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Systematic meta-analysis of genetic variants associated with osteosarcoma susceptibility

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Background: In the past decade, accumulated evidence has suggested that genetic variation is related to the pathogenesis of osteosarcoma. Although there are a large number of studies on the association between genetic variation and osteosarcoma, their results are inconsistent. To clarify these findings, we performed a systematic meta-analysis using allelic contrasts for each gene-specific single nucleotide variants with all available data in the field of osteosarcoma.

Methods: The literature search for relevant studies was conducted in PubMed, Embase, and Cochrane databases. Pooled ORs and 95% CI values were calculated by the random-effects model using the Comprehensive Meta-analysis version 2.0 software package. Heterogeneity between studies was examined by the Cochran's *Q*-test.

Results: The 32 genome-wide case–control population-based studies, involving 15,336 study subjects (6924 cases and 8412 controls), were included in this meta-analysis. We analyzed 24 single nucleotide variants (SNVs) in 14 genes. We identified 12 SNVs in *CTLA-4*, *IL-8*, *MDM2*, *PRCKG*, *RECQL5*, *TNF-a*, *TP53*, *XRCC3*, and *VEGF* that correlated with osteosarcoma susceptibility. The average pooled odds ratio for the 9 risk alleles was 2.082 (range: 1.585 to 3.262). These included *CTLA-4* rs231775, *CTLA-4* rs5742909, *PRCKG* rs454006, *RECQL5* rs820196, TNF-α rs1800629, *TP53* rs1042522, *XRCC3* rs861539, *VEGF* rs699947, and *VEGF* rs3025039. The average pooled odds ratio for the 3 protective alleles, *IL-8* rs4073, *MDM2* rs1690916, and *VEGF* rs2010963, was 0.606 (range: 0.510–0.719). Publication bias was not observed among the studies reporting positively correlated SNVs. The pooled odds ratios for the SNVs that correlated with osteosarcoma risk showed homogeneity.

Conclusion: Our results provide powerful information for tracking the most viable gene candidates. Further studies with larger multiethnicity populations and investigations of the potential biological roles of these genetic variants in osteosarcoma should be conducted.

Abbreviations: CIs = confidence intervals, HWE = Hardy–Weinberg equilibrium, ORs = odds ratios, OS = osteosarcoma, *P* = *P*-value, SNVs = single nucleotide variants.

Keywords: meta-analyses, osteosarcoma, polymorphism, single nucleotide variant, susceptibility

1. Introduction

Osteosarcoma (OS) is the most common primary bone tumor in children and adolescents.[1,2] It predominantly occurs in the metaphyseal area of the distal femur and proximal tibia, characterized by the formation of immature bone or osteoid tissue. Current treatment consists of surgical resection in combination with radiotherapy and chemotherapy, which improves long-term survival. However, high incidence of recurrence and metastasis results in poor prognosis of osteosarcoma.[3,4] Hence, elucidating the causative factors is critical to improve the therapeutic strategies to increase the overall survival rates of osteosarcoma patients.

Pathogenesis of osteosarcoma involves interactions between genetic and environmental factors.[5,6] Genetic variation plays an important role in the pathogenesis of osteosarcoma.[7,8] In population-based case–control studies, single nucleotide variants (SNVs) were identified as candidate risk factors associated with osteosarcoma.[9,10] Although a number of gene association studies identified osteosarcoma risk loci, they were not statistically significant. Meta-analysis improves statistical power by synthesizing association data from multiple studies regarding individual genetic loci or variants.[11]

In this meta-analysis, we systematically analyzed population-based case–control genetic association studies to identify all gene-specific SNVs associated with osteosarcoma risk.

2. Materials and methods

2.1. Search strategy

We conducted exhaustive literature searches from inception to August 2016 in the PubMed, Embase, and Cochrane databases using the following Keywords: *osteosarcoma* AND *polymorphism* OR *association* OR *variation* OR *variant* OR *risk* OR *susceptible* OR *susceptibility* OR *sequencing* OR *case–control* OR *gene*. Key studies and reviews were screened to identify additional relevant publications. Only studies published in English were included. To test ability of our search strategies

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to capture all of the published genetic association data targeted for osteosarcoma, we searched the Web of Science using SNVs combined with the keyword "osteosarcoma," respectively. This study was exempt from approval by the Institutional Review Board because it was a meta-analysis analyzing publically available data and did not need handle individual patient data.

2.2. Inclusion criteria

For inclusion in this meta-analysis, the studies had to meet the following criteria: studies assessed the association between the reported SNV and osteosarcoma; original studies published in a peer reviewed journal; the SNVs were represented in at least 2 independent population case–control studies, and studies reported sufficient genotype and other data necessary to calculate the OR and 95% CI. We contacted the corresponding authors by e-mail to obtain information that was not available in some studies. If the essential data were not obtained, the studies were excluded.

2.3. Data extraction

Two authors extracted data independently and reached consensus by discussion and re-examination. The following information was collected from each study: the first author, published year, ethnic group, total number of cases and controls, genotype or allele data, and PubMed ID. The extracted data were recorded in a standardized form. In this meta-analysis, SNVs were represented by their dbSNP identifiers (rs numbers).

2.4. Statistical analyses

Statistical analysis was conducted with the Comprehensive Meta-analysis version 2.0 software package (Biostat, Englewood, NJ). The strength of the association between SNVs and the risk of osteosarcoma was assessed using ORs with the corresponding 95% CIs. For all variants that had case–control genotype data in 2 or more independent samples, we calculated the crude ORs and 95% CI values from the allele distributions in each study. Pooled ORs and 95% CI values were calculated by the random-effects model (the DerSimonian and Laird method), which considered variability within or between studies.[12] A $P < .05$ was considered statistically significant for pooled ORs by a Z-test. Heterogeneity between studies was examined by the Cochran's Q-test and considered significant if $P < .1$. For each study, the Hardy-Weinberg equilibrium (HWE) was tested in the controls and $P < .05$ suggested deviation from HWE according to Chi-square test. If the number of included studies were more than 5 for a single SNV, the publication bias of the literature was assessed by Egger's regression and funnel plot analysis.[13] For variants with more than 3 studies, the sensitivity and stability of the meta-analyses was determined by one study removed analyses and meta-cumulative methods. In the one study removed analyses, pooled ORs were calculated after omitting one study at a time to determine if significance of the meta-analysis remained. The meta-cumulative method calculated pooled ORs iteratively by adding each study at a time.

3. Results

3.1. Study characteristics

We identified 11913 potentially relevant studies from the literature search including 8375 from PubMed, 3472 from Embase, and 66 from Cochrane. Further, 11 articles were added by manual search of the references of the relevant studies. After assessing titles and abstracts, 115 articles remained. After evaluating the full text of 115 articles, we identified 63 publications reporting on 125 genetic SNVs in 53 different genes (Fig. 1). Finally, 32 genome-wide association studies were included in this meta-analysis. These included 24 SNVs in 14 different genes with sufficient data for analysis. Among these, 3 studies were performed in Caucasian populations, 1 each in Russian and Mexican populations, and 26 in Chinese populations. No additional eligible publications from the Web of Science were included. Characteristics of the studies are summarized in Supplementary Tables S1 and S2, http://links.lww.com/MD/C507.

3.2. Meta-analyses of SNVs associated with osteosarcoma

We calculated the pooled odds ratio (OR) and 95% confidence interval (CI) values for the 24 included variants using random-effects model. Pooled ORs of 12 SNVs in 9 genes (*CTLA-4*, *IL-8*, *MDM2*, *PRCKG*, *RECQL5*, *TNF-a*, *TP53*, *XRCC3*, and *VEGF*) correlated with either increased or decreased risk for osteosarcoma. The 12 other variants that did not show significant pooled ORs were referred to as negatively associated, whereas those that correlated were referred to as positively correlated (Table 1).

3.3. Genetic polymorphisms positively correlated with osteosarcoma risk

Twelve polymorphisms showed positive correlation to osteosarcoma and included 15336 study subjects (6924 cases and 8412 controls). The average sample size was 1278 (range: 323 to 3056). An average of 3 independent studies was included for each of the 12 SNVs (range: 2 to 7). Nine of the 12 SNVs increased the risk for osteosarcoma by 2.082-fold (range: 1.585 to 3.262). These included *CTLA-4* rs231775, *CTLA-4* rs5742909, *PRCKG* rs454006, *RECQL5* rs820196, TNF-α rs1800629, *TP53* rs1042522, *XRCC3* rs861539, *VEGF* rs699947, and *VEGF* rs3025039. Three SNVs, namely, *IL-8* rs4073, *MDM2* rs1690916, and *VEGF* rs2010963 decreased risk of osteosarcoma by 39.4% and had an average pooled OR of 0.606 (range: 0.510–0.719). No heterogeneity was observed for any of the pooled ORs in the studies regarding the positive correlating SNVs (Fig. 2).

Seven SNVs reported in 10 studies showed deviation from Hardy-Weinberg equilibrium (HWE) among control subjects. After removing these 10 studies, the only SNVs with more than 2 studies available for analysis were *CTLA-4* rs231775 and *VEGF* rs3025039. The pooled ORs were still positive for *CTLA-4* rs231775 ($P = .007$; 327 cases and 347 controls; study heterogeneity analysis, $P = .980$) and *VEGF* rs3025039 ($P = .014$; 816 cases and 1008 controls; study heterogeneity analysis, $P = .244$; Supplementary Table S1 and Supplementary Figure S1, http://links.lww.com/MD/C507). Three variants, namely, *MDM2* rs1690916, *TNF-α* rs1800629, and *TP53* rs1042522 were represented by all ethnicities studied, but, the other 9 variants were found only in Chinese populations. Since the number of studies on positive variants in populations with different ethnicities was fewer than 3, we did not perform a subgroup analysis based on ethnicity.

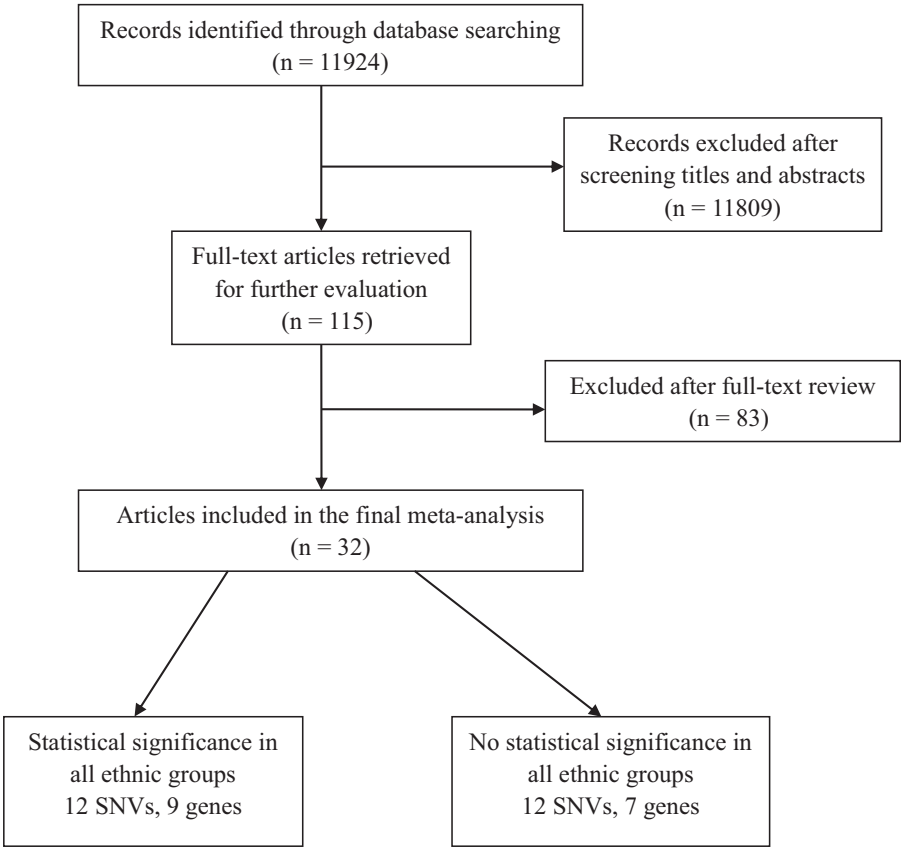

Figure 1. Flow diagram of the selection of eligible studies for this meta-analysis.

Table 1

Random-effects meta-analyses using allelic contrasts for SNVs showing summary ORs.

Gene	SNV	Putative function	Model	OR (95% CI) *P*-value	*Q*- value	Heterogeneity *P*-value	Cases versus controls (number of independent samples)
CTLA-4	rs231775	Missense (p.Thr17Ala)	A vs G	1.977 (1.384–2.823) 0.000	0.060	.970	594 vs 629 (3)
CTLA-4	rs5742909	Promoter	T vs C	2.298 (1.105–4.782) 0.026	0.004	.951	389 vs 413 (2)
ERCC2	rs13181	Missense (p.Lys751Gln)	C vs A	1.425 (0.691–2.936) 0.337	0.246	.620	169 vs 379 (2)
ERCC2	rs1799793	Missense (p.Asp312Tyr)	T vs C	0.791 (0.535–1.163) 0.233	0.235	.628	169 vs 379 (2)
GRM4	rs1906953	Intron	T vs C	0.995 (0.300–3.300) 0.994	11.139	.001	294 vs 384 (2)
IL-6	rs1800795	Intron	C vs G	1.305 (0.713–2.388) 0.388	2.297	.130	280 vs 376 (2)
IL-8	rs4073	Promoter	T vs A	0.590 (0.424–0.819) 0.002	0.050	.823	299 vs 299 (2)
IL-10	rs1800896	Promoter	G vs A	1.371 (0.777–2.417) 0.276	2.291	.130	338 vs 417 (2)
MDM2	rs1690916	3′UTR	A vs G	0.510 (0.270–0.965) 0.038	0.049	.826	164 vs 159 (2)
PRCKG	rs2242245	Intron	C vs T	1.413 (0.895–2.229) 0.138	0.031	.860	998 vs 998 (2)
PRCKG	rs454006	Intron	C vs T	1.989 (1.536–2.575) 0.000	0.281	.596	998 vs 998 (2)
PRCKG	rs8103851	Intron	G vs C	0.913 (0.660–1.264) 0.585	2.191	.139	998 vs 998 (2)
RECQL5	rs820196	Missense (p.Asp453Gly)	C vs T	2.152 (1.409–3.288) 0.000	0.149	.700	397 vs 441 (2)
TGF-β1	rs1800469	Upstream variant	T vs C	1.132 (0.789–1.624) 0.502	0.057	.811	326 vs 352 (2)
TGF-β1	rs1800470	Missense (p.Pro10Leu)	C vs T	1.104 (0.424–2.870) 0.840	6.916	0009	326 vs 352 (2)
TNF-α	rs1800629	Promoter	A vs G	3.262 (1.512–7.036) 0.003	0.292	.589	160 vs 259 (2)
TP53	rs1042522	Missense (p.Pro33)	G vs C	1.604 (1.201–2.142) 0.001	0.239	.625	410 vs 670 (2)
XRCC3	rs861539	Intron	A vs G	2.234 (1.399–3.567) 0.001	0.017	.896	287 vs 440 (2)
VEGF	rs833061	Promoter	C vs T	1.394 (0.898–2.163) 0.138	1.170	.279	358 vs 358 (2)
VEGF	rs699947	Promoter	A vs C	1.637 (1.236–2.168) 0.001	1.104	.776	709 vs 874 (4)
VEGF	rs3025039	3′UTR	T vs C	1.585 (1.209–2.078) 0.001	4.647	.590	1350 vs 1706 (7)
VEGF	rs2010963	5′UTR	C vs G	0.719 (0.596–0.866) 0.001	5.315	.379	1167 vs 1524 (6)
VEGF	rs10434	3′UTR	A vs G	1.175 (0.945–1.460) 0.146	0.420	.995	1166 vs 1524 (6)
VEGF	rs1570360	Promoter	A vs G	0.813 (0.644–1.025) 0.080	0.407	.816	529 vs 692 (3)

CIs = confidence intervals, ORs = odds ratios, SNVs = single nucleotide variants.

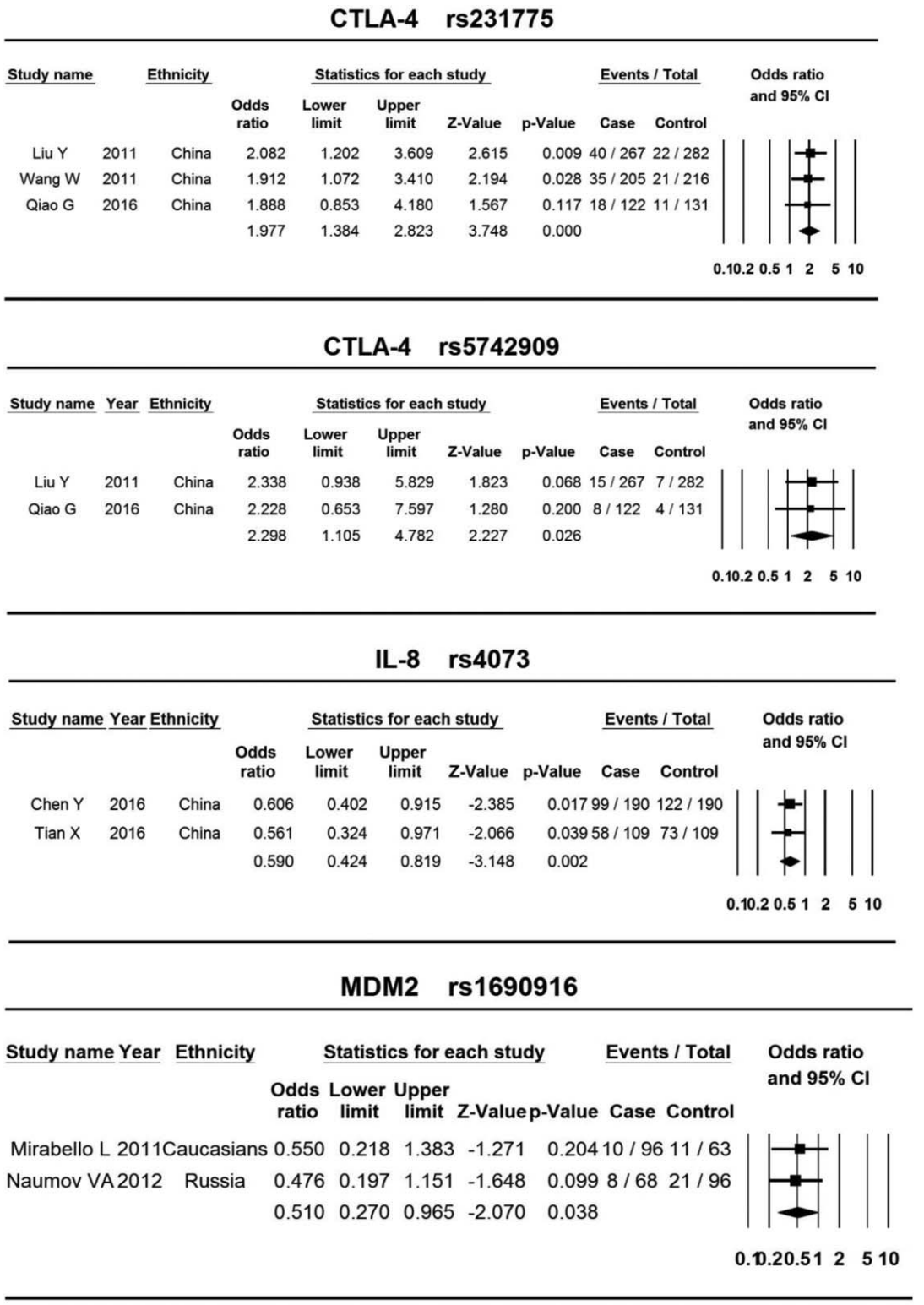

Figure 2. Graphical display of random-effects meta-analyses results showing significant summary ORs. Summary ORs and 95% CI values were calculated with all ethnic populations. CIs = confidence intervals, ORs = odds ratios.

3.4. Genetic polymorphisms not associated with osteosarcoma risk

The 12 polymorphisms that showed no correlation with osteosarcoma were *ERCC2* rs13181, *ERCC2* rs1799793, *GRM4* rs1906953, *IL-6* rs1800795, *IL-10* rs1800896, *PRCKG* rs2242245, *PRCKG* rs8103851, *TGF-β1* rs1800469, *TGF-β1* rs1800470, *VEGF* rs833061, *VEGF* rs10434, and *VEGF* rs1570360. The sample size was 13160 study subjects (5951 cases and 7209 controls) with an average sample size of 1097 (range: 548 to 2690). The pooled OR was 1.153 (range: 0.791–

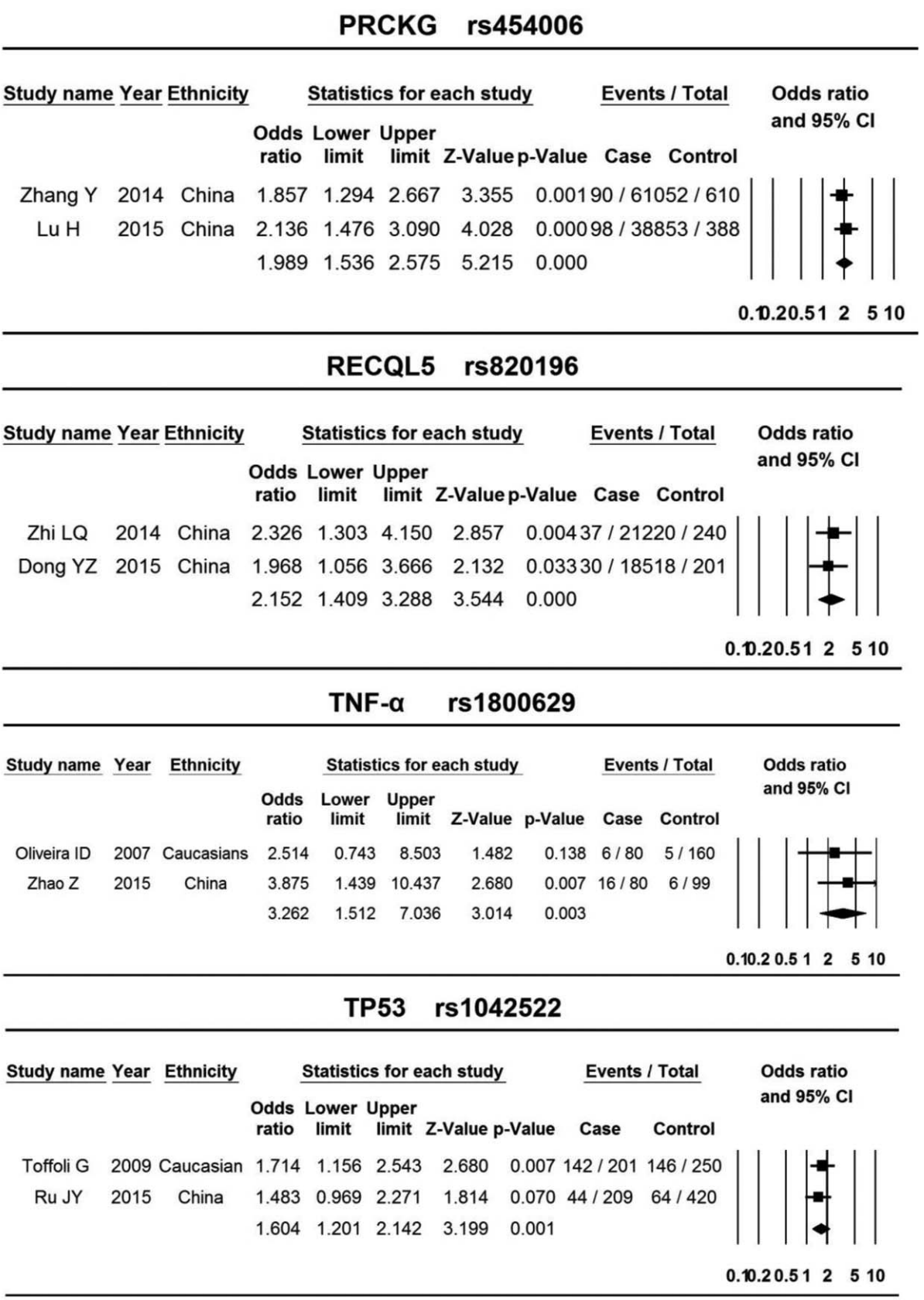

Figure 2. (Continued)

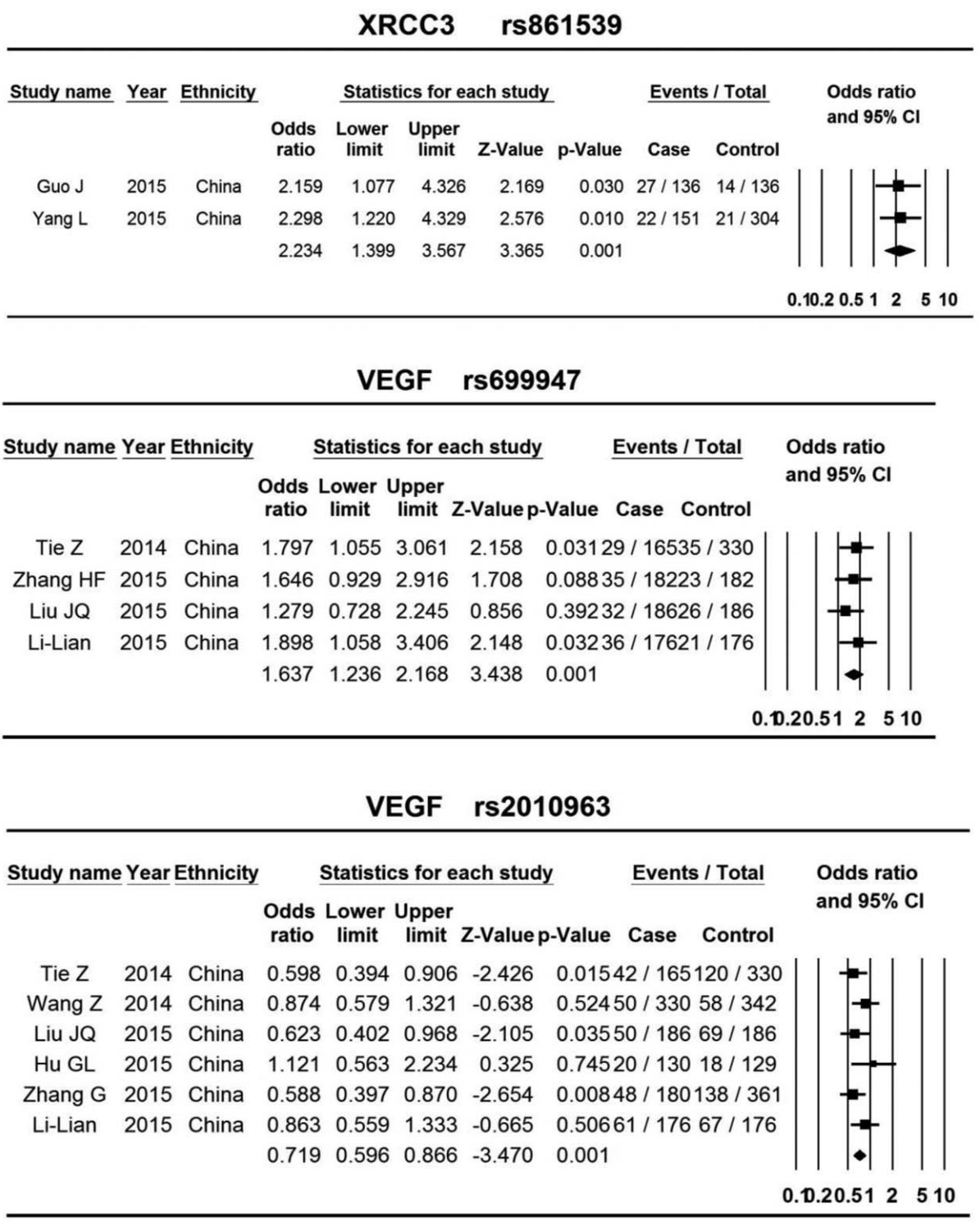

Figure 2. (Continued)

1.425). At least 2 independent studies were included for each of the 12 negative SNVs (range: 2 to 6). Heterogeneity was detected in studies related to *GRM4* rs1906953 and *TGF-β1* rs1800470 (Supplementary Figure S2, http://links.lww.com/MD/C507). Among the negative SNVs, 7 studies showed deviation from HWE in 6 SNVs in the control subjects. After removing the HWE-deviation studies, there were no variants with more than 2 studies for re-analysis. Hence, further analysis was not performed. Four negative variants, namely, *ERCC2* rs13181, *ERCC2* rs1799793, *IL-6* rs1800795, and *IL-10* rs1800896 were represented by all ethnicities, whereas the remaining 8 negative variants were found only in Chinese populations. Since the number of studies of negative variants with different ethnicities was <3, subgroup analyses based on ethnicity was not performed.

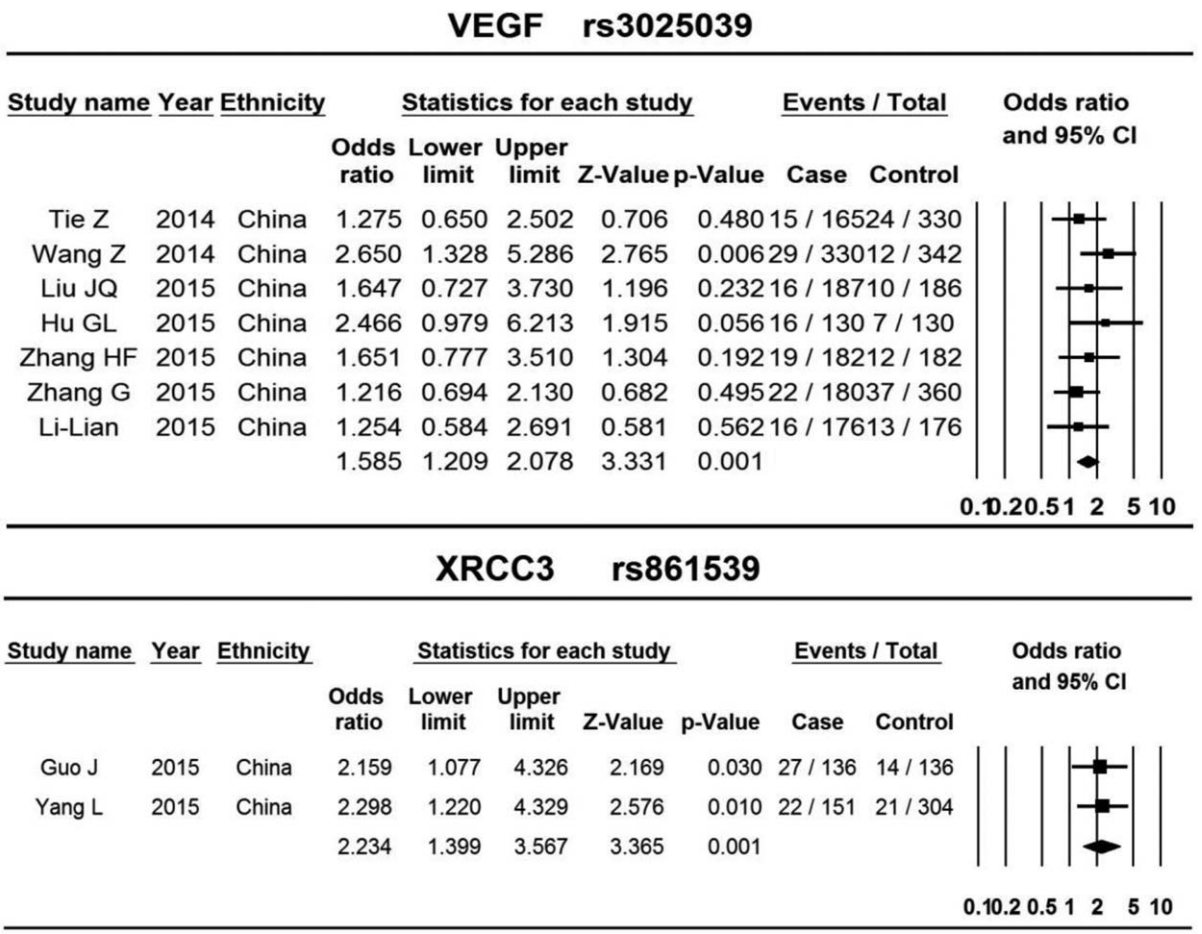

Figure 2. (Continued)

3.5. One-study removed and meta-cumulative analyses

Next, we conducted one-study-removed and meta-cumulative analyses for variants with more than 3 studies. These included *CTLA-4* rs231775, *VEGF* rs699947, *VEGF* rs3025039, *VEGF* rs2010963, *VEGF* rs10434, and *VEGF* rs1570360. The included studies were chronologically sorted to assess the influence of the individual data set on the pooled ORs. The pooled ORs did not change significantly for the positive variants, *CTLA-4* rs231775, *VEGF* rs699947, VEGF rs3025039, and *VEGF* rs2010963 in the one-study-removed tests. However, in meta-cumulative analysis, pooled ORs were not significant for *VEGF* rs3025039 and *VEGF* rs2010963 until the third study was added. Two SNVs, *CTLA-4* rs231775 and *VEGF* rs699947 showed significant pooled ORs from the first study. The negative variants, *VEGF* rs10434 and *VEGF* rs1570360 did not show significant pooled ORs in the one-study-removed tests and meta-cumulative analysis (Supplementary Figures S3 and S4, http://links.lww.com/MD/C507).

3.6. Egger's regression analyses of publication bias

Funnel plots and Egger's regression analyses were performed to assess the publication bias for 3 SNVs with more than 5 studies. The results suggested that the 3 variants, *VEGF* rs2010963, *VEGF* rs3025039, and *VEGF* rs10434 showed no significant publication bias (Supplementary Figure S5, http://links.lww.com/MD/C507).

4. Discussion

In this meta-analysis, we conducted a comprehensive evaluation of osteosarcoma related SNVs reported since the early 1950s. We systematically analyzed 24 polymorphisms from 14 genes with at least 2 independent case–control samples per variant. We discovered 12 polymorphisms (50%, 12/24) in 9 genes that showed significant pooled ORs in combined ethnicities. The average pooled odds ratio was 2.082 for risk alleles and 0.606 for protective alleles. Twelve SNVs (50%, 12/24) in 7 genes showed no correlation with osteosarcoma.

Among the 24 SNVs, 18 SNVs had only 2 included studies each, whereas 6 SNVs had more than 3 included studies. The small number of publications may have impacted the statistical significance of some of our findings. We also noticed that 26 (81.3%) of 32 studies that were analyzed in this meta-analysis were published in the last 3 years. Moreover, most of the published studies were conducted in Chinese populations. Among all positive variants, there were only 2 SNVs from other ethnicities. We speculated that the large of population of Chinese may contribute to attention for this rare disease. Therefore, further studies in other ethnicities are necessary to obtain a worldwide perspective of the gene variants that correlate with osteosarcoma.

The extent of heterogeneity is an important factor estimated in genetic association meta-analysis.[14] In our study, all positive variants showed homogeneity while 2 SNVs of negative variants

had some heterogeneity. However, heterogeneity could not be accurately estimated because most variants were reported in 3 or fewer studies. Although fixed-effects model was acceptable in the absence of heterogeneity between studies, we adopted the random-effects model since it will be more conservative and provide wider CIs than the fixed-effects model.[15] After removing studies that showed HWE-deviation, meta-analysis was performed only on *CTLA-4* rs231775 and *VEGF* rs3025039 because the study sizes were smaller for other variants. Egger's regression analysis showed no significant publication bias for 3 SNVs, *VEGF* rs2010963, *VEGF* rs3025039, and *VEGF* rs10434, which were evaluated in more than 5 studies.

In previous meta-analyses, the association of genetic polymorphisms in *CTLA-4*, *MDM2*, *VEGF*, *TNF-α*, *TNF-β1*, and *GST* with osteosarcoma susceptibility were investigated.[16–22] We employed different inclusion and exclusion criteria for this meta-analysis. We focused on the SNVs represented by population-based case–control studies and excluded studies based on family designs or quantitative trait analyses. Eleven (4 positive and 7 negative) of the 24 variants studied in this meta-analysis were not reported in previous meta-analysis. In addition, we added recent studies to all available population-based case–control genetic association studies in osteosarcoma to investigate the relationship between SNVs and osteosarcoma susceptibility comprehensively.

However, there are several limitations of the present study. First, we had few studies with small sample sizes. Most of the studies included Chinese populations, which contributed to bias. Hence, large sample size studies in different ethnic populations are required. Second, only studies published in English were selected. This may have introduced language bias and disproportionate exclusion of negative data leading to overestimation of pooled ORs. Third, we could not explain gene–environment interactions and the underlying mechanisms due to the lack of relevant data from the original studies. Osteosarcoma initiation and progression is a complex, multistep and multifactorial process. Based on the available data, our meta-analysis demonstrated that *CTLA-4, PRCKG, RECQL, TNF-α, TP53, XRCC3,* and *VEGF* genes contributed to osteosarcoma susceptibility. However, the underlying mechanisms need to be investigated in the future. In conclusion, our meta-analysis demonstrated 12 SNVs in 9 genes that correlated with osteosarcoma susceptibility.

Author contributions

Conceptualization: Zhenyu Liu, Xinjia Wang.
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Supervision: Zhenyu Liu.
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Writing – original draft: Xinjia Wang.
Writing – review & editing: Zhenyu Liu.
Conceptualization: zhenyu liu.
Data curation: Xinjia Wang.
Methodology: Zhenyu Liu.
Software: Xinjia Wang.
Supervision: Zhenyu Liu.
Writing – original draft: Xinjia Wang.
Writing – review & editing: Zhenyu Liu.

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