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Review Article

Association of genetic polymorphisms with osteosarcoma risk: a meta-analysis

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Abstract: Osteosarcoma (OS) is the most common pediatric and adult bone malignancy worldwide. Genetic polymorphisms may play critical roles in the development of OS. However, there present inconclusive results. The current study was to investigate the role of genetic polymorphisms in OS risk. Electronic databases were searched for relevant studies published between 2000 and 2014. The odds ratio (OR) with its 95% confidence interval (CI) were employed to estimate the associations. Total 7 studies containing 911 OS patients and 1145 matched controls were included. Our results found that CTLA-4 +49A/G G allele and TGF-β1 29T/C C allele were more frequent in OS patients than that in controls, indicating that these two alleles were significantly associated with increased the risk of OS (G vs. A: OR = 1.36, 95% CI = 1.13-1.64, P = 0.001; C vs. T: OR = 1.49, 95% CI = 1.17-1.90, P = 0.001) in a fixed-effect model. This significant relationship was also found under other three genetic models in both variants (P<0.05). While no association was found between TNF-α -308G/A or TNF-β +252A/G polymorphism and OS risk. In conclusion, our results demonstrated that CTLA-4 +49A/G and TGF-β1 29T/C variants were significantly associated with OS susceptibility. Although number of included studies is small, several polymorphisms appearing to significantly influence the OS risk should be focused. Moreover, further studies with gene-gene and gene-environmental interactions should be considered.

Keywords: Osteosarcoma, genetic polymorphism, meta-analysis, susceptibility

Introduction

Osteosarcoma (OS), which can occur in any bone (the frequent sites are the femur, the tibia and the humerus), is the most common primary malignant bone tumor in the world, representing about 56% of all bone cancers [1]. It results from primitive bone-forming mesenchymal cells, and is characterized by complex, unbalanced karyotypes and alterations in multiple genes and pathways [2]. The specific risk factors for OS incidence are age, gender, ethnicity, and site of disease [3]. According to Cancer Statistics Review, the incidence rate for all ages and all races is 0.9 per 100,000 persons per year, and the mortality rate is 0.4 per 100,000, with a 5-year overall survival rate of 67.9% [4]. Although modern chemotherapy in conjunction with surgery achieves the event-free survival [5], patients who successfully treated for OS may develop second malignant neoplasms, including an additional OS [3]. Hence, discussing the known and suspected risk factors of OS to gain insight into its etiology is very important.

This genetic background can lead to multiple malignant cell populations within the same tumor. Polymorphisms in genes that exert basic processes of regulatory systems and metabolic chains can affect the proliferation, differentiation, death of transformed and even malignant cells [6]. These alterations, especially involved in immune and inflammatory responses, have been reported to influence the level of secreted mediators, and unbalance the inflammatory cascade [7]. Arora found that genetic variants in the innate immunity pathway and its related inflammatory cascade are associated with some metabolic risk factors for type 2 diabetes mellitus [8]. Plantinga firstly demonstrated that ATG16L1 polymorphism increased the production of interleukin (IL)-1β and IL-6 in humans, acting a role on the inflammatory process in Crohn's disease [9]. Studies have also shown that the expression of most of the mediator

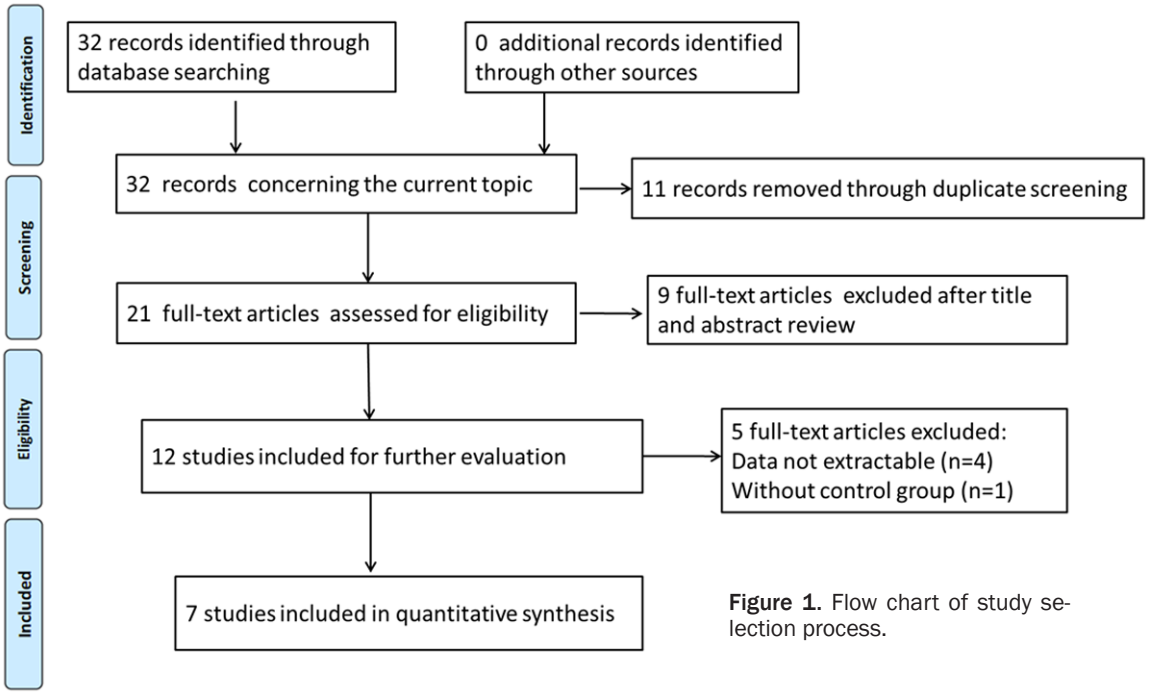

Figure 1. Flow chart of study selection process.

Table 1. Main characteristics of included studies

First author	Year	Country	Ethnicity	Total number		Genotype method	SNP
				Cases	Controls		
Patino-Garcia A	2000	Spain	Caucasian	63	111	PCR	TNF-α
Oliveira ID	2007	Brazil	Caucasian	80	160	PCR-RFLP	TNF-β, TNF-α
Xie JT	2008	China	Asian	52	60	PCR	TNF-β, TNF-α
Ma JF	2010	China	Asian	42	100	PCR	TGF-β1
Liu Y	2011	China	Asian	267	282	Sequencing	CTLA-4
Wang W	2011	China	Asian	205	216	Sequencing	CTLA-4
Xu SG	2014	China	Asian	202	216	PCR-RFLP	TGF-β1

SNP, single Nucleotide Polymorphism; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.

complex genes is altered in OS and may be associated with the development of OS risk [10]. Xiao suggested that tumor necrosis factor-alpha (TNF-α), IL-6, IL-1Ra and IL-8 were related with increased risk of OS, in which TNF-α and IL-8 may be further correlated with the progression of this disease [11]. Windsor showed that germline genetic polymorphisms may influence chemotherapy response and disease outcome in OS [12].

Recent meta-analysis have identified that murine double minute 2 and glutathione S-transferase polymorphisms have some effect on the risk of OS [13, 14]. Although several studies have already reported the genetic variants in OS risk, there is limited published evidences on polymorphic alleles of inflammatory genes. The aim of this study is systematically evaluate TNF-a, tumor necrosis factor-beta (TNF-β), transforming growth factor-beta (TGF-β1) and cytotoxic T-lymphocyte antigen-4 (CTLA-4) polymorphisms in the development of OS susceptibility.

Materials and methods

Literature search

We carried out a systematic search in the following electronic databases: PubMed, Embase, Medline and CNKI (China National Knowledge Infrastructure) for relating articles published between 2000 and 2014. The

Table 2. Distributions of genotypes and alleles of each gene in OS cases and controls in the individual studies included in the meta-analysis

Genotype	Cases					Controls				
TNF-α (-308G/A)	AA	AG	GG	A	G	AA	AG	GG	A	G
Patino-Garcia A	0	14	49	14	112	0	28	83	28	194
Oliveira ID	6	19	55	31	129	5	25	130	35	285
Xie JT	0	1	51	1	103	1	8	51	10	110
TNF-β +252A/G										
Oliveira ID	9	37	34	55	105	17	66	77	100	220
Xie JT	16	25	11	57	47	20	27	13	67	53
CTLA-4 +49G/A										
Liu Y	40	128	99	208	326	22	140	120	184	380
Wang W	35	106	64	176	234	21	108	87	150	282
TGF-β1 29T/C	TT	TC	CC	T	C	TT	TC	TCC	T	C
Ma JF	6	18	18	30	54	16	59	25	91	109
Xu SG	42	102	58	186	218	66	110	40	242	190

Table 3. Summary of the pooled results of genetic polymorphisms with risk in osteosarcoma in the meta-analysis

Comparisons	OR (95% CI)	P	I^2	Ph	Model
TNF-α-308G/A					
A vs. G	0.85 (0.29, 2.53)	0.77	79%	0.008	R
AA vs. GG	1.98 (0.66, 5.95)	0.23	33%	0.22	F
AA + AG vs. GG	0.84 (0.27, 2.57)	0.76	76%	0.01	R
AA vs. AG + GG	1.85 (0.62, 5.57)	0.27	15%	0.28	F
TGF-β +252A/G					
G vs. A	0.93 (0.67, 1.28)	0.65	0%	0.59	F
GG vs. AA	0.92 (0.47, 1.83)	0.82	0%	0.74	F
GG + AG vs. AA	1.03 (0.58, 1.86)	0.91	0%	0.76	F
GG vs. AG + AA	0.84 (0.53, 1.33)	0.45	0%	0.71	F
CTLA-4 +49G/A					
A vs. G	1.36 (1.13, 1.64)	0.001	0%	0.71	F
AA vs. GG	2.23 (1.45, 3.43)	0.0002	0%	0.95	F
AA + AG vs. GG	1.35 (1.04, 1.75)	0.02	0%	0.53	F
AA vs. AG + GG	2.00 (1.34, 2.98)	0.0007	0%	0.83	F
TGF-β1 29T/C					
C vs. T	1.49 (1.17, 1.90)	0.001	0%	0.98	F
CC vs. TT	2.20 (1.33, 3.63)	0.002	0%	0.79	F
CC + TC vs. TT	1.57 (1.05, 2.37)	0.03	0%	0.50	F
CC vs. TC + TT	1.88 (1.27, 2.79)	0.002	0%	0.60	F

OR, odd ratio; 95% CI, 95% confidence interval; P, *P*-value of ORs; I^2, and Ph, index of heterogeneity of included studies; F, fixed-effect model; R, random-effect model.

searching terms were: "osteosarcoma or osteogenic sarcoma or bone tumor", "genetic or TNF-a or TNF-β or TGF-β1 or CTLA-4" in combination with "polymorphism or mutation or variant". References of retrieved articles were also searched with no language restrictions.

Criteria for inclusion

The inclusion criteria were as follows: 1) the paper should be case-control studies; 2) the OS patients were confirmed histologically or pathologically; the controls were age-, sex- and race-matched; 3) each study included at least one of these polymorphisms, TNF-a, TNF-β, TGF-β1 and CTLA-4; 4) genotype distribution information and results of odds ratios (ORs) with its 95% confidence interval (CI) were available to extract; and 5) genotype distribution of control for a certain polymorphism should be in Hardy-Weinberg equilibrium.

The exclusion criteria were as follows: 1) studies were review reports or conference papers; 2) articles without control gorup; 3) controls were not race-matched; and 4) information of genotyp distribution was not available to extract.

Data extraction

According to the descriptions provided by the authors of the included studies, two experts assessed the quality independently. Any disagreement was subsequently discussed with a third expert. The following information was extracted from each article: first author, year of publication, country, ethnicity, total numbers, genotype methods, single nucleotide polymorphisms (SNPs), genotype distributions in OS cases and controls.

Statistical analysis

Overall associations between genetic polymorphisms and OS risk was measured by pooled OR and its 95% CI. A *P* value of the Z test which used to determine the OR less than 0.05 was

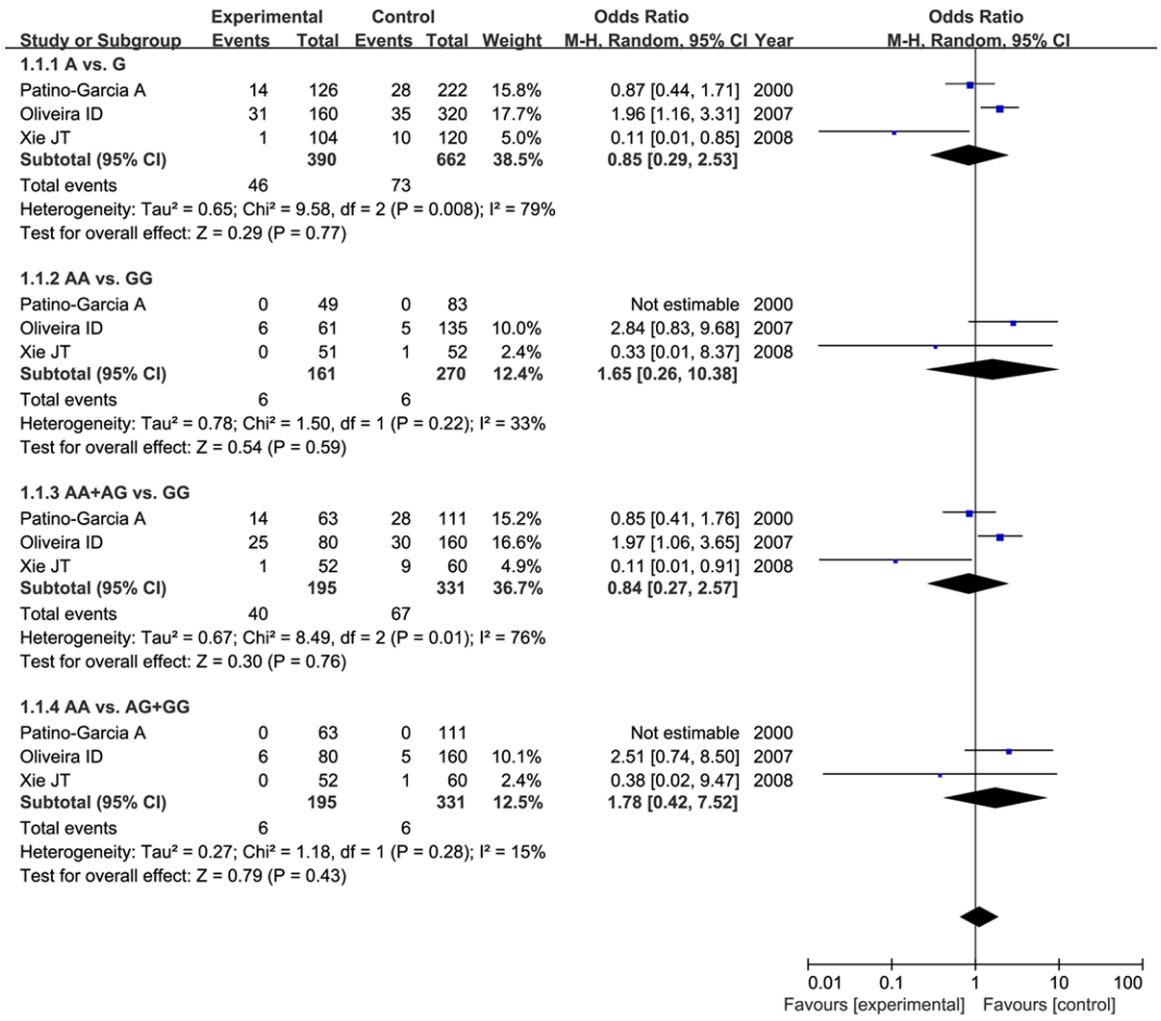

Figure 2. Meta-analysis of -308G/A polymorphism of TNF-α and osteosarcoma risk.

considered statistically significant. For all genetic polymorphisms, the allelic model and genetic models (co-dominant effects; dominant effect; and recessive effect) were examined. The Q-statistic test and the I^2 test were employed to assess the heterogeneity among studies. The random-effect model was used when the *P*-value less than 0.10 for the Q-test and I^2 more than 50% which was considered significant heterogenous among the studies; otherwise, the fixed-effect model was employed. The publication bias was assessed by visual funnel plot inspection. Statistical analyses were conducted in Review Manager (RevMan version 5.2, the Cochrane Colla boration, Oxford, England) [15] as described by Deeks [16]. All the tests were two-sided.

Results

Study characteristics

After applying the inclusion criteria, finally 7 articles were screened out, including 911 OS cases and 1145 matched controls. **Figure 1** showed the process of flow diagram. Of the seven articles, five were conducted in China, one in Spain and one in Brazil. Three articles concerned the TNF-α -308G/A variant [17-19], two articles in TNF-β +252A/G variant [18, 19], two in CTLA-4 +49A/G variant [20, 21], and two in TGF-β1 29T/C [22, 23]. The main characteristics of included studies were listed in **Table 1**. The alleles and genotypes of each gene distribution were presented in **Table 2**.

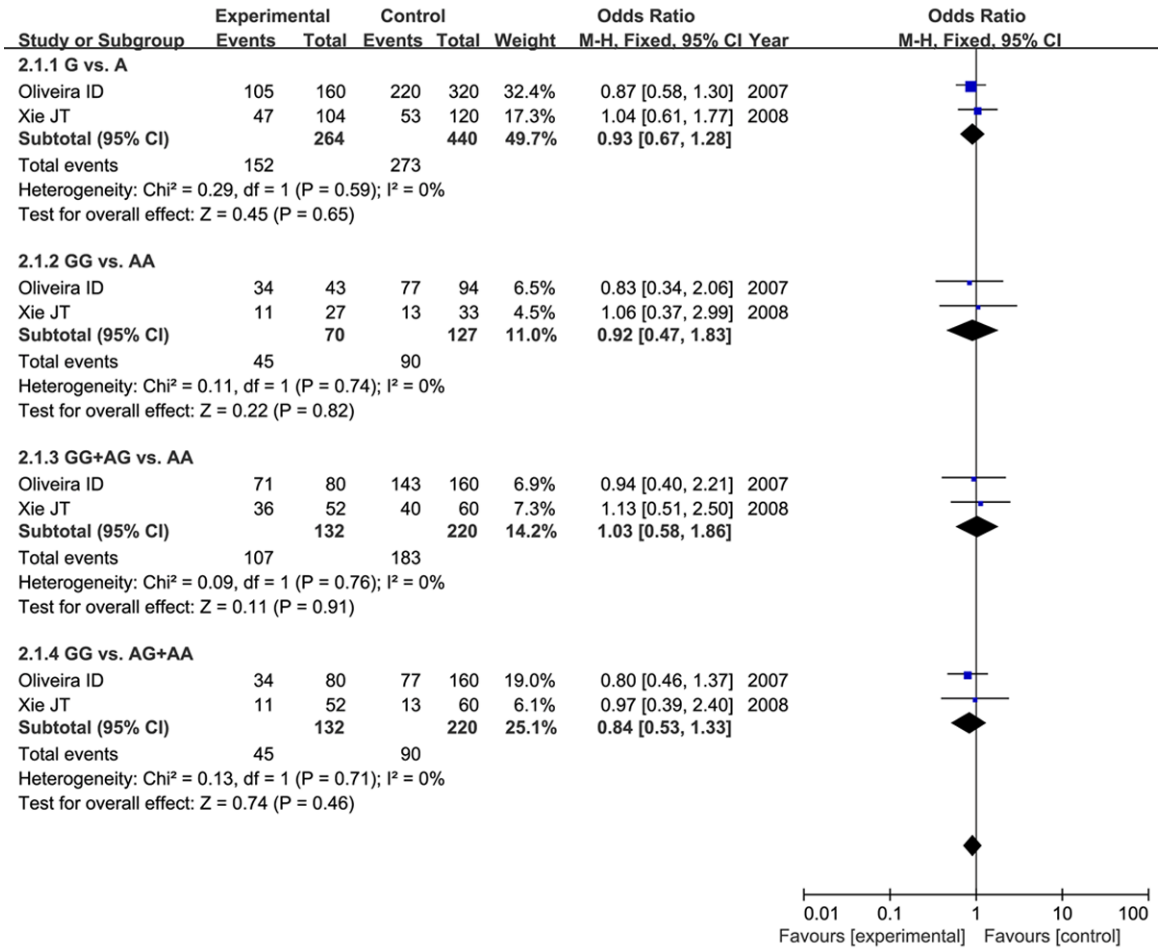

Figure 3. Meta-analysis of +252A/G polymorphism of TNF-β and osteosarcoma risk.

Association between polymorphisms of TNF-α-308G/A and TNF-β +252A/G and OS risk

For TNF-α-308G/A polymorphism, three studies contained 195 OS cases and 331 matched controls. For TNF-β +252A/G polymorphism, two articles included 132 OS patients and 220 controls. **Table 3** presented the results of pooled ORs and heterogeneity tests for the association of all genetic polymorphisms with OS risk. Overall, we found no significant association between polymorphisms of TNF-α-308G/A A allele or TNF-β +252A/G G allele and OS risk (A vs. G: OR = 0.85, 95% CI = 0.29-2.53, P = 0.77; G vs. A: OR = 0.93, 95% CI = 0.67-1.28, P = 0.65). Other genetic models of both variants were also not associated with OS risk (AA vs. GG: OR = 1.98, 95% CI = 0.66-5.95, P = 0.23; AA + AG vs. GG: OR = 0.84, 95% CI = 0.27-2.57, P = 0.76; AA vs. AG + GG: OR = 1.85, 95% CI = 0.62-5.57, P = 0.27 for TNF-α-308G/A variant as shown in **Figure 2**; GG vs. AA: OR = 0.92, 95% CI = 0.47-1.83, P = 0.82; GG + AG vs. AA: OR = 1.03, 95% CI = 0.58-1.86, P = 0.91; GG vs. AG+AA: OR = 0.84, 95% CI = 0.53-1.33, P = 0.45 for TNF-β +252A/G variant as shown in **Figure 3**).

Association between CTLA-4 +49A/G polymorphism and OS risk

Two articles reported the role of CTLA-4 +49A/G polymorphism in OS risk, including 472 OS cases and 498 controls. The frequency of G allele was higher in OS patients than that in controls (40.7% vs. 33.5%), indicating that G allele was significantly associated with increased the risk of OS (G vs. A: OR = 1.36, 95% CI = 1.13-1.64, P = 0.001). Furthermore, CTLA-4 +49A/G polymorphism was associated with OS risk under other three genetic models as well (AA vs. GG: OR = 2.23, 95% CI = 1.45-

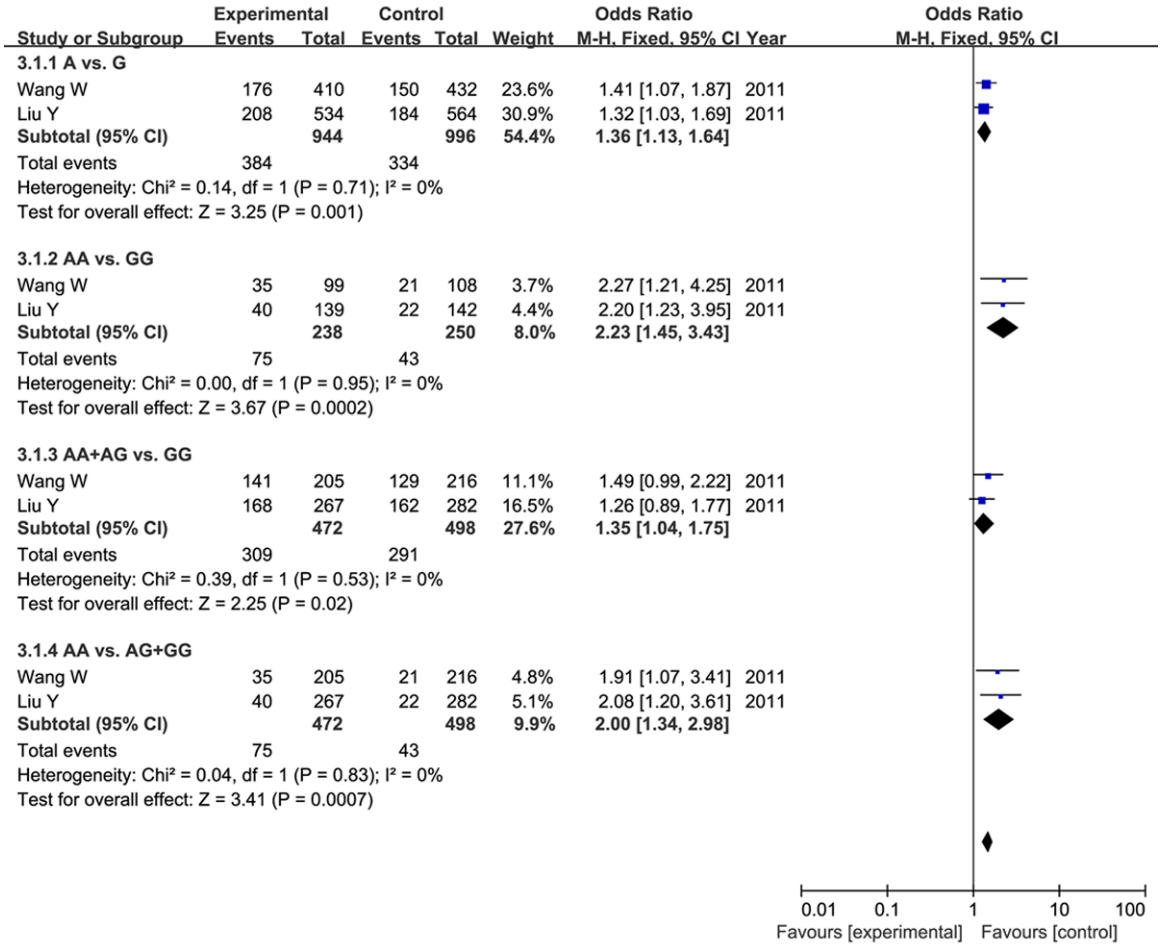

Figure 4. Meta-analysis of +49A/G polymorphism of CTLA-4 and osteosarcoma risk.

3.43, P = 0.0002; AA + AG vs. GG: OR = 1.35, 95% CI = 1.04-1.75, P = 0.02; AA vs. AG + GG: OR = 2.00, 95% CI = 1.34-2.98, P = 0.0007) in a fixed-effect model as shown in **Figure 4**.

Association between TGF-β1 +29T/C polymorphism and OS risk

Two articles estimated the association of TGF-β1 +29T/C polymorphism and OS risk, including 244 OS cases and 316 controls. The frequency of C allele was higher in OS patients than that in healthy controls (55.7% vs. 47.3%). Our result found that C allele was significantly associated with OS risk (OR = 1.49, 95% CI = 1.17-1.90, P = 0.001) in a fixed-effect model. This association was also found in other genetic models (CC vs. TT: OR = 2.20, 95% CI = 1.33-3.63, P = 0.002; CC + TC vs. TT: OR = 1.57, 95% CI = 1.05-2.37, P = 0.03; CC vs. TC + TT: OR = 1.88, 95% CI = 1.27-2.79, P = 0.002) as shown in **Figure 5**.

Publication bias

The funnel plot was approximately symmetrical, thus no publication bias in the current meta-analysis might exist (**Figures 6** and **7**).

Discussion

OS is the most common bone malignancy in children and adolescents worldwide. Inflammatory genes polymorphisms have drawn increasing attentions and have been indicated as candidates in the risk of OS, but the results are still inconsistent. In our present meta-analysis, we assessed the roles of TNF-α -308G/A, TNF-β +252A/G CTLA-4 +49A/G and TGF-β1 29T/C polymorphisms in OS risk. Our results showed that CTLA-4 +49A/G and TGF-β1 29T/C

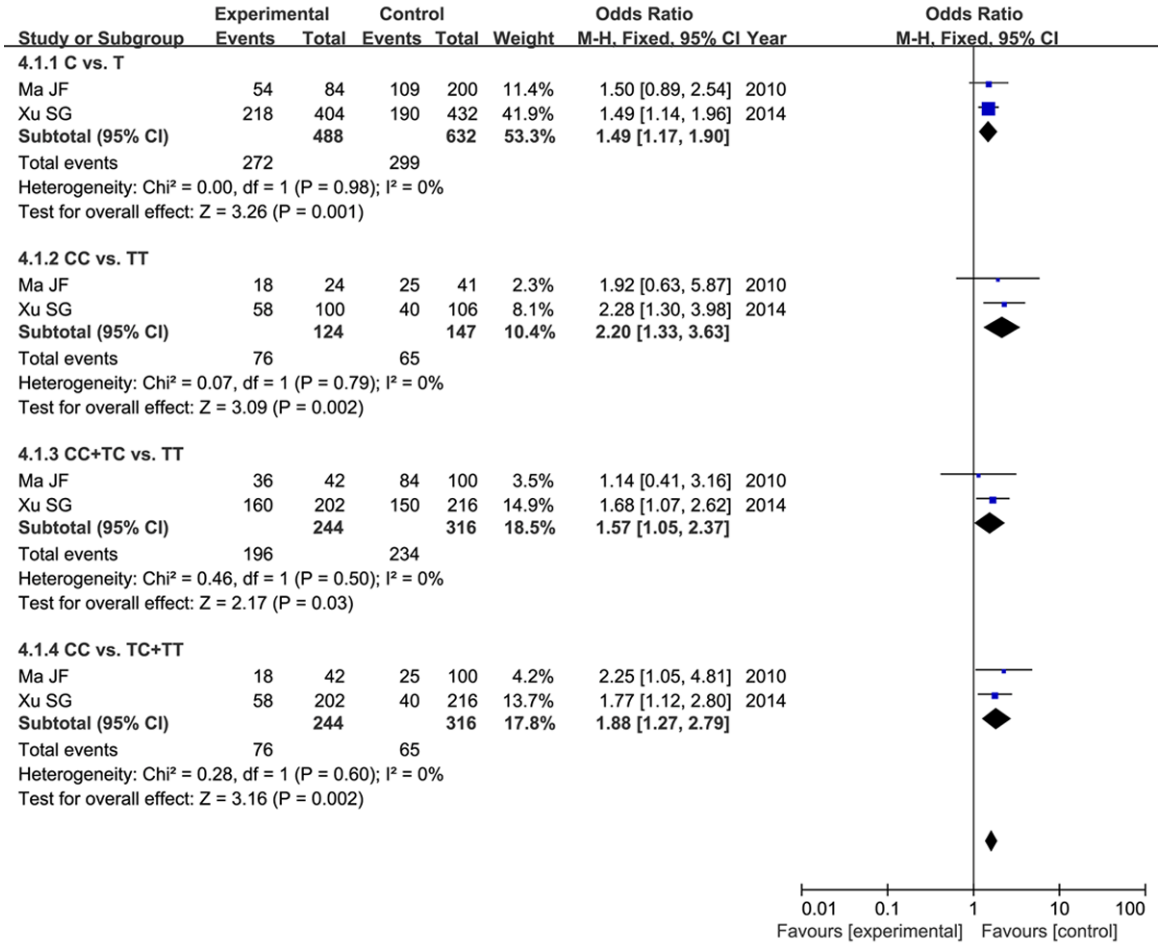

Figure 5. Meta-analysis of +29T/C polymorphism of TGF-β1 and osteosarcoma risk.

variants were significantly associated with increased the risk of OS under each comparison models, while no association was found between TNF-α -308G/A or TNF-β +252A/G polymorphism and OS risk.

CTLA-4, also known as CD152, is one member of the immunoglobulin superfamily, and a costimulatory molecule mapped to chromosome 2q33. It is expressed by activated T cells, and plays an important role in down-regulating the T cell proliferation and activation [24]. Previous studies have identified that CTLA-4 is one of the most important candidate genes for influencing the risk of several autoimmune diseases [25], and also able to affect T cell responses in animal tumor models and cancer immunotherapy trials in humans [26]. Many SNPs have been identified in the CTLA-4 gene region [27], in which the +49G/A mutation, results in alanine exchange to threonine, is the most studied. This mutation is correlated with high expression of the CTLA-4 protein [28]. CTLA-4 (+49) A/G polymorphism is associated with the coronary artery lesions formation of Kawasaki Disease particularly in female patients [29] and is involved in susceptibility to developing pancreatic cancer [30]. It is indicated that this polymorphism alone and in a haplotype with -318C allele may confer susceptibility to chronic HBV infection in Chinese Han patients [31]. Previous meta-analysis suggested that the CTLA-4 +49A/G polymorphism may be a risk factor for primary biliary cirrhosis in Asians [32] and is significantly associated with bladder cancer risk [33]. However, other studies found that this polymorphism was not associated with human diseases. A meta-analysis conducted by Gyu Song did not find an association between the CTLA-4 +49A/G polymorphism and susceptibility to multiple sclerosis in Caucasian, Asian, and Arab populations [34].

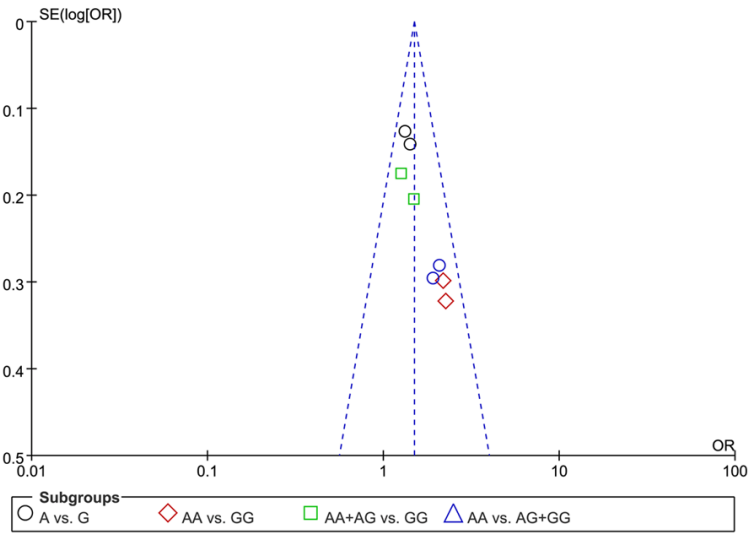

Figure 6. Forest plot on the association of CTLA-4 +49A/G variant and OS in a fixed-effect model.

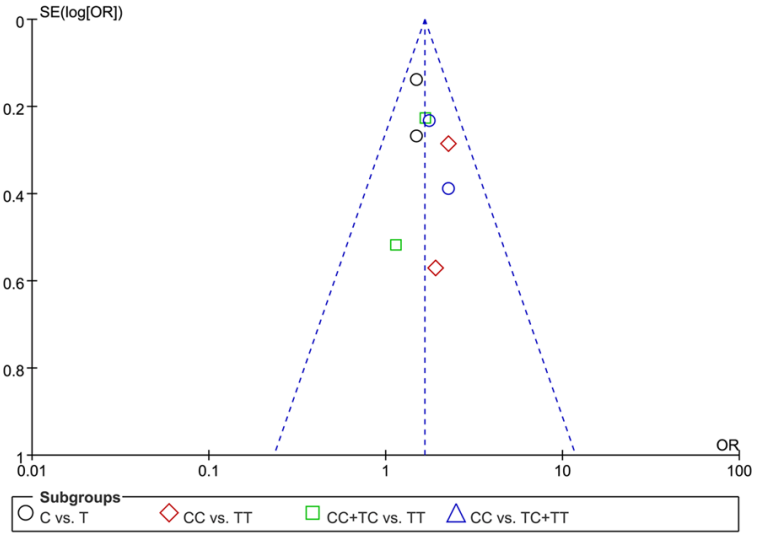

Figure 7. Forest plot on the association of TGF-β1 +29T/C variant and OS.

Several published studies demonstrated that the SNPs in CTLA-4 gene are potentially associated with OS and might have influences on the risk of OS. Liu found that +49A/G polymorphism of CTLA-4 may play an important role in carcinogenesis of OS [35]. Yu showed that CTLA-4 +49A/G polymorphism is associated with risk of malignant bone tumors, including OS and Ewing's sarcoma [36]. Chang indicated that there may be an association between CD152 polymorphisms and risk of osteosarcoma in Chinese population [37]. Our result is consistent with these evidences.

TGF-β, which was isolated in 1978, is a polypeptide which plays an important role in cell proliferation, differentiation and apoptosis, extracellular matrix synthesis [38]. TGF-β1, one of the five isomeric forms existing in TGF-β, is the predominant isoform in humans and is synthesized by several cells. It is approximately 25 kDa, and is a multifunctional cytokine: it regulates cell proliferation, growth, differentiation and cells movement; it has immunomodulatory effects; it has profibrogenic effects [39]. TGF-β1 is located at chromosome 19q13 and contains several variants. A replacement of Leucine by Proline at position 29 (TGF-β1 29T/C) is the most studied and has been shown to result in an increased secretion of the cytokine [40]. Studies showed that the TGF-β1 +29C/C genotypes, which appear to affect the cytokine production, may be associated with susceptibility to chronic hepatitis C infection and resistance to combined antiviral therapy [41]. TGF-β1 SNP are associated with susceptibility to chronic periodontitis in the population studied [42]. However, Amani found no association between the studied SNPs of TGFβ1 and breast cancer among Iranian women [43]. Our result found that TGF-β1 +29T/C was associated with OS risk.

TNF-α and TNF-β are important cytokines in the tumor microenvironment, and play multiple roles in inflammatory and immunomodulatory activities. They both can induce inflammatory response by activating NF-kB nuclear protein upon to binding to TNF receptor and expressed in atherosclerotic plaques [44, 45]. TNF-α -308G/A polymorphism was wildly studied and a functional research suggested that the A allele of -308G/A polymorphism was associat-

ed with increased TNF-α production [46]. This SNP was also associated with many human diseases. Studies showed that TNF-α -308G/A polymorphism was an independent risk factor for suicide attempts in major depressive disorder [47] and nasopharyngeal carcinoma development [48]. Previous meta-analysis demonstrated that the TNF-α polymorphism was associated with sepsis [49]. Wang found that the polymorphism of TNF-α -308G/A participates in modifying the susceptibility to ulcerative colitis and Crohn's disease in Europeans and Asians [50]. TNF-β may play critical roles in bone tissues, and is growth stimulatory for mesenchymal cells such as osteoblasts [51]. Studies have shown that TNF-β genetic polymorphism is especially interesting since variations in the region responsible for transcriptional regulation may have implications for the TNF-α expression and variability on TNF-α synthesis. A SNP at position +252 located in the first intron of the TNF-β (TNF-β G252A) consists of a guanine in the allele TNF-β1 and of an adenine in the variant allele TNF-β2 [52]. TNF-β G252A is associated with inflammatory and metabolic markers in acute ischemic stroke [53] and multiple sclerosis patients [54]. However, our results did not find significant associations between these two variants and OS risk.

Several limitations should be focused on in present meta-analysis. Firstly, studies included was relatively small, thus, the statistical power may be undermined on a given SNP. Secondly, studies were mainly conducted in Asian or Caucasian population, other ethnicities should also be included. Thirdly, these polymorphisms may interact with other gene- or environment-based risk factors which should be considered.

In conclusion, our meta-analysis demonstrated that CTLA-4 +49A/G and TGF-β1 29T/C variants were associated with an increased risk of developing OS. However, no significant association was found between TNF-α -308G/A or TNF-β +252A/G polymorphism and OS risk. Further studies with large sample sizes are needed to assess associations between the genetic polymorphisms and risk of OS.

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Disclosure of conflict of interest

None.

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