## Original Article
# Associations between *CTLA-4* +49 A/G (rs231775) polymorphism and cancer risk: a meta-analysis based on 52 case-control studies

Lu Wang[1,2*], Zhiwei Jiang[1,2*], Hao Qiu[2*], Weifeng Tang[3], Tanghai Duan[4], Lixin Wang[1,2]

[1]Department of Clinical Laboratory, Danyang People's Hospital, Medical School of Southeast University, Danyang 212300, China; [2]Department of Microbiology and Immunology, Medical School of Southeast University, Nanjing 210009, China; [3]Department of Cardiothoracic Surgery, Affiliated People's Hospital of Jiangsu University, Zhenjiang 212000, China; [4]Department of Clinical Laboratory, Pu'ai Hospital Affiliated to Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China. *Equal contributors.

Abstract: Objectives: To date, a number of epidemiological studies have explored the association between *CTLA-4* +49 A/G polymorphism and cancer risk with elusive results. To address this gap, we carried out a comprehensive meta-analysis. Design and methods: Two reviewers independently searched the PubMed, EMBASE, CBM (Chinese BioMedical Disc) and China National Knowledge Infrastructure (CNKI) Databases for relevant studies up to December 20, 2014. Odds ratios (ORs) with 95% confidence intervals (CIs) for *CTLA-4* +49 A/G polymorphism and cancer risk were used to evaluate the strength of association. Results: A total of 52 case-control studies were ultimately recruited. Our results showed the statistical evidence of an association between the *CTLA-4* +49 A/G polymorphism and decreased risk of overall cancer in all the comparison models. In stratified analyses by cancer type, ethnicity and the origin of cancer, significant decreases in cancer risk were observed in breast cancer, lung cancer, other cancers epithelial tumor and Asians. In addition, in a stratified analysis by the system of cancer, significant decreases in cancer risk were found for reproductive and breast cancer, respiratory system cancer, and malignant bone tumor in all the genetic models, and other system cancer in two genetic models: GG+AG vs. AA and GG vs. AA. Conclusions: This meta-analysis suggests that the *CTLA-4* +49 A/G polymorphism may be a protective factor for cancer.

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

## Introduction

Cancer is one of the most common types of human disease which results from interactions between multiple genetic and environmental factors [1-4]. It is estimated that more than fourteen million cancer cases and eight million cancer deaths occurred in 2012 worldwide [5]. The etiology of cancer is very complicated and not fully known as yet. Of late, a considerable number of studies have focused on a vital role of human immune system in cancer development and progression. T lymphocytes and natural killer (NK) cells play an important role in antitumor immunity and are regulated by some costimulatory and coinhibitory molecules [6]. Thus, the mutations of cellular immune function genes that regulate the activation and pro-liferation of T lymphocytes and NK cells may alter cancer susceptibility [7, 8]. Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), also named CD152, is a member of the immunoglobulin superfamily and mainly expressed on activated T cells. Through engagement of the T-cell antigen receptor, CD28 plays a key role in increasing and maintaining the T-cell response, whereas the CTLA-4-ligand acts as a negative regulator of T-cell activation [9]. Mice deficient in *CTLA-4* gene have been shown to develop lymphoproliferative disorders and severe autoimmune diseases [10, 11]. In addition, application of CTLA-4 blockade in the tumor-transplanted mice leads to enhancement of the immune response *in vivo*, resulting in rejection of tumors and long-lasting antitumor immunity [12]. The *CTLA-4* gene locates on

**Figure 1.** Flow diagram of articles selection process for *CTLA-4* +49 A/G (rs231775) polymorphism and cancer risk meta-analysis.

relevant to the risk of cancer, whereas in that analysis, only 22 case-control studies were recruited [16]. However, up to now, 52 investigations have been carried out regarding the association of *CTLA-4* +49 A/G polymorphism with cancer risk and these studies reported inconsistent results rather than conclusive. Thus, we conducted a comprehensive meta-analysis to assess whether this polymorphism was associated with cancer risk.

**Materials and methods**

This meta-analysis is reported according to the Preferred Reporting Items for Meta-analyses (PRISMA) guideline (Table S1. PRISMA checklist) [17].

*Search Strategy, inclusion criteria and exclusion criteria*

A systematic literature searching was performed on PubMed, EMBASE, CBM (Chinese Bio-Medical Disc) as well as CNKI (Chinese National Knowledge Infrastructure) on December 20, 2014. The search strategy was based on combinations of 'CTLA-4', 'CTLA4', or 'cytotoxic T-lymphocyte antigen-4'; 'polymorphism', 'mutation', 'variant', or 'SNP'; 'cancer', 'carcinoma', 'tumor', or 'malignance'. References of retrieved articles, comments, letters and reviews were manually searched for supplemental studies. The language of publication was restricted to English or Chinese. The major inclusion criteria were (a) evaluated the *CTLA-4* +49 A/G polymorphism and cancer risk, (b) designed as a case-control study or cohort studies, (c) sufficient data for evaluation of the frequencies of various genotypes in case groups and control groups, (d) provided the genotyping method and ethnicity and (e) genotype distributions of controls consistent with Hardy-Weinberg equilibrium (HWE). The major reasons for exclusion of studies were (a) overlapping data, (b) case report, not designed as case-control studies or cohort studies and (c) review publication, comment and letter.

chromosome 2q33 in humans and consists of four exons which encode several domains of CTLA-4 protein. The *CTLA-4* single nucleotide polymorphisms (SNPs) are considered to influence the expression of the protein and/or the functional activity of CTLA-4 [13]. There are more than 100 SNPs in *CTLA-4* gene which have been identified in the previous investigations, such as +49 A/G, -318 C/T, CT60, -1611 G/A, -1722 T/C, 10223 G/T (Jo31) polymorphisms, etc [14]. Variations of *CTLA-4* gene have been implicated in the etiology of breast cancer [15], cervical cancer [16], and so on. Among *CTLA-4* SNPs, *CTLA-4* +49 A/G polymorphism was the most widely investigated for susceptibility of cancer. In the past decade, a number of investigations were performed to evaluate the association between *CTLA-4* +49 A/G polymorphism and cancer risk. A meta-analysis conducted by Zhang *et al.* suggested that *CTLA-4* +49 A/G polymorphism might be

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

| Study | Year | Country | Ethnicity | Cancer type | No. of case/control | Genotype Method |
|---|---|---|---|---|---|---|
| Queirolo et al. | 2013 | Italy | Caucasians | Melanoma | 14/45 | PCR-RFLP |
| Gokhale et al. | 2013 | India | Asians | Cervical cancer | 100/101 | PCR-RFLP |
| Song et al. | 2013 | China | Asians | Lung cancer | 158/72 | PCR-RFLP |
| Bharti et al. | 2013 | India | Asians | Oral cancer | 130/180 | PCR-RFLP |
| Fan et al. | 2012 | China | Asians | Colorectal cancer | 291/352 | PCR-RFLP |
| Yang et al. | 2012 | China | Asians | Pancreatic cancer | 368/926 | PCR-RFLP |
| Yang et al. | 2012 | China | Asians | Ewing's Sarcoma | 223/302 | PCR-RFLP |
| Qi et al. | 2012 | China | Asians | Gastric cancer | 118/96 | PCR-RFLP |
| Erfani et al. | 2012 | Iran | Caucasians | Head and neck cancer | 80/85 | PCR-RFLP |
| Li et al. | 2012 | China | Asians | Breast cancer | 581/566 | PCR-RFLP |
| Karabon et al. | 2012 | Poland | Caucasians | Myeloma | 200/380 | PCR-RFLP, TaqMan |
| Karabon et al. | 2011 | Poland | Caucasians | Lung cancer | 208/326 | PCR-RFLP, TaqMan |
| Jiang et al. | 2011 | China | Asians | Cervical cancer | 100/100 | MALDI-TOF-MS |
| Cai et al. | 2011 | China | Caucasians | Esophageal cancer | 125/250 | PCR-RFLP |
| Li et al. | 2011 | China | Asians | Cervical cancer | 314/320 | PCR-RFLP |
| Wang et al. | 2011 | China | Asians | Osteosarcoma | 205/216 | PCR-RFLP, DNA sequencing |
| Wu et al. | 2011 | China | Asians | Glioma | 670/680 | PCR-LDR; DNA sequencing |
| Rahimifar et al. | 2010 | Iran | Caucasians | Cervical cancer | 55/110 | PCR-RFLP, PCR-ARMS |
| Kammerer et al. | 2010 | German | Caucasians | Oral cancer | 83/40 | RT-PCR |
| Bouwhuis et al. | 2010 | German | Caucasians | Melanoma | 763/734 | DNA sequencing |
| Khaghanzadeh et al. | 2010 | Iran | Caucasians | Lung cancer | 127/124 | PCR-RFLP, PCR-ARMS |
| Gogas et al. | 2010 | Greece | Caucasians | Melanoma | 286/288 | DNA sequencing |
| Pawlak et al. | 2010 | Poland | Caucasians | Cervical cancer | 147/225 | PCR-RFLP |
| Gu et al. | 2010 | China | Asians | Hepatocellular carcinoma | 375/419 | PCR-LDR |
| Hu et al. | 2010 | China | Asians | Cervical, hepatocellular cancer | 1549/1563 | TaqMan |
| Qi et al. | 2010 | China | Asians | Colorectal cancer | 124/407 | PCR-LDR |
| Xiao et al. | 2010 | China | Asians | Nasopharyngeal carcinoma | 457/485 | PCR-RFLP |
| Dehaghani et al. | 2009 | Iran | Caucasians | Gestational trophoblastic neoplasms | 92/295 | PCR-RFLP, PCR-SSCP |
| Castro et al. | 2009 | Sweden | Caucasians | Cervical cancer | 973/1763 | Multiplex PCR with hybridization |
| Shi et al. | 2009 | China | Asians | Pancreatic cancer | 138/278 | PCR-RFLP |
| Suwalska et al. | 2008 | Poland | Caucasians | Leukemia | 178/336 | SNaPshot |
| Mahajan et al. | 2008 | Poland | Caucasians | Gastric cancer | 301/411 | TaqMan |
| Sun et al. | 2008 | China | Asians | Lung, breast, esophageal, gastric cancer | 5832/5831 | PCR-RFLP, Sequenom |
| Cozar et al. | 2007 | Spain | Caucasians | Colorectal, renal cell cancer | 223/196 | TaqMan |
| Hadinia et al. | 2007 | Iran | Caucasians | Colorectal, gastric cancer | 155/190 | RFLP, PCR-ARMS |
| Wang et al. | 2007 | China | Asians | Breast cancer | 117/148 | PCR-RFLP |
| Nearman et al. | 2007 | USA | Caucasians | Leukemia | 26/96 | RT-PCR |
| Su et al. | 2007 | China | Asians | Cervical cancer | 144/378 | PCR-RFLP |
| Cheng et al. | 2006 | China | Asians | Lymphoma | 62/250 | PCR-RFLP |
| Wong et al. | 2006 | China | Asians | Oral cancer | 118/147 | PCR-RFLP |
| Piras et al. | 2005 | Italy | Caucasians | Lymphoma | 100/128 | PCR-RFLP |
| Solerio et al. | 2005 | Italy | Caucasians | Colorectal cancer | 132/238 | PCR-RFLP |
| Ghaderi et al. | 2004 | Iran | Caucasians | Breast cancer | 197/151 | PCR-SSCP |
| Monne et al. | 2004 | Italy | Caucasians | Lymphoma | 44/76 | PCR-RFLP |

MALDI-TOF-MS, matrix-assisted laser desorption/ionization time of flight mass spectrometry; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; MLPA, multiplex ligation dependent probe amplification; PCR-LDR, polymerase chain reaction-ligase detection reaction; PCR-ARMS, amplification refractory mutation system-polymerase chain reaction; PCR-SSCP, polymerase chain reaction-single-strand conformation polymorphism.

*Data extraction*

Two reviewers (L. Wang and Z. Jiang) extracted information from all the eligible articles independently with a standard form and reached a consensus on all items. In case of conflicting evaluations, the disagreements were settled by further discussion among all authors. The

**Table 2.** Distribution of *CTLA-4* +49 A/G polymorphisms genotype and allele among multiple cancer patients and controls

| | Case | | | Control | | | Case | | Control | | HWE | Quality scores |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AA | AG | GG | AA | AG | GG | A | G | A | G | | |
| Queirolo et al. | 6 | 8 | 0 | 26 | 16 | 3 | 20 | 8 | 68 | 22 | Yes | 7.5 |
| Bharti et al. | 67 | 63 | 0 | 75 | 80 | 25 | 197 | 63 | 230 | 130 | Yes | 7.0 |
| Gokhale et al. | 32 | 46 | 26 | 27 | 87 | 48 | 110 | 98 | 141 | 183 | Yes | 7.0 |
| Song et al. | 22 | 66 | 70 | 7 | 31 | 34 | 110 | 206 | 45 | 99 | Yes | 6.5 |
| Qi et al. | 8 | 45 | 65 | 21 | 45 | 30 | 61 | 175 | 87 | 105 | Yes | 7.5 |
| Yang et al. | 32 | 114 | 77 | 26 | 149 | 127 | 178 | 268 | 201 | 403 | Yes | 7.0 |
| Yang et al. | 50 | 178 | 140 | 70 | 374 | 482 | 278 | 458 | 514 | 1338 | Yes | 7.5 |
| Li et al. | 49 | 281 | 246 | 54 | 243 | 256 | 379 | 773 | 351 | 755 | Yes | 7.0 |
| Karabon et al. | 48 | 103 | 48 | 124 | 169 | 75 | 199 | 199 | 417 | 319 | Yes | 7.0 |
| Erfani et al. | 41 | 35 | 4 | 50 | 29 | 6 | 117 | 43 | 129 | 41 | Yes | 5.5 |
| Fan et al. | 123 | 146 | 22 | 170 | 138 | 44 | 392 | 190 | 478 | 226 | Yes | 6.5 |
| Li et al. | 30 | 144 | 140 | 18 | 129 | 173 | 204 | 424 | 165 | 475 | Yes | 6.0 |
| Karabon et al. | 68 | 106 | 34 | 107 | 145 | 72 | 242 | 174 | 359 | 289 | Yes | 6.0 |
| Jiang et al. | 13 | 42 | 45 | 19 | 49 | 42 | 68 | 132 | 87 | 133 | Yes | 7.5 |
| Wang et al. | 35 | 106 | 64 | 21 | 108 | 87 | 176 | 234 | 150 | 282 | Yes | 6.5 |
| Wu et al. | 97 | 259 | 297 | 70 | 295 | 300 | 453 | 853 | 435 | 895 | Yes | 8.5 |
| Cai et al. | 27 | 68 | 30 | 47 | 133 | 70 | 122 | 128 | 227 | 273 | Yes | 8.5 |
| Gogas et al. | 132 | 128 | 26 | 152 | 111 | 25 | 392 | 180 | 415 | 161 | Yes | 7.0 |
| Rahimifar et al. | 28 | 27 | 0 | 58 | 45 | 7 | 83 | 27 | 161 | 59 | Yes | 3.5 |
| Hu et al. | 106 | 380 | 367 | 79 | 376 | 399 | 592 | 1114 | 534 | 1174 | Yes | 9.5 |
| Hu et al. | 80 | 290 | 326 | 56 | 300 | 353 | 450 | 942 | 412 | 1006 | Yes | 9.5 |
| Kammerer et al. | 35 | 32 | 16 | 11 | 23 | 6 | 102 | 64 | 45 | 35 | Yes | 5.5 |
| Khaghanzadeh et al. | 66 | 44 | 13 | 68 | 47 | 7 | 176 | 70 | 183 | 61 | Yes | 5.0 |
| Xiao et al. | 57 | 195 | 205 | 38 | 201 | 246 | 309 | 605 | 277 | 693 | Yes | 7.5 |
| Pawlak et al. | 43 | 72 | 26 | 71 | 103 | 43 | 158 | 124 | 245 | 189 | Yes | 6.5 |
| Qi et al. | 4 | 60 | 60 | 45 | 179 | 183 | 68 | 180 | 269 | 545 | Yes | 7.5 |
| Gu et al. | 51 | 166 | 150 | 45 | 179 | 183 | 268 | 466 | 269 | 545 | Yes | 7.5 |
| Bouwhuis et al. | 289 | 369 | 104 | 283 | 345 | 106 | 947 | 577 | 911 | 557 | Yes | 7.5 |
| Dehaghani et al. | 42 | 34 | 7 | 45 | 37 | 2 | 118 | 48 | 127 | 41 | Yes | 3.5 |
| Castro et al. | 252 | 449 | 252 | 456 | 825 | 434 | 953 | 953 | 1737 | 1693 | Yes | 10 |
| Shi et al. | 26 | 71 | 41 | 43 | 113 | 122 | 123 | 153 | 199 | 357 | Yes | 6.0 |
| Mahajan et al. | 89 | 153 | 59 | 152 | 189 | 70 | 331 | 271 | 493 | 329 | Yes | 7.5 |
| Sun et al. | 101 | 485 | 474 | 65 | 446 | 559 | 687 | 1433 | 576 | 1564 | Yes | 7.0 |
| Sun et al. | 100 | 455 | 482 | 73 | 451 | 546 | 655 | 1419 | 597 | 1543 | Yes | 7.0 |
| Sun et al. | 128 | 434 | 448 | 73 | 406 | 529 | 690 | 1330 | 552 | 1464 | Yes | 7.0 |
| Sun et al. | 60 | 235 | 235 | 39 | 209 | 282 | 355 | 705 | 287 | 773 | Yes | 7.0 |
| Sun et al. | 135 | 519 | 509 | 81 | 488 | 563 | 789 | 1537 | 650 | 1614 | Yes | 7.0 |
| Sun et al. | 125 | 439 | 468 | 90 | 438 | 493 | 689 | 1375 | 618 | 1424 | Yes | 7.0 |
| Suwalska et al. | 56 | 84 | 30 | 71 | 106 | 47 | 196 | 144 | 248 | 200 | Yes | 4.0 |
| Hadinia et al. | 52 | 47 | 6 | 117 | 59 | 14 | 151 | 59 | 293 | 87 | Yes | 6.5 |
| Hadinia et al. | 24 | 13 | 6 | 117 | 59 | 14 | 61 | 25 | 293 | 87 | Yes | 6.5 |
| Wang et al. | 48 | 59 | 10 | 55 | 70 | 23 | 155 | 79 | 180 | 116 | Yes | 7.0 |
| Cozar et al. | 46 | 44 | 6 | 78 | 77 | 21 | 136 | 56 | 233 | 119 | Yes | 7.0 |
| Cozar et al. | 73 | 43 | 9 | 78 | 77 | 21 | 189 | 61 | 233 | 119 | Yes | 7.0 |
| Su et al. | 17 | 62 | 60 | 42 | 155 | 178 | 96 | 182 | 239 | 511 | Yes | 6.5 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Nearman et al. | 14 | 8 | 4 | 26 | 51 | 19 | 36 | 16 | 103 | 89 | Yes | 2.0 |
| Cheng et al. | 2 | 26 | 34 | 29 | 102 | 119 | 30 | 94 | 160 | 340 | Yes | 7.5 |
| Wong et al. | 12 | 58 | 48 | 25 | 64 | 58 | 82 | 154 | 114 | 180 | Yes | 6.5 |
| Piras et al. | 74 | 23 | 3 | 74 | 43 | 11 | 171 | 29 | 191 | 65 | Yes | 5.0 |
| Solerio et al. | 76 | 43 | 13 | 128 | 91 | 19 | 195 | 69 | 347 | 129 | Yes | 6.5 |
| Ghaderi et al. | 84 | 104 | 9 | 60 | 72 | 19 | 272 | 122 | 192 | 110 | Yes | 5.0 |
| Monne et al. | 36 | 7 | 1 | 38 | 32 | 6 | 79 | 9 | 108 | 44 | Yes | 5.0 |

HWE: Hardy-Weinberg equilibrium.

following information from each included study was extracted: first author, year of publication, country, cancer type, ethnicity, sample size, genotyping method, allele and genotype frequency.

*Methodological quality assessment*

The quality of investigations included in the current study was carefully assessed by two reviewers (L. Wang and Z. Jiang) according to the "methodological quality assessment scale" (Table S2. Scale for methodological quality assessment.) [18, 19]. Six items, comprising representativeness of cases, source of controls, sample size, ascertainment of relevant cancer, quality control of genotyping, and HWE, were assessed. Scores range from 0 to 10 and a high score suggests good quality of this meta-analysis. In case of disagreements between the two reviewers, a final consensus shall be reached following elaborate discussion. If the quality scores ≥ 6, studies were categorized as "high quality", and others were defined as "low quality".

*Statistical analysis*

The ORs of *CTLA-4* +49 A/G polymorphism and cancer risk were calculated for each study. The pooled ORs were determined in six genetic comparison models. The *I*-squared value was an index of between-study heterogeneity, with $I^2$ less than 25% indicating low, $25\% \le I^2 \le 50\%$ indicating moderate, and $I^2 > 50\%$ indicating high heterogeneity. A chi-squared-based Q statistic test was also conducted to measure heterogeneity. If $I^2 > 50\%$ or $P < 0.10$, ORs were pooled according to the random-effect model (DerSimonian and Laird) [20]; otherwise, the fixed-effect model (Mantel-Haenszel) was applied [21]. Stratified analyses were carried out based on ethnicity, cancer type, origin and system to evaluate ethnicity-specific, cancer

type-specific (we combined the cancer type which was investigated by less than three individual studies into the group of "other cancers"), origin-specific and system-specific effects (if one system cancer was investigated by less than two individual studies, it was included into "other system cancers"). The HWE was evaluated by an internet-based HWE calculator in controls (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). Sensitivity analysis was performed to assess the stability of our results. The funnel plot and Egger's test were conducted to detect latent publication bias and a $P < 0.1$ was considered significant. Publication bias was also measured by visual inspection of funnel plots. Statistical analysis was performed using STATA (v12.0) software (Stata Corporation, Texas, USA).

**Results**

*Characteristics of studies*

The combined search yielded 345 papers following an initial search. After we abstract-screened and full-text assessed these papers, a total of 285 papers were excluded (85 for title duplication, nine for not being case-control study design, 47 for cancer treatment, 95 for irrelevance to *CTLA-4* +49 A/G polymorphism and cancer risk, 48 reviews, comments or letters, and one for overlapping data), thereby 60 remaining papers met the inclusion criteria. Another two articles were then included via a detailed manual search of the reference lists from all retrieved articles (**Figure 1**). Afterwards, online HWE test was performed on genotype distribution of the controls in each recruited study, and the results showed that the distribution in the control group from 10 studies deviated from HWE ($P < 0.05$). Since more than one study group was included in some publications [15, 22-24], we treated them separately. Therefore, in total, 52 studies in 44 publica-

**Table 3.** Summary of results of the meta-analysis from different comparative genetic models in overall and the subgroup analysis

| | No. (cases/ controls) | G vs. A | | | GG vs. AA | | | GG+AG vs. AA | | | GG vs. AG+AA | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 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 | 16,594/20,283 | 0.89 (0.84-0.95) | 0.000 | 0.000 | 0.76 (0.67-0.88) | 0.000 | 0.000 | 0.85 (0.76-0.95) | 0.004 | 0.000 | 0.85 (0.78-0.91) | 0.000 | 0.000 |
| Ethnicity | | | | | | | | | | | | | |
| Asians | 12,143/13,845 | 0.85 (0.79-0.91) | 0.000 | 0.000 | 0.68 (0.58-0.80) | 0.000 | 0.000 | 0.76 (0.66-0.88) | 0.000 | 0.000 | 0.82 (0.75-0.89) | 0.000 | 0.000 |
| Caucasians | 4,451/6,438 | 0.96 (0.87-1.06) | 0.455 | 0.001 | 0.93 (0.77-1.12) | 0.446 | 0.024 | 0.98 (0.85-1.12) | 0.753 | 0.000 | 0.96 (0.86-1.06) | 0.423 | 0.108 |
| Cancer type | | | | | | | | | | | | | |
| Breast cancer | 2,987/2,992 | 0.83 (0.77-0.89) | 0.000 | 0.445 | 0.63 (0.46-0.86) | 0.004 | 0.068 | 0.78 (0.66-0.92) | 0.003 | 0.140 | 0.78 (0.70-0.87) | 0.000 | 0.139 |
| Cervical cancer | 2,502/3,718 | 0.90 (0.80-1.02) | 0.108 | 0.055 | 0.78 (0.58-1.05) | 0.103 | 0.029 | 0.84 (0.67-1.07) | 0.155 | 0.045 | 0.92 (0.82-1.03) | 0.148 | 0.190 |
| Colorectal cancer | 748/1,363 | 1.06 (0.92-1.22) | 0.389 | 0.254 | 1.01 (0.56-1.82) | 0.971 | 0.047 | 1.24 (0.88-1.76) | 0.225 | 0.035 | 0.87 (0.66-1.13) | 0.300 | 0.160 |
| Gastric cancer | 992/1,227 | 1.28 (0.80-2.05) | 0.307 | 0.000 | 1.62 (0.66-4.02) | 0.295 | 0.000 | 1.33 (0.72-2.46) | 0.362 | 0.001 | 1.37 (0.73-2.56) | 0.331 | 0.000 |
| Lung cancer | 2,684/2,671 | 0.84 (0.78-0.92) | 0.000 | 0.340 | 0.66 (0.54-0.79) | 0.000 | 0.169 | 0.74 (0.63-0.88) | 0.000 | 0.103 | 0.84 (0.75-0.93) | 0.002 | 0.308 |
| Lymphoma | 206/454 | 0.61 (0.24-1.57) | 0.307 | 0.000 | 0.62 (0.08-4.60) | 0.642 | 0.010 | 0.64 (0.19-2.24) | 0.491 | 0.004 | 0.63 (0.20-2.00) | 0.430 | 0.070 |
| Melanoma | 1,062/1,067 | 1.04 (0.92-1.18) | 0.504 | 0.486 | 1.00 (0.76-1.32) | 0.983 | 0.767 | 1.11 (0.93-1.32) | 0.233 | 0.349 | 0.95 (0.73-1.23) | 0.706 | 0.814 |
| Oral cancer | 331/367 | 0.82 (0.50-1.32) | 0.411 | 0.015 | 0.50 (0.07-3.36) | 0.474 | 0.002 | 0.85 (0.43-1.68) | 0.640 | 0.042 | 0.61 (0.15-2.56) | 0.500 | 0.008 |
| Other cancers | 5,082/6,424 | 0.82 (0.74-0.91) | 0.000 | 0.001 | 0.64 (0.53-0.78) | 0.000 | 0.006 | 0.73 (0.61-0.87) | 0.001 | 0.001 | 0.78 (0.70-0.88) | 0.000 | 0.030 |
| The origin of cancer cells | | | | | | | | | | | | | |
| Epithelial tumor | 13,850/16,891 | 0.89 (0.83-0.95) | 0.000 | 0.000 | 0.76 (0.65-0.89) | 0.000 | 0.000 | 0.86 (0.77-0.97) | 0.012 | 0.000 | 0.83 (0.76-0.91) | 0.000 | 0.000 |
| Non-epithelial tumor | 2,744/3,392 | 0.88 (0.74-1.04) | 0.143 | 0.000 | 0.78 (0.57-1.07) | 0.130 | 0.003 | 0.80 (0.59-1.07) | 0.131 | 0.000 | 0.92 (0.81-1.04) | 0.181 | 0.291 |
| System of cancer | | | | | | | | | | | | | |
| Digestive system cancer | 4,932/6,680 | 0.93 (0.81-1.07) | 0.300 | 0.000 | 0.86 (0.64-1.16) | 0.329 | 0.000 | 0.95 (0.76-1.18) | 0.640 | 0.000 | 0.86 (0.72-1.02) | 0.089 | 0.000 |
| Respiratory system cancer | 3,141/3,156 | 0.83 (0.77-0.90) | 0.000 | 0.415 | 0.64 (0.54-0.76) | 0.000 | 0.231 | 0.72 (0.62-0.84) | 0.000 | 0.127 | 0.83 (0.75-0.92) | 0.000 | 0.422 |
| Reproductive and breast cancer | 5,572/6,794 | 0.88 (0.81-0.95) | 0.002 | 0.030 | 0.73 (0.58-0.92) | 0.007 | 0.002 | 0.84 (0.72-0.98) | 0.026 | 0.032 | 0.84 (0.74-0.95) | 0.007 | 0.025 |
| Hematopoietic malignancy | 601/1142 | 0.77 (0.50-1.16) | 0.213 | 0.000 | 0.81 (0.39-1.67) | 0.563 | 0.006 | 0.73 (0.38-1.40) | 0.339 | 0.000 | 0.99 (0.77-1.29) | 0.957 | 0.202 |
| Malignant bone tumor | 428/518 | 0.73 (0.61-0.88) | 0.001 | 0.755 | 0.47 (0.30-0.72) | 0.001 | 0.803 | 0.54 (0.36-0.81) | 0.003 | 0.859 | 0.70 (0.54-0.92) | 0.010 | 0.780 |
| Skin cancer | 1,062/1,067 | 1.04 (0.92-1.18) | 0.504 | 0.486 | 1.00 (0.76-1.32) | 0.983 | 0.767 | 1.11 (0.93-1.32) | 0.233 | 0.349 | 0.95 (0.73-1.23) | 0.706 | 0.814 |
| Other system cancer | 858/926 | 0.88 (0.76-1.01) | 0.078 | 0.101 | 0.67 (0.49-0.92) | 0.013 | 0.609 | 0.72 (0.56-0.92) | 0.008 | 0.072 | 0.97 (0.79-1.19) | 0.745 | 0.364 |
| Publication year | | | | | | | | | | | | | |
| ≤ 2006 | 653/990 | 0.81 (0.56-1.16) | 0.250 | 0.000 | 0.79 (0.35-1.80) | 0.575 | 0.004 | 0.82 (0.48-1.40) | 0.469 | 0.001 | 0.78 (0.46-1.31) | 0.343 | 0.032 |
| 2007-2011 | 13,680/16,152 | 0.88 (0.83-0.94) | 0.000 | 0.000 | 0.74 (0.65-0.85) | 0.000 | 0.000 | 0.83 (0.74-0.93) | 0.001 | 0.000 | 0.84 (0.79-0.90) | 0.000 | 0.034 |
| ≥ 2012 | 2,261/3,141 | 0.94 (0.75-1.17) | 0.565 | 0.000 | 0.78 (0.48-1.27) | 0.316 | 0.000 | 0.98 (0.70-1.37) | 0.892 | 0.000 | 0.84 (0.62-1.14) | 0.259 | 0.000 |
| Sample sizes | | | | | | | | | | | | | |
| ≤ 1000 | 5,901/8,296 | 0.93 (0.84-1.02) | 0.108 | 0.000 | 0.84 (0.68-1.03) | 0.094 | 0.000 | 0.93 (0.80-1.07) | 0.320 | 0.000 | 0.87 (0.76-0.99) | 0.033 | 0.000 |
| > 1000 | 10693/11987 | 0.84 (0.78-0.90) | 0.000 | 0.000 | 0.67 (0.57-0.79) | 0.000 | 0.000 | 0.72 (0.62-0.83) | 0.000 | 0.000 | 0.83 (0.76-0.90) | 0.000 | 0.005 |
| Country | | | | | | | | | | | | | |
| China | 12,034/13,753 | 0.86 (0.80-0.92) | 0.000 | 0.000 | 0.70 (0.60-0.82) | 0.000 | 0.000 | 0.78 (0.68-0.90) | 0.001 | 0.000 | 0.82 (0.76-0.89) | 0.000 | 0.001 |
| German | 845/774 | 0.98 (0.85-1.13) | 0.804 | 0.460 | 0.95 (0.70-1.29) | 0.751 | 0.823 | 0.98 (0.80-1.20) | 0.869 | 0.115 | 0.96 (0.73-1.28) | 0.796 | 0.498 |
| India | 234/342 | 0.62 (0.49-0.80) | 0.000 | 0.448 | 0.13 (0.00-3.82) | 0.235 | 0.018 | 0.58 (0.40-0.83) | 0.003 | 0.290 | 0.17 (0.00-9.22) | 0.382 | 0.005 |
| Iran | 686/932 | 1.07 (0.91-1.26) | 0.381 | 0.317 | 1.05 (0.52-2.12) | 0.900 | 0.028 | 1.17 (0.95-1.43) | 0.142 | 0.694 | 0.97 (0.47-2.01) | 0.944 | 0.015 |

| | | | F | R | | F | R | | F | R | | F | R |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Italy | 290/487 | 0.64 (0.36-1.13) | 0.124 | 0.008 | 0.64 (0.35-1.16) | 0.141 | 0.153 | 0.61 (0.31-1.19) | 0.147 | 0.011 | 0.74 (0.41-1.32) | 0.306 | 0.217 |
| Poland | 1,019/1,544 | 1.08 (0.97-1.21) | 0.170 | 0.113 | 1.12 (0.89-1.41) | 0.326 | 0.110 | 1.22 (1.03-1.45) | 0.023 | 0.283 | 0.97 (0.80-1.19) | 0.796 | 0.258 |
| Spain | 221/352 | 0.71 (0.55-0.92) | 0.010 | 0.365 | 0.47 (0.25-0.89) | 0.020 | 0.932 | 0.69 (0.49-0.97) | 0.031 | 0.224 | 0.54 (0.29-0.99) | 0.047 | 0.812 |
| Other countries | 1,265/2,099 | 0.99 (0.77-1.29) | 0.963 | 0.064 | 1.04 (0.85-1.27) | 0.730 | 0.280 | 0.92 (0.59-1.44) | 0.714 | 0.013 | 1.05 (0.89-1.25) | 0.562 | 0.835 |
| Quality score | | | | | | | | | | | | | |
| < 6.0 | 961/1,116 | 0.81 (0.64-1.02) | 0.071 | 0.008 | 0.66 (0.40-1.12) | 0.123 | 0.051 | 0.76 (0.56-1.03) | 0.076 | 0.006 | 0.77 (0.47-1.25) | 0.290 | 0.054 |
| ≥ 6.0 | 15,633/19,167 | 0.90 (0.84-0.96) | 0.002 | 0.000 | 0.77 (0.67-0.89) | 0.000 | 0.000 | 0.87 (0.77-0.98) | 0.020 | 0.000 | 0.85(0.79-0.92) | 0.000 | 0.000 |

F indicates fixed model; R indicates random model.

tions were finally identified with 16,594 cases and 20,283 controls [8, 15, 16, 22-62]. The detailed characteristics of the selected studies are presented in **Table 1**. Among the 52 case-control studies, eight investigated cervical cancer [16, 22, 26, 29, 35, 50, 53, 54], five investigated breast cancer [15, 27, 36, 61], five investigated lung cancer [15, 39, 45, 49], five investigated colorectal cancer [23, 24, 33, 41, 59], four investigated gastric cancer [15, 24, 40, 62], three investigated melanoma [43, 48, 52], three investigated oral cancer [25, 38, 51] and three investigated lymphoma [37, 58, 60]. The other studies investigated esophageal cancer [15, 47], renal cell carcinoma [23], pancreatic cancer [8, 42], osteosarcoma [30], nasopharyngeal carcinoma [34], myeloma [46], leukemia [56, 57], head and neck cancer [44], glioma [31], gestational trophoblastic neoplasms [55], hepatocellular carcinoma [22, 32] and Ewing's Sarcoma [28]. When come to subjects in these studies, 28 were Asian [8, 15, 16, 22, 25-42] and 24 were Caucasian [23, 24, 43-62]. The genotype numbers of *CTLA-4* +49 A/G polymorphism are showed in **Table 2**. Results of the meta-analysis are listed in **Table 3**.

*Quantitative synthesis*

A total of 16,594 cancer cases and 20,283 controls from 52 original case-control studies in 44 publications were included for the present meta-analysis concerning the association between the *CTLA-4* +49 A/G polymorphism and cancer risk [8, 15, 16, 22-62]. Divided by ethnicity, 28 case-control studies focused on Asian subjects [8, 15, 16, 22, 25-42], and 24 case-control studies focused on Caucasian subjects [23, 24, 43-62]. After combining all eligible studies, there was statistical evidence of an association between the *CTLA-4* +49 A/G polymorphism and decreased overall cancer risk in four genetic models: GG+AG vs. AA (OR, 0.85; 95% CI, 0.76-0.95; *P* = 0.004), GG vs. AG+AA (OR, 0.85; 95% CI, 0.78-0.91; *P* = 0.000), GG vs. AA (OR, 0.76; 95% CI, 0.67-0.88; *P* = 0.000) and G vs. A (OR, 0.89; 95% CI, 0.84-0.95; *P* = 0.000) (**Table 3**; **Figures 2**, **3**). In a stratified analysis by cancer type, there was an decreased risk of breast cancer in four genetic models: GG+AG vs. AA (OR, 0.78; 95% CI, 0.66-0.92; *P* = 0.003), GG vs. AG+AA (OR, 0.78; 95% CI, 0.70-0.87; *P* = 0.000), GG vs. AA (OR, 0.63; 95% CI, 0.46-0.86; *P* = 0.004) and G vs. A (OR, 0.83; 95% CI, 0.77-0.89; *P* = 0.000), of lung

cancer in four genetic models: GG+AG vs. AA (OR, 0.74; 95% CI, 0.63-0.88; *P* = 0.000), GG vs. AG+AA (OR, 0.84; 95% CI, 0.75-0.93; *P* = 0.002), GG vs. AA (OR, 0.66; 95% CI, 0.54-0.79; *P* = 0.000) and G vs. A (OR, 0.84; 95% CI, 0.78-0.92; *P* = 0.000), and of other cancers in four genetic model: GG+AG vs. AA (OR, 0.73; 95% CI, 0.61-0.87; *P* = 0.001), GG vs. AG+AA (OR, 0.78; 95% CI, 0.70-0.88; *P* = 0.000), GG vs. AA (OR, 0.64; 95% CI, 0.53-0.78; *P* = 0.000), and G vs. A (OR, 0.82; 95% CI, 0.74-0.91; *P* = 0.000) (**Table 3**). In a stratified analysis by ethnicity, significant decreases in cancer risk were observed for Asians, but not Caucasians, for four genetic models: GG+AG vs. AA (OR, 0.76; 95% CI, 0.66-0.88; *P* = 0.000), GG vs. AG+AA (OR, 0.82; 95% CI, 0.75-0.89; *P* = 0.000), GG vs. AA (OR, 0.68; 95% CI, 0.58-0.80; *P* = 0.000) and G vs. A (OR, 0.85; 95% CI, 0.79-0.91; *P* = 0.000) (**Table 3**; **Figures 2**, **3**). A significant decrease in cancer risk was identified in epithelial in four genetic models: GG+AG vs. AA (OR, 0.86; 95% CI, 0.77-0.97; *P* = 0.012), GG vs. AG+AA (OR, 0.83; 95% CI, 0.76-0.91; *P* = 0.000), GG vs. AA (OR, 0.76; 95% CI, 0.65-0.89; *P* = 0.000) and G vs. A (OR, 0.89; 95% CI, 0.83-0.95; *P* = 0.000). In a stratified analysis by the system of cancer, a decreased risk of respiratory system cancer was noticed in four genetic models: GG+AG vs. AA (OR, 0.72; 95% CI, 0.62-0.84; *P* = 0.000), GG vs. AG+AA (OR, 0.83; 95% CI, 0.75-0.92; *P* = 0.000), GG vs. AA (OR, 0.64; 95% CI, 0.54-0.76; *P* = 0.000) and G vs. A (OR, 0.83; 95% CI, 0.77-0.90; *P* = 0.000), of reproductive and breast cancer in four genetic models: GG+AG vs. AA (OR, 0.84; 95% CI, 0.72-0.98; *P* = 0.026), GG vs. AG+AA (OR, 0.84; 95% CI, 0.74-0.95; *P* = 0.007), GG vs. AA (OR, 0.73; 95% CI, 0.58-0.92; *P* = 0.007) and G vs. A (OR, 0.88; 95% CI, 0.81-0.95; *P* = 0.002), of malignant bone tumor in four genetic models: GG+AG vs. AA (OR, 0.54; 95% CI, 0.36-0.81; *P* = 0.003), GG vs. AG+AA (OR, 0.70; 95% CI, 0.54-0.92; *P* = 0.010), GG vs. AA (OR, 0.47; 95% CI, 0.30-0.72; *P* = 0.001) and G vs. A (OR, 0.73; 95% CI, 0.61-0.88; *P* = 0.001), and of other cancers in two genetic model: GG+AG vs. AA (OR, 0.72; 95% CI, 0.56-0.92; *P* = 0.008) and GG vs. AA (OR, 0.67; 95% CI, 0.49-0.92; *P* = 0.013) (**Table 3**), but not digestive system cancer, hematopoietic malignancy and skin cancer. After the Bonferroni correction, our results showed statistical evidence of an association between the *CTLA-4* +49 A/G polymorphism and decreased risk of overall cancer in all the genetic comparison models (P < 0.05).
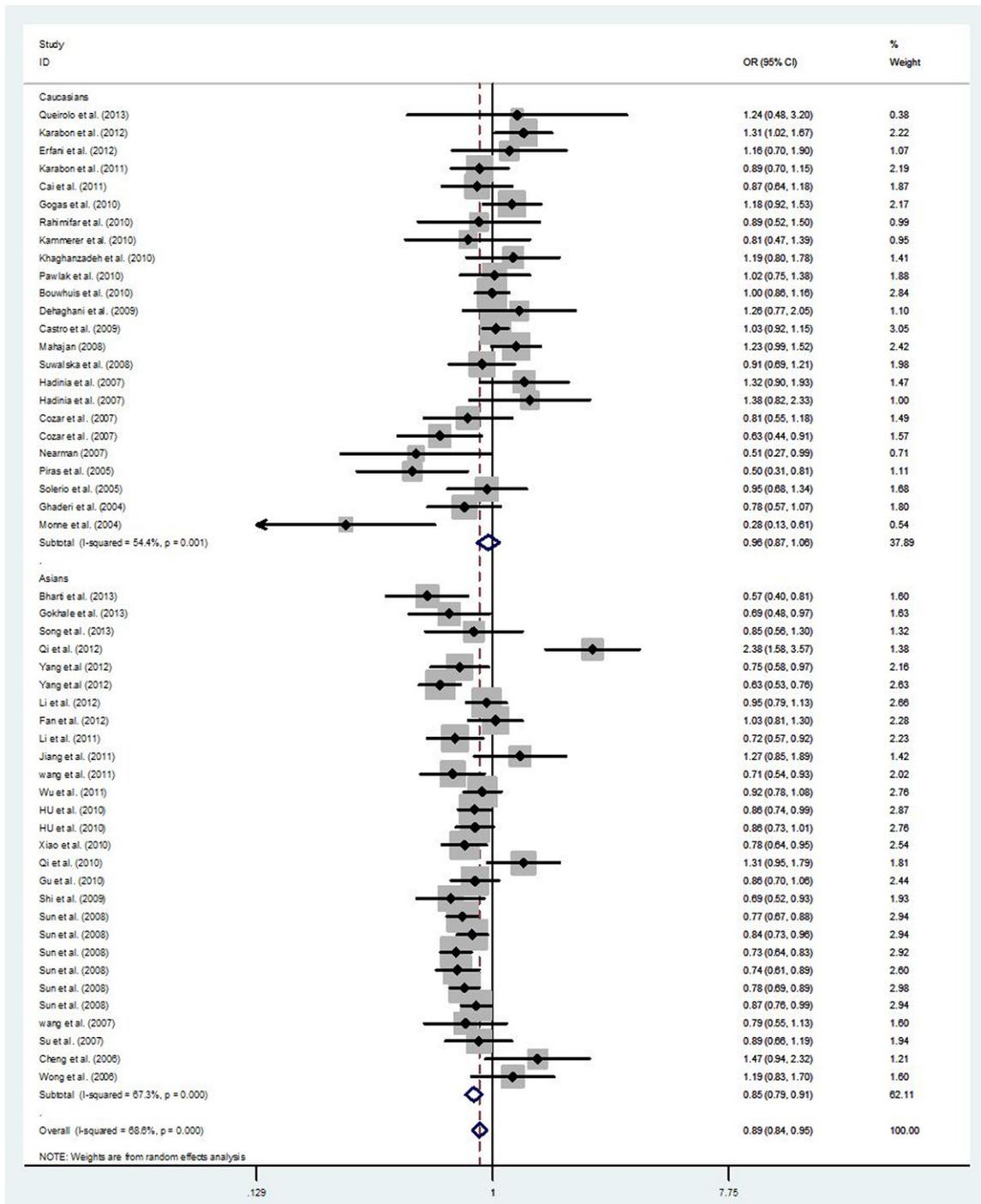
**Figure 2.** Meta-analysis with a random-effects model for the association between the risk of cancer and the *CTLA-4* +49 A/G polymorphism (G vs. A).

*Tests for publication bias, sensitivity analysis, and heterogeneity*

In this meta-analysis, the shape of funnel plot did not show the evidence of asymmetry in all the genetic models. In addition, the results of Egger's test indicated that there were no publication bias (GG+AG vs. AA: Begg's test *P* = 0.407, Egger's test *P* = 0.842; GG vs. AG+AA: Begg's test *P* = 0.734, Egger's test *P* = 0.939; GG vs. AA: Begg's test *P* = 0.390, Egger's test *P* = 0.665; G vs. A: Begg's test *P* = 0.398, Egger's
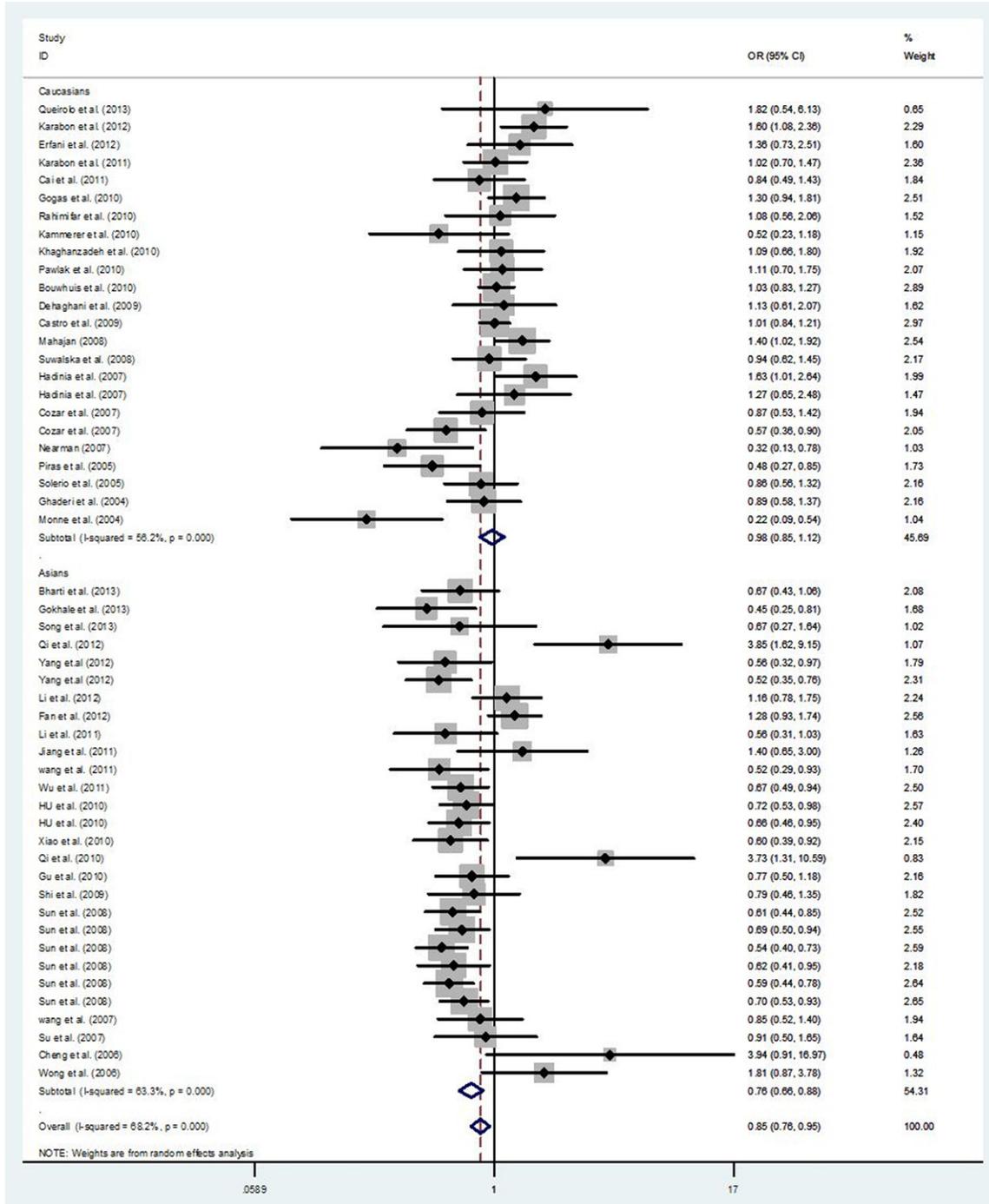
**Figure 3.** Meta-analysis with a random-effects model for the association between the risk of cancer and the *CTLA-4* +49 A/G polymorphism (GG+AG vs. AA).

test *P* = 0.356) (**Figure 4**). Sensitivity analyses were implemented to assess the influence of each individual dataset on the pooled OR by omitting one study at a time and consistent statistical significances of the results were observed across all the genetic comparisons, confirming that our results were robust enough (data not shown). Nonparametric "trim-and-fill" method was also applied to sensitivity analysis. The results showed that the findings of this meta-analysis were reliable (GG+AG vs. AA: adjusted pooled OR = 0.85, 95% CI: 0.76-0.94,
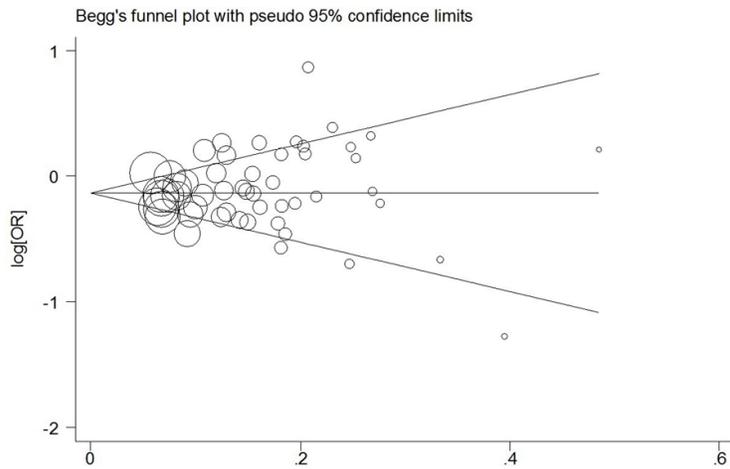
**Figure 4.** Begg's funnel plot of meta-analysis of between the *CTLA-4* +49 A/G polymorphism and the risk of cancer in the allele model (G vs. A).
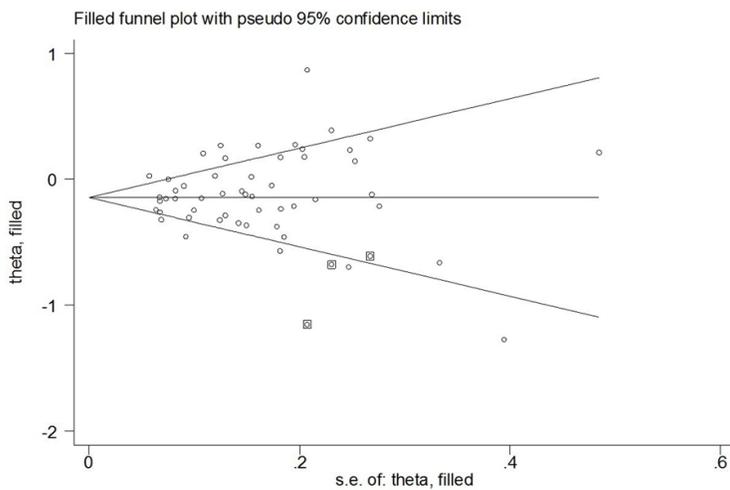


**Figure 5.** Filled funnel plot of meta-analysis of between the *CTLA-4* +49 A/G polymorphism and the risk of cancer in the allele model (G vs. A).
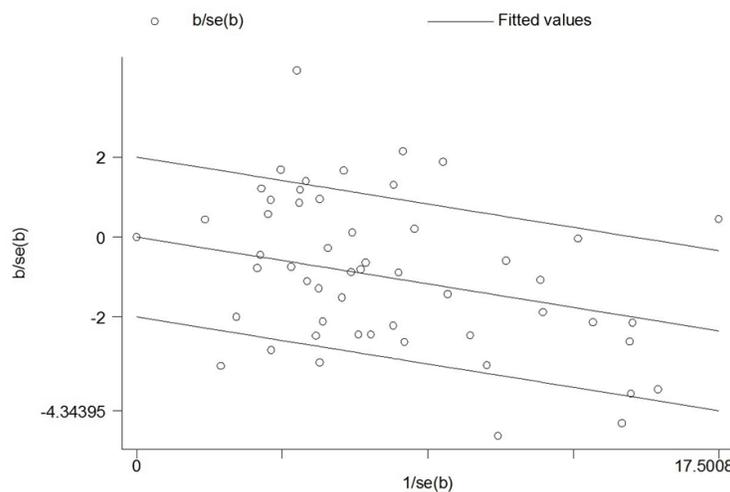


**Figure 6.** Galbraith radial plot of meta-analysis (G vs. A compare genetic model).

*P* = 0.003; GG vs. AG+AA: adjusted pooled OR = 0.85, 95% CI: 0.78-0.91, *P* = 0.000; GG vs. AA: adjusted pooled OR = 0.76, 95% CI: 0.67-0.88, *P* = 0.000; G vs. A: adjusted pooled OR = 0.87, 95% CI: 0.82-0.93, *P* = 0.000) (**Figure 5**). Since large heterogeneities among the studies were observed, we performed subgroup analyses by cancer type and ethnicity (**Table 3**). The results showed that cervical cancer, gastric cancer, lymphoma, oral cancer, other cancers and Asian population subgroup may contribute to the major heterogeneity. As shown in **Table 3**, heterogeneity was significant in allele comparison. Afterwards, Galbraith radial plot was conducted to analyze the source of heterogeneity and the results indicated that 12 outliers might contribute to the major source of heterogeneity [8, 15, 25, 33, 37, 40, 46, 52, 58, 60, 62] (**Figure 6**). Further stratified analyses were conducted by publication year, country (if one system cancer investigated by less than two individual studies, it was included into "other countries"), sample size and quality score of this meta-analysis and the results showed an association of studies published ≤ 2006 [37, 58, 60] or ≥ 2012 [8, 25, 40, 46], conducted in Chinese population [8, 15, 33, 37, 40], Italian population and high quality score studies [58, 60] with more prominent heterogeneity (**Table 3**).

## Discussion

*CTLA-4* gene is one of the most important immunoglobulin superfamily genes. Mutations in *CTLA-4* genes may alter predisposition to cancer [15]. So far, many previous investigations have focused on the association of *CTLA-4* +49 A/G polymorphism with cancer, but the results were inconsistent. In this meta-analysis, a total of 52 eligible and original case-control

studies, including 16,594 cancer cases and 20,283 controls, were recruited and analyzed the association. Our results suggested that the *CTLA-4* +49 A/G polymorphism was associated with a statistically decreased risk of cancer, especially for breast cancer, lung cancer, and other cancers, epithelial tumor, respiratory system cancer, reproductive and breast cancer, malignant bone tumor, and other system cancer subgroup such effect was still found in Asian populations. After the Bonferroni correction, in all the genetic comparison models, the results are still positive. CTLA-4 is a costimulatory molecule expressed mainly by activated T cells [63]. CTLA-4 acts as a crucial negative regulator of activated of T cells through reducing both interleukin (IL)-2 and IL-2 receptor productions, retarding T cells at the G1 phase in cell cycle or inducing Fas-independent apoptosis of activated T cells [64, 65] Targeting CTLA-4 in immunotherapy with a type of monoclonal antibodies (mAbs) was utilized as a therapeutic approach in metastatic melanoma and other tumors, with the aim to enhance antitumor T cell activation and expansion [66, 67]. Thus, CTLA-4 may be involved in carcinogenesis. Our results showed a decreased risk of overall cancer for carriers of the G allele. The *CTLA-4* +49 A/G polymorphism (A→G), a SNP in exon 1 of *CTLA-4*, causes a threonine (Thr) to alanine (Ala) substitution in the CTLA-4 receptor and guanine at this position is correlated with low expression of the CTLA-4 protein [11, 13]. A previous study conducted by Sun *et al.* has reported that the 49G allele reduces messenger RNA efficiency and attenuates CTLA-4 expression compared with the 49A allele, and individuals carrying the GG genotype may have higher T cell proliferation and activation than those with the AA genotype under the condition of suboptimal stimulation [11]. In this meta-analysis, our results show that the A→G change in *CTLA-4* may lead to increase of both IL-2 and IL-2 receptor productions, accelerating T cells at the G1 phase in cell cycle or restraining apoptosis of activated T cells and dramatic decline of the CTLA-4 expression, which may lower cancer risk. Since the results from meta-analysis can be affected by tumor type, subgroup analysis was performed according to various cancer types for the *CTLA-4* +49 A/G polymorphism. The results suggest that the *CTLA-4* +49 A/G polymorphism is associated with an decreased risk of lung cancer, breast

cancer and other cancers, but not cervical cancer, colorectal cancer, gastric cancer, lymphoma, melanoma and oral cancer (shown in **Table 3**). However, these results of the current meta-analysis should be interpreted with very caution. For lymphoma, melanoma, and oral cancer, in every subgroup, only three investigations were included for the analysis, which may weaken statistical power and therefore increase the uncertainty. More studies with large sample sizes are demanded to validate these results. When stratified by ethnics, we found the *CTLA-4* +49 A/G polymorphism is associated with the decreased risk of cancer in Asians but not Caucasians (shown in **Table 3**). This meta-analysis confirmed that ethnic mutation of genetic background would be modified by multiple environmental factors, such as age, sex, smoking, drinking status, diet, lifestyle and so on [68]. In the current analysis, the possible reason of the inconsistent results among different populations could be that different level exposure of genetic and environmental factors might have an impact on cancer risk. In the future, further studies with detailed environment data and a larger sample size are required to corroborate or refute these results. Furthermore, in a stratified analysis by the origin of cancer, a significant decrease in cancer risk was identified for epithelial tumor, but not non-epithelial tumor (shown in **Table 3**). Only 12 case-control studies were included in non-epithelial tumor subgroup and the sample size was small in most of these studies, which might restrict power to obtain a credible result. In the future, additional studies with large sample sizes should be carried out to confirm these results. Additionally, in a stratified analysis by the system of cancer, significant decreases in cancer risk were found for respiratory system cancer, reproductive and breast cancer, malignant bone tumor and other system cancers, but not digestive system cancer, hematopoietic malignancy and skin cancer (shown in **Table 3**). Considering only two studies with small sample size were conducted in malignant bone tumor, there is an indication of possible benefit, so further numerous studies are needed to achieve an accurate result. Two significant issues, heterogeneity and publication bias, should be further addressed in this study. Both Begg's Funnel plot and Egger's test were implemented to evaluate publication bias and no significant publication bias was detected, suggesting the

reliability of our findings. The relatively large heterogeneity was detected between publications recruited in this meta-analysis. Important sources of heterogeneity involve ethnicity, origin of cancer, system of cancer, country, the publication year, cancer type, sample size, quality score, and so on. When stratified by ethnics, origin of cancer, system of cancer and cancer type, this heterogeneity was effectively reduced or almost removed in some subgroups, suggesting different gene-environment factors effect on different populations and cancer types, even for the same polymorphism. And then, further subgroup analyses were conducted by publication year, country, quality score, and sample size. The pooled subgroup analysis of a subset of studies published ≤ 2006 or ≥ 2012, high quality studies, epithelial tumor, digestive system cancer, reproductive and breast cancer, hematopoietic malignancy, cervical cancer, gastric cancer, lymphoma, oral cancer, other cancers, Asian population, studies conducted in Chinese population and Italian population, suggested an association with more prominent heterogeneity. The reason might be attributed to multiple mixed environmental factors, design bias in the study or the different predisposition to cancer in different race. From the forest plot in allele genetic model (**Figure 2**), we can confirm that 12 studies are the main sources of heterogeneity. There are some drawbacks in several publications included in this meta-analysis, for instance, some investigations with small sample sizes (≤ 1000 subjects) [25, 33, 37, 40, 46, 52, 58, 60, 62]. Publication year may be another source of heterogeneity. Some investigations published ≤ 2006 [37, 58, 60] or ≥ 2012 [8, 25, 40, 46] were detected and went on to show significant heterogeneity. When come to country origins, studies conducted in Chinese populations [8, 15, 33, 37, 40] and Italian populations [58, 60], contribute the major outlier. In this meta-analysis, the power (α = 0.05) was measured using an internet-based power and sample size calculator (PS, version 3.0, 2009, http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize). The power was 1.000 in four genetic models (GG+AG vs. AA, GG vs. AG+AA, GG vs. AA and G vs. A). Here, we should mention the following. First, to date, this meta-analysis is the most comprehensive synthesis assessing the association of *CTLA-4* +49 A/G polymorphism with cancer risk.

Second, the shape of funnel plot showed no publication bias in the meta-analysis. Third, 52 case-control studies were considered for analysis; the sample size regarding *CTLA-4* +49 A/G polymorphism was large. Fourth, after the Bonferroni correction, for *CTLA-4* +49 A/G polymorphism, the association was confirmed in all the comparison models. Fifth, although there were several low quality studies (the quality score < 6) in this meta-analysis, we excluded or recruited them, the results were similar, suggesting that our results were stable (shown in **Table 3**). In addition, some limitations in our study should be acknowledged when interpreting our findings. First, all the case-control studies in current meta-analysis were from Asians and Caucasians; thus, these results might only fit for these two ethnicities. Second, only published investigations were recruited in this analysis; therefore, publication bias might have occurred ineluctably. Third, in some subgroups, the number of cases and controls was relatively small, which might have limited the statistical power. Fourth, due to the lack of uniform gene-environment interactions data for recruited studies, we failed to perform the further stratified analysis by other factors (e.g., age, sex, alcohol consumption, smoking, diet, and other lifestyle factors). Given the low-penetrance cancer susceptibility gene effects from SNP, the vital gene-environment factors should not be ignored. To sum up, this meta-analysis demonstrated that the *CTLA-4* +49 A/G polymorphism was a risk factor for cancer, especially in Asians, lung cancer, breast cancer, other cancers, epithelial tumor, respiratory system cancer, reproductive and breast cancer, malignant bone tumor, and other system cancer subgroup. Nevertheless, for practical reasons, more well-designed studies with functional evaluations should be carried out in order to further investigate the molecular mechanisms by which variations of *CTLA-4* gene modify cancer risk with detailed gene-gene and gene-environment interactions data.

### Acknowledgements

### Disclosure of conflict of interest

None.

## Abbreviations

CI, confidence interval; OR, odds ratio; CTLA-4, Cytotoxic T-lymphocyte-associated antigen 4; IL-2, interleukin-2; NK, natural killer; HWE, Hardy-Weinberg equilibrium; SNP, single nucleotide polymorphism.

**Address correspondence to:** Dr. Lixin Wang, Department of Microbiology and Immunology, Medical School of Southeast University, #87 Ding-Jia-Qiao Road, Nanjing 210009, Jiangsu Province, China. Tel: +86-25-83272503; Fax: +86-25-83324887; E-mail: lxwang@seu.edu.cn

## References

[1] Ihsan R, Chauhan PS, Mishra AK, Yadav DS, Kaushal M, Sharma JD, Zomawia E, Verma Y, Kapur S and Saxena S. Multiple analytical approaches reveal distinct gene-environment interactions in smokers and non smokers in lung cancer. PLoS One 2011; 6: e29431.

[2] Liu L, Wu C, Wang Y, Zhong R, Wang F, Zhang X, Duan S, Lou J, Yu D, Tan W, Yuan J, Wu T, Nie S, Miao X and Lin D. Association of candidate genetic variations with gastric cardia adenocarcinoma in Chinese population: a multiple interaction analysis. Carcinogenesis 2011; 32: 336-342.

[3] Andrew AS, Nelson HH, Kelsey KT, Moore JH, Meng AC, Casella DP, Tosteson TD, Schned AR and Karagas MR. Concordance of multiple analytical approaches demonstrates a complex relationship between DNA repair gene SNPs, smoking and bladder cancer susceptibility. Carcinogenesis 2006; 27: 1030-1037.

[4] Allin KH, Nordestgaard BG, Zacho J, Tybjaerg-Hansen A and Bojesen SE. C-reactive protein and the risk of cancer: a mendelian randomization study. J Natl Cancer Inst 2010; 102: 202-206.

[5] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.

[6] Chen L. Co-inhibitory molecules of the B7-CD28 family in the control of T-cell immunity. Nat Rev Immunol 2004; 4: 336-347.

[7] Yu F and Miao J. Significant association between cytotoxic T lymphocyte antigen 4 +49 G > A polymorphism and risk of malignant bone tumors. Tumour Biol 2013;

[8] 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.

[9] Tivol EA, Schweitzer AN and Sharpe AH. Co-stimulation and autoimmunity. Curr Opin Immunol 1996; 8: 822-830.

[10] Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA and Sharpe AH. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. Immunity 1995; 3: 541-547.

[11] Sun T, Hu Z, Shen H and Lin D. Genetic polymorphisms in cytotoxic T-lymphocyte antigen 4 and cancer: the dialectical nature of subtle human immune dysregulation. Cancer Res 2009; 69: 6011-6014.

[12] Leach DR, Krummel MF and Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. Science 1996; 271: 1734-1736.

[13] Ligers 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.

[14] 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.

[15] 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.

[16] 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.

[17] Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Ann Intern Med 2009; 151: 264-269, W264.

[18] 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.

[19] Qiu MT, Hu JW, Ding XX, Yang X, Zhang Z, Yin R and Xu L. Hsa-miR-499 rs3746444 polymor-

phism contributes to cancer risk: a meta-analysis of 12 studies. PLoS One 2012; 7: e50887.

[20] DerSimonian R and Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986; 7: 177-188.

[21] Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 1959; 22: 719-748.

[22] 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.

[23] 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.

[24] Hadinia A, Hossieni SV, Erfani N, Saberi-Firozi M, Fattahi MJ and Ghaderi A. CTLA-4 gene promoter and exon 1 polymorphisms in Iranian patients with gastric and colorectal cancers. J Gastroenterol Hepatol 2007; 22: 2283-2287.

[25] Bharti V, Mohanti BK and Das SN. Functional genetic variants of CTLA-4 and risk of tobacco-related oral carcinoma in high-risk North Indian population. Hum Immunol 2013; 74: 348-352.

[26] 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.

[27] Li D, Zhang Q, Xu F, Fu Z, Yuan W, Li D and Pang D. Association of CTLA-4 gene polymorphisms with sporadic breast cancer risk and clinical features in Han women of northeast China. Mol Cell Biochem 2012; 364: 283-290.

[28] 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.

[29] 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.

[30] Wang W, Wang J, Song H, Liu J, Song B and Cao X. Cytotoxic T-lymphocyte antigen-4 +49 G/A polymorphism is associated with increased risk of osteosarcoma. Genet Test Mol Biomarkers 2011; 15: 503-506.

[31] Wu Q, Zhan X, Dou T, Chen H, Fan W, Zhou K, Zhang H, Zheng H, Cai Y, Zhao Y, Huang F, Zhou L, Mao Y and Lu D. CTLA4 A49G polymorphism shows significant association with glioma risk

in a Chinese population. Biochem Genet 2011; 49: 190-201.

[32] Gu X, Qi P, Zhou F, Ji Q, Wang H, Dou T, Zhao Y and Gao C. +49 G > A polymorphism in the cytotoxic T-lymphocyte antigen-4 gene increases susceptibility to hepatitis B-related hepatocellular carcinoma in a male Chinese population. Hum Immunol 2010; 71: 83-87.

[33] Qi P, Ruan CP, Wang H, Zhou FG, Xu XY, Gu X, Zhao YP, Dou TH and Gao CF. CTLA-4 +49 A>G polymorphism is associated with the risk but not with the progression of colorectal cancer in Chinese. Int J Colorectal Dis 2010; 25: 39-45.

[34] Xiao M, Qi F, Chen X, Luo Z, Zhang L, Zheng C, Hu S, Jiang X, Zhou M and Tang J. Functional polymorphism of cytotoxic T-lymphocyte antigen 4 and nasopharyngeal carcinoma susceptibility in a Chinese population. Int J Immunogenet 2010; 37: 27-32.

[35] 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.

[36] 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.

[37] 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.

[38] Wong YK, Chang KW, Cheng CY and Liu CJ. Association of CTLA-4 gene polymorphism with oral squamous cell carcinoma. J Oral Pathol Med 2006; 35: 51-54.

[39] Song P. The Research on Blood Micrometastasis and CTLA-4 Gene Polymorphism for Patients with Non Small Cell. China: Shandong University; 2013. pp. 100.

[40] Qi Y, Si J, Cui J and Ji L. Polymorphism of CTLA-4 gene promoter region and extron region in gastric cancer. Chinese Clinical Oncolog 2012; 17: 828-831.

[41] Fan Z, Fu J, Chen T and Wang G. The Association Between CTLA 4 +49 A/G Polymorphism and Colorectal Cancer. Hei Long Jiang Medical Journal 2012; 36: 810-811.

[42] Shi H. The Screening for Pancreatic Cancer in Asymptomatic Community Populations Using The High Risk Scoring Model and The Preliminary Study of SNP Applications in High Risk Population. China: Peking Union Medical College; 2009. pp. 113.

[43] 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.

[44] Erfani N, Haghshenas MR, Hoseini MA, Hashemi SB, Khademi B and Ghaderi A. Strong Association of CTLA-4 Variation (CT60A/G) and CTLA-4 Haplotypes with Predisposition of Iranians to Head and Neck Cancer. Iran J Immunol 2012; 9: 188-198.

[45] 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.

[46] 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.

[47] Cai J. The screening value of immune-related gene and metabolic-related gene detection for Kazakh's esophageal cancer in xinjiang. China: Shi Hezhi University; 2011. pp. 53.

[48] 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.

[49] 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.

[50] Rahimifar S, Erfani N, Sarraf Z and Ghaderi A. ctla-4 gene variations may influence cervical cancer susceptibility. Gynecol Oncol 2010; 119: 136-139.

[51] Kammerer PW, Toyoshima T, Schoder F, Kammerer P, Kuhr K, Brieger J and Al-Nawas B. Association of T-cell regulatory gene polymorphisms with oral squamous cell carcinoma. Oral Oncol 2010; 46: 543-548.

[52] 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.

[53] 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.

[54] 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.

[55] Dehaghani AS, Kashef MA, Ghaemenia M, Sarraf Z, Khaghanzadeh N, Fattahi MJ and Ghaderi A. PDCD1, CTLA-4 and p53 gene polymorphism and susceptibility to gestational trophoblastic diseases. J Reprod Med 2009; 54: 25-31.

[56] 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.

[57] Nearman ZP, Wlodarski M, Jankowska AM, Howe E, Narvaez Y, Ball E and Maciejewski JP. Immunogenetic factors determining the evolution of T-cell large granular lymphocyte leukaemia and associated cytopenias. Br J Haematol 2007; 136: 237-248.

[58] Piras G, Monne M, Uras A, Palmas A, Murineddu M, Arru L, Bianchi A, Calvisi A, Curreli L, Gaviano E, Lai P, Murgia A, Latte GC, Noli A and Gabbas A. Genetic analysis of the 2q33 region containing CD28-CTLA4-ICOS genes: association with non-Hodgkin's lymphoma. Br J Haematol 2005; 129: 784-790.

[59] Solerio E, Tappero G, Iannace L, Matullo G, Ayoubi M, Parziale A, Cicilano M, Sansoe G, Framarin L, Vineis P and Rosina F. CTLA4 gene polymorphism in Italian patients with colorectal adenoma and cancer. Dig Liver Dis 2005; 37: 170-175.

[60] Monne M, Piras G, Palmas A, Arru L, Murineddu M, Latte G, Noli A and Gabbas A. Cytotoxic T-lymphocyte antigen-4 (CTLA-4) gene polymorphism and susceptibility to non-Hodgkin's lymphoma. Am J Hematol 2004; 76: 14-18.

[61] 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.

[62] Mahajan R, El-Omar EM, Lissowska J, Grillo P, Rabkin CS, Baccarelli A, Yeager M, Sobin LH, Zatonski W, Channock SJ, Chow WH and Hou L. Genetic variants in T helper cell type 1, 2 and

3 pathways and gastric cancer risk in a Polish population. Jpn J Clin Oncol 2008; 38: 626-633.

[63] Rudd CE and Schneider H. Unifying concepts in CD28, ICOS and CTLA4 co-receptor signalling. Nat Rev Immunol 2003; 3: 544-556.

[64] Appleman LJ, Berezovskaya A, Grass I and Boussiotis VA. CD28 costimulation mediates T cell expansion via IL-2-independent and IL-2-dependent regulation of cell cycle progression. J Immunol 2000; 164: 144-151.

[65] 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.

[66] Page DB, Yuan J and Wolchok JD. Targeting cytotoxic T-lymphocyte antigen 4 in immunotherapies for melanoma and other cancers. Immunotherapy 2010; 2: 367-379.

[67] Peggs KS, Quezada SA, Chambers CA, Korman AJ and Allison JP. Blockade of CTLA-4 on both effector and regulatory T cell compartments contributes to the antitumor activity of anti-CTLA-4 antibodies. J Exp Med 2009; 206: 1717-1725.

[68] Hirschhorn JN, Lohmueller K, Byrne E and Hirschhorn K. A comprehensive review of genetic association studies. Genet Med 2002; 4: 45-61.

**Table S1.** PRISMA checklist, checklist of items to include when reporting a systematic review or meta-analysis (diagnostic review consisting of cohort studies)

| Section/topic | # | Checklist item | Reported on section # |
|---|---|---|---|
| **TITLE** | | | |
| Title | 1 | Identify the report as a systematic review, meta-analysis, or both. | Title page |
| **ABSTRACT** | | | |
| Structured summary | 2 | Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number. | Abstract page |
| **INTRODUCTION** | | | |
| Rationale | 3 | Describe the rationale for the review in the context of what is already known. | Introduction section |
| Objectives | 4 | Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS). | Introduction section |
| **METHODS** | | | |
| Protocol and registration | 5 | Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number. | N/A |
| Eligibility criteria | 6 | Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale. | Materials and methods section, Inclusion and exclusion criteria |
| Information sources | 7 | Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched. | Materials and methods section, Search Strategy |
| Search | 8 | Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated. | Materials and methods section, Search Strategy |
| Study selection | 9 | State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis). | Materials and methods section, Data extraction |
| Data collection process | 10 | Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators. | Materials and methods section, Data extraction |
| Data items | 11 | List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made. | Materials and methods section, Data extraction |
| Risk of bias in individual studies | 12 | Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis. | Materials and methods section, Statistical analysis |
| Summary measures | 13 | State the principal summary measures (e.g., risk ratio, difference in means). | Materials and methods section, Statistical analysis |
| Synthesis of results | 14 | Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., $I^2$) for each meta-analysis. | Materials and methods section, Statistical analysis |
| Section/topic | # | Checklist item | Reported on page # |
| Risk of bias across studies | 15 | Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies). | Materials and methods section, Statistical analysis |
| Additional analyses | 16 | Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified. | Materials and methods section, Statistical analysis |

**RESULTS**

| Study selection | 17 | Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram. | **Figure 1** |
|---|---|---|---|
| Study characteristics | 18 | For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations. | **Table 1** |
| Risk of bias within studies | 19 | Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12). | Results section, Tests for publication bias, sensitivity analysis, and heterogeneity; **Figure 4** |
| Results of individual studies | 20 | For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot. | **Figures 2**, **3** |
| Synthesis of results | 21 | Present results of each meta-analysis done, including confidence intervals and measures of consistency. | Results section, Quantitative synthesis; **Table 3** |
| Risk of bias across studies | 22 | Present results of any assessment of risk of bias across studies (see Item 15). | Results section, Tests for publication bias, sensitivity analysis, and heterogeneity; **Figure 4** |
| Additional analysis | 23 | Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]). | Results section, Tests for publication bias, sensitivity analysis, and heterogeneity |

**DISCUSSION**

| Summary of evidence | 24 | Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers). | Discussion section |
|---|---|---|---|
| Limitations | 25 | Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias). | Discussion section |
| Conclusions | 26 | Provide a general interpretation of the results in the context of other evidence, and implications for future research. | Discussion section |

**FUNDING**

| Funding | 27 | Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review. | N/A |
|---|---|---|---|

2

**Table S2.** Scale for methodological quality assessment

| Criteria | Score |
|---|---|
| 1. Representativeness of cases | |
|     Selected from cancer registry or multiple cancer center sites | 2 |
|     Selected from oncology department or cancer institute | 1 |
|     Not described | 0 |
| 2. Source of controls | |
|     Population or community based | 2 |
|     Hospital-based cancer-free controls | 1.5 |
|     Healthy volunteers without total description | 1 |
|     Cancer-free controls with related diseases | 0.5 |
|     Not described | 0 |
| 3. Ascertainment of relevant cancer | |
|     Histopathologic confirmation | 2 |
|     Patient medical record | 1 |
|     Not described | 0 |
| 4. Sample size | |
|     $\geq 1000$ | 2 |
|     200-1000 | 1 |
|     < 200 | 0 |
| 5. Quality control of genotyping methods | |
|     Repetition of partial/total tested samples with a different method | 1 |
|     Repetition of partial/total tested samples with the same method | 0.5 |
|     Not described | 0 |
| 6. Hardy-Weinberg equilibrium (HWE) | |
|     Hardy-Weinberg equilibrium in control subjects | 1 |
|     Hardy-Weinberg disequilibrium in control subjects | 0 |