RESEARCH ARTICLE

Genetic Association between ERCC2, NBN, RAD51 Gene Variants and Osteosarcoma Risk: a Systematic Review and Meta-Analysis

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Abstract

Background: To date, only a few studies have investigated associations between ERCC2, NBN, and RAD51 variants and risk of developing osteosarcoma. In this systematic review and meta-analysis, we focused on clarifying links. **Materials and Methods:** We systematically searched PubMed, Google Scholar, and ISI web of knowledge databases to identify relevant studies. Odds ratios (ORs) with 95% confidence intervals (CIs) were used to calculate the strength of associations with fixed effect models. **Results:** No statistical evidence of association was found between ERCC2 rs13181 (G vs. T: OR= 1.224, 95% CI: 0.970-1.545, p= 0.088; GT vs. TT: OR= 1.135, 95% CI: 0.830-1.552, p= 0.428; GG vs. TT: OR= 1.247, 95% CI: 0.738-2.108, p= 0.409; GG+GT vs. TT: OR= 1.174, 95% CI: 0.929-1.484, p= 0.179; GG vs. GT+TT: OR= 1.476, 95% CI: 0.886-2.460, p= 0.135), ERCC2 rs1799793 (GA+AA vs. GG: OR= 1.279, 95% CI: 0.912-1.793, p= 0.154), NBN rs709816 (OR= 1.047, 95% CI: 0.763-1.437, p= 0.775), NBN rs1805794 (OR= 1.126, 95% CI: 0.789-1.608, p= 0.513), RAD51 rs1801320 (OR= 0.977, 95% CI: 0.675-1.416, p= 0.904), RAD51 rs1801321 (TT+GT vs. GG: OR= 1.167, 95% CI: 0.848-1.604, p= 0.343), RAD51 rs12593359 (GG+GT vs. TT: OR= 0.761, 95% CI: 0.759-1.470, p= 0.744) polymorphisms and osteosarcomas. The lack of the original data limited our further evaluation of the adjusted ORs concerning age and gender; however, the previous individual studies results indicated the age- and gender-specific effects of two ERCC2 rs1799793 and NBN rs1805794 variants on osteosarcoma risk. **Conclusion:** The results suggested a lack of association between the ERCC2 (rs13181 and rs1799793), NBN (rs709816 and rs1805794), and RAD51 (rs1801320, rs1801321, and rs12593359) variants with osteosarcoma risk. Further comprehensive and well-designed studies are required to assess the role for ERCC2, NBN, RAD51 variants in osteosarcoma development more adequately.

Keywords: Osteosarcoma- ERCC2- NBN- RAD51- variant- meta-analysis

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Introduction

Osteosarcoma is the most common primary bone tumor in children and adolescents (Arndt et al., 2012). According to the statistics, osteosarcoma representing approximately 3.4% of all childhood cancers and 56% of malignant bone tumors in children (Geller et al., 2010). It was reported that osteosarcoma is the second most frequent cause of cancer-related death in adolescents (Liu et al., 2016; Sobhan et al., 2017). In humans, the peak incidence of osteosarcoma occurs in the second decade of life, during periods of rapid skeletal growth (Gorlick et al., 2003; Geller et al., 2010). Based on epidemiological studies, osteosarcoma predominantly occurred in the femur (42%, with 75% of tumors in the distal femur), followed by the tibia (19%, with 80% of tumors in the proximal tibia), and the humerus (10%, with 90% of tumors in the proximal humerus) (Ottaviani et al., 2009).

Osteosarcoma is a complex, multistep, and multifactorial disease (Sobhan et al., 2017). The molecular mechanism involved in the tumorigenesis of osteosarcoma is not well understood yet (Li et al., 2016). Many environmental factors (such as radiation and chemicals exposure) and genetic (family history and several putative single genes) are known to contribute to the development of osteosarcoma (Ottaviani et al., 2009; Tang et al., 2016; Sobhan et al., 2017).

To date, several studies identified different putative genetic susceptibility polymorphisms for risk of osteosarcoma development, which participates in several critical cellular functions such as repair of damaged DNA; however, the statistical power of these studies was limited by small sample sizes. Recently, a few studies have investigated the genetic link between the important DNA

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repair genes variants, including ERCC2, NBN, and RAD51 and increased risk of developing osteosarcoma. However, the results have been inconsistent. In addition, the data regarding the role of those genetic polymorphisms in osteosarcoma risk are sparse. It is clear that meta-analysis can be used to increase power and answer questions not posed by the individual studies. Therefore, the objective of this systematic review and meta-analysis was to detect the potential association between osteosarcoma susceptibility and ERCC2 rs13181 and rs1799793, NBN rs709816 and rs1805794, RAD51 rs1801320, rs1801321, and rs12593359 polymorphisms.

Materials and Methods

Search Strategy

We searched PubMed, Google Scholar, and ISI web of knowledge electronic databases to identify eligible studies that were published before February 2017. The used search terms were: ‘‘DNA repair gene’’ and ‘‘ERCC2’’, ‘‘NBN, ‘‘RAD51’’ and ‘‘bone tumors’’, ‘‘bone malignancy’’, ‘‘osteosarcomas’’, and ‘‘polymorphism’’, ‘‘polymorphisms’’, ‘‘variant’’, or ‘‘mutation’’. In addition, we checked references of all those retrieved articles to identify more relevant papers.

Selection Criteria

We used the following inclusion criteria to identify articles for our meta-analysis: (1) the studies assessed the association between the ERCC2, NBN, RAD51 polymorphisms, and osteosarcoma risk; (2) studies with a case-control or cohort design; and (3) studies provided sufficient published data to estimate an odds ratio (OR) with a 95% confidence interval (95% CI). In case of more than one eligible article with overlapping data by the same authors, we included the most recent or the largest one.

Data Extraction

In the current meta-analysis, two authors independently searched and identified the eligible articles based on the inclusion criteria. The following information were extracted from each study: first author, year of publication, country (ethnicity), number of cases and controls, and the genotype frequencies of the cases and controls.

Statistical Analysis

The statistical analysis for the current meta-analysis study was performed using the comprehensive meta-analysis (CMA) V2 software (Biostat, USA). In the current meta-analysis, all P values were considered two-sided, and P = 0.05 was set as the threshold value for statistical significance. To evaluate the associations between ERCC2 rs13181 polymorphism and osteosarcoma risk, the pooled odds ratio (OR) and associated 95% confidence interval (CI) were calculated under five genetic contrasts, including allelic (G vs. T), heterozygote (GT vs. TT), homozygote (GG vs. TT), dominant (GG+GT vs. TT), and recessive (GG vs. GT+TT). In addition, due to insufficient data, we used only the dominant genetic contrast to calculate ORs for ERCC2 rs1799793 (GA+AA vs. GG), NBN rs709816 (TT+TC vs. CC) and rs1805794 (CC+CG vs. GG), RAD51 rs1801320 (CC+CG vs. GG), rs1801321 (TT+GT vs. GG), and rs12593359 (GG+GT vs. TT) polymorphisms. The I2 statistics was also employed to assess the risks of heterogeneity. Therefore, in the current meta-analysis the I2 values of 25, 50, and 75% meant a low, moderate, and high heterogeneity, respectively, (Higgins et al., 2003; Khoram-Abadi et al., 2016). Moreover, a random effects model using the DerSimonian was utilized to calculate the OR and 95% CI for comparisons with moderate to high heterogeneity (P-value > 0.1 and $I^2 > 25\%$) (DerSimonian et al., 1986). If the Q test and the I2 statistics (P-value < 0.1 and $I^2 < 25\%$) both indicated no existence of heterogeneity, a fixed model with the Mantel-Haenszel method would be employed to pool data. We removed each study in turn for sensitivity analyses, and the remaining studies were reanalyzed to assess the stability of the meta-analysis results. Funnel plots and Egger’s test were used to examine publication bias (P<0.05) (Egger et al., 1997). Chi-Square test was used to determine the frequency distribution of genotypes, which were met the Hardy–Weinberg equilibrium (HWE).

Results

Characteristics of the Pooled Case-Control Ctudies

Through electronic search and considering the inclusion criteria, a total of seven relevant studies concerning ERCC2, NBN, and RAD51 gene polymorphisms and osteosarcoma were initially identified. However, three studies were excluded because they were about DNA repair genes association with osteosarcoma treatment. The final pool of eligible studies included four articles (Goričar et al., 2015; Jin et al., 2015; Ma et al., 2015; Wang et al., 2016) with 540 cases, and 1228 controls for ERCC2, NBN, and RAD51 gene polymorphisms in a sample of Slovenian population (Goricar et al. 2015) and three in Chinese population (Jin et al. 2015; Ma et al. 2015; Wang et al. 2016). All studies were case–control in design. In searching reference lists, relevant meta-analyses and reviews, we did not find additional papers. General characteristics of the included studies are summarized in Table 1.

Meta-Analysis Results

Table 2 lists the main results of the meta-analysis for ERCC2, NBN, and RAD51 polymorphisms. For ERCC2 rs13181 polymorphism, no significant association was found between this polymorphism and osteosarcoma when all eligible studies were pooled into meta-analysis in any genetic contrast (G vs. T: OR= 1.224, 95% CI: 0.970-1.545, p= 0.088; GT vs. TT: OR= 1.135, 95% CI: 0.830-1.552, p= 0.428; GG vs. TT: OR= 1.247, 95% CI: 0.738-2.108, p= 0.409; GG+GT vs. TT: OR= 1.174, 95% CI: 0.929-1.484, p= 0.179; GG vs. GT+TT: OR= 1.476, 95% CI: 0.886-2.460, p= 0.135).

In the current meta-analysis only the dominant genetic contrast was determined for ERCC2 rs1799793, NBN rs709816 and rs1805794, RAD51 rs1801320, rs1801321, and rs12593359 polymorphisms, due to insufficient data. However, we did not find a significant association between

Table 1. Characteristics of Studies Included in the Meta-Analysis of ERCC2, NBN, and RAD51 Gene Polymorphisms and Osteosarcoma.

First author	Country (Ethnicity)	Case/Control	Cases					Controls					HWE
			Genotype			Allele		Genotype			Allele		
ERCC2 rs13181			TT	TG	GG	T	G	TT	TG	GG	T	G	
Jin et al. 2015	China (Asian)	148/298	86	62				181	117				
Ma et al. 2015	China (Asian)	141/282	96	32	13	224	58	206	58	18	470	94	<0.001
Wang et al. 2016	China (Asian)	172/275	90	66	16	246	98	155	103	18	413	139	0.873
ERCC2 rs1799793			AA	AG	GG			AA	AG	GG	A	G	
Jin et al. 2015	China (Asian)	148/298	84	64				201	97				
Ma et al. 2015	China (Asian)	141/282	60	62	19	182	100	134	117	31	385	179	0.475
NBN rs709816			CC	CT	TT			CC	CT	TT			
Goricar et al. 2015	Slovenia (Caucasian)	79/373	29	50				139	234				
Jin et al. 2015	China (Asian)	148/298	55	93				115	183				
NBN rs1805794			GG	GC	CC			GG	GC	CC			
Goricar et al. 2015	Slovenia (Caucasian)	79/373	11	68				33	340				
Jin et al. 2015	China (Asian)	148/298	52	96				127	171				
RAD51 rs1801320			GG	GC	CC			GG	GC	CC			
Goricar et al. 2015	Slovenia (Caucasian)	78/373	69	9				304	69				
Jin et al. 2015	China (Asian)	148/298	101	47				213	85				
RAD51 rs1801321			GG	GT	TT			GG	GT	TT			
Goricar et al. 2015	Slovenia (Caucasian)	79/373	25	54				133	240				
Jin et al. 2015	China (Asian)	148/298	88	60				187	111				
RAD51 rs12593359			TT	TG	GG			TT	TG	GG			
Goricar et al. 2015	Slovenia (Caucasian)	79/373	22	57				103	270				
Jin et al. 2015	China (Asian)	148/298	49	99				105	193				

Table 2. Results of Meta-Analysis for ERCC2, NBN, and RAD51 Gene Polymorphisms and Osteosarcoma

Polymorphism	Genetic Model	Type of Model	Heterogeneity		Odds ratio		
			I^2 (%)	PH	OR	95% CI	POR
ERCC2 rs13181							
	G vs. T	Fixed	0	0.696	1.224	0.970-1.545	0.088
	GT vs. TT	Fixed	0	0.829	1.135	0.830-1.552	0.428
	GG vs. TT	Fixed	0	0.432	1.247	0.738-2.108	0.409
	GG+GT vs. TT	Fixed	0	0.909	1.174	0.929-1.484	0.179
	GG vs. GT+TT	Fixed	0	0.974	1.476	0.886-2.460	0.135
ERCC2 rs1799793	Dominant	Fixed	70.49	0.066	1.279	0.912-1.793	0.154
NBN rs709816	Dominant	Fixed	0	0.911	1.047	0.763-1.437	0.775
NBN rs1805794	Dominant	Fixed	73.32	0.053	1.126	0.789-1.608	0.513
RAD51 rs1801320	Dominant	Fixed	61.85	0.105	0.977	0.675-1.416	0.904
RAD51 rs1801321	Dominant	Fixed	0	0.902	1.167	0.848-1.604	0.343
RAD51 rs12593359	Dominant	Fixed	0	0.761	1.057	0.759-1.470	0.744

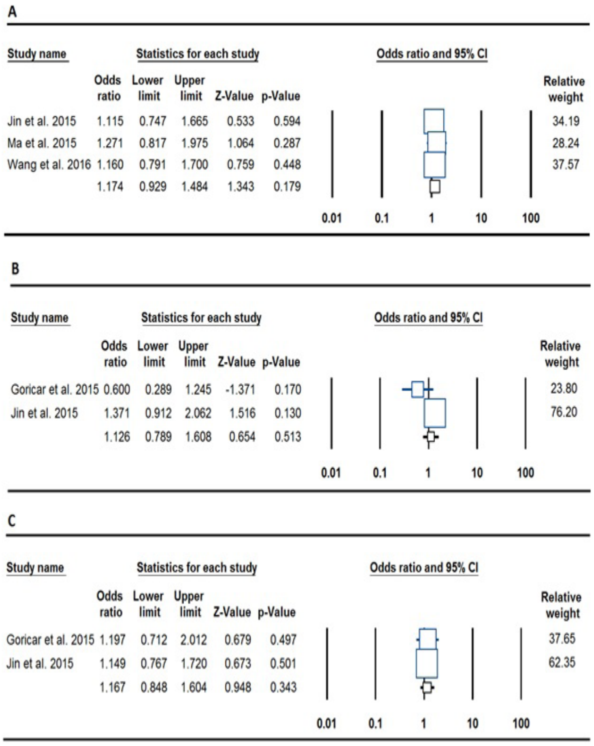

Figure 1. Forest Plot for the Association of the ERCC2, NBN, and RAD51 Gene Polymorphisms and Osteosarcoma. A: ERCC2 rs13181 (dominant: GG+GT vs. TT), B: NBN rs1805794 (dominant: TT+TC vs. CC) and C: RAD51 rs1801321 (GG+GT vs. TT).

the ERCC2 rs1799793 (GA+AA vs. GG: OR= 1.279, 95% CI: 0.912-1.793, p= 0.154), NBN rs709816 (OR= 1.047, 95% CI: 0.763-1.437, p= 0.775), NBN rs1805794 (OR= 1.126, 95% CI: 0.789-1.608, p= 0.513), RAD51 rs1801320 (OR= 0.977, 95% CI: 0.675-1.416, p= 0.904), RAD51 rs1801321 (TT+GT vs. GG: OR= 1.167, 95% CI: 0.848-1.604, p= 0.343), RAD51 rs12593359 (GG+GT vs. TT: OR= 0.761, 95% CI: 0.759-1.470, p= 0.744) polymorphisms and osteosarcomas.

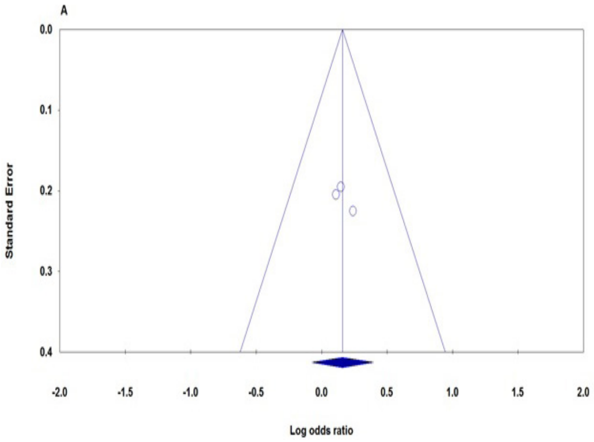

Figure 2. Begg's Funnel Plots of the ERCC2 rs13181 Polymorphism and Osteosarcoma Risk for Publication Bias Test in Dominant Genetic Contrast (GG+GT vs. TT). Each point represents a separate study for the indicated association.

Heterogeneity Test

We used Q-test and I^2 statistics to test the heterogeneity among the studies. No heterogeneity was observed in either polymorphisms (A vs. G) as well as the dominant genotype contrast for overall cancer, which was included for the analysis (Overall allele, A vs. G: Q=31.08, $P_{heterogeneity}$ = 0.186, I^2= 19.58; Overall dominant contrast, AA+GG vs. GG: Q= 25.76, $P_{heterogeneity}$ = 0.11, I^2 = 25.76).

Publication Bias

Funnel plot and Egger's linear regression were performed to assess the publication bias of the included studies. The shapes of the funnel plots did not reveal any evidence of obvious asymmetry (Figure 2). The results of Egger's test also showed no strong statistical evidence of publication bias (P_{Beggs} = 1.000, P_{Eggers}= 0.417, Figure 2).

Discussion

ERCC2, NBN, and RAD51 genes play an important role in DNA repair pathways (Damaraju et al., 2006). Several important single nucleotide polymorphisms (SNPs) have been identified in the loci. Recently, several case control epidemiological studies have evaluated the association between RAD51, ERCC2 and NBN polymorphisms and risk of a large range of cancers (Ji et al., 2015).

The Human RAD51 gene, one of the key proteins for homologous recombination (HR), plays a crucial role in maintaining the genetic stability of the cell through HR repair of DNA double-strand breaks (DSBs) (Richardson, 2005). It is well known that RAD51 is the major component of the HR pathway in combination of BRCA1 and BRCA2 (Roy et al., 2011). However, the nature of interaction between BRCA1 and RAD51 is still unknown. It is postulated that the association is likely to be indirect and possibly mediated by BRCA2 (O'Donovan et al., 2010). RAD51 is involved in the search for homology and strand pairing stages of the process. RAD51 overcomes internal DNA bonding forces to unwind and separate DNA strands and also to compete with other DNA binding proteins (Thacker, 2005). The human RAD51 gene is found to be located on chromosome 15q15.1, span >39 kb, contains 10 exons and encodes a 339 amino acid protein (Chen et al., 2016). RAD51 gene belongs to a region which exhibits a high loss of heterozygosity (LOH) in a large range of malignancies such lung, the colorectum, and the breast (Lose et al., 2006).

The excision repair cross-complementing rodent repair deficiency, group 2 (ERCC2) or XPD (Xeroderma Pigmentosum Complementary group D) plays an important role in transcription-coupled nucleotide excision repair (NER) pathway (Vashisht et al., 2015). ERCC2 is a 5'-3' ATP-dependent helicase as a subunit of the basal transcription factor TFIIH (Fukuda et al., 2001; Compe et al., 2012). Human XRCC1 gene maps to chromosome 19q13.32 and is composed of 23 exons. It spans approximately 54336 bp and encodes a protein of 760 amino acids with a molecular weight of 86,900 (Li et al., 2013; Forat-Yazdi et al., 2015). Several validated single nucleotide polymorphisms (SNPs) in ERCC2 are listed in the dbSNP database, which play a causative role in DNA repair-deficiency diseases, including Xeroderma Pigmentosum (XP), Trichothiodystrophy (TTD), and Cockayne Syndrome (CS) (Lehmann 2003). These diseases characterized by high ultraviolet-light hypersensitivity, a high mutation frequency, and cancer-proneness, as well as some mental and growth retardation and probably aging (Benhamou ET AL., 2005). Additionally, it is reported that different mutations in the ERCC2 (XPD) gene may lead to one of 6 different clinical disorders: XP, XP neurological disease, TTD, the XP/CS complex, XP/TTD complex or a severe form of CS known as COFS (cerebral, ocular, facial, skeletal syndrome) (Kraemer et al., 2007).

The NBN gene encoding a tumor suppressor protein belongs to the DNA DSBs repair genes and encodes a protein called nibrin (also known NBS1, p95 or NBN), which participates in several critical cellular functions, including the repair of damaged DNA (Czornak et al., 2008; Berardinelli et al., 2013). The gene is located on chromosome 8 at 8q21and consists of 16 exons, which spans a region of more than 50 kb. Its product nibrin is a 95-kDa protein, which forms a multiprotein complex with hMRE11 and hRAD50 (MRN) (OMIM; Uzunoglu et al., 2016). Mutations in the NBN gene lead to an autosomal recessive disorder named Nijemen breakage syndrome (NBS), a radiation-sensitivity disorder with multiple deficiencies, which may result in susceptibility to cancers such as breast cancer (Schröder-Heurich et al., 2014; Uzunoglu et al., 2016).

Considering the functional significance of ERCC2, NBN, and RAD51 genes in carcinogenesis, it is speculated that ERCC2, RAD51, and NBN polymorphisms may be a potential susceptibility factor for osteosarcoma. However, a few studies evaluated the association between ERCC2, NBN, and RAD51 polymorphisms and osteosarcoma risk (Goričar et al., 2015; Jin et al., 2015; Ma et al., 2015; Wang et al., 2016). To the best of our knowledge, this meta-analysis represents the first one investigating the association between ERCC2 (rs13181 and rs1799793), NBN (rs709816 and rs1805794), RAD51 (rs1801320, rs1801321, and rs12593359) polymorphisms and osteosarcoma risk. Notwithstanding, our results suggested that ERCC2 (rs13181 and rs1799793), NBN (rs709816 and rs1805794), RAD51 (rs1801320, rs1801321, and rs12593359) polymorphisms might not be associated with osteosarcoma risk. The finding was consistent with the individual study by Jin et al., reported the association of ERCC2 rs1799793 and NBN rs1805794 polymorphisms with risk of osteosarcoma (2015). Additionally, they held that these two polymorphisms are associated with an increased risk of osteosarcoma in females (Jin et al., 2015). However, the other included study, with 79 osteosarcoma cases and 373 controls from Slovenia, by Goricar et al. found no association between NBN rs709816 and rs1805794, RAD51 rs1801320, rs1801321, and rs12593359 polymorphisms and risk of osteosarcoma (2015). However, they demonstrated that the NBN rs1805794 polymorphism is significantly associated with risk of osteosarcoma in younger patients (<30 years) (Goričar et al., 2015; Jin et al., 2015). Therefore, these two individual studies indicated the age- and gender-specific effects of ERCC2 rs1799793 polymorphism and in particular NBN rs1805794 polymorphism on risk of the osteosarcoma development.

Although we conducted the first meta-analysis investigating the association between ERCC2, NBN and RAD51 genes polymorphism and risk of osteosarcoma, there were some limitations. First, the present meta-analysis involved only four studies, the meta-analysis was relatively small in comparison with other meta-analyses; therefore, we cannot generalize our findings. To validate the present meta-analysis results, more comprehensive studies with larger sample size must be included in future investigations. Second, we only included published articles and also searched literature written in English; hence, the existence of high risk of potential bias must be considered. Finally, the current meta-analysis was based on unadjusted ORs estimates, the eligible studies did not describe the age and gender distribution between cases and controls. Therefore, further evaluation of adjustments of risk factors such as age, gender and the potential interactions among gene–gene and gene–environment was not possible for us.

In summary, the current meta-analysis findings indicated no significant association between ERCC2 (rs13181 and rs1799793), NBN (rs709816 and rs1805794), RAD51 (rs1801320, rs1801321, and rs12593359) polymorphisms and osteosarcoma risk. Due to small sample size, this conclusion should be interpreted with caution. Further well-designed and large-scale studies are recommended considering gene–gene and gene–environment interactions for investigation of such associations.

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