validate the association of these gene polymorphism with cancer susceptibility.

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