RESEARCH ARTICLE

Association of Cytotoxic T-lymphocyte Antigen-4 Polymorphisms with Malignant Bone Tumors Risk: A Meta-analysis

Chao Zhang, Wei-Hua Hou, Xuan-Xi Ding, Xiong Wang, Hui Zhao, Xing-Wen Han, Wen-Ji Wang*

Abstract

Background: Previous studies have assessed the association between the Cytotoxic T-lymphocyte Antigen-4(CTLA-4) polymorphism with the risk of malignant bone tumor, but the conclusions were inconsistent. We aimed to clarify association of cytotoxic T-lymphocyte antigen-4 polymorphisms with malignant bone tumors risk by performing a meta-analysis. Materials and Methods: The databases including PubMed, EMBase databases and the Cochrane Library were searched to identify the eligible studies prior to January 30 2016. Odds ratio (OR) with 95% confidence interval (95%CI) were used to estimate the strengths of the association between the CTLA-4 polymorphism and the malignant bone tumor risks. The meta-analysis was performed by STATA 12.0. Results: Four individual studies with a total of 1003 cases with malignant bone tumor and 1162 controls were included in our meta-analysis. The results of meta-analysis on those data demonstrated that CTLA-4 +49G>A polymorphism was associated with the risk of Ewing's sarcoma and osteosarcoma strongly (A *vs.* G: OR=1.36, 95%CI:1.20-1.54, *p*=0.000; AA+AG *vs.* GG: OR=1.35, 95%CI:1.14-1.61, *p*=0.001; AA *vs.* GG: OR=2.24, 95%CI:1.67-2.99, *p*=0.000; AA *vs.* AG+GG: OR=2.00, 95%CI:1.53-2.62, *p*=0.000), but CTLA-4 -318C/T polymorphism was not associated with the risk of malignant bone tumor (C *vs.* T: OR=0.76, 95%CI:0.76-1.08, *p*= 0.262; CC+CT *vs.* TT: OR=0.70, 95%CI:0.41-1.20, *p*= 0.198; CC *vs.* TT: OR=0.69, 95%CI:0.40-1.19, *p*= 0.183; CC *vs.* CT+TT: OR=0.92, 95%CI:0.75-1.13, *p*= 0.419). Subgroup analysis showed that there are significantly positive correlations between CTLA-4 +49G>A polymorphism and increased risks of malignant bone tumors in large size of sample (A *vs.* G: OR=1.347, 95%CI: 1.172,1.548, *p*=0.000; AA *vs.* GG: OR=2.228, 95%CI: 1.608,3.085, *p*=0.000), Ewing's Sarcoma or Osteosarcoma (A *vs.* G: OR=1.361, 95%CI: 1.201,1.540, *p*=0.000; AA *vs.* GG: OR=2.236, 95%CI: 1.674,2.986, *p*=0.000), and PCR-RFLP or Sequencing(A *vs.* G: OR=1.361, 95%CI: 1.201,1.540, *p*=0.000; AA *vs.* GG: OR=2.236, 95%CI: 1.674,2.986, *p*=0.000), but CTLA-4 -318C/T polymorphism was not associated with the risk of malignant bone tumors in diagnosis, genotype method, and sample size (all *p*>0.05). Conclusions: CTLA-4 +49A/G variant was associated with an increased risk of developing the malignant bone tumors, such as Ewing's sarcoma and osteosarcoma. However, it failed to show the association between CTLA-4 -318C/T polymorphism and the risk of malignant bone tumors. Future large-scale studies remain to be done to confirm our conclusions.

Keywords: Cytotoxic T-lymphocyte antigen-4 (CTLA-4) - Osteosarcoma - Ewing's sarcoma - Meta-analysis

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Introduction

Osteosarcoma and Ewing's sarcoma are the two most common primary malignant bone tumors in children, adolescents and young adults, which cause serious damage to human health (Balamuth et al., 2010). Although osteosarcoma and Ewing's sarcoma are orphan and rare disease, they have an overall incidence of 0.88-11/100 000 per year in the age group 15-19years (Bielack et al., 2009). Estimates of its cancer mortality are commonly presented as 5-year overall survival from the time of initial diagnosis, while metastatic disease carried a poor prognosis (Kager et al., 2003; Miller et al., 2013). The exact mechanisms and molecular pathogenesis of Osteosarcoma and Ewing's sarcoma are not well understood (Gil et al., 2015). However, the etiology of Osteosarcoma and Ewing's sarcoma is still indistinct. Accumulating researches suggests environmental factors, genetic components and gene-environment interactions may play an important role in the development and progression of osteosarcoma and Ewing's sarcoma.

Cytotoxic T-lymphocyte antigen 4 (CTLA-4) is one of the most vital members of the immunoglobulin superfamily, which is a restraining regulator of T-cell activation and proliferation. CTLA-4 gene is located on chromosome 2q33 and is one of candidate genes

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for influencing the risk of several diseases, including Osteosarcoma and Ewing's sarcoma (Alfadhli S et al., 2013; Hao Q 2013). CTLA-4 consists of four exons that encode separate functional domains (leader sequence, extracellular domain, transmembrane domain, cytoplasmic domain) (Hironori et al., 2003). Recently, several studies have showed that CTLA-4 +49G/A (rs231775), -1661A/G (rs4553808), -318C/T (rs5742909), and CT60A/G (rs3087243) polymorphisms are associated with different cancers (Sun T et al., 2008; Zhang Y et al., 2011), but the relationship between them on osteosarcoma and Ewing's sarcoma is not clear. Therefore, in present study, we aim to investigate the associations of CTLA-4 +49G/A, -318C/T SNPs with osteosarcoma and Ewing's sarcoma in Chinese population.

Materials and Methods

Search Strategy

To identify all publications relevant to the association between CTLA-4 polymorphisms malignant bone tumors, our 2 investigators performed the comprehensive literature search by using the PubMed, EMBase databases and the Cochrane Library (last search was updated on January 30 2016), with the following search strategy: CTLA-4 [All Fields] AND ("polymorphism, genetic"[MeSH Terms] OR ("polymorphism" [All Fields] AND "genetic" [All Fields]) OR "genetic polymorphism" [All Fields] OR "polymorphism" [All Fields]) AND ("osteosarcoma"[MeSH Terms] OR "osteosarcoma"[All Fields] OR "sarcoma, ewing"[MeSH Terms] OR "ewing sarcoma"[All Fields] OR "ewing's sarcoma"[All Fields] OR "malignant bone tumors" [All Fields]). A further manual search of extra eligible studies cited in published article was also carried out. Our research was limited to English-language article, but not to time period.

Study Selection

(1) Inclusion Criteria: The identified studies may met following criteria: 1) the aim of studies was on the association of CTLA-4 polymorphisms (+49G/A, -1661A/G, -318C/T, and CT60A/G) between osteosarcoma and Ewing's sarcoma; 2) the type of article was case-control study and reported the frequencies of genotype; 3) inclusion of sufficient data to perform meta-analysis; 4) can access the full-text articles on publications.

(2)Exclusion Criteria: The studies meet following criteria was excluded: 1) the other SNPs about assessing the association between CTLA-4 and malignant bone tumors; 2) review articles, case reports, abstracts, editorials, letters and meta-analysis; 3) the article without sufficient data to analysis after contacting study authors.

Data Extraction

Two investigators performed the data extraction independently, and asking the third author to resolve the disagreements if there was a different results. Information from each study including following criteria: the first author, the year of publication, country, ethnicity, sources of controls, diagnosis, the number of cases and controls, genotype method, weather controls meet the Hardy-Weinberg Equilibrium (HWE), and allele frequencies.

Quality Assessment

The qualities of included studies were assessed by modified Newcastle-Ottawa Scale (NOS) (Lo et al., 2014): (1) Representativeness of the exposed cohort; (2) Selection of the non-exposed cohort; (3) Ascertainment of exposure; (4) Demonstration that outcome of interest was not present at start of study; (5) Comparability of cohorts on the basis of the design or analysis; (6) Assessment of outcome; (7) Was follow-up long enough for outcomes to occur? (8) Adequacy of follow up of cohorts. Interpretation of the NOS is performed by awarding points or "stars" for high-quality elements. In the end, the stars were added up and used to compare the quality of study in a quantitative manner.

Statistical analysis

The strength of association between each CTLA-4 polymorphism (-318 C/T and +49 G/A) and risk of osteosarcoma and ewing's sarcoma was assessed by calculating the pooled OR and 95%CI in different genetic models, including allele model (C *vs*. T for -318 C/T and G *vs*. A for +49 G/A), a dominant model (CC+CT *vs*. TT for -318 C/T and GG+GA *vs*. AA for +49 G/A), a co-dominant model (CC *vs*. TT for -318 C/T and GG *vs*. AA for +49 G/A), a recessive model (CC *vs*. CT+TT for -318 C/T and GG *vs*. GA+AA for +49 G/A). The HWE was recalculated in meta-analysis according to the principle of HWE (Rachel et al., 2014): if *p*-value was more than 0.05, the study was considered to meet the HWE.

Heterogeneity assumption was evaluated with a χ^2-based Q-test: if the *p* value was more than 0.1 or I^2 was less than 50%, it demonstrated that all included studies were lack of heterogeneity, thus the Mantel-Haenszel method (fixed effect model) was used to merge the studies, or else the random effect model was adopted. Subgroup analyses were performed for different ethnicity, diagnosis, genotype method et al. In addition, potential publication bias was diagnosed and measured by Begg's test and Funnel plots.

Results

Characteristics of eligible studies

According to the search strategy, a total of 167 studies were identified by searching PubMed, EMBase and the Cochrane Library. Following the inclusion and exclusion criteria, 4 case-control studies (Yang et al., 2011; Wang W et al., 2011; Yang S et al., 2012; Feng et al., 2016) that consisted of 1003 malignant bone tumors patients and 1162 control subjects were included in this meta-analysis. The detail information of flow chart of study selection process was showed in Figure1. All of included studies were conducted in China and all of them met the HWE (all $p>0.05$). In these included studies, two of which are about Ewing's Sarcoma and the other of which are about the Osteosarcoma. The main characteristics of eligible studies were listed in Table1 and Table2. The NOS score of 4 included studies was range from 7 two 8, the mean score was 7.50±0.58.

Meta-analysis results

Association between CTLA-4 +49A/G polymorphism and Ewing's Sarcoma and Osteosarcoma risk

Allele model (A *vs*. G): For CTLA-4 +49A/G allele model (A *vs*. G), 4 case-control studies (Feng et al., 2016; Liu et al., 2011; Wang et al., 2011; Yang et al., 2012) were analyzed in the current meta-analysis. There was no between-study heterogeneity among those four studies in allele model (A *vs*. G) (I^2=0.00%, *p*=0.98). The pooled results showed that CTLA-4 +49A/G A allele was significantly associated with Ewing's Sarcoma and Osteosarcoma risk (OR=1.36, 95%CI:1.20-1.54, *p*=0.000). (Figure2)

Dominant model (AA+AG *vs*. GG): For CTLA-4 +49A/G dominant model (AA+AG *vs*. GG), 4 case-control studies (Feng et al., 2016; Liu et al., 2011; Wang et al., 2011; Yang et al., 2012) were analyzed in the current meta-analysis. There was no between-study heterogeneity among those four studies in dominant model (AA+AG *vs*. GG) (I^2=0.00%, *p*=0.94). The pooled results showed that CTLA-4 +49A/G dominant model was significantly associated with Ewing's Sarcoma and Osteosarcoma risk (OR=1.35, 95%CI:1.14-1.61, *p*=0.001) (Figure3).

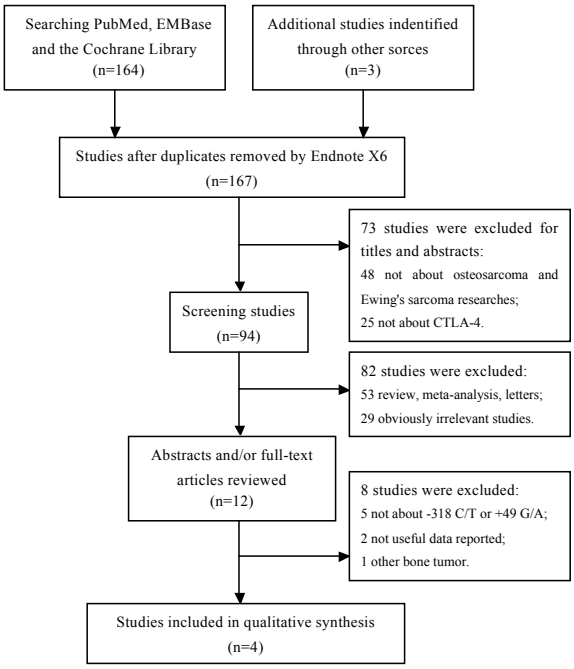

Figure 1. Flow chart of study selection process

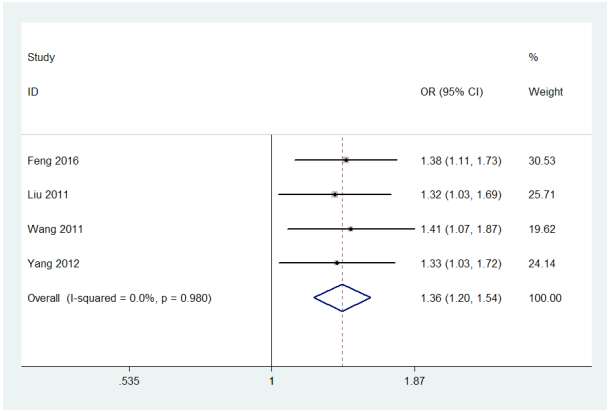

Figure 2. Meta-analysis of the association between CTLA-4 +49G>A polymorphism and the risk of malignant bone tumors (fixed-effects model, A allele versus G allele)

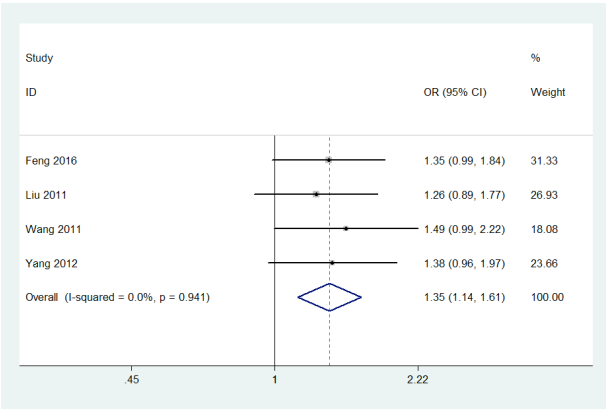

Figure 3. Meta-analysis of the association between CTLA-4 +49G>A polymorphism and the risk of malignant bone tumors (fixed-effects model, AA+AG *vs*. GG)

Table 1. Characteristics of Eligible Studies

Study ID	Country	Ethnicity	Sources of controls	Diagnosis	No. of cases	No. of controls	HWE	Genotype method
Feng 2016	China	ASian (Han Chinese)	HCC	Ewing's Sarcoma	308	362	Yes	PCR-RFLP
Liu 2011	China	ASian (Han Chinese)	HCC	Osteosarcoma	267	282	Yes	Sequencing
Wang 2011	China	ASian (Han Chinese)	HCC	Osteosarcoma	205	216	Yes	Sequencing
Yang 2012	China	ASian (Han Chinese)	HCC	Ewing's Sarcoma	223	302	Yes	PCR-RFLP

HCC, hospital-based case–control study; HWE : hardy-weinberg equilibrium

Table 2. Clinical Characteristics of Included Studies

Study ID	Age(n)				Mean age(Year)		Gender(n)				Tumor location (n)
	No. of cases		No. of controls		case	control	No. of cases		No. of controls		Long tubular bones
	≤20	>20	≤20	>20			Male	Female	Male	Female	
Feng 2016	227	81	271	91	NR		189	119	226	136	122
Liu 2011	187	80	181	101	25.1±18.2	29.4±17.7	172	95	156	126	213
Wang 2011	132	73	116	100	27.1±17.7	32.4±18.2	119	86	113	103	152
Yang 2012	169	54	221	81	NR		140	83	193	109	99

NR, not reported

Co-dominant model (AA *vs*. GG): For CTLA-4 +49A/G co-dominant model (AA *vs*. GG), 4 case-control studies (Liu et al., 2011; Wang et al., 2011; Yang et al., 2012; Feng et al., 2016) were analyzed in the current meta-analysis. There was no between-study heterogeneity among those four studies in co-dominant model (AA *vs*. GG) (I^2=0.00%, p=0.98). The pooled results showed that CTLA-4 +49A/G co-dominant model was significantly associated with Ewing's Sarcoma and Osteosarcoma risk (OR=2.24, 95%CI:1.67-2.99, p=0.000) (Figure4).

Recessive model (AA *vs*. AG+GG): For CTLA-4 +49A/G recessive model (AA *vs*. AG+GG), 4 case-control studies (Feng D et al., 2016; Liu Y et al., 2011; Wang W et al., 2011; Yang S et al., 2012) were analyzed in the current meta-analysis. There was no between-study heterogeneity among those four studies in recessive model (AA *vs*. AG+GG) (I^2=0.00%, p=0.95). The pooled results showed that CTLA-4 +49A/G recessive model was significantly associated with Ewing's Sarcoma and Osteosarcoma risk (OR=2.00, 95%CI:1.53-2.62, p=0.000) (Figure5).

Association between CTLA-4 -318C/T polymorphism and Ewing's Sarcoma and Osteosarcoma risk

Allele model (C *vs*. T): For CTLA-4 -318C/T allele model (C *vs*. T), 3 case-control studies (Liu et al., 2011; Yang et al., 2012; Feng et al., 2016) were analyzed in the current meta-analysis. There was no between-study heterogeneity among those 3 studies in allele model (C *vs*. T) (I^2=0.00%, p=0.40). The pooled results showed that CTLA-4 -318C/T C allele was not significantly associated with Ewing's Sarcoma and Osteosarcoma risk (OR=0.76, 95%CI:0.76-1.08, p= 0.262) (Figure 6).

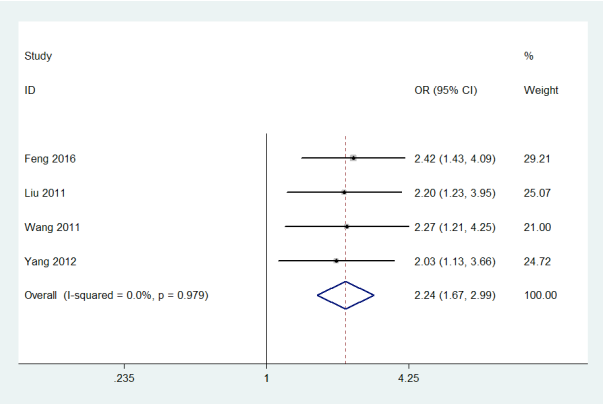

Figure 4. Meta-analysis of the association between CTLA-4 +49G>A polymorphism and the risk of malignant bone tumors (fixed-effects model, AA *vs*. GG)

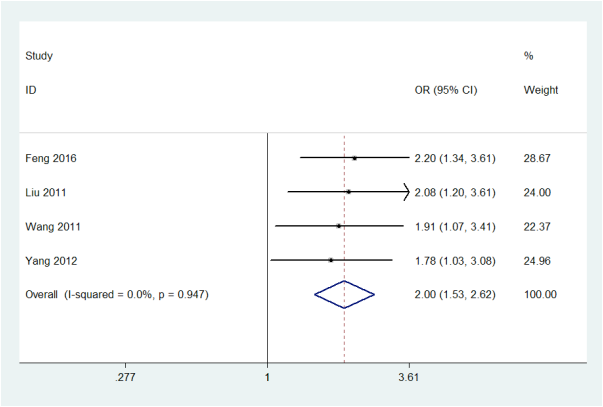

Figure 5. Meta-analysis of the association between CTLA-4 +49G>A polymorphism and the risk of malignant bone tumors (fixed-effects model, AA *vs*. AG+GG)

Table 3. Meta-Analysis of the Association Between CTLA-4 +49G>A Polymorphism and Risk of Malignant Bone Tumors

Subgroup		No. of studies	A vs. G			AA vs. GG			AA+AG vs. GG			AA vs. AG+GG		
			OR	95%CI	p value	OR	95%CI	p value	OR	95%CI	p value	OR	95%CI	p value
Sample size	n>500	3	1.347	1.172,1.548	0.000	2.228	1.608,3.085	0.000	1.404	1.155,1.706	0.001	1.268	0.930,1.727	0.133
	n≤500	1	1.414	1.070,1.868	0.015	2.266	1.207,4.254	0.011	1.359	0.913,2.025	0.131	1.265	0.703,2.276	0.433
	Overall	4	1.36	1.201,1.540	0.000	2.236	1.674,2.986	0.000	1.395	1.171,1.662	0.000	1.267	0.964,1.666	0.090
Diagnosis	Ewing's Sarcoma	2	1.361	1.150,1.610	0.000	2.239	1.512,3.314	0.000	1.507	1.188,1.911	0.001	1.254	0.865,1.819	0.232
	Osteosarcoma	2	1.359	1.130,1.636	0.001	2.232	1.454,3.426	0.000	1.273	0.982,1.649	0.068	1.282	0.855,1.921	0.229
	Overall	4	1.361	1.201,1.540	0.000	2.236	1.674,2.986	0.000	1.395	1.17,1.662	0.000	1.267	0.964,1.666	0.090
Genotype method	PCR-RFLP	2	1.361	1.150,1.610	0.000	2.239	1.512,3.314	0.000	1.507	1.188,1.911	0.001	1.254	0.865,1.819	0.232
	Sequencing	2	1.359	1.130,1.636	0.001	2.232	1.454,3.426	0.000	1.273	0.982,1.649	0.068	1.282	0.855,1.921	0.229
	Overall	4	1.361	1.201,1.540	0.000	2.236	1.674,2.986	0.000	1.395	1.17,1.662	0.000	1.267	0.964,1.666	0.090

Table 4. Meta-Analysis of the Association Between CTLA-4 -318C/T Polymorphism and Risk of Malignant Bone Tumors

Subgroup		No. of studies	C vs. T			CC vs. TT			CC+CT vs. TT			CC vs. CT+TT		
			OR	95%CI	p value	OR	95%CI	p value	OR	95%CI	p value	OR	95%CI	p value
Sample size	n>500	2	0.925	0.750,1.141	0.466	0.758	0.397,1.445	0.400	0.767	0.404,1.457	0.417	0.936	0.735,1.192	0.594
	n≤500	1	0.857	0.622,1.182	0.348	0.552	0.201,1.515	0.249	0.564	0.207,1.539	0.264	0.882	0.609,1.279	0.508
	Overall	3	0.904	0.759,1.078	0.262	0.691	0.401,1.190	0.183	0.702	0.409,1.204	0.198	0.921	0.751,1.126	0.419
Diagnosis	Ewing's Sarcoma	2	0.105	0.790,1.405	0.721	0.941	0.469,1.886	0.863	0.953	0.477,1.903	0.891	0.956	0.747,1.222	0.718
	Osteosarcoma	1	0.826	0.662,1.031	0.090	0.419	0.167,1.051	0.064	0.428	0.172,1.066	0.068	0.849	0.594,1.213	0.368
	Overall	3	0.904	0.759,1.078	0.262	0.691	0.401,1.190	0.183	0.702	0.409,1.204	0.198	0.921	0.751,1.126	0.419
Genotype method	PCR-RFLP	1	0.105	0.790,1.405	0.721	1.568	0.570,4.312	0.383	1.577	0.577,4.316	0.375	0.108	0.733,1.413	0.918
	Sequencing	2	0.826	0.662,1.031	0.090	0.472	0.240,0.930	0.031	0.483	0.246,0.946	0.034	0.865	0.668,1.118	0.268
	Overall	3	0.904	0.759,1.078	0.262	0.691	0.401,1.190	0.183	0.702	0.409,1.204	0.198	0.921	0.751,1.126	0.419

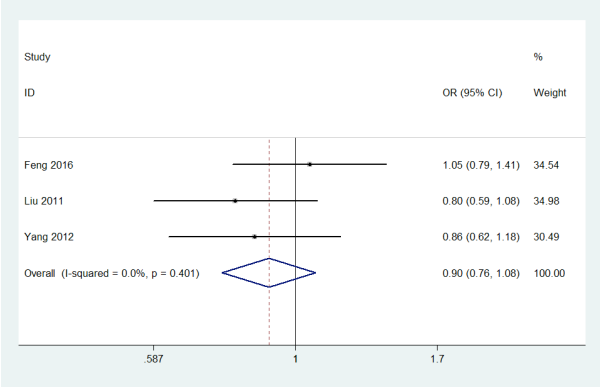

Figure 6. Meta-analysis of the association between CTLA-4 -318C/T polymorphism and the risk of malignant bone tumors (fixed-effects model, C *vs*. T)

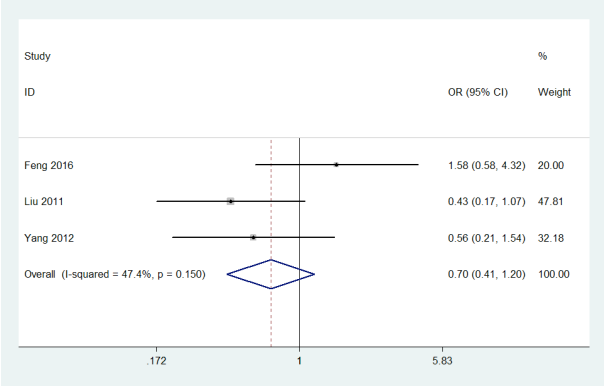

Figure 7. Meta-analysis of the association between CTLA-4 -318C/T polymorphism and the risk of malignant bone tumors (fixed-effects model, CC+CT *vs*. TT)

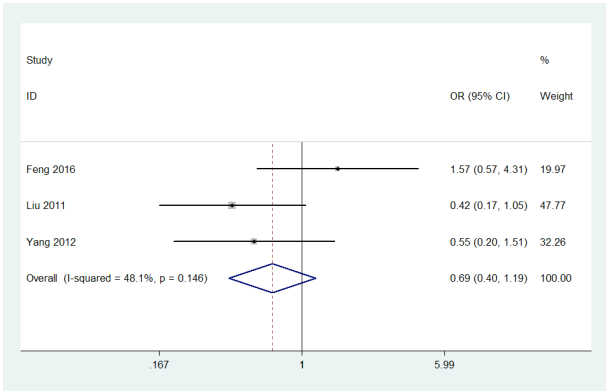

Figure 8. Meta-analysis of the association between CTLA-4 -318C/T polymorphism and the risk of malignant bone tumors (fixed-effects model, CC *vs*. TT)

Dominant model (CC+CT *vs*. TT): For CTLA-4 -318C/T dominant model (CC+CT *vs*. TT), 3 case-control studies (Feng D et al., 2016; Liu Y et al., 2011; Yang S et al., 2012) were analyzed in the current meta-analysis. There was no between-study heterogeneity among those 3 studies in dominant model (CC+CT *vs*. TT) (I^2=47.4%, p=0.15). The pooled results showed that CTLA-4 -318C/T dominant model was not significantly associated with Ewing's Sarcoma and Osteosarcoma risk (OR=0.70, 95%CI:0.41-1.20, p= 0.198). (Figure 7)

Figure 9. Meta-analysis of the association between CTLA-4 -318C/T polymorphism and the risk of malignant bone tumors (fixed-effects model, CC *vs*. CT+TT)

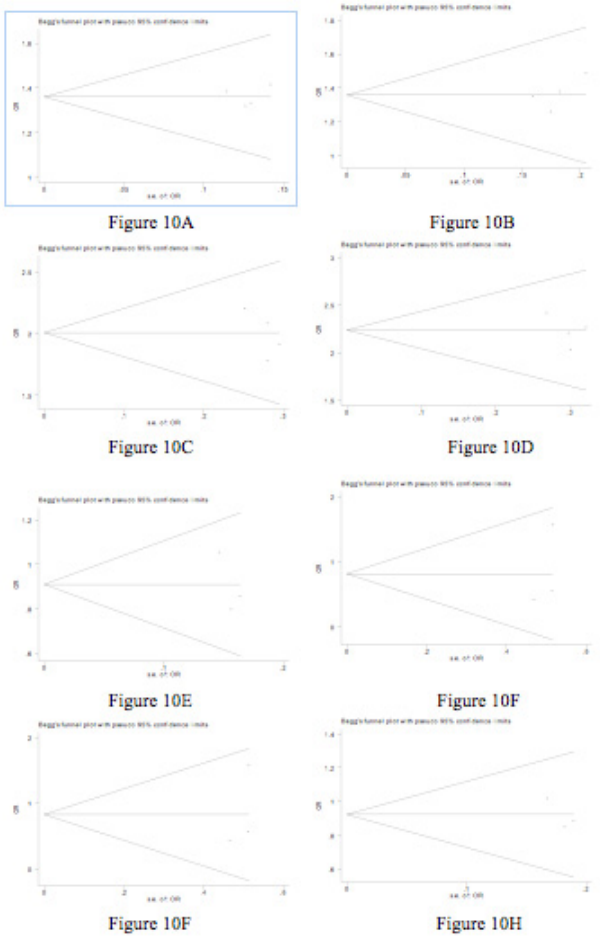

Figure 10. Begg's funnel plot for publication bias test

Co-dominant model (CC *vs*. TT): For CTLA-4 -318C/T co-dominant model (CC *vs*. TT), 3 case-control studies (Feng D et al., 2016; Liu Y et al., 2011; Yang S et al., 2012) were analyzed in the current meta-analysis. There was no between-study heterogeneity among those 3 studies in co-dominant model (CC *vs*. TT) (I^2=48.1%, p=

0.146). The pooled results showed that CTLA-4 -318C/T co-dominant model was not significantly associated with Ewing's Sarcoma and Osteosarcoma risk (OR=0.69, 95%CI:0.40-1.19, p= 0.183). (Figure 8)

Recessive model (CC *vs*. CT+TT): For CTLA-4 -318C/T recessive model (CC *vs*. CT+TT), 3 case-control studies (Feng D et al., 2016; Liu Y et al., 2011; Yang S et al., 2012) were analyzed in the current meta-analysis. There was no between-study heterogeneity among those 3 studies in recessive model (CC *vs*. CT+TT) (I^2=0.00%, p= 0.738). The pooled results showed that CTLA-4 -318C/T recessive model was significantly associated with Ewing's Sarcoma and Osteosarcoma risk (OR=0.92, 95%CI:0.75-1.13, p= 0.419). (Figure 9)

Subgroup analysis

Subgroup analysis of CTLA-4 +49G>A polymorphism was performed for diagnosis, genotype method and sample size. The results of meta-analysis were showed in Table 2. There was no between-study heterogeneity observed (all p>0.1). We found significantly positive correlations between CTLA-4 +49G>A polymorphism and increased risks of malignant bone tumors in large size of sample (A *vs*. G: OR=1.347, 95%CI: 1.172, 1.548, p=0.000; AA *vs*. GG: OR=2.228, 95%CI: 1.608, 3.085, p=0.000), Ewing's Sarcoma or Osteosarcoma (A *vs*. G: OR=1.361, 95%CI: 1.201, 1.540, p=0.000; AA *vs*. GG: OR=2.236, 95%CI: 1.674, 2.986, p=0.000), and PCR-RFLP or Sequencing (A *vs*. G: OR=1.361, 95%CI: 1.201, 1.540, p=0.000; AA *vs*. GG: OR=2.236, 95%CI: 1.674, 2.986, p=0.000).

Subgroup analysis of CTLA-4 -318C/T polymorphism was also performed for diagnosis, genotype method and sample size. There was no between-study heterogeneity observed (all p>0.1). The results of meta-analysis were indicated that there were no association between diagnosis, genotype method, sample size and the risk of malignant bone tumors (all p>0.05) (Table 3).

Publication bias

Publication bias was valuated by performed Begg's funnel plot. All demonstrated in Figure 10, the funnel plots did not display any evidence of obvious asymmetry under all models (Figure 10A. A *vs*. G: z=0.34, p=0.734; Figure 10B. AA *vs*. GG: z=0.34, p=0.734; Figure 10C. AA+AG vs GG: z=1.02, p=0.308; Figure 10D. AA *vs*. AG+GG: z=0.34, p=0.734; Figure 10E. C vs T: z=0.00, p=1.000; Figure 10F. CC *vs*. TT: z=1.04, p=0.296; Figure 10G. CC+CT *vs*. TT: z=1.04, p=0.296; Figure 10H. CC *vs*. CT+TT: z=0.00, p=1.000).

Discussion

CTLA-4 was expressed by cytotoxic T-lymphocytes on the surface of antigen presenting cells, which effects on cell cycle arrest and down-regulates the proliferation of T-cell (Lesterhuis W J et al., 2013; Salvi S et al., 2012). Previous evidence demonstrated that CTLA-4 could elevate the T-cell activation threshold, attenuate the response of anti-tumor, and increase the host's susceptibility to the same tumors including the Ewing's sarcoma and osteosarcoma (Yuan J et al., 2011). Thus, the host genetic factors, especially the variants of genes influence the tumorigenesis of Ewing's sarcoma and osteosarcoma, may affect the host's difference in the response to tumors (Postel-Vinay S et al., 2012; Huang H J et al., 2011). In fact, the interest in the genetic susceptibility to Ewing's sarcoma and osteosarcoma has led to an increasing number of studies on the association between the CTLA-4 genetic polymorphism and the malignant bone tumor (Silva D S B S et al., 2012; Mackintosh C et al., 2012). CTLA-4 +49G>A and CTLA-4 -318C/T gene polymorphism are commonly studied in CTLA-4 gene (Benhatchi K et al., 2011). Recent studies have showed that CTLA-4 +49G>A and CTLA-4 -318C/T polymorphism are involved in the risk of breast cancer and pancreatic cancer (Yang M et al., 2012; Lang C et al., 2012).

However, there are several studies which have assessed the association between CTLA-4 +49G>A and -318C/T polymorphism and the malignant bone tumors risks, the results were inconclusive due to the limited sample of single-study. We performed a pooled meta-analysis of current published studies to further evaluate the association between CTLA-4 gene polymorphism and the risk of Ewing's sarcoma and osteosarcoma. Four individual studies with a total of 1003 cases with malignant bone tumor and 1162 controls were included in our meta-analysis. The results of meta-analysis on those data demonstrated that CTLA-4 +49G>A polymorphism was associated with the risk of Ewing's sarcoma and osteosarcoma strongly (A *vs*. G: OR=1.36, 95%CI:1.20-1.54, p=0.000; AA+AG *vs*. GG: OR=1.35, 95%CI:1.14-1.61, p=0.001; AA *vs*. GG: OR=2.24, 95%CI:1.67-2.99, p=0.000; AA *vs*. AG+GG: OR=2.00, 95%CI:1.53-2.62, p=0.000), but CTLA-4 -318C/T polymorphism was not associated with the risk of malignant bone tumor (C *vs*. T: OR=0.76, 95%CI:0.76-1.08, p= 0.262; CC+CT *vs*. TT: OR=0.70, 95%CI:0.41-1.20, p= 0.198; CC *vs*. TT: OR=0.69, 95%CI:0.40-1.19, p= 0.183; CC *vs*. CT+TT: OR=0.92, 95%CI:0.75-1.13, p= 0.419). Current evidence on CTLA-4 +49G>A polymorphism and the risk of digestive system tumor was indicated that homozygote comparison (OR=1.433, 95%CI: 1.100-1.866) and dominant model (OR=1.360, 95%CI: 1.059-1.746) were linked to higher risks for hepatocellular cell carcinoma (Liu X et al., 2015). Furthermore, a meta-analysis (He L et al., 2014) including 1180cases and 2110controls demonstrated that CTLA-4 +49A/G polymorphism significantly increase the risk of colorectal (dominant model: OR=1.63, 95% CI: 1.09-2.43; AG *vs*. AA: OR=1.69, 95% CI: 1.15-2.48), especially for Asian descent (dominant model: OR=2.42, 95% CI: 1.40-4.16; AG *vs*. AA: OR=2.39, 95% CI: 1.52-3.76). In addition, the CTLA-4 +49A/G polymorphism has been proved to be associated with the risk of malignant bone tumors, but how much correlation between them was not perfectly clear (Yu F et al., 2013; Bian Z et al., 2014). Otherwise, there is little studies about CTLA-4 -318C/T on the association between its polymorphism and the risk of tumors.

There are several limitations that should be considered in the present meta-analysis. Firstly, our result may be applicable only to the Asian population because the included studies are on the Asian group. Thus, the

association between CTLA-4 +49A/G and CTLA-4 -318C/T polymorphism and the risk of bone cancers in other populations should be needed to study further. What's more, the number of included studies was really small so that statistical power may be undermined on the given SNP. Thirdly, more original data on gene-gene or gene-environment interactions between CTLA-4 polymorphism and risk of malignant bone cancers should be analyzed to get a more scientific conclusion about it.

In conclusion, our pooled meta-analysis suggested that CTLA-4 +49A/G variant was association with an increased risk of developing the malignant bone tumors, such as Ewing's sarcoma and osteosarcoma. However, it failed to show the association between CTLA-4 -318C/T polymorphism and the risk of malignant bone tumors. Future large-scale studies remain to be done to confirm our conclusions.

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