Contribution of the *GSTP1* gene polymorphism to the development of osteosarcoma in a Chinese population

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ABSTRACT. We conducted a case-control study to investigate the associations between *GSTT1*, *GSTM1*, and *GSTP1* gene polymorphisms and development of osteosarcoma in a Chinese population. Between January 2013 and February 2015, 153 patients diagnosed with osteosarcoma and 252 control subjects were enrolled in the current study from the Orthopedic Hospital of the Second Hospital of Jilin University. The *GSTM1*, *GSTT1*, and *GSTP1* gene polymorphisms were detected by polymerase chain reaction coupled with restriction fragment length polymorphism analysis. As determined by a multiple-logistic regression analysis, the Val/Val genotype of *GSTP1* was associated with a significantly increased risk of osteosarcoma

compared to that of the Ile/Ile genotype, with an odds ratio (OR) = 3.39, and a 95% confidence interval (CI) = 1.45-8.13. Moreover, the Ile/Val+Val/Val genotype of *GSTP1* was correlated with a marginally significant increased risk of osteosarcoma compared to that of the Ile/Ile genotype (OR = 1.65, 95%CI = 1.08-2.53). However, we did not find any significant associations between the *GSTM1* and *GSTT1* gene polymorphisms and osteosarcoma risk. In conclusion, our results suggest that the *GSTP1* gene polymorphism is associated with an increased risk of osteosarcoma, whereas the *GSTM1* and *GSTT1* gene polymorphisms may not influence the development of this cancer.

Key words: *GSTM1*; *GSTT1*; *GSTP1*; *GSTs*; Polymorphism; Osteosarcoma

INTRODUCTION

Osteosarcoma is the leading cause of bone malignancy in adolescents. The etiology of osteosarcoma is not well understood, and it is reported that many environmental factors including ionizing radiation and a family history of osteosarcoma may contribute to the development of this cancer (Ottaviani and Jaffe, 2009). Previous studies have suggested that many gene polymorphisms may be involved in the development of osteosarcoma, such as those in the angiogenic growth factor genes *VEGF*, *FGF2*, *ERCC1*, *ERCC2*, and *GSTs* (Zhang et al., 2015a,b; Wang et al., 2015a; Jin et al., 2015; Han et al., 2015; Bian et al., 2015).

Glutathione S transferases (GSTs) comprise a homologous dimer enzyme gene family of detoxifying enzymes that catalyze the conjugation of glutathione with a broad spectrum of endogenous and exogenous compounds. Gene variations in *GSTs* may alter the expression levels of enzymes and affect their detoxification abilities. Previous studies have reported that the *GSTT1*, *GSTM1*, and *GSTP1* gene polymorphisms are involved in the development of several kinds of cancers, such as lung cancer, acute lymphoblastic leukemia, breast cancer, skin cancer, head and neck cancer, and oral cancer (Sharma et al., 2015; Guven et al., 2015; Jaramillo-Rangel et al., 2015; Lei et al., 2015; Choudhury et al., 2015; Krüger et al., 2015). Here, we conducted a case-control study to investigate the association between the *GSTT1*, *GSTM1*, and *GSTP1* gene polymorphisms and development of osteosarcoma in a Chinese population.

MATERIAL AND METHODS

Subjects

Between January 2013 and February 2015, 153 patients diagnosed with osteosarcoma from the Orthopedic Hospital of the Second Hospital of Jilin University and the Affiliated Southeast Hospital of Xiamen University were enrolled in the current study. All osteosarcoma diagnoses were confirmed by histopathologic examination. Patients who had primary tumors other than osteosarcoma were excluded. During the same period, 252 subjects were randomly selected from those who obtained a health examination at

the Second Hospital of Jilin University and the Affiliated Southeast Hospital of Xiamen University as the control group. Subjects who had a history of cancers were excluded from the study.

The demographic and clinical data from the osteosarcoma patients and control subjects were obtained from their medical records. The demographics included gender, age, smoking status, alcohol use, and familial history of cancer. The clinical data included tumor location and histological subtypes. Written informed consent was obtained from the participants prior to their enrollment in the study. The protocol of this study was previously approved by the Institutional Research Ethics Committee of the Orthopedic Hospital of the Second Hospital of Jilin University.

DNA extraction and genotyping

Peripheral blood (5 mL) was obtained from each patient and control subject after enrollment in this study. DNA was extracted from peripheral blood samples using the TIANamp Blood DNA Kit according to manufacturer instruction (Tiangen Biotech, Beijing, China). The *GSTM1*, *GSTT1*, and *GSTP1* gene polymorphisms were detected by polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP) analysis. Primer sequences for *GSTM1*, *GSTT1*, and *GSTP1* were designed using the Primer Premier v5.0 software (Premier Biosoft International, Palo Alto, CA, USA) (Table 1). The PCR product lengths for *GSTM1*, *GSTT1*, and the β-globin locus were 215, 480, and 268 bp, respectively. The product lengths for the *GSTP1* Val allele were 176, 91, and 85 bp, and they were 91 and 85 bp for the Val allele.

Table 1. Primers for *GSTM1*, *GSTT1*, and *GSTP1*, and β-globin locus.

Genes	Primers (5'-3')
GSTM1	CTG CCC TAC TTG ATT GAT GGG CTG GAT TGT AGC AGA TCA TGC
GSTT1	TTC CTT ACT GGT CCT CAC ATC TC TCA CCG GAT CAT GGC CAG CA
GSTP1	ACCAGGGCTCTATGGCCAA TGA CCC GAG AAG AAC GGG T
β-globin locus	GAA GAG CCA AGG ACA GGT AC CAA CTT CAT CCA CGT TCA CC

Statistical analysis

The association between demographic and clinical data and development of osteosarcoma were analyzed using univariate-logistic regression analysis. The Hardy-Weinberg equilibrium (HWE) was assessed with the goodness-of-fit chi-squared (χ^2) test. The associations between *GSTM1*, *GSTT1*, and *GSTP1* gene polymorphisms and risk of osteosarcoma were estimated using multiple-logistic regression analysis, and the results are reported using odds ratios (ORs) and 95% confidence intervals (95%CIs). All statistical analyses were conducted using the SPSS statistical software package, version 17.0 (SPSS Inc., Chicago, IL, USA). All tests were two-sided, with P values less than 0.05 considered as statistically significant.

RESULTS

The demographic and clinical characteristics of osteosarcoma patients and control subjects are shown in Table 2. No significant differences were found between osteosarcoma patients and control subjects in terms of gender, smoking status, alcohol use, or familial history of cancer. However, we found that patients with osteosarcoma were significantly younger than the control subjects (OR = 0.33, 95%CI = 0.21-0.51). Of the 153 samples obtained from patients with osteosarcoma, 86 (56.21%) were of the osteoblastic type, 31 (20.26%) were of the chondroblastic type, 26 (16.99%) were of the fibroblastic type, and 10 (6.54%) of other types, 105 (68.63%) were at the extremities, and 48 (31.37%) were at other locations.

Table 2. Demographic and clinical characteristics of osteosarcoma patients and control subjects.

Characteristics	Patients (N = 153)	%	Controls (N = 252)	%	OR (95%CI)	P value
Age (years)						
<20	103	67.32	102	40.48	1.0 (Ref.)	
≥20	50	32.68	150	59.52	0.33 (0.21-0.51)	<0.001
Gender						
Female	61	39.87	114	45.24	1.0 (Ref.)	
Male	92	60.13	138	54.76	1.25 (0.81-1.91)	0.29
Smoking status						
No	128	83.66	218	86.51	1.0 (Ref.)	
Yes	25	16.34	34	13.49	1.25 (0.68-2.27)	0.43
Alcoholuse						
No	123	80.39	219	86.90	1.0 (Ref.)	
Yes	30	19.61	33	13.10	1.62 (0.91-2.88)	0.08
Family history of cancer						
No	134	87.58	235	93.25	1.0 (Ref.)	
Yes	19	12.42	17	6.75	1.96 (0.93-4.16)	0.05
Histological subtype						
Osteoblastic	86	56.21				
Chondroblastic	31	20.26				
Fibroblastic	26	16.99				
Others	10	6.54				
Tumor location						
Extremities	105	68.63				
Others	48	31.37				

The genotype frequencies of *GSTM1*, *GSTT1*, and *GSTP1* genes are shown in Table 3. As determined by χ^2 test, a significant difference in the frequency of *GSTP1* was found between osteosarcoma patients and control subjects (χ^2 = 10.77, P = 0.01). However, no significant differences were found in the frequencies of *GSTM1* (χ^2 = 10.77, P = 0.01) or *GSTT1* (χ^2 = 10.77, P = 0.01) between patients and controls. As determined by a multiple-logistic regression analysis, the Val/Val genotype of *GSTP1* was associated with a significantly increased risk of osteosarcoma compared to that of the Ile/Ile genotype (OR = 3.39, 95%CI = 1.45-8.13). Moreover, the IIe/Val+Val/Val genotype of *GSTP1* was correlated with a marginally significant increased risk of osteosarcoma compared to that of the Ile/Ile genotype (OR = 1.65, 95%CI = 1.08-2.53). However, we did not find any significant associations between the *GSTM1* and *GSTT1* gene polymorphisms and osteosarcoma risk.

Table 3. Association between *GSTM1*, *GSTT1*, and *GSTP1* gene polymorphisms and risk of osteosarcoma.

Gene	Patients	%	Controls	%	χ^2 test	P value	OR (95%CI)[1]	P value
GSTM1								
Present	88	57.52	157	62.30			1.0 (Ref.)	
Null	65	42.48	95	37.70	0.91	0.34	1.22 (0.79-1.88)	0.34
GSTT1								
Present	85	55.56	145	57.54			1.0 (Ref.)	
Null	68	44.44	107	42.46	0.15	0.70	1.08 (0.71-1.66)	0.70
GSTP1								
Ile/Ile	64	41.17	135	53.57			1.0 (Ref.)	
Ile/Val	71	46.41	105	41.67			1.45 (0.93-2.27)	0.09
Val/Val	19	12.42	12	4.76	10.77	0.01	3.39 (1.45-8.13)	0.002
Ile/Val+Val/Val	90	58.82	117	46.43			1.65 (1.08-2.53)	0.02

[1]Adjusted for gender, age, smoking, drinking, and familial history of cancer.

DISCUSSION

In the current study, we investigated whether the *GSTM1*, *GSTT1*, and *GSTP1* gene polymorphisms influenced the development of osteosarcoma in a Chinese population, and found that the *GSTP1* gene polymorphism did indeed affect osteosarcoma susceptibility.

Previous studies have reported on the correlation between the *GSTP1* gene polymorphism and several kinds of cancers, such as lung cancer, acute lymphoblastic leukemia, breast cancer, skin cancer, head and neck cancer, and oral cancer (Choudhury et al., 2015; Guven et al., 2015; Jaramillo-Rangel et al., 2015; Krüger et al., 2015; Lei et al., 2015; Sharma et al., 2015). For example, Sharma et al. (2015) conducted a study in an Indian population, and reported that the null *GSTT1* and wild-type *GSTP1* genotypes were associated with an increased risk of lung cancer. However, Guven et al. (2015) suggested that the *GST* variants might not influence the risk of developing childhood acute lymphoblastic leukemia in a Turkish population. Furthermore, Jaramillo-Rangel et al. (2015) reported that the *GSTM1*-null genotype was associated with breast cancer risk in a Mexican population. Lei et al. (2015) demonstrated that the *GSTP1* 105Val polymorphism might contribute to the development of skin cancer in a Chinese population. Choudhury et al. (2015) reported that the *GSTM1*-null genotypes were associated with head and neck cancer susceptibility in an Indian population. Lastly, Krüger et al. (2015) reported that the *GSTM1* deletion could help to identify German patients at risk for oral cancer development.

For the reported association between *GST* gene polymorphisms and the development and prognosis of osteosarcoma (Li et al., 2014), two meta-analyses have further evaluated their correlations (Wang et al., 2015b; Han et al., 2015). Specifically, Li et al. (2014) reported that the GG genotype of *GSTP1* was significantly associated with overall survival of osteosarcoma in a Chinese population. Wang et al. (2015b) conducted a meta-analysis of six case-control studies, and did not find any association between *GSTT1* polymorphisms and osteosarcoma risk. However, Han et al. (2015) performed a pooled meta-analysis of three case-control studies, and their results revealed a significant association between the *GSTT1*-null genotype and risk of developing osteosarcoma. The results herein indicate that the *GSTP1* gene polymorphism may affect osteosarcoma susceptibility in a Chinese population. The discrepancies between these different studies may be due to different study populations, sample sizes, and/or selection criteria for patients and controls.

In conclusion, the results of the current study indicate that the *GSTP1* gene

polymorphism is associated with an increased risk of osteosarcoma. Further studies are needed to elucidate the impact of *GST* gene polymorphisms in the risk of osteosarcoma.

Conflicts of interest

The authors declare no conflict of interest.

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