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Association between *XRCC3* Thr241Met polymorphism and risk of osteosarcoma in a Chinese population

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ABSTRACT. Osteosarcoma is one of the most common bone malignancies in adolescents, and hereditary factors may influence its susceptibility. We assessed the association between *XRCC3* Thr241Met polymorphism and susceptibility to osteosarcoma in a Chinese population. Between May 2012 and May 2014, a total of 136 osteosarcoma patients and 136 healthy control subjects were included in our study. The *XRCC3* Thr241Met polymorphism was analyzed using a polymerase chain reaction restriction fragment length polymorphism assay. By multiple logistic regression analysis, individuals carrying the Met/Met genotype of *XRCC3* Thr241Met were at significantly increased risk of osteosarcoma when compared with the Thr/Thr (OR = 2.50, 95%CI = 1.13-5.66). The Thr/Met+Met/Met genotype of *XRCC3* Thr241Met was furthermore found to be correlated with an elevated increased risk of osteosarcoma when compared with the Thr/Thr genotype (OR = 1.71, 95%CI = 1.03-2.87), and Met/Met genotype of *XRCC3* Thr241Met was associated with an increased risk of osteosarcoma compared to the Thr/Thr (OR = 3.50, 95%CI = 1.51-8.79). In conclusion, our study firstly reports

that *XRCC3* Thr241Met gene polymorphism is associated with an elavated risk of osteosarcoma.

Key words: *XRCC3* Thr241Met; Polymorphism; Osteosarcoma; Chinese population

INTRODUCTION

Osteosarcoma is one of the most common bone malignancies in adolescents. Despite the application of multimodal treatment strategies of osteosarcoma (OS), the overall five years survival rate for osteosarcoma is 68%. The development of osteosarcoma is a complex and multifactorial process, involving various environmental and genetic factors (Powers et al., 2010;). Previous studies have reported that radiation, chemicals exposure and child history of hernias have been suggested as risk factors for this disease (Burningham et al., 2012; Thiagarajan and Iyer, 2014). Not all people exposed to similar risk factors develop osteosarcoma, however, which suggests that hereditary factors may influence susceptibility. Several previous studies have reported that gene polymorphisms play an important role in osteosarcoma, including variations in VEGF, FGF2, GSTs, NFKB1, RECQL5 and CTLA-4 genes (He et al., 2014; Zhi et al., 2014; Han et al., 2015; Li et al., 2015; Zhang et al., 2015; Wang et al., 2015).

It is well known that unrepaired DNA damage can cause cell apoptosis and unregulated cell growth, and thus increase the cancer susceptibility. The DNA repair genes are responsible for maintaining cellular functions and homeostasis, and the capacity of DNA repair could be changed by DNSA sequence variations. X-ray repair cross-complementing group 3 *(XRCC3)* is a member of the DNA repair genes and is involved in the process of homologous recombination repair pathway (Griffin et al., 2000). The function of *XRCC3* is to maintain the stability of the genome and repair DNA damage (Griffin et al., 2000). *XRCC3* gene is localized on human chromosomes 14q32.3. Codon 241 (Thr241Met) is the most frequent polymorphism in *XRCC3*, and its polymorphism results in amino acid from C to T transition (Shen et al., 1998). Polymorphism in the *XRCC3* Thr241Met can influence the function of the encoded protein and thus change the capacity of DNA repair (Matullo et al., 2001). Currently, an association between *XRCC3* Thr241Met polymorphism and the risk of osteosarcoma, however, has not been reported. The aim of this study was to the association between *XRCC3* Thr241Met polymorphism and susceptibility to osteosarcoma.

MATERIAL AND METHODS

Study population

This study was a hospital-based case-control study. Between May 2012 and May 2014, patients with pathologically diagnosed osteosarcoma were enrolled from the Second Hospital of Inner Mongolia Medical University. A total of 136 patients were newly diagnosed and included in our study. A total of 136 control subjects without cancers were selected from the Second Hospital of Inner Mongolia Medical University during the same period. The control subjects were frequently matched to cases by age (± 5 years).

Demographic and clinical information of patients were collected from medical records, including histological type, tumor location and stage. The clinical features of the study subjects are described in Table 1. A signed informed consent form was obtained from every participant (test and

control) before their inclusion in the study. This protocol was approved by the Second Hospital of Inner Mongolia Medical University.

DNA extraction and SNP genotyping

Each subject was asked to provide a 5-mL peripheral venous blood for DNA extraction. According to the manufacture's instructions, the TIANamp Blood DNA kit (Tiangen Biotech Co., Ltd., Beijing, China) was taken to extract genomic DNA from peripheral blood samples. The *XRCC3* Thr241Met polymorphism was analyzed using a polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay. The forward and reverse primers for the *XRCC3* Thr241Met were forward 5'-GGTCGACAGAGTGTCCAAAC-3' and reverse 5'-CTACCCGCAGGAGGGCCACC-3', respectively. The PCR amplification conditions were as follows: a 8 minutes denaturation step at 94°C followed by 30 cycles of 95°C for 60 seconds (denaturation), 60°C for 60 seconds (annealing), and 72°C for 1 minute (extension). Amplified products were digested with restriction enzyme of 10 U FastDigest *Nla*III at 37°C for 10 min, yielding products of 205 bp in length for *XRCC3* Thr241Met. The resulting DNA fragments were analyzed by electrophoresis on a 2% agarose gel stained with ethidium bromide and visualized under UV light. Three patterns were observed: 104, 141 and 170bp band for Met allele, and 141 and 274bp bands for Thr allele.

Statistical analysis

Statistical differences between osteosarcoma patients and controls regarding demographic and clinical characteristics were assessed by a Chi-squared test or a Fisher's exact test. Departures from the Hardy-Weinberg equilibrium (HWE) were analyzed using a Chi-squared test with one degree of freedom (Genetic Data Analysis program version 1.1). Multiple logistic regression models were established to estimate the association between the *XRCC3* Thr241Met polymorphism and osteosarcoma risk. The quantification of statistically significant association was estimated by adjusted odds ratio (OR) and their associated 95% confidence intervals (CIs). The most common control homozygote of XRCC3 Thr241Met was taken as reference for analysis. Statistical analysis was conducted using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Differences with two-tailed P values < 0.05 were considered statistically significant.

RESULTS

The distributions of demographic and clinical characteristics are shown in Table 1. The mean ages of osteosarcoma patients and controls were 17.82 ± 5.40 and 17.31 ± 5.52 years, respectively. There were no significant differences in the sexes (χ^2 = 0.75, P = 0.39) and ages (χ^2 = 0.06, P = 0.80) between osteosarcoma group and the control group. Among the 136 patients, 83 (61.03%) had I-II Enneking stage, 53 (38.97%) had III Enneking stage, 80 (58.82%) were osteoblastic type, 21 (15.44%) were chondroblastic type, 17 (12.50%) were fibroblastic type, 86 (63.24%) were located in the extremities and 50 (36.76%) were located elsewhere.

Genotype frequencies of the *XRCC3* Thr241Met gene in osteosarcoma patients and controls are summaried in Table 2. The observed genotype frequencies of *XRCC3* Thr241Met in osteosarcoma patients and controls conformed with the Hardy-Weinberg equilibrium, and the P values of HWE were 0.28 and 0.95 for osteosarcoma patients and controls, respectively. By

means of a χ^2 test, significant differences were found in the genotype frequencies of *XRCC3* Thr241Met between patients and controls in codominant (χ^2 = 6.27, P = 0.04), dominant (χ^2 = 4.78, P = 0.03) and recessive models (χ^2 = 10.37, P = 0.001). By multiple logistic regression analysis, individuals carrying the Met/Met genotype of *XRCC3* Thr241Met were at significantly increased risk of osteosarcoma when compared with the Thr/Thr genotype (OR = 2.50, 95%CI = 1.13-5.66). The Thr/Met+Met/Met genotype of *XRCC3* Thr241Met was furthermore found to be correlated with an elevated increased risk of osteosarcoma when compared with the Thr/Thr genotype (OR = 1.71, 95%CI = 1.03-2.87), and the Met/Met genotype of *XRCC3* Thr241Met was associated with an increased risk of osteosarcoma compared to the Thr/Thr genotype (OR = 3.50, 95%CI = 1.51-8.79).

Stratification analysis showed no significant correlation of *XRCC3* Thr241Met polymorphism with Enneking stage, tumor location, or histological subtype in the risk of osteosarcoma (Table 3). No interaction was moreover identified between *XRCC3* Thr241Met polymorphism and genders and ages (P for interaction > 0.05).

Table 1. The demographic and clinical characteristics of patients with osteosarcoma and control subjects.

Characteristics	Patients	%	Controls	%	χ^2 test	P value
Gender						
Female	52	38.24	59	43.60		
Male	84	61.76	77	56.40	0.75	0.39
Age, years						
<20	85	62.50	87	63.97		
≥20	51	37.50	49	36.03	0.06	0.80
Enneking stage						
I-II	83	61.03				
III	53	38.97				
Histological subtype						
Osteoblastic	80	58.82				
Chondroblastic	21	15.44				
Fibroblastic	17	12.50				
Other	18	13.24				
Tumor location						
Extremities	86	63.24				
Other	50	36.76				

Table 2. Association between *XRCC3* Thr241Met gene polymorphism and osteosarcoma risk.

XRCC3 Thr241Met	Patients	%	Controls	%	χ^2 value	P value	OR (95%CI)[1]	P value
Codominant								
Thr/Thr	54	39.71	70	51.47			1.0 (Ref.)	-
Thr/Met	55	40.44	52	38.24			1.37 (0.79-2.38)	0.23
Met/Met	27	19.85	14	10.29	6.27	0.04	2.50 (1.13-5.66)	0.01
Dominant								
Thr/Thr	54	39.71	70	51.47			1.0 (Ref.)	-
Thr/Met + Met/Met	82	60.29	62	45.59	4.78	0.03	1.71 (1.03-2.87)	0.03
Recessive								
Thr/Thr + Thr/Met	109	80.15	127	93.38			1.0 (Ref.)	-
Met/Met	27	19.85	9	6.62	10.37	0.001	3.50 (1.51-8.79)	0.001

[1]Adjusted for sex and age. ORR: odds ratio; CI: confidence interval.

Table 3. Stratification analysis of the association between *XRCC3* Thr241Met polymorphism and osteosarcoma risk by clinical characteristics.

Variables	Number	%	Thr/Thr	%	Thr/Met + Met/Met	%	OR(95%CI)[1]	P value
Enneking stage			54		82			
I-II	83	61.03	31	57.41	52	63.41	1.0 (Ref.)	
III	53	38.97	23	42.59	30	36.59	1.29 (0.60-2.75)	0.48
Tumor location								
Other	50	36.76	19	35.19	31	37.80	1.0 (Ref.)	
Extremities	86	63.24	35	64.81	51	62.20	1.12 (0.52-2.45)	0.76
Tumor metastasis								
Osteoblastic	80	58.82	31	57.41	49	59.76	1.0 (Ref.)	
Chondroblastic	21	15.44	9	16.67	12	14.63	1.19 (0.39-3.48)	0.73
Fibroblastic	17	12.5	14	25.93	21	25.61	1.05 (0.43-2.55)	0.9

OR: odds ratio; CI: confidence interval.

DISCUSSION

In this hospital-based case-control study, the role of three important polymorphisms of the *XRCC3* Thr241Met gene in osteosarcoma risk as well as their interaction of this polymorphism with clinical and demographic factors in the development of osteosarcoma were assessed. The results of this study revealed that *XRCC3* Thr241Met polymorphism is associated with an increased risk of osteosarcoma in codominant, dominant and recessive models, suggesting that variants in *XRCC3* Thr241Met are involved in susceptibility to this disease.

It is well known that *XRCC3* gene is a member of the Rad51-related protein in human, which plays an important role in homologous recombination to maintain genome integrity and repair DNA damage. Defective double-strand break repair of cells in human could contribute to carcinogenesis. Polymorphisms in *XRCC3* result in alteration in DNA repair efficiency, and thus influence the cancer susceptibility in human. Several recent studies have investigated the association between *XRCC3* Thr241Met polymorphism and different types of cancers, but the results are contradictory. Some studies have found that *XRCC3* Thr241Met polymorphism is associated with a high risk of cancer, such as glioma, hepatocellular carcinoma, colorectal cancer, breast cancer, head and neck cancer and gastric cancer (Duan et al., 2013; Luo et al., 2013; Kayani et al., 2014; Nissar et al., 2014; Qureshi et al., 2014). Duan and colleagues investigated the role of *XRCC3* Thr241Met polymorphism in hepatocellular carcinoma, and reported that the Met/Met and Thr/Met+Met/Met genotypes were significantly associated with hepatocellular carcinoma in a Chinese population (Duan et al., 2013). Luo amd colleagues conducted a case-control study with 297 glioma cancer patients and 458 cancer-free controls in a Chinese population, and reported that *XRCC3* Thr241Met polymorphism was associated with an elevated risk of gliomas and meningiomas (Luo et al., 2013). Kayani and colleagues investigated the role of *XRCC3* Thr241Met gene polymorphism in head and neck cancer and reported that *XRCC3* Thr241Met polymorphism was an independent risk factor for head and neck cancer (Kayani et al., 2014). Nissar and colleagues conducted a study with 120 colorectal cancer and 150 healthy subjects in an Indian population, and reported that Thr/Met and Met/Met Genotypes of *XRCC3* Thr241Met were corrected with colorectal cancer (Nissar et al., 2014).

However, some studies have shown different results (Xu et al., 2013; Bănescu et al., 2014; Xing et al., 2014; Bashir et al., 2015). Bănescu and colleagues reported that the *XRCC3* Thr241Met polymorphism was not associated with the risk of chronic myeloid leukemia (Bănescu et al., 2014). Xu and colleagues revealed that *XRCC3* Thr241Met polymorphism was not associated with the development of lung cancer in a meta-analysis (Xu et al., 2014). Bashir and colleagues conducted

a study with 80 gastric cancer patients and 70 healthy controls, and reported that the Thr/Met and Met/Met genotypes of *XRCC3* Thr241Met may not play a role in the development of gastric cancer (Bashir et al., 2015). These discrepancies between different studies may result from differences in the cancer types, and study designs between studies as well as sample sizes.

To date, no association between *XRCC3* Thr241Met and risk of osteosarcoma has been reported. Our study firstly reports that *XRCC3* Thr241Met gene polymorphism is associated with an elevated risk of osteosarcoma. These findings certainly warrant further investigation using larger sample sizes for confirmation.

Conflicts of interest

The authors declare no conflict of interest.

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