RESEARCH COMMUNICATION

Glutathione S-transferase Polymorphisms and Bone Tumor Risk in China

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Abstract

Aim: We aimed to study the potential role of GSTM1 and GSTT1 in the risk of osteosarcoma in Chinese population. Methods: We collected 110 osteosarcomas by pathologic examination and 226 controls from the First Affiliated Hospital of Harbin Medical University during December 2008 to December 2010. Genotyping was based upon duplex polymerase-chain-reaction with the PCR-CTPP method. Results: Individuals carrying null GSTM1 and GSTT1 had 1.50 and 2.07 fold risks of osteosarcoma when compared with non-null genotypes, respectively. The increased risk associated with the GSTT1 polymorphism seemed more evident among males (Null GSTT1 genotype vs. non-null genotype, adjusted OR= 2.43, 95% CI: 1.29-3.30) than females (adjusted OR =1.66, 95% CI: 1.02-2.78). The increased risk was also more evident among individuals aged 15 years or less (adjusted OR for null GSTT1 genotype vs. non-null genotype = 2.24, 95% CI: 1.20-3.24) than those aged more than 15 years (adjusted OR = 1.82, 95% CI: 1.07-2.95). Conclusion: Our study of the association between polymorphisms in GSTM1 and GSTTI and the risk of osteosarcoma in a Chinese population provided evidence that null GSTTI might be a useful marker of susceptibility to osteosarcoma development, especially for male sand young age individuals.

Keywords: GSTM1 - GSTT1 - polymorphisms - osteosarcoma

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Introduction

Osteosarcomas derive from primitive bone-forming mesenchymal cells and is the most common primary bone malignancy, and which is the most common malignant bone tumor in children and adolescents. The risk of being diagnosed with cancer increases as an individual ages, and 77% of all cancers are diagnosed in persons aged 55 years and above. As a lifetime risk, the probability that an individual, over the course of a lifetime, will develop a cancer is slightly less than one in two for men and a little more than one in three for women (ACS, 2007; US Cancer Statistics Working Group, 2007; Ries et al., 2009). The etiology of OS still remains unknown, it may be considered to be caused by an interaction of environmental results and genetic susceptibility. Studies to determine the etiology of osteosarcoma involve epidemiologic and environmental factors, and genetic impairments.

Recently evidence indicated that carcinogen-metabolizing genes and DNA-repair genes may play critical roles in determining individual susceptibility to cancers. Polymorphisms in these genes encoding the enzymes, possibly by altering their expression and function, may increase or decrease carcinogen activation or detoxication and modulate DNA repair.

Xenobiotics can be detoxified by phase II enzymes, such as GSTM1 and GSTT1 which have been suggested to be involved in detoxification of polycyclic aromatic hydrocarbons (PAHs) and benzo(a)pyrene (Schneider et al., 2004), which could detoxify carcinogens and reactive oxygen species (Rebbeck, 1997). Individuals who have homozygous deletions for GSTM1 and GSTT1 gene have no GSTM1 and GSTT1 enzyme activity. Lack of these enzymes may potentially increase cancer susceptibility because of a decreased ability to detoxify carcinogens such as benzo[α]pyrene-7,8-diol epoxide, the activated form of benzo[α]pyrene. Previous published studies have focused on relationship between GSTM1 and GSTTI and the risk of osteosarcoma patients (Barnette et al., 2004). However, no evidence from Chinese populations about the relationship between GSTM1 and GSTTI and osteosarcoma patients. Therefore, we conducted a hospital-based case-control study, a province in north China, to evaluate the association between polymorphisms in GSTM1 and GSTTI genes and the risk of osteosarcoma.

Materials and Methods

This case-control study was conducted in the First Affiliated Hospital of Harbin Medical University, a province in North China. 124 Chinese cases with newly diagnosed osteosarcoma between January 2008 and January 2011 in these hospitals were invited participate within two months after diagnosis. All cases recruited in

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Table 1. Demographic Characteristics Among Cases and Controls

Variable	Cases (N=110)	Controls (N=226)	p value*
Age, years, mean ± SD	13.6±3.2	13.8±2.9	0.716
Sex, N (%)			
Male	66 (60.0)	130 (57.5)	0.66
Female	44 (40.0)	96 (42.5)	
Family history of cancer, N (%)			
Yes	15(13.6)	3 (1.3)	<0.05
No	95 (86.4)	223 (98.7)	

this study were histologically confirmed. Among a total of 124 eligible cases, 110 were successfully interviewed and donoted blood samples with a participation rate of 88.7%. Controls were randomly selected from outpatients without cancer history in the same hospital during the same period. Controls were required to be without any history of any type of cancer and frequency matched by five-year age groups. Among a total of 241 eligible controls, 226 were successfully interviewed and donated blood samples with a participation rate of 93.8%. Informed consent was obtained before each interview and blood taking. Trained interviewers conducted face-to-face interviews using a structured questionnaire to collect information on sociodemographic characteristics, family history of cancer, and other potential confounders. Approval to conduct this study was granted by the Ethics Committee of the First Affiliated Hospital of Harbin Medical University. All interviews and blood samples collection were conducted after obtaining signed informed consent from participants.

Genotyping

Genomic DNA was extracted from whole-blood samples using the Qiagen Blood Kit (Qiagen, Chastworth, CA). Genotyping was conducted using TaqMan assays (Applied Biosystems, Foster City, CA). Primer and probe sets were designed and manufactured using Applied Biosystems 'Assay-by-Design' custom service (Applera, Austria). General TaqMan reaction conditions were as described previously (Salinas-Souza et al., 2010). We also performed the genotyping of internal positive control samples, use of no template controls, and use of replicates for 10% samples for quality control.

Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software 13.0 for windows. Chi square or t tests were used to test differences of sociodemographic factors and potential confounders between the cases and controls. Deviation of genotype frequency distribution in controls from those expected under Hardy-Weinberg Equilibrium (HWE) was assessed using chi square tests. Unconditional logistic regression was used to estimate the odds ratios (ORs) and corresponding 95% confidence intervals (CIs) for each polymorphism. The associations between each polymorphism and risk of osteosarcoma were further examined after adjusting for potential confounders using multivariate logistic regression models. Inclusion of potential confounders was based on biological and statistical considerations.

Table 2. Associations Between Polymorphisms in GSTM1 and GSTT1 Genes and the Risk of Osteosarcoma

Genotype	Cases N=110(%)	Controls N=226(%)	Crude OR	Adjusted OR[1]
GSTM1				
Non-null	49 (44.5)	122(54.0)	1.46(0.90-2.37)	1.50(0.92-2.41)
Null	61(55.5)	104(46.0)		
GSTT1				
Non-null	40(36.4)	115(51.9)	1.81(1.11-2.98)	2.07(1.19-3.04)
Null	70(63.6)	111(49.1)		

[1]Adjusted for age, sex and family history

Results

The distribution basic characteristics among cases and controls are shown in Table 1. The mean age was 14.3 years among cases and 15.1 years among controls. There were no significant differences between cases and controls in age and sex distribution. More cases than controls had family cancer history ($p<0.05$).

Table 2 showed the genotype frequencies of the two gene polymorphisms in cases and controls and their corresponding ORs with CIs. Polymorphisms in Non-null and Null GSTM1 showed no statistically significant difference between glioma cases and controls. However, the genotype distribution of Non-null and Null GSTT1 differed between glioma cases and controls. Individuals with the Null GSTT1 genotype had a significantly increased risk of developing osteosarcoma compared with

Table 3. Stratified Analyses Between GSTM1 and GSTT1 Gene Polymorphism and the Risk of Osteosarcoma

Variables	Cases/Controls 110/226	Adjusted OR[1] (95% CI) GSTM1		Adjusted OR[1] (95% CI) GSTT1	
		Non-null	Null	Non-null	Null
Age					
< 15 years	59/124	1.0(Reference)	1.53(0.94-1.85)	1.0(Reference)	2.24(1.20-3.24)
≥ 15 years	51/102	1.0(Reference)	1.42(0.88-1.79)	1.0(Reference)	1.82(1.07-2.95)
Sex					
Male	66/130	1.0(Reference)	1.60(0.97-2.11)	1.0(Reference)	2.43(1.29-3.30)
Female	44/96	1.0(Reference)	1.37(0.75-1.63)	1.0(Reference)	1.66(1.02-2.78)
Family history of cancer, N (%)					
Yes	3/15	1.0(Reference)	1.33(0.06-91.6)	1.0(Reference)	2.28(0.94-151)
No	95/223	1.0(Reference)	1.48(0.65-2.04)	1.0(Reference)	1.69(0.83-1.85)

[1]Adjusted for age, sex and family history

those with the non-null genotype (adjusted OR = 2.07, 95% CI: 3.04). The distributions of the three genotype frequencies were in agreement with those expected from the HWE model at the 0.05 level for controls (P=0.45, and 0.13 for GSTM1 and GSTTI, respectively).

We further performed subgroup analyses stratified by age, sex and family history of cancer for GSTM1 and GSTTI polymorphism in table 3. The increased risk associated with the GSTT1 polymorphism seemed more evident among males (Null GSTT1 genotype vs. non-null genotype, adjusted OR= 2.43, 95% CI: 1.29-3.30) than females (adjusted OR =1.66, 95% CI: 1.02-2.78). The increased risk was also more evident among individuals aged 15 years or less (adjusted OR for null GSTT1 genotype vs. non-null genotype = 2.24, 95% CI: 1.20-3.24) than those aged more than 15 years (adjusted OR = 1.82, 95% CI: 1.07-2.95). The increased risk associated with GSTT1 polymorphism varied significantly across different family history.

Discussion

To our knowledge, the present study was the first one which examined associations between polymorphisms in GSTM1 and GSTTI and the risk of osteosarcoma in Chinese population. The observed significant association between GSTTI polymorphism and the risk of osteosarcoma suggested that null GSTTI might be a useful susceptibility and detective biomarker for osteosarcoma.

This is the first report that has focused on the impact of GSTM1 and GSTT1 genotypes in risk of osteosarcoma in Chinese population. Although the polymorphisms of the two genotypes are associated with a modification in cancer risk, however, we only find a significant difference regarding GSTT1 genotype frequencies between osteosarcoma patients and control group. Only one previous study in USA reported an increased risk of osteosarcoma for patients carrying at least one non-null allele of GSTM1 and/or GSTT1. Our present study shows null GSTT1 associate with risk of osteosarcoma. Null mutations of GSTM1 and GSTT1, one of the phase II enzymes, are known to abolish enzyme activities and therefore have been linked with increasing incidence of certain cancers. Previous meta-analysis studies indicated that null genotypes of GSTM1 and GSTT1 might have a significant association with increased risks of breast cancer, lung cancer and gastric cancer in Chinese population (Sull et al., 2005; Saadat, 2006; Hosgood et al., 2007; Shi et al., 2008). In the present study , GSTT1 deficiency is like to act as a risk factor for osteosarcoma, in line with previous meta-analyses concerning esophageal cancer (Yang et al., 2005), prostate cancer (Ntais et al., 2005) and breast cancer (Vogl et al., 2004), respectively.

Regarding the GSTM1 null genotype, the significant association with breast cancer, lung cancer and gastric cancer and risk of osteosarcoma suggested that this enzyme could increased susceptibilities to environmental toxins and carcinogens. Although we could not find a significant relationship between GSTT1 genotypes and osteosarcoma risk, as we have a limited number of patients to draw further conclusions. Therefore, further large sample size is warranted.

There were two limitations in our study. Firstly, the controls were selected from the outpatients in the same hospital, which may be a threat to validity of the results and bring selection bias. There might be a certain risk of selection bias if they had any difference in terms of the studied exposures. However, diseases of most of the control subjects in our study were flu or diseases of digestive system. Second, because of the rarity of osteosarcoma, we only had limited number of cases. Increasing the number of controls to some extent to increase the study power needs consideration in future studies.

In summary, as the first study to investigate the association between polymorphisms in GSTM1 and GSTTI and the risk of osteosarcoma in a Chinese population, and this study found suggestive evidence that the null GSTTI might be a useful susceptibility and detective biomarker for osteosarcoma, especially for male and young age individuals.

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