

## Original Article

# Relationship between cytotoxic T-lymphocyte antigen 4 (CTLA-4) rs5742909 C>T polymorphism and cancer risk: a meta-analysis based on thirty case-control studies

Weifeng Tang<sup>1</sup>, Yafeng Wang<sup>2</sup>, Boyang Chen<sup>1</sup>, Wenwei Lin<sup>1</sup>, Heping Jiang<sup>3</sup>, Chao Liu<sup>4</sup>, Mingqiang Kang<sup>1</sup>

<sup>1</sup>Department of Thoracic Surgery, The Union Clinical Medical College of Fujian Medical University, Fuzhou, Fujian Province, China; <sup>2</sup>Department of Cardiology, The People's Hospital of Xishuangbanna Dai Autonomous Prefecture, Jinghong, Yunnan Province, China; <sup>3</sup>Department of Emergency, Affiliated Jintan People's Hospital of Jiangsu University, Jintan, China; <sup>4</sup>Department of Cardiothoracic Surgery, Affiliated People's Hospital of Jiangsu University, Zhenjiang, Jiangsu Province, China

Received March 13, 2016; Accepted May 26, 2016; Epub August 15, 2016; Published August 30, 2016

**Abstract:** The association between cytotoxic T-lymphocyte antigen-4 (CTLA-4) rs5742909 C>T (-318C/T) polymorphism and cancer risk has been widely investigated, while many publications have reported controversial results. The aim of this study was to carry out a meta-analysis evaluating the association between CTLA-4 rs5742909 C>T polymorphism and cancer risk. The PubMed, EMBASE and CBM (China Biology Medicine), as well as CNKI (China National Knowledge Infrastructure) databases were extensively searched up to June 22, 2015 for relevant articles investigating the relationship between CTLA-4 rs5742909 C>T polymorphism and cancer risk. A meta-analysis was conducted using STATA software (version 12.0). Thirty case-control studies from twenty-nine publications were recruited for analysis. The results indicated a significant difference in genotype and allele distributions of CTLA-4 rs5742909 C>T polymorphism between cancer cases and controls. A slight publication bias was detected in this study; therefore, non-parametric "trim-and-fill" method was used to check the stability of our results. The adjusted ORs and CIs indicated that CTLA-4 rs5742909 C>T polymorphism might be a risk factor for cancer, suggesting the reliability of our results. This meta-analysis suggests that CTLA-4 rs5742909 C>T polymorphism may be associated with cancer susceptibility.

**Keywords:** CTLA-4, polymorphism, cancer susceptibility, meta-analysis

## Introduction

The evidence is mounting suggesting that cancer is a multifactorial disorder results from interactions between genetic background and environmental factors [1, 2]. A number of studies have been implemented to unravel the genetic basis of cancer; whereas, these genetic determinants and driving genes that attribute to the development and progression of cancer so far remain inconsistent.

Recently, many investigations suggested that the gene encoding cytotoxic T-lymphocyte antigen 4 (CTLA-4) was a promising candidate in the pathogenesis of cancer [3, 4]. CTLA-4, a vital member of the immunoglobulin superfamily, induces Fas-independent apoptosis of activated T cells and results in the negative regula-

tion of T-cell proliferation and activation [4, 5]. Single nucleotide polymorphisms (SNPs) of CTLA-4 have been widely investigated, and many investigations have demonstrated that mutations of the CTLA-4 gene may be relevant to the risk of multiple cancers [4, 6].

CTLA-4 is composed of 4 exons which possess a number of important SNPs, such as the +49 G/A (rs231775), -318C/T (rs5742909), CT60 G/A (rs3087243), and -1722 T>C (rs733618) etc [4, 7]. A meta-analysis conducted by Zhang *et al.* showed that CTLA-4 rs5742909 C>T polymorphism might not be relevant to the risk of overall cancer, whereas in the subgroup analyses, a significantly increased risk of cancer was identified among Europeans, but not among any cancer types [3]. Recently, Xia *et al.* reported a meta-analysis with null association be-

tween the *CTLA-4* rs5742909 C>T polymorphism and cancer risk in overall and in subgroup analyses based on cancer types, ethnicity, epithelial tumors to non-epithelial tumors and solid tumors to non-solid tumors [8]. Liu *et al.* reported that *CTLA-4* rs5742909 C>T polymorphism was significantly associated with the risk of cervical cancer in a recent meta-analysis [9]. However, these studies may have some limitations to obtain a comprehensive and reliable conclusion. Up to now, more studies focused on the association between the *CTLA-4* rs5742909 C>T polymorphism and cancer risk, and the results remained controversial. To further address this potential relationship, we conducted a comprehensive meta-analysis to derive a more precise estimation.

## Materials and methods

### Search strategy

All studies investigating the association between the *CTLA-4* rs5742909 C>T polymorphism and cancer risk were identified by an exhaustive electronic search of the PubMed, EMBASE, CBM (China Biology Medicine), and CNKI (China National Knowledge Infrastructure) databases with combinations of the following key words: 'CTLA-4' or 'CTLA4' or 'Cytotoxic T-lymphocyte antigen 4' or 'rs5742909' and 'polymorphism' or 'SNP' or 'variation' or 'mutation' annexed 'tumor' or 'carcinoma' or 'cancer', or 'neoplasm' or 'malignance' up to June 22, 2015 without language restrictions. The electronic search results were supplemented by manual search all bibliographies listed in these publications, letters and published reviews.

### Inclusion and exclusion criteria

The following inclusion criteria were utilized for selecting the eligible studies: (1) investigate the associations of the *CTLA-4* rs5742909 C>T polymorphism with cancer risk, (2) use a case-control design, (3) provided sufficient published data for evaluating an odds ratio (OR) with 95% confidence interval (CI), distribution of genotypes, alleles, sample sizes and other information that could help us infer the results and (4) provided the genotyping method and ethnicity. The major exclusion criteria were: (1) duplication of a previous publication, (2) not a case-control study, (3) animal studies, abstracts, letters and review publications, and (4) distribu-

tion of control genotypes deviated from Hardy-Weinberg equilibrium (HWE). For publications that did not offer raw data, we attempted to get this information by correspondence with the authors. If this information could not be obtained, the literatures were excluded.

### Data extraction

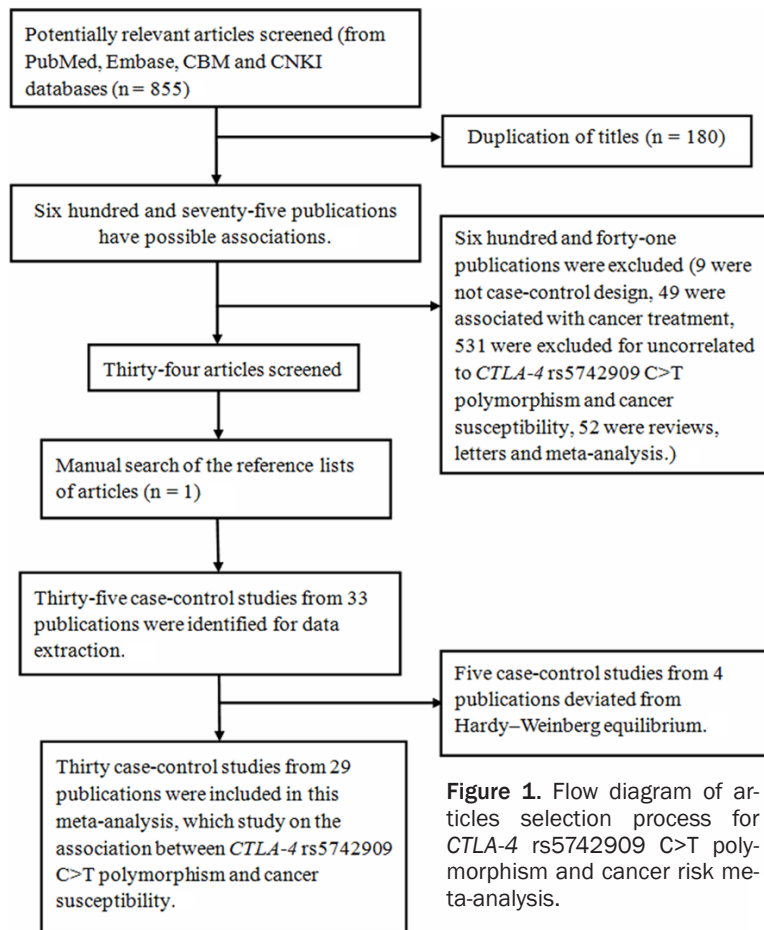
According to the inclusion and exclusion criteria, two authors (W. Tang and Y. Wang) reviewed and extracted information from recruited publications independently. If they generated different results, discrepancies were adjudicated by further discussion among all reviewers. The detailed data were considered: name of first author, published year, country, ethnicity, cancer type, number of cancer cases and controls, allele and genotype frequency, genotyping method, and evidence of HWE in controls.

### Methodological quality assessment

The quality of eligible studies was carefully assessed by two reviewers (W. Tang, and Y. Wang) according to a "methodological quality assessment scale" [10-12]. Six items, such as representativeness of cases, source of controls, sample size, ascertainment of relevant cancer, quality control of genotyping, and HWE, were evaluated. Scores ranged from 0 to 10 and a high score showed good quality of this meta-analysis. The inconsistencies were settled by further discussion among all reviewers. If the quality scores  $\geq 6$ , studies were categorized as "high quality", otherwise, studies were defined as "low quality".

### Statistical analysis

The crude ORs with the corresponding 95% CIs were calculated to assess the strength of association between the *CTLA-4* rs5742909 C>T polymorphism and risk of cancer and  $P < 0.05$  was considered statistically significant. Between-study variations and heterogeneities were checked by a Chi-square-based  $I^2$  test.  $I^2 > 50\%$  or  $P < 0.10$  was defined to be manifestation of statistically significant heterogeneity, and the summary ORs were calculated by the random-effects model (the DerSimonian-Laird method) [13], otherwise, the fixed-effects model was utilized (the Mantel-Haenszel method) [14]. We implemented subgroup analyses to evaluate ethnicity-specific and cancer type-



seven investigated cervical cancer [17, 20, 24, 30, 32, 33, 38], three investigated melanoma [26, 29, 34], three investigated lung cancer [4, 27, 31], two investigated lymphoma [15, 21], two investigated Ewing's Sarcoma [16, 22], two investigated breast cancer [19, 41], two investigated skin cancer [35] and two investigated leukemia [37, 39]. Other studies investigated colorectal cancer [36], myeloma [28], oral cancer [23], osteosarcoma [18], gastric cancer [25], bladder cancer [40] and pancreatic cancer [42]. As for subjects, sixteen studies were performed in Asian populations [4, 15-25, 39-42] and fourteen in Caucasian populations [26-38]. Characteristics of all recruited studies are exhaustively listed in **Table 1**. The distribution of the CTLA-4 rs5742909 C>T variants and alleles is presented in **Table 2**. Results of the meta-analysis are shown

specific effects (any cancer type evaluated by less than two individual studies was combined into "other cancers"). One-way sensitivity analysis and nonparametric "trim-and-fill" method were both performed to determine the stability of our results. The source of heterogeneity was detected by Galbraith radial plot. The funnel plot and Egger's test were created to evaluate publication bias and statistical significance was defined as  $P < 0.1$  for the interpretation of Egger's test. Evidence of the HWE was assessed by an internet HWE calculator (<http://ihg.gsf.de/cgi-bin/hw/hwa1.pl>). All statistical analyses were done with STATA (v12.0) statistical software.

## Results

### Characteristics

A total of thirty case-control studies from twenty-nine publications met the inclusion criteria [4, 15-42]. The detail process of selecting was presented in **Figure 1**. Among thirty studies,

seven investigated cervical cancer [17, 20, 24, 30, 32, 33, 38], three investigated melanoma [26, 29, 34], three investigated lung cancer [4, 27, 31], two investigated lymphoma [15, 21], two investigated Ewing's Sarcoma [16, 22], two investigated breast cancer [19, 41], two investigated skin cancer [35] and two investigated leukemia [37, 39]. Other studies investigated colorectal cancer [36], myeloma [28], oral cancer [23], osteosarcoma [18], gastric cancer [25], bladder cancer [40] and pancreatic cancer [42]. As for subjects, sixteen studies were performed in Asian populations [4, 15-25, 39-42] and fourteen in Caucasian populations [26-38]. Characteristics of all recruited studies are exhaustively listed in **Table 1**. The distribution of the CTLA-4 rs5742909 C>T variants and alleles is presented in **Table 2**. Results of the meta-analysis are shown

### Quantitative synthesis

A total of 9,224 cancer cases and 11,201 controls from thirty eligible case-control studies were recruited in the pooled analysis. After combining all eligible investigations, statistical evidence of an association between the CTLA-4 rs5742909 C>T polymorphism and cancer risk was observed in three genetic models: TT+CT vs. CC (OR, 1.21; 95% CI, 1.08-1.35;  $P = 0.001$ ), TT vs. CC (OR, 1.37; 95% CI, 1.08-1.75;  $P = 0.010$ ) and T vs. C (OR, 1.19; 95% CI, 1.07-1.31;  $P = 0.001$ ) (**Table 3; Figures 2 and 3**). When stratified by ethnicity, as showed in **Table 3**, significant increases in cancer risk were detected for Asians in four genetic models: TT+CT vs. CC (OR, 1.28; 95% CI, 1.07-1.54;  $P = 0.008$ ), TT vs. CT+CC (OR, 1.38; 95% CI, 1.02-1.87;  $P = 0.036$ ), TT vs. CC (OR, 1.44; 95% CI, 1.06-1.95;  $P = 0.020$ ), and T vs. C (OR, 1.26; 95% CI, 1.07-1.49;  $P = 0.006$ ), for Caucasians

## CTLA-4 rs5742909 C>T polymorphism and cancer risk

**Table 1.** Characteristics of populations and cancer types of the individual studies included in the meta-analysis

Study	Year	Ethnicity	Country	Cancer type	Sample size (case/control)	Genotype method
Liang et al.	2014	China	Asians	Leukemia	86/112	DNA sequencing
Jaiswal et al.	2014	India	Asians	Bladder cancer	200/200	PCR-ARMS
Gokhale et al.	2013	Asians	India	Cervical cancer	100/101	PCR-RFLP
Feng et al.	2013	Asians	China	Ewing's Sarcoma	308/362	PCR-RFLP
Queirolo et al.	2013	Caucasians	Italy	Melanoma	14/45	PCR-RFLP
Bharti et al.	2013	Asians	India	Oral cancer	130/180	PCR-RFLP
Liu et al.	2013	Asians	China	Lymphoma	291/300	PCR-LDR
Yang et al.	2012	Asians	China	Ewing's Sarcoma	223/302	PCR-RFLP
Karabon et al.	2012	Caucasians	Poland	Myeloma	200/380	PCR-RFLP, TaqMan
Jiang et al.	2011	Asians	China	Cervical cancer	100/100	MALDI-TOF-MS
Karabon et al.	2011	Caucasians	Poland	Lung cancer	208/326	PCR-RFLP, TaqMan
Liu et al.	2011	Asians	China	Osteosarcoma	267/282	PCR-RFLP
Kong et al.	2010	Asians	China	Breast cancer	315/322	PCR-RFLP
Ivansson et al.	2010	Caucasians	Sweden	Cervical cancer	1306/811	TaqMan
Pawlak et al.	2010	Caucasians	Poland	Cervical cancer	147/225	PCR-RFLP
Rahimifar et al.	2010	Caucasians	Iran	Cervical cancer	55/110	PCR-RFLP, PCR-ARMS
Khaghanzadeh et al.	2010	Caucasians	Iran	Lung cancer	127/124	PCR-RFLP, PCR-ARMS
Bouwhuis et al.	2010	Caucasians	German	Melanoma	763/734	DNA sequencing
Gogas et al.	2010	Caucasians	Greece	Melanoma	286/288	DNA sequencing
Castro et al.	2009	Caucasians	Sweden	Cervical cancer	973/1763	Multiplex PCR with hybridization
Li et al.	2009	Asians	China	Gastric cancer	121/236	PCR-RFLP, PCR-ARMS
Shi et al.	2009	Asians	China	Pancreatic cancer	138/278	PCR-RFLP
Welsh et al.	2009	Caucasians	USA	Skin cancer	930/849	TaqMan
Welsh et al.	2009	Caucasians	USA	Skin cancer	713/849	TaqMan
Dilmec et al.	2008	Caucasians	Turkey	Colorectal cancer	56/162	PCR-RFLP
Suwalska et al.	2008	Caucasians	Poland	Leukemia	178/336	SNaPshot
Sun et al.	2008	Asians	China	Lung cancer	800/800	MALDI-TOF-MS
Su et al.	2007	Asians	China	Cervical cancer	144/378	PCR-RFLP
wang et al.	2007	Asians	China	Breast cancer	117/148	PCR-RFLP
Cheng et al.	2006	Asians	China	Lymphoma	62/250	PCR-RFLP

PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism; PCR-LDR: polymerase chain reaction-ligase detection reaction; MALDI-TOF MS: Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry; PCR-ARMS: amplification refractory mutation system-polymerase chain reaction.

in one genetic model: TT+CT vs. CC (OR, 1.11; 95% CI, 1.00-1.23;  $P = 0.046$ ). In a stratified analysis by cancer type, as showed in **Table 3**, there was an increased risk of leukemia in four genetic models: TT+CT vs. CC (OR, 1.86; 95% CI, 1.31-2.63;  $P < 0.001$ ), TT vs. CT+CC (OR, 3.38; 95% CI, 1.34-8.54;  $P = 0.010$ ), TT vs. CC (OR, 3.96; 95% CI, 1.56-10.05;  $P = 0.004$ ) and T vs. C (OR, 1.86; 95% CI, 1.37-2.51;  $P < 0.001$ ) and breast cancer in two genetic models: TT+CT vs. CC (OR, 1.98; 95% CI, 1.44-2.73;  $P < 0.001$ ) and T vs. C (OR, 1.81; 95% CI, 1.35-2.42;  $P < 0.001$ ). In a stratified analysis by system, there was an increased risk of reproductive and breast cancer in two genetic models: TT+CT vs. CC (OR, 1.49; 95% CI, 1.05-2.13;  $P = 0.027$ )

and T vs. C (OR, 1.40; 95% CI, 1.05-1.86;  $P = 0.020$ ), and of hematopoietic malignancy in one genetic model: TT vs. CC (OR, 2.05; 95% CI, 1.05-4.00;  $P = 0.036$ ) (**Table 3**).

### *Tests for publication bias, sensitivity analyses, and heterogeneity*

Begg's Funnel plot and Egger's test were both created to assess the potential publication bias of included studies and the results confirmed that there was a slight publication bias in current meta-analysis (TT+CT vs. CC: Begg's test  $P = 0.269$ , Egger's test  $P = 0.336$ ; TT vs. CT+CC: Begg's test  $P = 0.835$ , Egger's test  $P = 0.057$ ; TT vs. CC: Begg's test  $P = 0.868$ , Egger's test  $P = 0.057$ ).

## CTLA-4 rs5742909 C>T polymorphism and cancer risk

**Table 2.** Distribution of CTLA-4 rs5742909 C>T polymorphisms genotype and allele among multiple cancer patients and controls

	Case			Control			Case		Control		HWE	Quality scores
	CC	CT	TT	CC	CT	TT	C	T	C	T		
Jaiswal et al.	106	89	5	112	81	7	301	99	305	95	Yes	7.0
Liang et al.	48	31	7	84	25	3	127	45	193	31	Yes	6.0
Liu et al.	222	64	5	222	73	5	508	74	517	83	Yes	7.5
Queirolo et al.	10	4	0	35	9	1	24	4	79	11	Yes	7.5
Feng et al.	213	89	6	249	102	11	515	101	600	124	Yes	7.0
Bharti et al.	112	8	0	170	10	0	232	8	350	10	Yes	7.0
Gokhale et al.	93	7	0	94	7	0	193	7	195	7	Yes	7.0
Yang et al.	149	65	9	210	85	7	363	83	505	99	Yes	7.0
Karabon et al.	155	40	0	297	68	2	350	40	662	72	Yes	7.0
Karabon et al.	160	46	2	261	61	1	366	50	583	63	Yes	6.0
Jiang et al.	75	24	1	92	8	0	174	26	192	8	Yes	7.5
Liu et al.	175	77	15	195	80	7	427	107	470	94	Yes	5.0
Gogas et al.	229	55	2	230	57	1	513	59	517	59	Yes	7.0
Rahimifar et al.	51	3	0	89	20	1	105	3	198	22	Yes	3.5
Khaghanzadeh et al.	107	17	2	105	16	1	231	21	226	18	Yes	5.0
Ivansson et al.	1044	228	9	666	138	4	2316	246	1470	146	Yes	9.5
Pawlak et al.	99	38	3	180	35	1	236	44	395	37	Yes	6.5
Bouwhuis et al.	619	136	8	596	130	8	1374	152	1322	146	Yes	7.5
Kong et al.	225	83	7	263	54	5	533	97	580	64	Yes	7.0
Welsh et al.	745	152	7	682	132	7	1642	166	1496	146	Yes	9.0
Welsh et al.	561	120	6	682	132	7	1242	132	1496	146	Yes	9.0
Li et al.	99	17	5	206	27	3	215	27	439	33	Yes	6.5
Castro et al.	5	124	819	6	223	1471	134	1762	235	3165	Yes	10.0
Shi et al.	95	38	5	187	82	9	228	48	456	100	Yes	6.0
Sun et al.	562	212	26	577	204	19	1336	264	1358	242	Yes	7.0
Dilmeç et al.	48	8	0	149	12	1	104	8	310	14	Yes	4.5
Suwalska et al.	121	42	7	267	62	4	284	56	596	70	Yes	4.0
wang et al.	84	33	0	129	19	0	201	33	277	19	Yes	7.0
Su et al.	105	38	1	306	67	5	248	40	679	77	Yes	6.5
Cheng et al.	59	3	0	209	40	1	121	3	458	42	Yes	7.5

HWE: Hardy-Weinberg equilibrium.

= 0.699; T vs. C: Begg's test  $P = 0.134$ , Egger's test  $P = 0.197$ ) (**Figure 4**).

One-way sensitivity analysis was utilized to measure the influence of each single dataset on the pooled OR by sequentially excluding individual studies and the results confirmed that our findings were robust enough (**Figure 5**) (data not shown). Nonparametric "trim-and-fill" method was performed and the adjusted ORs and CIs were not changed, suggesting that our results are statistically robust (TT+CT vs. CC: adjusted pooled OR = 1.19, 95% CI: 1.05-1.34,  $P = 0.005$ ; TT vs. CT+CC: adjusted pooled OR = 1.07, 95% CI: 0.90-1.26,  $P = 0.446$ ; TT vs. CC:

adjusted pooled OR = 1.35, 95% CI: 1.05-1.72,  $P = 0.019$  and T vs. C: adjusted pooled OR = 1.15, 95% CI: 1.03-1.29,  $P = 0.010$ ) (**Figure 6**).

Large heterogeneities were detected among the studies enrolled. We conducted subgroup analyses by ethnicity, cancer type, system and quality score to detect the source of heterogeneity (**Table 3**). The results indicated that Asian population, cervical cancer, lymphoma, reproductive and breast cancer, hematopoietic malignancy and high quality score study subgroups might contribute to the major source of heterogeneity. We found significant heterogeneity in allele comparison model (**Table 3**).

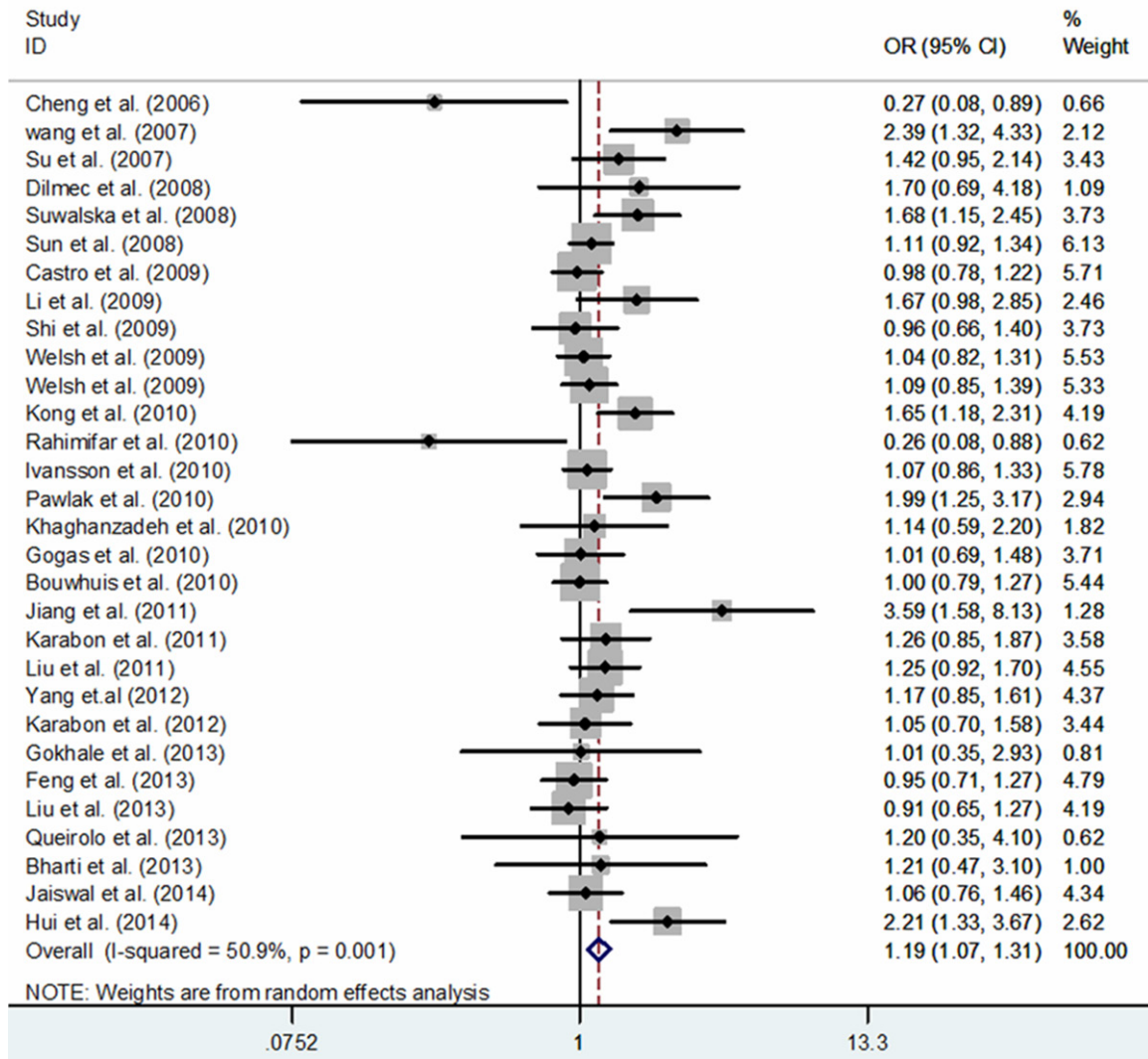
CTLA-4 rs5742909 C>T polymorphism and cancer risk

**Table 3.** Summary of results of the meta-analysis

	No. of studies	T vs. C			TT vs. CC			TT+CT vs. CC			TT vs. CT+CC		
		OR (95% CI)	P	P (Q-test)	OR (95% CI)	P	P (Q-test)	OR (95% CI)	P	P (Q-test)	OR (95% CI)	P	P (Q-test)
Total	30	<b>1.19 (1.07-1.31)</b>	<b>0.001</b>	0.001	<b>1.37 (1.08-1.75)</b>	<b>0.010</b>	0.849	<b>1.21 (1.08-1.35)</b>	<b>0.001</b>	0.002	1.15 (0.97-1.37)	0.100	0.831
Ethnicity													
Asians	16	<b>1.26 (1.07-1.49)</b>	<b>0.006</b>	0.001	<b>1.44 (1.06-1.95)</b>	<b>0.020</b>	0.601	<b>1.28 (1.07-1.54)</b>	<b>0.008</b>	0.001	<b>1.38 (1.02-1.87)</b>	<b>0.036</b>	0.676
Caucasians	14	1.12 (0.99-1.26)	0.065	0.093	1.27 (0.85-1.90)	0.237	0.824	<b>1.11 (1.00-1.23)</b>	<b>0.046</b>	0.157	1.06 (0.87-1.30)	0.564	0.856
Cancer type													
Cervical cancer	7	1.26 (0.92-1.74)	0.154	0.001	1.17 (0.60-2.29)	0.637	0.579	1.30 (0.83-2.02)	0.249	0.002	1.02 (0.81-1.27)	0.882	0.701
Lung cancer	3	1.14 (0.96-1.34)	0.130	0.841	1.50 (0.85-2.65)	0.158	0.782	1.13 (0.93-1.36)	0.212	0.841	1.48 (0.84-2.60)	0.176	0.790
Melanoma	3	1.01 (0.83-1.23)	0.938	0.962	1.08 (0.45-2.59)	0.861	0.857	1.01 (0.81-1.25)	0.943	0.889	1.07 (0.45-2.56)	0.873	0.855
Breast cancer	2	<b>1.81 (1.35-2.42)</b>	<b>&lt; 0.001</b>	0.284	1.64 (0.51-5.23)	0.406	-	<b>1.98 (1.44-2.73)</b>	<b>&lt; 0.001</b>	0.280	1.44 (0.45-4.59)	0.537	-
Lymphoma	2	0.57 (0.18-1.83)	0.343	0.052	1.02 (0.32-3.29)	0.973	0.927	0.55 (0.17-1.80)	0.322	0.054	1.06 (0.33-3.43)	0.917	0.885
Leukemia	2	<b>1.86 (1.37-2.51)</b>	<b>&lt; 0.001</b>	0.399	<b>3.96 (1.56-10.05)</b>	<b>0.004</b>	0.953	<b>1.86 (1.31-2.63)</b>	<b>&lt; 0.001</b>	0.325	<b>3.38 (1.34-8.54)</b>	<b>0.010</b>	0.922
Skin cancer	2	1.06 (0.90-1.26)	0.497	0.773	0.97 (0.46-2.08)	0.946	0.867	1.07 (0.89-1.29)	0.451	0.784	0.96 (0.45-2.05)	0.920	0.876
Ewing's Sarcoma	2	1.04 (0.84-1.29)	0.721	0.349	1.06 (0.53-2.13)	0.863	0.152	1.05 (0.82-1.34)	0.718	0.572	1.05 (0.53-2.10)	0.891	0.156
Other cancers	7	1.15 (0.98-1.35)	0.083	0.643	1.49 (0.89-2.52)	0.131	0.453	1.15 (0.96-1.38)	0.130	0.814	1.46 (0.87-2.45)	0.152	0.438
System of cancer													
Hematopoietic malignancy	5	1.16 (0.75-1.81)	0.509	0.001	<b>2.05 (1.05-4.00)</b>	<b>0.036</b>	0.346	1.15 (0.72-1.84)	0.552	0.003	1.92 (0.98-3.75)	0.057	0.467
Reproductive and breast cancer	9	<b>1.40 (1.05-1.86)</b>	<b>0.020</b>	< 0.001	1.28 (0.72-2.28)	0.409	0.667	<b>1.49 (1.05-2.13)</b>	<b>0.027</b>	< 0.001	1.03 (0.83-1.28)	0.792	0.766
Digestive system cancer	4	1.20 (0.91-1.58)	0.208	0.335	1.62 (0.71-3.71)	0.249	0.448	1.18 (0.86-1.61)	0.307	0.415	1.62 (0.71-3.68)	0.250	0.476
Respiratory system cancer	3	1.14 (0.96-1.34)	0.130	0.841	1.50 (0.85-2.65)	0.158	0.782	1.13 (0.93-1.36)	0.212	0.841	1.48 (0.84-2.60)	0.176	0.790
Skin system cancer	5	1.04 (0.91-1.18)	0.570	0.990	1.02 (0.57-1.81)	0.949	0.985	1.05 (0.91-1.20)	0.534	0.974	1.01 (0.57-1.79)	0.977	0.985
Malignant bone tumor	3	1.11 (0.93-1.32)	0.262	0.401	1.45 (0.84-2.49)	0.183	0.146	1.09 (0.89-1.33)	0.419	0.738	1.43 (0.83-2.45)	0.198	0.150
Quality score													
≥ 6.0	25	<b>1.17 (1.06-1.30)</b>	<b>0.003</b>	0.002	1.25 (0.97-1.63)	0.089	0.878	<b>1.20 (1.07-1.35)</b>	<b>0.003</b>	0.004	1.09 (0.92-1.30)	0.315	0.908
< 6.0	5	1.23 (0.84-1.80)	0.284	0.060	<b>2.41 (1.24-4.67)</b>	<b>0.009</b>	0.809	1.19 (0.78-1.81)	0.415	0.063	2.34 (1.21-4.52)	0.012	0.857

Bold values are statistically significant ( $P < 0.05$ ).

## CTLA-4 rs5742909 C>T polymorphism and cancer risk



**Figure 2.** Meta-analysis with a random-effects model for the association between the risk of cancer and the *CTLA-4* rs5742909 C>T polymorphism (T vs. C).

Thus, Galbraith radial plot also was also utilized to detect the source of heterogeneity. As shown in **Figure 7**, the results showed that seven outliers might contribute to the major sources of heterogeneity.

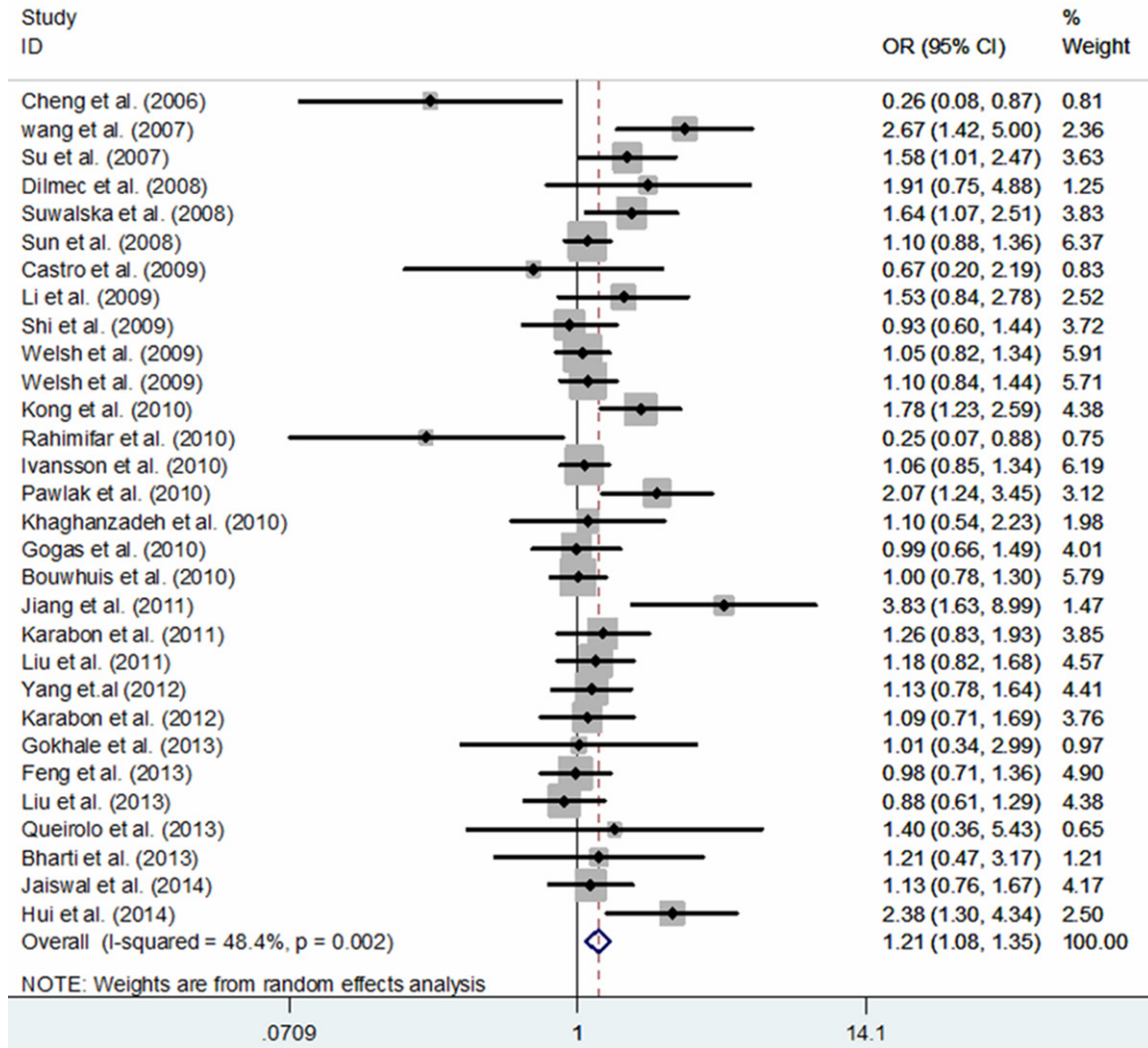
### Discussion

*CTLA-4* is a member of immunoglobulin super-family genes. Mutations in *CTLA-4*, such as SNPs, may alter the predisposition to cancer [4]. So far, many investigations have focused on the association between *CTLA-4* rs5742909 C>T polymorphism and cancer susceptibility, and the results remain inconsistent. In current meta-analysis, our results highlighted the association between *CTLA-4* rs5742909 C>T poly-

morphism and the risk of cancer, especially in Asian populations.

*CTLA-4*, also known as a cluster of differentiation (CD) 152, usually acts as an negative immune regulator of T-cell proliferation and activation, and induces Fas-independent apoptosis of activated T cells, thereby indirectly controlling effector T cells [4, 5]. *CTLA-4*, composed of four exons encoding different functional domain proteins, is located on chromosome 20q33 and close to genes of other immune regulatory molecules, such as CD28 and inducible co-stimulator [43-45]. Functional activities and expression level of *CTLA-4* are affected by SNPs of *CTLA-4* [4]. A number of SNPs, such as the -318C/T, +49A/G, +6230G/A (CT60),

## CTLA-4 rs5742909 C>T polymorphism and cancer risk

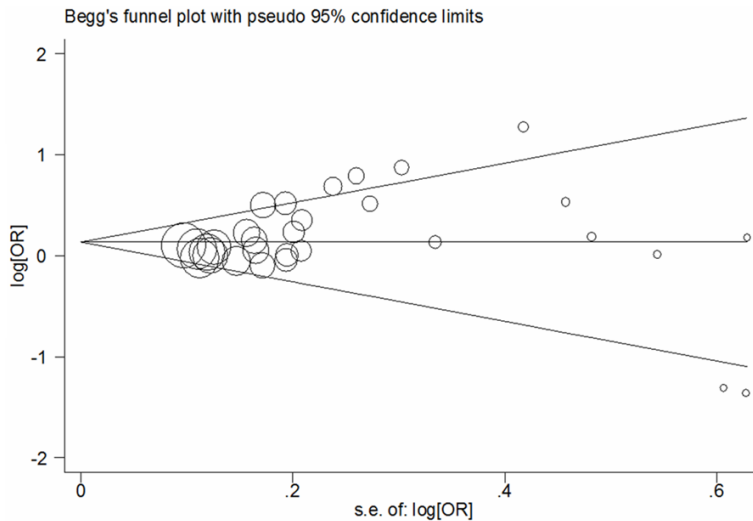


**Figure 3.** Meta-analysis with a random-effects model for the association between the risk of cancer and the *CTLA-4* rs5742909 C>T polymorphism (TT+CT vs. CC).

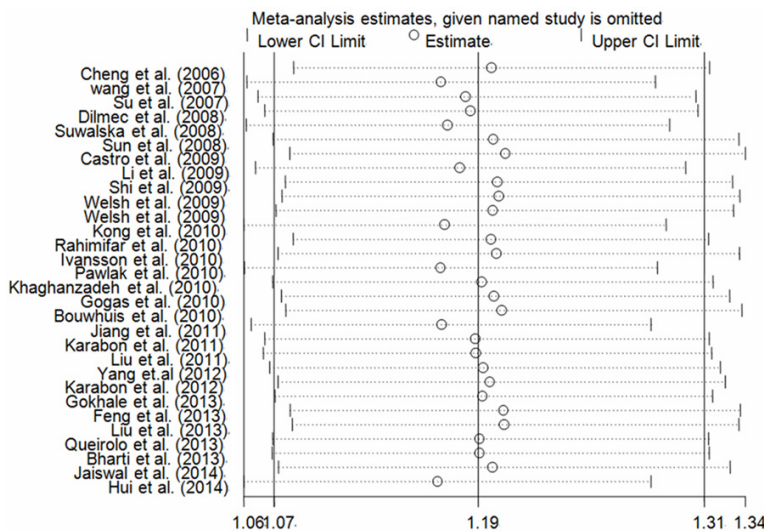
-1722T/C and -1611G/A etc, have been identified that these SNPs may associate with cancer susceptibility [4, 16, 19, 37, 46, 47]. Individuals carrying the homozygous for adenine at position 49 and thymine at position -318 showed significantly over-expression of both *CTLA-4* mRNA and cell surface *CTLA-4* [44]. According to our findings, these results indicate that the C→T change in *CTLA-4* rs5742909 C>T polymorphism may result in over-expression of *CTLA-4*, elevate the T-cell activation threshold and thereby attenuate the antitumor response, which may increase the risk of multiple cancers.

Since the results of meta-analysis can be affected by cancer origins, stratified analyses

were performed for the *CTLA-4* rs5742909 C>T polymorphism. The results show that the *CTLA-4* rs5742909 C>T polymorphism is associated with the risk of breast cancer and leukemia. However, all results should be interpreted with caution. For breast cancer and leukemia, only two investigations were included in each group, which may restrict the statistical power to confirm a real influence. When stratified by ethnicity, the *CTLA-4* -318C/T polymorphism is associated with the risk of cancer in both Asians and Caucasians. In the allele genetic comparison model, a borderline statistically increased risk of cancer was observed in Caucasian populations. In the future, larger association studies or multi-centric case-control studies should be carried out to confirm or refute these asso-



**Figure 4.** Begg's funnel plot of meta-analysis of between the *CTLA-4* rs5742909 C>T polymorphism and the risk of cancer in the dominant model (T vs. C).



**Figure 5.** Sensitivity analysis of the influence of T vs. C in overall cancer meta-analysis (random-effects estimates).

ciations, particularly regarding Caucasian populations.

Two significant issues, heterogeneity and publication bias, should be addressed here. We identified a slight publication bias in the recessive model; however, the following nonparametric “trim-and-fill” method indicated the adjusted pooled ORs and CIs were not materially changed, suggesting the reliability of our results. Significant heterogeneity was detected among publications for *CTLA-4* rs5742909 C>T

polymorphism in the allele model. In combination with the Galbraith radial plot and **Figure 2**, one can identify that seven investigations contribute to the main sources of heterogeneity [17, 19, 21, 30, 33, 39, 41]. In these publications, some of these studies were designed as small sample sizes ( $\leq 1,000$  subjects) [17, 19, 21, 30, 33, 39, 41] and five studies conducted in Asian populations [17, 19, 21, 39, 41].

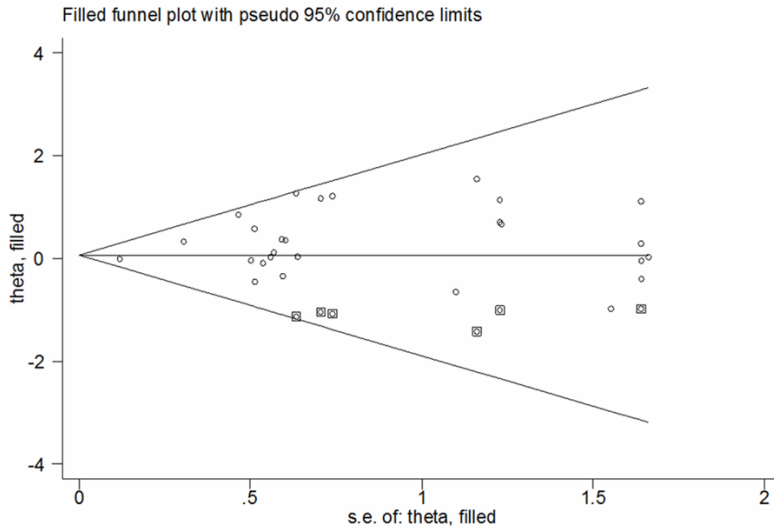
An internet-based Power and Sample Size Calculator (PS, Version 3.0, 2009, <http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize>) was used to provide the power of meta-analysis ( $\alpha = 0.05$ ). The power was 1.000 in three genetic models (TT+CT vs. CC, TT vs. CC and T vs. C) and 0.945 in TT vs. CT+CC genetic model.

Certain merit in the current meta-analysis should be addressed. First, this meta-analysis was the most extensive synthesis probing the association of *CTLA-4* rs5742909 C>T polymorphism with cancer susceptibility. Second, our results first confirmed the association between *CTLA-4* rs5742909 C>T polymorphism and cancer risk. The third, although there were five low quality studies (the quality

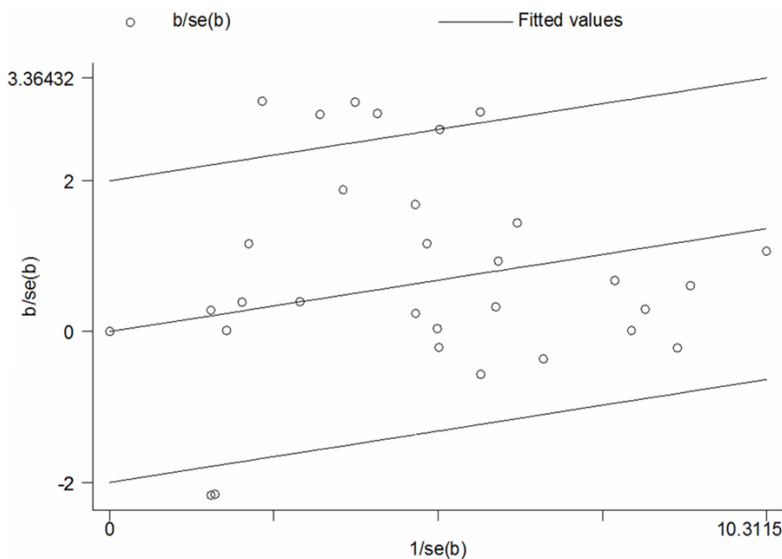
score < 6) in this meta-analysis, we excluded or included them, the results were similar (except TT vs. CC genetic model), suggesting our results were relatively stable (shown in **Table 3**).

Although our results were suggestive, certain limitations in our study should be acknowledged. In our study, relatively large heterogeneity was observed in some genetic model, which means the results should be interpreted with caution. Additionally, all recruited investigations were conducted in Asians and Caucasians;

## CTLA-4 rs5742909 C>T polymorphism and cancer risk



**Figure 6.** Filled funnel plot of meta-analysis of between the *CTLA-4* rs5742909 C>T polymorphism and the risk of cancer in the recessive model.



**Figure 7.** Galbraith radial plot of meta-analysis (T vs. C compare genetic model).

data investigated in some other ethnicities (e.g., African), were not available. Furthermore, only published studies were recruited and a slight publication bias in current meta-analysis was observed; thus, bias might inevitably occur. As well, due to lack of plenty and uniform information in recruited investigations, our study was based on single-factor evaluation without adjustment for other factors (e.g., age, smoking, alcohol consumption, and other lifestyle factors). The statistical power might be limited. Finally, in this meta-analysis, any type of cancer

investigated by less than two case-control studies was combined into “other cancers”, which might lead to heterogeneity in this subgroup.

In summary, this meta-analysis strongly suggests that the *CTLA-4* rs5742909 C>T polymorphism is a low risk factor for cancer. Due to the limitations of current meta-analysis, in the future, extensive studies should be performed to confirm or refute our results.

### Acknowledgements

This study was supported in part by National Natural Science Foundation of China (81472332, 81341006), Fujian Province Natural Science Foundation (2013J011-26, 2013J05116), Fujian Medical University professor fund (JS12008), The Fund of Union Hospital (2015TC-1-048 and 2015TC-2-004), Fujian Province Science and Technology Programmed Fund (2012Y0030), Fujian Medical Innovation Fund (2014-CX-15) and Jiangsu University Clinical Medicine Science and Technology Development Fund (JLY20140-012).

### Disclosure of conflict of interest

None.

**Address correspondence to:** Mingqiang Kang, Department of Thoracic Surgery, The Union Clinical Medical College of Fujian Medical University, Fuzhou 350001, China. E-mail: Mingqiang\_Kang@126.com

### References

- [1] Liu L, Zhong R, Wei S, Yin JY, Xiang H, Zou L, Chen W, Chen JG, Zheng XW, Huang LJ, Zhu BB, Chen Q, Duan SY, Rui R, Yang BF, Sun JW, Xie DS, Xu YH, Miao XP and Nie SF. Interactions

## CTLA-4 rs5742909 C>T polymorphism and cancer risk

- between genetic variants in the adiponectin, adiponectin receptor 1 and environmental factors on the risk of colorectal cancer. *PLoS One* 2011; 6: e27301.
- [2] Reeves GK, Pirie K, Green J, Bull D, Beral V; Million Women Study Collaborators. Comparison of the effects of genetic and environmental risk factors on in situ and invasive ductal breast cancer. *Int J Cancer* 2012; 131: 930-937.
- [3] Zhang Y, Zhang J, Deng Y, Tian C, Li X, Huang J and Fan H. Polymorphisms in the cytotoxic T-lymphocyte antigen 4 gene and cancer risk: a meta-analysis. *Cancer* 2011; 117: 4312-4324.
- [4] Sun T, Zhou Y, Yang M, Hu Z, Tan W, Han X, Shi Y, Yao J, Guo Y, Yu D, Tian T, Zhou X, Shen H and Lin D. Functional genetic variations in cytotoxic T-lymphocyte antigen 4 and susceptibility to multiple types of cancer. *Cancer Res* 2008; 68: 7025-7034.
- [5] Scheipers P and Reiser H. Fas-independent death of activated CD4(+) T lymphocytes induced by CTLA-4 crosslinking. *Proc Natl Acad Sci U S A* 1998; 95: 10083-10088.
- [6] Hu L, Liu J, Chen X, Zhang Y, Liu L, Zhu J, Chen J, Shen H, Qiang F and Hu Z. CTLA-4 gene polymorphism +49 A/G contributes to genetic susceptibility to two infection-related cancers-hepatocellular carcinoma and cervical cancer. *Hum Immunol* 2010; 71: 888-891.
- [7] Ueda H, Howson JM, Esposito L, Heward J, Snook H, Chamberlain G, Rainbow DB, Hunter KM, Smith AN, Di Genova G, Herr MH, Dahlman I, Payne F, Smyth D, Lowe C, Twells RC, Howlett S, Healy B, Nutland S, Rance HE, Everett V, Smink LJ, Lam AC, Cordell HJ, Walker NM, Bordin C, Hulme J, Motzo C, Cucca F, Hess JF, Metzker ML, Rogers J, Gregory S, Allahabadia A, Nithiyananthan R, Tuomilehto-Wolf E, Tuomilehto J, Bingley P, Gillespie KM, Undlien DE, Ronningen KS, Guja C, Ionescu-Tirgoviste C, Savage DA, Maxwell AP, Carson DJ, Patterson CC, Franklyn JA, Clayton DG, Peterson LB, Wicker LS, Todd JA and Gough SC. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature* 2003; 423: 506-511.
- [8] Xia W, Shi R, Zheng WL and Ma WL. Lack of association between cytotoxic T-lymphocyte antigen-4 -318C/T polymorphism and cancer risk: a meta-analysis of case-control studies. *Technol Cancer Res Treat* 2013; 12: 565-574.
- [9] Liu P, Xu L, Sun Y and Wang Z. The association between cytotoxic T lymphocyte-associated antigen-4 and cervical cancer. *Tumour Biol* 2014; 35: 2893-2903.
- [10] Guo J, Jin M, Zhang M and Chen K. A genetic variant in miR-196a2 increased digestive system cancer risks: a meta-analysis of 15 case-control studies. *PLoS One* 2012; 7: e30585.
- [11] Qiu MT, Hu JW, Ding XX, Yang X, Zhang Z, Yin R and Xu L. Hsa-miR-499 rs3746444 polymorphism contributes to cancer risk: a meta-analysis of 12 studies. *PLoS One* 2012; 7: e50887.
- [12] Wang L, Jiang Z, Qiu H, Tang W, Duan T and Wang L. Associations between CTLA-4 +49 A/G (rs231775) polymorphism and cancer risk: a meta-analysis based on 52 case-control studies. *Int J Clin Exp Med* 2015; 8: 6835-6851.
- [13] DerSimonian R and Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; 7: 177-188.
- [14] Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst* 1959; 22: 719-748.
- [15] Liu J, Liu J, Song B, Wang T, Liu Y, Hao J and Yu J. Genetic variations in CTLA-4, TNF-alpha, and LTA and susceptibility to T-cell lymphoma in a Chinese population. *Cancer Epidemiol* 2013; 37: 930-934.
- [16] Yang S, Wang C, Zhou Y, Sun G, Zhu D and Gao S. Cytotoxic T-lymphocyte antigen-4 polymorphisms and susceptibility to Ewing's sarcoma. *Genet Test Mol Biomarkers* 2012; 16: 1236-1240.
- [17] Jiang L, Luo RY, Zhang W, Wang LR, Wang F and Cheng YX. [Single nucleotide polymorphisms of CTLA4 gene and their association with human cervical cancer]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 2011; 28: 313-317.
- [18] Liu Y, He Z, Feng D, Shi G, Gao R, Wu X, Song W and Yuan W. Cytotoxic T-lymphocyte antigen-4 polymorphisms and susceptibility to osteosarcoma. *DNA Cell Biol* 2011; 30: 1051-1055.
- [19] Wang L, Li D, Fu Z, Li H, Jiang W and Li D. Association of CTLA-4 gene polymorphisms with sporadic breast cancer in Chinese Han population. *BMC Cancer* 2007; 7: 173.
- [20] Su TH, Chang TY, Lee YJ, Chen CK, Liu HF, Chu CC, Lin M, Wang PT, Huang WC, Chen TC and Yang YC. CTLA-4 gene and susceptibility to human papillomavirus-16-associated cervical squamous cell carcinoma in Taiwanese women. *Carcinogenesis* 2007; 28: 1237-1240.
- [21] Cheng TY, Lin JT, Chen LT, Shun CT, Wang HP, Lin MT, Wang TE, Cheng AL and Wu MS. Association of T-cell regulatory gene polymorphisms with susceptibility to gastric mucosa-associated lymphoid tissue lymphoma. *J Clin Oncol* 2006; 24: 3483-3489.
- [22] Feng D, Yang X, Li S, Liu T, Wu Z, Song Y, Wang J, Gao W, Huang Q, Huang W, Zheng W and Xiao J. Cytotoxic T-lymphocyte antigen-4 genetic variants and risk of Ewing's sarcoma. *Genet Test Mol Biomarkers* 2013; 17: 458-463.
- [23] Bharti V, Mohanti BK and Das SN. Functional genetic variants of CTLA-4 and risk of tobacco-related oral carcinoma in high-risk North

## CTLA-4 rs5742909 C>T polymorphism and cancer risk

- Indian population. *Hum Immunol* 2013; 74: 348-352.
- [24] Gokhale P, Kerkar S, Tongaonkar H, Salvi V and Mania-Pramanik J. CTLA-4 gene polymorphism at position +49 A>G in exon 1: a risk factor for cervical cancer in Indian women. *Cancer Genet* 2013; 206: 154-161.
- [25] Li R, Xia B, Xiao H, Jiang Y and Zhou F. Association between CTLA4 gene promoter region polymorphisms at positions -1661 and -318 and gastric cancer. *Chinese Journal of Gastroenterology* 2009; 14: 332-336.
- [26] Queirolo P, Morabito A, Laurent S, Lastraioli S, Piccioli P, Ascierto PA, Gentilcore G, Serra M, Marasco A, Tornari E, Dozin B and Pistillo MP. Association of CTLA-4 polymorphisms with improved overall survival in melanoma patients treated with CTLA-4 blockade: a pilot study. *Cancer Invest* 2013; 31: 336-345.
- [27] Karabon L, Pawlak E, Tomkiewicz A, Jedynak A, Passowicz-Muszynska E, Zajda K, Jonkisz A, Jankowska R, Krzakowski M and Frydecka I. CTLA-4, CD28, and ICOS gene polymorphism associations with non-small-cell lung cancer. *Hum Immunol* 2011; 72: 947-954.
- [28] Karabon L, Pawlak-Adamska E, Tomkiewicz A, Jedynak A, Kielbinski M, Woszczyk D, Potoczek S, Jonkisz A, Kuliczkowski K and Frydecka I. Variations in suppressor molecule *ctla-4* gene are related to susceptibility to multiple myeloma in a Polish population. *Pathol Oncol Res* 2012; 18: 219-226.
- [29] Gogas H, Dafni U, Koon H, Spyropoulou-Vlachou M, Metaxas Y, Buchbinder E, Pectasides E, Tsoutsos D, Polyzos A, Stratigos A, Markopoulos C, Panagiotou P, Fountzilas G, Castana O, Skarlos P, Atkins MB and Kirkwood JM. Evaluation of six CTLA-4 polymorphisms in high-risk melanoma patients receiving adjuvant interferon therapy in the He13A/98 multicenter trial. *J Transl Med* 2010; 8: 108.
- [30] Rahimifar S, Erfani N, Sarraf Z and Ghaderi A. *ctla-4* gene variations may influence cervical cancer susceptibility. *Gynecol Oncol* 2010; 119: 136-139.
- [31] Khaghanzadeh N, Erfani N, Ghayumi MA and Ghaderi A. CTLA4 gene variations and haplotypes in patients with lung cancer. *Cancer Genet Cytogenet* 2010; 196: 171-174.
- [32] Ivansson EL, Juko-Pecirep I and Gyllensten UB. Interaction of immunological genes on chromosome 2q33 and IFNG in susceptibility to cervical cancer. *Gynecol Oncol* 2010; 116: 544-548.
- [33] Pawlak E, Karabon L, Wlodarska-Polinska I, Jedynak A, Jonkisz A, Tomkiewicz A, Kornafel J, Stepien M, Ignatowicz A, Lebioda A, Dobosz T and Frydecka I. Influence of CTLA-4/CD28/ICOS gene polymorphisms on the susceptibility to cervical squamous cell carcinoma and stage of differentiation in the Polish population. *Hum Immunol* 2010; 71: 195-200.
- [34] Bouwhuis MG, Gast A, Figl A, Eggermont AM, Hemminki K, Schadendorf D and Kumar R. Polymorphisms in the CD28/CTLA4/ICOS genes: role in malignant melanoma susceptibility and prognosis? *Cancer Immunol Immunother* 2010; 59: 303-312.
- [35] Welsh MM, Applebaum KM, Spencer SK, Perry AE, Karagas MR and Nelson HH. CTLA4 variants, UV-induced tolerance, and risk of non-melanoma skin cancer. *Cancer Res* 2009; 69: 6158-6163.
- [36] Dilmec F, Ozgonul A, Uzunkoy A and Akkafa F. Investigation of CTLA-4 and CD28 gene polymorphisms in a group of Turkish patients with colorectal cancer. *Int J Immunogenet* 2008; 35: 317-321.
- [37] Suwalska K, Pawlak E, Karabon L, Tomkiewicz A, Dobosz T, Urbaniak-Kujda D, Kuliczkowski K, Wolowiec D, Jedynak A and Frydecka I. Association studies of CTLA-4, CD28, and ICOS gene polymorphisms with B-cell chronic lymphocytic leukemia in the Polish population. *Hum Immunol* 2008; 69: 193-201.
- [38] Castro FA, Haimila K, Sareneva I, Schmitt M, Lorenzo J, Kunkel N, Kumar R, Forsti A, Kjellberg L, Hallmans G, Lehtinen M, Hemminki K and Pawlita M. Association of HLA-DRB1, interleukin-6 and cyclin D1 polymorphisms with cervical cancer in the Swedish population—a candidate gene approach. *Int J Cancer* 2009; 125: 1851-1858.
- [39] Hui L, Lei Z, Peng Z, Ruobing S and Fenghua Z. Polymorphism analysis of CTLA-4 in childhood acute lymphoblastic leukemia. *Pak J Pharm Sci* 2014; 27: 1005-1013.
- [40] Jaiswal PK, Singh V and Mittal RD. Cytotoxic T lymphocyte antigen 4 (CTLA4) gene polymorphism with bladder cancer risk in North Indian population. *Mol Biol Rep* 2014; 41: 799-807.
- [41] Kong F. Association between polymorphisms of CTLA-4, IL-10 gene and breast cancer in Chinese Han population: Fourth Military Medical University; 2010. 65 pp.
- [42] Loh M, Koh KX, Yeo BH, Song CM, Chia KS, Zhu F, Yeoh KG, Hill J, Iacopetta B and Soong R. Meta-analysis of genetic polymorphisms and gastric cancer risk: variability in associations according to race. *Eur J Cancer* 2009; 45: 2562-2568.
- [43] Ghaderi A, Yeganeh F, Kalantari T, Talei AR, Pezeshki AM, Doroudchi M and Dehaghani AS. Cytotoxic T lymphocyte antigen-4 gene in breast cancer. *Breast Cancer Res Treat* 2004; 86: 1-7.
- [44] Ligiers A, Teleshova N, Masterman T, Huang WX and Hillert J. CTLA-4 gene expression is influenced by promoter and exon 1 polymorphisms. *Genes Immun* 2001; 2: 145-152.

## CTLA-4 rs5742909 C>T polymorphism and cancer risk

- [45] Buonavista N, Balzano C, Pontarotti P, Le Paslier D and Golstein P. Molecular linkage of the human CTLA4 and CD28 Ig-superfamily genes in yeast artificial chromosomes. *Genomics* 1992; 13: 856-861.
- [46] Cozar JM, Romero JM, Aptsiauri N, Vazquez F, Vilchez JR, Tallada M, Garrido F and Ruiz-Cabello F. High incidence of CTLA-4 AA (CT60) polymorphism in renal cell cancer. *Hum Immunol* 2007; 68: 698-704.
- [47] Yang M, Sun T, Zhou Y, Wang L, Liu L, Zhang X, Tang X, Zhou M, Kuang P, Tan W, Li H, Yuan Q and Yu D. The functional cytotoxic T lymphocyte-associated Protein 4 49G-to-A genetic variant and risk of pancreatic cancer. *Cancer* 2012; 118: 4681-4686.