# Association of The Cytotoxic T Lymphocyte Antigen-4 +49G/A Gene Polymorphism with Susceptibility to Malignant Bone Tumors

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**Research article**

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# Abstract

**Background** Earlier research works have studied the part that cytotoxic T-lymphocyte antigen-4 (CTLA-4) plays in the carcinogenesis of malignant bone tumors, nonetheless, findings had inconsistency. The current investigation aims at clarifying the association between CTLA-4 polymorphism and malignant bone tumor susceptibility through the meta-analysis.

**Methods** We searched pertinent research works in not just PubMed, but also EMBASE, Cochrane library, and Chinese National Knowledge Infrastructure (CNKI) databases in humans published before October 2019. The use of the pooled odds ratio (OR) with its 95% confidence interval (95%CI) was made for estimating the strengths of the correlation existing between the CTLA-4 genetic polymorphism and malignant bone tumors susceptibility. An aggregate of six research works with 1191 malignant bone tumors patients and 1418 controls were selected eventually. The pooled results shed light on the fact that CTLA-4 +49G/A polymorphism had a significant correlation with an augmented vulnerability to the malignant bone tumors (A vs. G: OR=1.37, 95%CI=1.22-1.54; GA vs. GG: OR=1.20, 95%CI=1.01-1.42; AA vs. GG: OR=2.13, 95%CI=1.63-2.78; GA+AA vs. GG: OR=1.35, 95%CI=1.15-1.59; AA vs. GG+GA: OR=2.02, 95%CI=1.60-2.56). Subgroup analysis indicated that there exists a statistically significant correlation between CTLA-4 +49G/A polymorphism and augmented susceptibility to the malignant bone tumor in the population-based or hospital-based samples, and Ewing's sarcoma or osteosarcoma. Moreover, there was also not observed any considerable heterogeneity across the research works.

**Results** Our results suggest that the CTLA-4 +49G/A polymorphism may play a pivotal part in the carcinogenesis of malignant bone tumors.

**Conclusions** More research works, on the basis of the large sample sizes as well as homogeneous specimens, are needed in order to verify these results.


# Background

Malignant bone tumors refer to the rare human sarcomas, accounting for no more than 0.2% of all of the types of tumors[1]. Osteosarcoma, Ewing sarcoma, and chondrosarcoma are termed as the most common malignant bone tumors[2]. In the year 2019, as estimated, there were reports of 3500 fresh cases of cancer, besides 1660 deaths, in the US; osteosarcoma, being specific, is indicated as the third most common cancer faced in youth, besides being the 8th most common cancer in kids, in general [3–5]. Pain refers to the most commonly present symptom for the patients, who have bone tumors, nonetheless, delayed diagnosis is quite common and patients are mandatorily required to be termed as specialist centers for diagnosis as well as management. Despite considerable development in treating the tumors during the previous decade, the patients having malignant bone tumors prognosis continue to be unsatisfactory, the 5-year relative survival rate is almost 60%[1, 3]. Nevertheless, it is quite likely that there are gene-environment interactions in the carcinogenesis of the malignant bone tumors; also, the genetic susceptibility is also likely to make contribution to the variable individual to the malignant bone tumors susceptibility[6–8].

Cytotoxic T-lymphocyte antigen-4 (CTLA-4) refers to a co-inhibitory molecule that is expressed by the activated cytotoxic T cells, besides being a particular surface marker of Treg[9] as well. CTLA-4 gene has a location on chromosome 2q33, together with comprising 4 exons[10]. CTLA-4 is capable of competing with CD28 for binding the common ligands B7 with the larger affinities as compared with CD28 on the antigen-presenting cells[11,12]. By means of the blockage of the engaging CD28 on T cells, CTLA-4 has the potential of preventing the T-cell activation, accordingly lowering the degrees of the interferon-γ (IFN-γ), matrix metalloproteinases (MMPs), and interleukin-6 (IL-6)[13–15]. That is the reason that CTLA-4 quite critically contributes to regulating the T cell proliferation and activation, and the loss of CTLA-4 in natural Treg cells is likely to exert an impact on the immune response and occurrence of malignant bone tumors.

Even though previous research works have investigated the correlation existing between the + 49G/A of the CTLA-4 gene and the vulnerability to the malignant bone tumors, nevertheless, these findings from the published articles remained controversial as well as inconclusive. We make a hypothesis that these inconsistent findings are likely to have been a result of the limited discrete outcome and sample size. Accordingly, we implemented a meta-analysis of epidemiological research works with a larger sample size, aimed at investigating the correlation of CTLA-4 + 49G/A genetic polymorphisms with the susceptibility to the malignant bone tumors.

# Methods

### Search strategy

We carried out a search of the pertinent research works not just in PubMed, but also in EMBASE, Cochrane library, and Chinese National Knowledge Infrastructure (CNKI) databases in humans published before the month of October 2019. We carried out electronic searches with the use of the terms (“osteosarcoma” or “Ewing sarcoma” or “malignant bone tumor” or “bone sarcoma”), (“Cytotoxic T lymphocyte antigen protein 4” or “CTLA-4” or “rs231775” or “+49G/A) and (“polymorphism” or “variant” or “mutation”). Our analysis merely placed emphasis on the research works, which were written in Chinese and English.

### Inclusion and exclusion criteria

For the purpose of having the eligibility to include in the meta-analysis, a research work mandatorily requires meeting the criteria as hereunder: (i) evaluating the correlation existing between CTLA-4 genetic variants and malignant bone tumors susceptibility; (ii) case-control studies dealing with human beings; (iii) having an available genotype for the evaluation of the odds ratio (OR) with its 95% confidence interval (95%CI). On the other hand, case reports, letters, review articles, and duplication of published articles were not included.

### Data extraction

There were 2 scholars, who, in an independent manner, performed the careful extraction of the relevant data from all of the retrieved investigations. In addition, the extraction of the following data associated with all of the eligible studies was carried out, which included not just the first author name, but also the year of publication, country, ethnicity, sample size, kinds of cancer, the source of controls, and HWE status in controls.

Any disagreements existing between the two authors were dissolved through the consultation with a third scholar.

### Statistical analysis

Each analysis was conducted through the use of STATA15.1 (StataCorp, College Station, TX, USA). The use of the pooled odds ratio (OR), having its 95% confidence interval (95%CI) was made for the purpose of estimating the strengths of the correlation existing between CTLA-4 +49G/A genetic polymorphism and malignant bone tumors susceptibility. Moreover, the stratified analyses were conducted in accordance with the types of cancer and the source of controls. The evaluation of the heterogeneity of between-study was carried out by the chi-square-based Cochrane's *Q* test and $I^2$ statistics. The use of fixed-effects model was made in a case where there was mild heterogeneity among between-studies ($P > 0.1$, $I^2 < 50\%$)[16]; or else, the random-effects framework was put to use for pooling the data[17]. The sensitivity analysis was employed by omitting the sequentially individual study, aimed at evaluating the stability of these results. The latent publication bias was estimated by making use of Begg's funnel plot as well as Egger's test. A *P*-value below 0.05 was termed as statistical significance.

## Results

### Characteristics of studies

For the process of selection, as evident from **Figure 1**, an aggregate of 300 research works was retrieved on the preliminary literature search, associated with the correlation existing between CTLA-4 +49G/A genetic polymorphism and malignant bone tumors susceptibility. Subsequent to removing the duplicated articles, after the scanning of the titles as well as the abstracts of these research works, with an aggregate of 50 research works remained. Thereafter, we carried out the evaluation of the full-text, together with excluding the ineligible studies; also, 6 case-control research works, having an aggregate of 2609 participants, were counted on in the present meta-analysis[6, 18-22]. The key features of the included research works are demonstrated in **Table 1**.

### Association of CTLA-4 +49G/A polymorphism with malignant bone tumor risk

6 research works, having an aggregate of 1191 patients as well as 1418 controls, were counted on in the present meta-analysis in order to evaluate the correlation of the CTLA-4 +49G/A genetic polymorphisms with the susceptibility to malignant bone tumors. In addition, any considerable heterogeneity was not observed to exist between these research works ($I^2=0$, $P = 0.996$), indicating that these studies were homogeneous, and thereby the use of the fixed-effects model was made for pooling the data (**Figure 5**). The cumulated findings shed light on the fact that the CTLA-4 +49G/A polymorphism had a significant correlation with an augmented susceptibility to malignant bone tumors subjected to all of the genetic contrast models (A vs. G: OR=1.37, 95%CI=1.22-1.54, $P < 0.001$; GA vs. GG: OR=1.20, 95%CI=1.01-1.42, $P = 0.037$; AA vs. GG: OR=2.13, 95%CI=1.63-2.78, $P < 0.001$; GA+AA vs. GG: OR=1.35, 95%CI=1.15-1.59, $P < 0.001$; AA vs. GG+GA: OR=2.02, 95%CI=1.60-2.56, $P < 0.001$) (**Figure 2** and **Table 2**).

In the course of the evaluation of the impact of the CTLA-4 +49G/A polymorphism by source of controls, we discovered significant correlation in both of the population-based populations (A vs. G: OR=1.42, 95%CI=1.16-1.74, *P* = 0.001; AA vs. GG: OR=1.94, 95%CI=1.22-3.08, *P* = 0.005; AA vs. GG+GA: OR=2.02, 95%CI=1.39-2.93, *P* < 0.001) and hospital-based populations (A vs. G: OR=1.35, 95%CI=1.17-1.55, *P* < 0.001; AA vs. GG: OR=2.23, 95%CI=1.61-3.09, *P* < 0.001; GA+AA vs. GG: OR=1.33, 95%CI=1.09-1.61, *P* = 0.004; AA vs. GG+GA: OR=2.03, 95%CI=1.50-2.75, *P* < 0.001). besides that, no considerable heterogeneity was observed to exist between these research works ($I^2$=0%, *P* = 0.955) (**Figure 5**), so, the use of the fixed-effects model was made for pooling the data (**Figure 3** and **Table 2**).

For the purpose of further exploring the correlation of the CTLA-4 +49G/A genetic polymorphisms with the susceptibility to the malignant bone tumors, we carried out the subgroup analysis in accordance with the type of cancer. With regard to osteosarcoma, there were four research works that had an aggregate of 660 patients as well as 754 controls, and no significant heterogeneity between these studies was found ($I^2$=0%, *P* = 0.960) (**Figure 5**). That was why the use of the fixed-effects model was made for pooling the data. The cumulated findings indicate that the CTLA-4 +49G/A polymorphism had a significant correlation with an augmented susceptibility to osteosarcoma (A vs. G: OR=1.38, 95%CI=1.18-1.61, *P* < 0.001; AA vs. GG: OR=2.04, 95%CI=1.42-2.93, *P* < 0.001; GA+AA vs. GG: OR=1.34, 95%CI=1.07-1.68, *P* = 0.012; AA vs. GG+GA: OR=2.04, 95%CI=1.50-2.77, *P* < 0.001). With regard to Ewing's sarcoma, the pooled results also shed light on the fact that the CTLA-4 +49G/A polymorphism had a considerable correlation with an increased the susceptibility to Ewing's sarcoma (**Figure 4** and **Table 2**).

### Publication bias and sensitivity analysis

As evident from **Figure 6**, the funnel plots appeared as approximately symmetrical, and the findings from Egger's test also extended support to the symmetry. The results suggested that the possible publication bias could be excluded. The sensitivity analysis suggested that our results were statistically robust as well as credible while omitting the studies one following the other.

## Discussion

CTLA-4 refers to an inhibitory molecule, which is expressed on the activated T cells, besides critically contributing to the balance existing between the pro- and anti-immune responses by downregulating the T cell signaling[23]. It has been reported that CTLA-4 overexpressed had a correlation with the poor prognosis in autoimmune diseases as well as the specific kinds of cancer, including osteosarcoma and Ewing's sarcoma[24–27]; however, the latent part played by CTLA-4 polymorphism in malignant bone tumors patients continue to be unclear. There are a number of research works that have investigated the correlation existing between the + 49G/A of the CTLA-4 gene and the susceptibility to malignant bone tumors, nevertheless, these results from published articles remained not just controversial but inconclusive as well. Wang et al., in an investigation, carried out in China, shed light on the fact that the CTLA-4 + 49AA genotype had a significant correlation with the augmented susceptibility to osteosarcoma (OR = 2.27, 95%CI = 1.21–4.25, *P* = 0.010) as compared with the ones carrying the CTLA-4 + 49GG genotype[20]. In the year 2012, Yang et al. demonstrated the fact that the CTLA-4 + 49AA genotype had a 2.03-fold increased to the susceptibility to Ewing's sarcoma[19]. Bilbao-Aldaiturriaga et al. discovered that the AG + GG genotype might be a safeguarding impact to

osteosarcoma susceptibility[6]. Here upon, we therefore carried out the current meta-analysis for the purpose of investigating the more precise correlation existing between the + 49G/A of the CTLA-4 gene and the susceptibility to the malignant bone tumors.

In the current research work, six research works, having an aggregate of 1191 patients as well as 1418 controls, were counted on in the present meta-analysis in order to evaluate the correlation of the CTLA-4 + 49G/A genetic polymorphisms with the susceptibility to the malignant bone tumors. The pooled results highlighted the fact that the CTLA-4 + 49G/A polymorphism had a significant correlation with the augmented susceptibility to the malignant bone tumors in the population overall. The stratification by the source of controls, an increased the susceptibility to malignant bone tumors was found in not just the population-based but also hospital-based population. In addition, the stratification analysis by cancer types indicated that the CTLA-4 + 49G/A polymorphism had a significant correlation with the augmented susceptibility to osteosarcoma as well as Ewing's sarcoma. Owing to the fact that only one research work evaluated the patients among non-Asian population, we accordingly failed in making use of the further meta-analysis stratified by ethnicity.

A number of limitations exist in the present research work. At first, only one study performed the evaluation of the patients among the non-Asian population, and more populations from diverse ethnicities are required in the prospective research. Secondly, a select bias might have occurred since only studies, which were published in the English or Chinese language, were counted on in the present study, so some of the relevant articles in other languages were published in the specific journals were overlooked. Thirdly, the impacts exerted by the other pertinent parts, for instance, age, in addition to gender, life-style and their contacts with CTLA-4 + 49G/A polymorphism on the malignant bone tumors occurrence were not analyzed for the lacking original data.

# Conclusions

In conclusion, the findings of our investigation indicate that the CTLA-4 + 49G/A polymorphism is likely to significantly contributes to the carcinogenesis of malignant bone tumors. Nevertheless, for the above-mentioned limitations, our conclusions are required to be verified by the large-scale multi-center studies on the basis of the multiple ethnic groups. Moreover, the further large-scale multi-center and well-conducted investigations are required for the purpose of verifying the correlation existing between the age, gender, life-style, and malignant bone tumors susceptibility.

# List Of Abbreviations

CTLA-4, cytotoxic T-lymphocyte antigen-4; CNKI, Chinese National Knowledge Infrastructure; OR, odds ratio; 95%CI, 95% confidence interval; IFN-γ, interferon-γ; MMPs, matrix metalloproteinases; IL-6, interleukin-6.

# Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Availability of data and materials

Not applicable.

### Competing Interests

The authors declare there is no conflicts of interest.


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### Authors' contributions

NF contributed to the study design and wrote the article. YJ conducted the literature search, required the data, performed data analysis and drafted. YM revised the article and gave the final approval of the version to be submitted.


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# Tables

Table 1
Main characteristics of studies included in the meta-analysis

| Author | Year | Country | Ethnicity | Cancer type | Source of Control | Sample size | | HWE |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Case | Control | |
| Bilbao-Aldaiturriaga | 2017 | Spain | non-Asian | osteosarcoma | PB | 66 | 125 | 0.9718 |
| Qiao | 2016 | China | Asian | osteosarcoma | PB | 122 | 131 | 0.7986 |
| Liu | 2011 | China | Asian | osteosarcoma | HB | 267 | 282 | 0.8957 |
| Wang | 2011 | China | Asian | osteosarcoma | PB | 205 | 216 | 0.1302 |
| Yang | 2012 | China | Asian | ewing's sarcoma | HB | 223 | 302 | 0.3256 |
| Feng | 2013 | China | Asian | ewing's sarcoma | HB | 308 | 362 | 0.7902 |

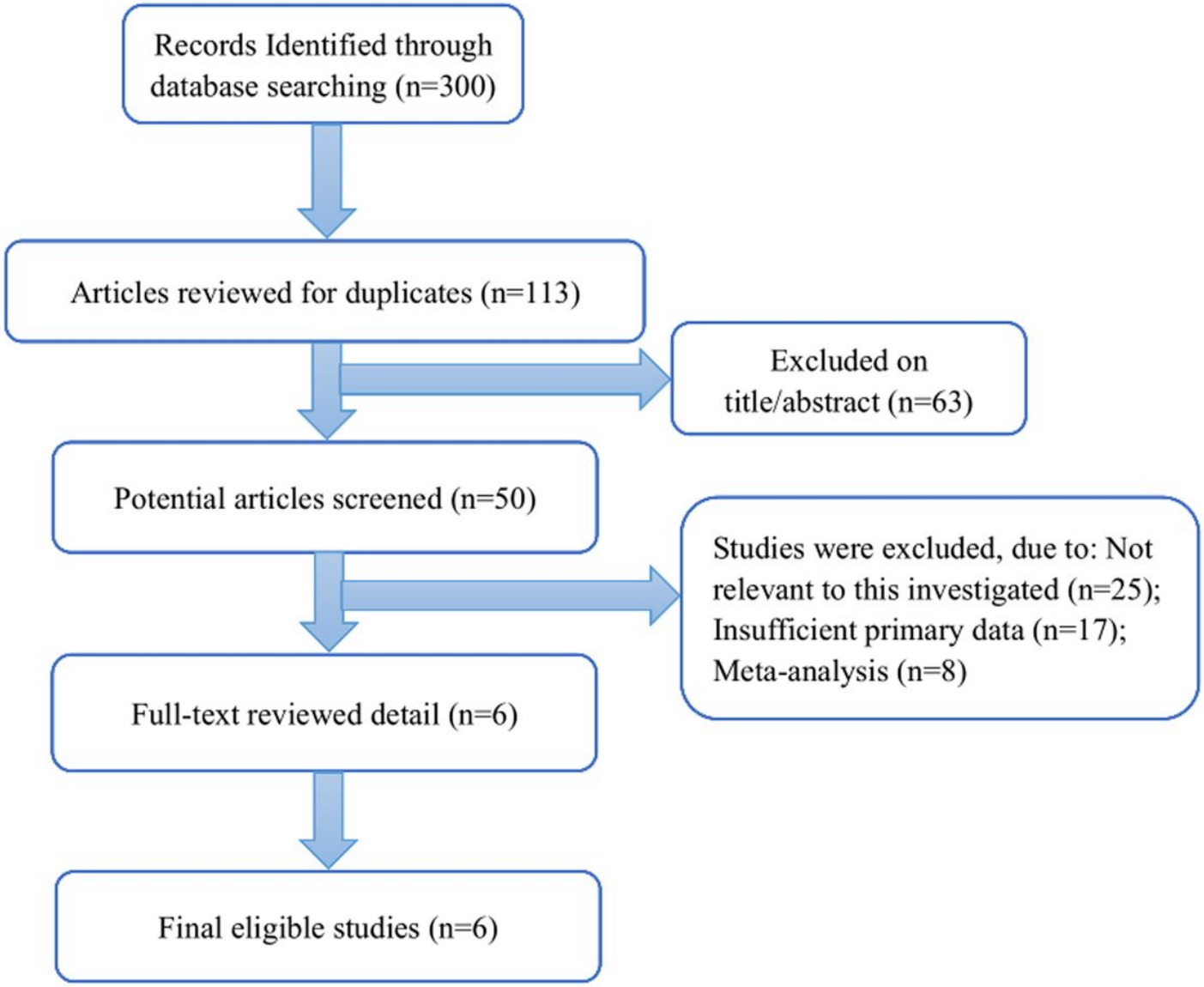
**HWE, Hardy-Weinberg equilibrium; PB, Population-based; HB, hospital-based**

Table 2
Meta-analysis of the association between CTLA-4 + 49G/A polymorphism and malignant bone tumor susceptibility.

| Variable | Subgroup | Heterogeneity test | | Association test | | Model | Publication bias |
|---|---|---|---|---|---|---|---|
| | | p-Value | $I^2$(%) | OR (95%CI) | p-Value | | Egger |
| A vs. G | Overall | 0.996 | 0 | 1.37(1.22–1.54) | < 0.001 | F | 0.431 |
| | osteosarcoma | 0.96 | 0 | 1.38(1.18–1.61) | < 0.001 | F | |
| | Ewing's | 0.825 | 0 | 1.36(1.15–1.61) | < 0.001 | F | |
| | PB | 0.961 | 0 | 1.42(1.16–1.74) | 0.001 | F | |
| | HB | 0.955 | 0 | 1.35(1.17–1.55) | < 0.001 | F | |
| GA vs. GG | Overall | 0.301 | 17.50% | 1.20(1.01–1.42) | 0.037 | F | 0.134 |
| | osteosarcoma | 0.113 | 49.70% | 1.18(0.93–1.50) | 0.164 | F | |
| | Ewing's | 0.793 | 0 | 1.22(0.95–1.55) | 0.12 | F | |
| | PB | 0.057 | 65.20% | 1.25(0.91–1.71) | 0.174 | R | |
| | HB | 0.886 | 0 | 1.18(0.96–1.45) | 0.11 | R | |
| AA vs. GG | Overall | 0.687 | 0 | 2.13(1.63–2.78) | < 0.001 | F | 0.26 |
| | osteosarcoma | 0.426 | 0 | 2.04(1.42–2.93) | < 0.001 | F | |
| | Ewing's | 0.666 | 0 | 2.24(1.51–3.31) | < 0.001 | F | |
| | PB | 0.262 | 25.40% | 1.94(1.22–3.08) | 0.005 | F | |
| | HB | 0.91 | 0 | 2.23(1.61–3.09) | < 0.001 | F | |
| GA + AA vs. GG | Overall | 0.507 | 0 | 1.35(1.15–1.59) | < 0.001 | F | 0.142 |
| | osteosarcoma | 0.233 | 29.90% | 1.34(1.07–1.68) | 0.012 | F | |

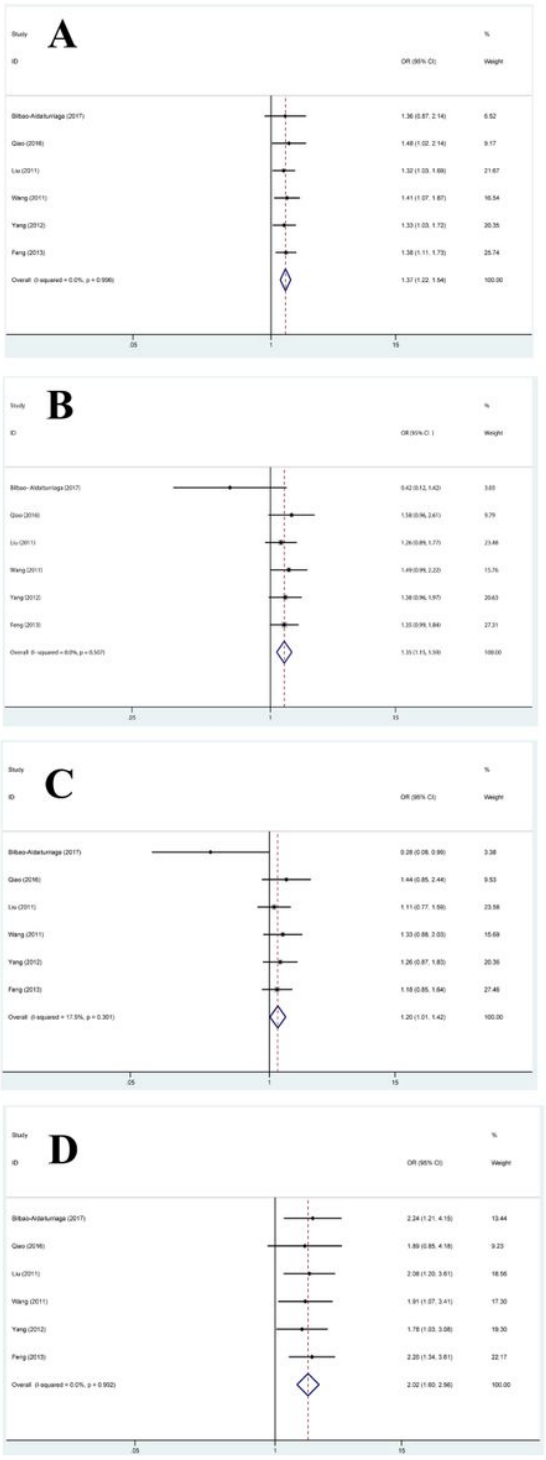
| Variable | Subgroup | Heterogeneity test | | Association test | | Model | Publication bias |
|---|---|---|---|---|---|---|---|
| | | p-Value | $I^2$(%) | OR (95%CI) | p-Value | | Egger |
| | Ewing's | 0.932 | 0 | 1.36(1.07–1.72) | 0.011 | F | |
| | PB | 0.132 | 50.70% | 1.29(0.79–2.11) | 0.311 | R | |
| | HB | 0.93 | 0 | 1.33(1.09–1.61) | 0.004 | R | |
| AA vs. GG + GA | Overall | 0.992 | 0 | 2.02(1.60–2.56) | < 0.001 | F | 0.598 |
| | osteosarcoma | 0.981 | 0 | 2.04(1.50–2.77) | < 0.001 | F | |
| | Ewing's | 0.571 | 0 | 2.01(1.39–2.89) | < 0.001 | F | |
| | PB | 0.919 | 0 | 2.02(1.39–2.93) | < 0.001 | F | |
| | HB | 0.846 | 0 | 2.03(1.50–2.75) | < 0.001 | F | |
| OR, odds ratio; CI, confidence interval; F, fixed-effects model; R, random-effects model | | | | | | | |

# Figures



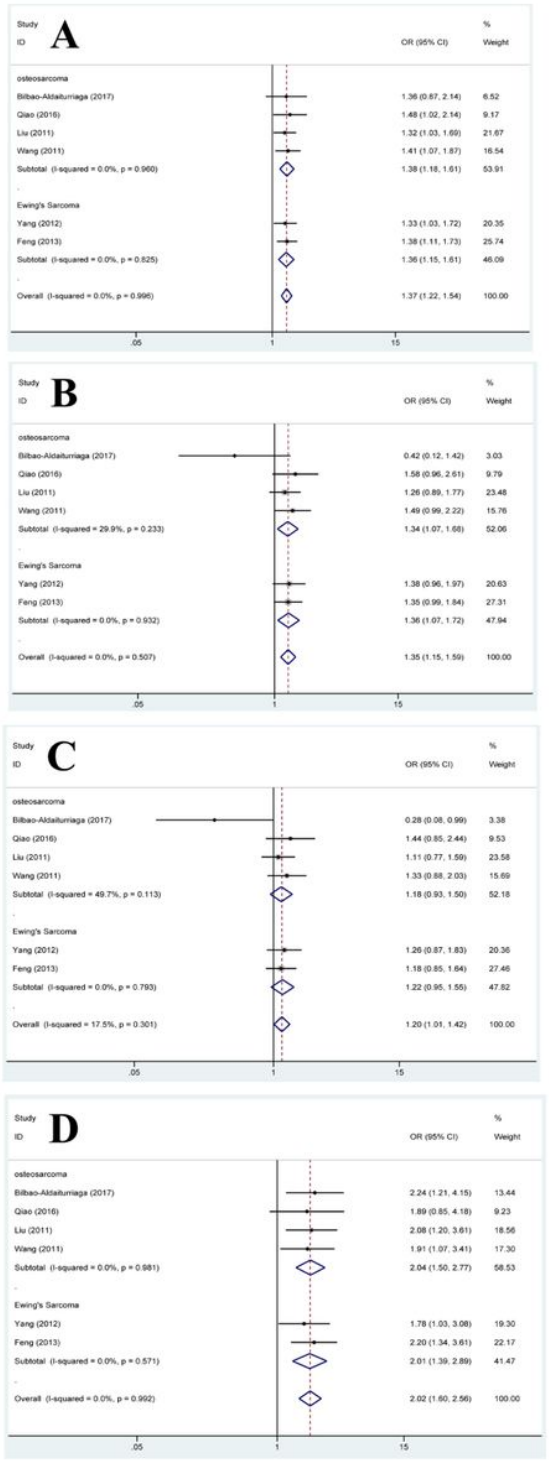

## Figure 1

Flow chart of study selection in this meta-analysis.



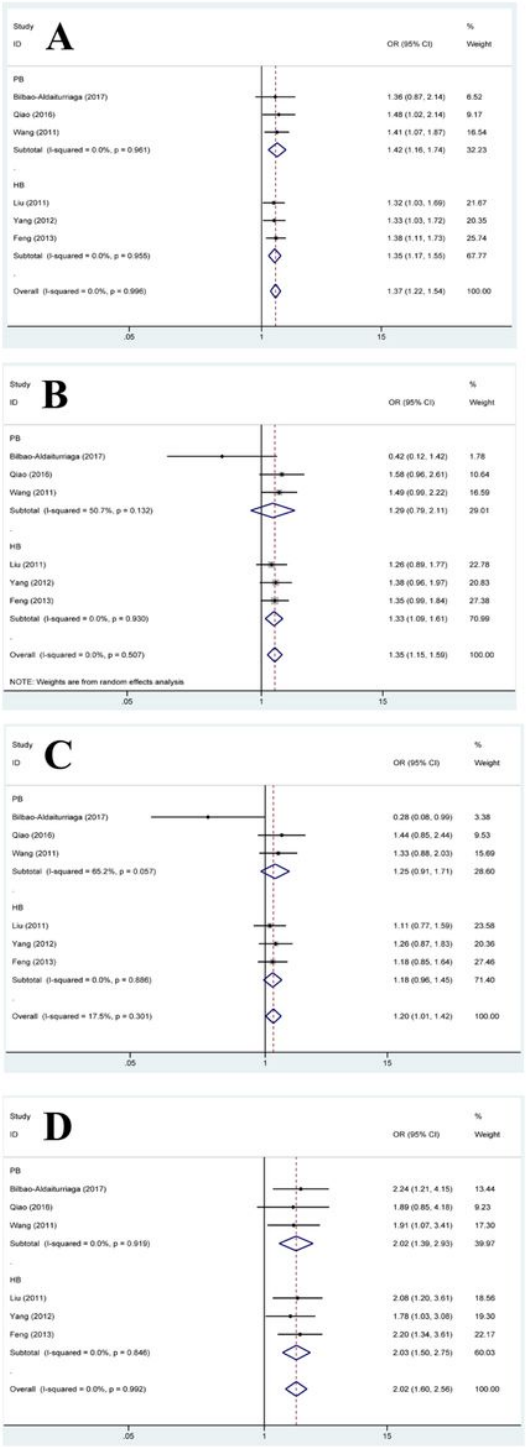

## Figure 2

Forest plot of the association between CTLA-4 +49G/A polymorphism and malignant bone tumor susceptibility in the overall populations. (A) allelic model; (B) dominant model; (C) heterozygous model and (D) recessive model.



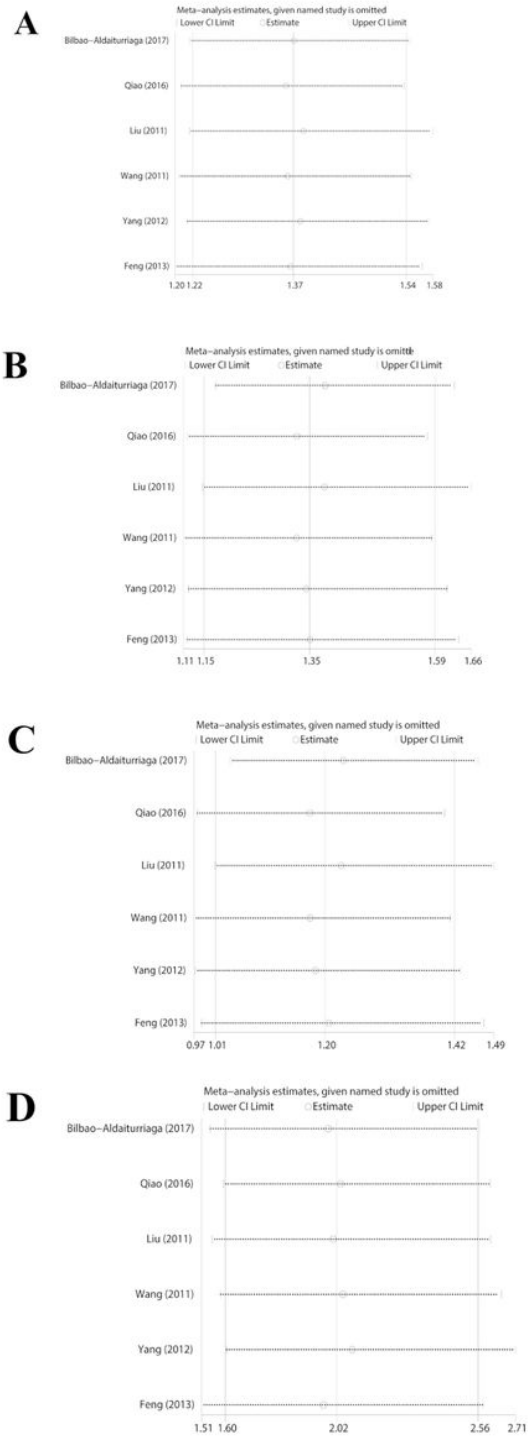

## Figure 3

Forest plot of the association between CTLA-4 +49G/A polymorphism and malignant bone tumor susceptibility stratified by cancer type. (A) allelic model; (B) dominant model; (C) heterozygous model and (D) recessive model.



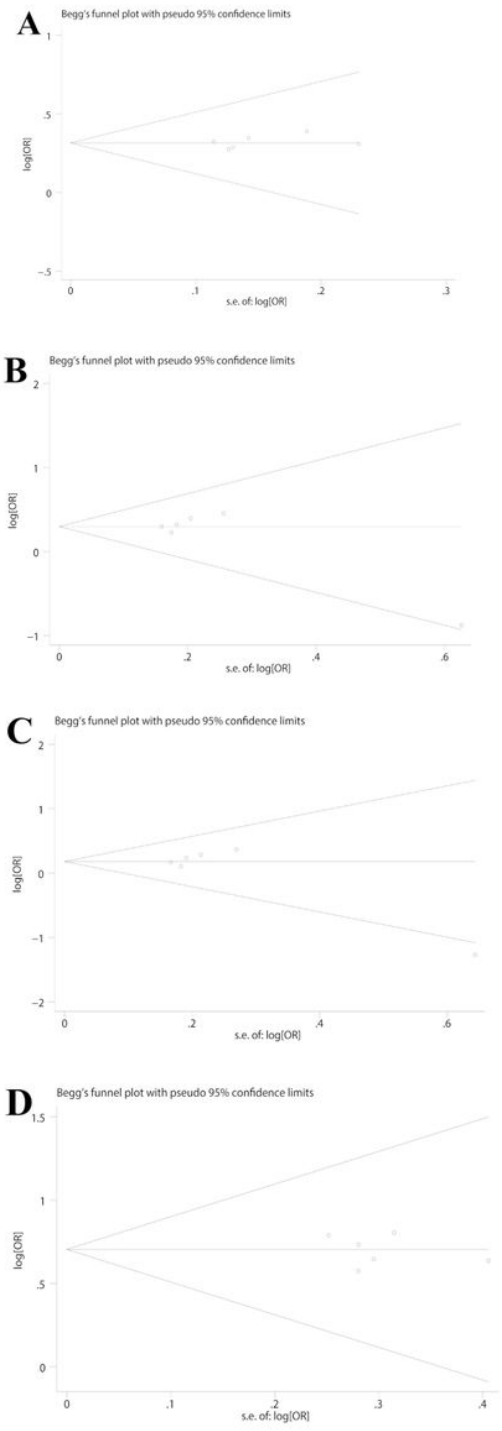

## Figure 4

Forest plot of the association between CTLA-4 +49G/A polymorphism and malignant bone tumor susceptibility stratified by sources of control. (A) allelic model; (B) dominant model; (C) heterozygous model and (D) recessive model.




## Figure 5

Sensitivity analysis of studies on CTLA-4 +49G/A genetic polymorphism and malignant bone tumor susceptibility: (A) allelic model; (B) dominant model; (C) heterozygous model and (D) recessive model.




## Figure 6

Begg's funnel plots of publication bias for the association between CTLA-4 +49G/A genetic polymorphism and malignant bone tumor: (A) allelic model; (B) dominant model; (C) heterozygous model and (D) recessive model.