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Effect of *CYP1A1* and *GSTM1* genetic polymorphisms on bone tumor susceptibility

L. Li[1], J.G. Li[2], C.Y. Liu[2] and Y.J. Ding[1]

[1]Division of Bone and Joint Surgery,
Jinan Central Hospital Affiliated to Shandong University, Jinan, China
[2]Division of Bone and Joint Surgery, Zhangqiu People's Hospital, Jinan, Shandong, China

Corresponding author: J.G. Li
E-mail: shishangniu456@163.com

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ABSTRACT. Tumor gene polymorphisms are often associated with individual susceptibility to genetic diseases. Cytochrome P4501A1 (*CYP1A1*) and glutathione S-transferase mu 1 (*GSTM1*) gene polymorphisms are closely related to the susceptibility of the body to chemical carcinogens in the environment. Therefore, we explored the relationship between *CYP1A1* and *GSTM1* gene polymorphisms and susceptibility to bone tumors. Multiplex-polymerase chain reaction (PCR), allelic-specific PCR, and PCR-restriction fragment length polymorphism techniques were used to analyze *CYP1A1* and *GSTM1* gene polymorphisms in 52 bone tumor patients and 100 healthy subjects. The allelic variation frequency of the *CYP1A1* gene at exon 7 (Ile 462 Val) in bone tumor patients was 0.462, which was significantly higher than that in the normal controls (0.223). The frequency of the absence of the *GSTM1* homozygous genotype in the patients (0.65) was also markedly higher than that in the control group (0.41). Subjects with *CYP1A1* Val/Val homozygous mutations and absence of the *GSTM1* homozygous genotype were at markedly increased risk of developing bone tumors [ORs 4.15 (95%CI: 1.268-13.30) and 2.35 (95%CI: 1.15-

4.85), respectively]. The OR for the combined effect of the *CYP1A1* and *GSTM1* gene polymorphisms was 8.55 (95%CI: 1.75-41.50). *CYP1A1* and *GSTM1* polymorphisms are genetic risk factors in patients with bone tumors, and the allelic variation of these genes increases the risk of bone tumor occurrence.

Key words: Bone tumor; *CYP1A1*; *GSTM1*; Genetic susceptibility; Genotype

INTRODUCTION

Osteosarcoma, also known as osteogenic sarcoma, is characterized by tumor cells that can produce the osteoid matrix. It is the most common primary malignant bone tumor and accounts for about 35% of all such tumors (Ottaviani and Jaffe, 2009). Although osteosarcoma appears in all age groups, it is most prevalent in adolescents (Chou and Gorlick, 2006). Osteosarcoma can occur in any part of the skeleton. Though bone sarcoma is uncommon compared with other malignant tumors, it nevertheless has a high incidence rate and features early distant metastases. It is the second most common fatal tumor in individuals under 20 years of age (Wittig et al., 2002; Gorlick et al., 2003; Jaffe, 2009). Following the emergence of neoadjuvant chemotherapy and the improvement of surgical techniques, the 5-year survival rate for osteosarcoma has increased from 20-30 to 70% (Wittig et al., 2002; Sánchez-Tilló et al., 2012). In recent years, however, the treatment of osteosarcoma has not improved significantly. About 30% of patients without metastasis at the first visit die of lung metastases (Sánchez-Tilló et al., 2012).

The majority of environmental carcinogens do not directly cause cancer. They can only form carcinogenic electrophilic compounds with strong polarity after catalysis by phase I metabolism enzymes such as cytochrome P450, while such compounds can be changed to non-toxic hydrophilic compounds through catalysis by phase II metabolism enzymes such as glutathione S-transferases (GSTs). Metabolic enzyme gene polymorphisms may change enzyme function, leading to increased vulnerability to external environmental risk factors and increased cancer risk. Environmental carcinogens may correlate with most of the susceptibility to tumors, genetic polymorphisms leads to a significant difference in host in response to different carcinogenic factors. Genetic polymorphism of biotransformation enzymes plays a key role in the carcinogenic effect of environmental carcinogens by initiating the processes involved in cancer (Taningher et al., 1999). Relevant research has indicated that the interaction between multiple metabolic enzymes and substrates is still not clear owing to the complicated carcinogenic process involving environmental carcinogens. Along with regional, individual, and ethnic differences, as well as differences in the method of detection used, conclusions about the relationship between tumor susceptibility and metabolic enzyme gene polymorphisms also differ (Nakazato et al., 2003). It has been found that phase I and II metabolic enzyme polymorphisms are associated with susceptibility to several types of cancer such as lung, breast, head and neck, and bladder cancer (Wang et al., 2003).

GSTs are phase II metabolic enzymes belonging to the multi-function dimer protein family. They are encoded by the GST-mu family of genes that locates in the chromosomal locus 1p13.3 (Abu-Amero et al., 2006). CYP450 is a phase I metabolic enzyme that belongs to a super family of proteins. It can convert procarcinogens into electrophilic compounds that attack DNA or proteins in cells; this can alter oncogenes or tumor suppressor genes and eventually lead to cancer (Jonsson et al., 2004). It has been reported that the *GSTM1* gene polymorphism is associated with an

increase in susceptibility to a variety of tumors (Mannervik and Danielson, 1988; Kiyohara et al., 1998). Moreover, *GSTM1* homozygosity rates vary in different populations, which leads to varying cancer probability (Hayes and Strange 2000; Schabath et al., 2005).

CYP1A1 is a subtype of CYP450. Studies have shown that *CYP1A1* and *GSTM1* polymorphisms are closely associated with susceptibility to mutagenic materials in the environment. Therefore, cell canceration is closely related to the activity of these two enzymes and their relative levels (Yang et al., 2005; Gonzalez and Yu, 2006; Yang et al., 2007).

Therefore, we explored the relationship between *CYP1A1* and *GSTM1* genetic polymorphisms and bone tumor susceptibility in patients, with the aim of providing a basis for diagnosis and treatment.

MATERIAL AND METHODS

Clinical samples

Fifty-two bone cancer patients were enrolled from our hospital, and their diagnoses were confirmed by pathological investigation. Another 100 healthy subjects were selected as controls. The age, gender, nationality, and place of residence of the subjects were compared.

The study protocol was approved by the Research Ethics Committee of our hospital, and all patients gave their informed consent before study commencement.

DNA extraction

Venous blood (5 mL) was extracted from each subject and the serum was separated by centrifugation. DNA was extracted from the blood according to the method described in the literature (Jaffe, 2009) After confirming that the optical density was above 1.8, the sample was diluted to 200 µg/mL and stored at -20°C.

Genotype detection

Primers were synthesized by the Shanghai Sangon Biological Company. The buffer and Taq enzyme were bought from the Shanghai Bocai Biotechnology Company. The polymerase chain reaction (PCR) was performed on a Bio-Rad 700 gene amplification system (Hercules, California, USA). The PCR-restriction fragment length polymorphism (RFLP) method was applied to detect the CYP1A1d Msp1 polymorphism, and the specific allele primer was as follows: forward, 5'-ACAGTGAAGAGGTGTAGCCGCTG-3'; reverse, 5'-TTAGGAGTCTTGTCTCATGCCTA-3'. The cycling conditions for the amplification of the *CYPIA1* gene comprised an initial single cycle of 5 min at 94°C; 30 cycles of 30 s at 94°C, 30 s at 54°C, and 30 s at 72°C; and an extension of 10 min at 72°C. The product was stored at -4°C. The PCR product of the Msp1 loci on the *CYPIA1* gene was digested using Msp1 enzyme at 37°C for 14 h. Allele-specific PCR was used to detect the 7th exon A4889G polymorphism in *CYP1A1*. The primers used were as follows: forward, 5'-AAGACCTCCCAGCGGGCAAT-3' and 5'-AAGACCTCCCAGCGGGCAAC-3'; reverse, 5'-CTCTGGTTACAGGAAGCTAT-3'. The cycling conditions comprised an initial single cycle of 5 min at 95°C; 35 cycles of 60 s at 95°C, 60 s at 64°C, and 120 s at 72°C; and an extension of 5 min at 72°C. Multi-PCR was performed to determine *GSTM1* allelic loss, and β-globin was selected as a control. The primers used were as follows: *GSTM1* forward, 5'-GTTGGGCTCAAATATACGG

TGG-3'; reverse, 5'-CAACTCCCTGAAAAGCTAAAGC-3'; β-globin forward, 5'-GAAGAGCCAAGG ACAGGTAC-3'; reverse, 5'-CAACTTCATCCACGTTCACC-3'. The cycling conditions comprised an initial single cycle of 5 min at 95°C; 35 cycles of 60 s at 95°C, 60 s at 64°C, and 120 s at 72°C; and an extension of 5 min at 72°C.

Statistical analysis

All statistical analyses were performed using SPSS11.5 software (Chicago, IL, USA). Differences between multiple groups were analyzed using the *t*-test or the chi-square test. The chi-square test was used to calculate odds ratios (ORs) and 95% confidence intervals (95%CIs). A P value < 0.05 was considered a statistically significant difference.

RESULTS

CYP1A1 polymorphism distribution

The PCR product length of the *CYPIA1* gene Msp1 loci was 343 bp. There were three digestion types: TT, T/C, and C/C. The *CYP1A1* exon 7 polymorphism distribution was significantly different in the two groups (P < 0.01). *CYP1A1* Val/Val homozygosity mutation frequency (0.462) in the bone tumor patients was significantly higher than in the control group (0.223), while the subjects carrying the *CYP1A1* Val/Val genotype had 4.13 times higher risk of suffering bone cancer than those carrying the *CYP1A1* Ile/Ile genotype (Figure 1 and Table 1).

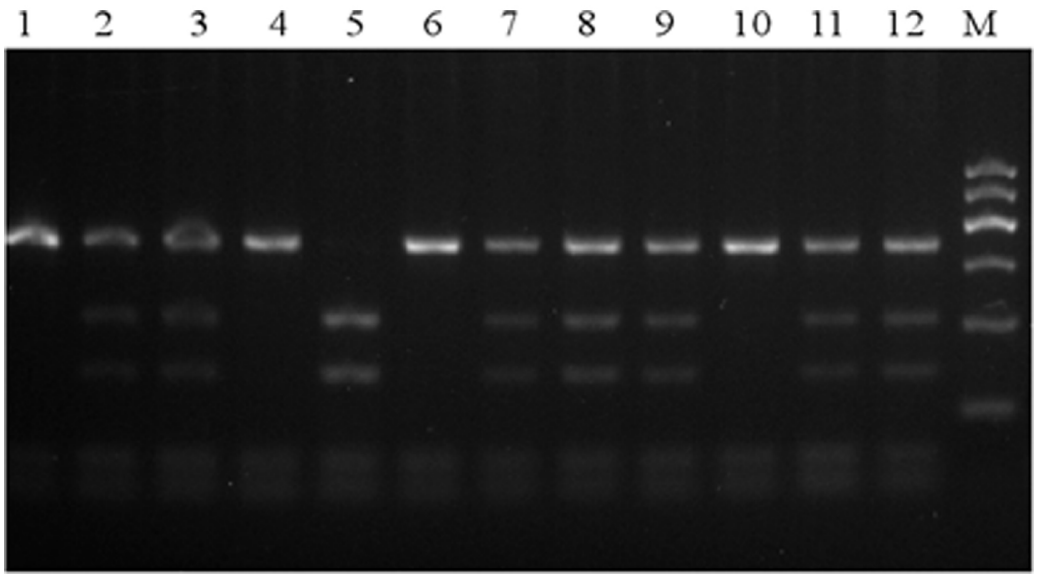

Figure 1. PCR detection of CYP1A1 gene in patients with bone cancer. *Lanes 1*, *4*, *6*, *10* (type TT); *lanes 2*, *3*, *7*, *8*, *9*, *11*, *12* (type T/C); *lane 5* (type C/C).

Table 1. *CYP1A1* polymorphism distribution.

CYP1A1 genotype	Case	Control	OR (95%CI)
Val/Val	7	6	4.15 (1.26-13.30)
Val/Ile	25	34	2.35 (1.15-4.85)
Ile/Ile	20	60	

GSTM1 polymorphism distribution

The *GSTM1* gene polymorphism was either present or absent. Gene deletion could not be amplified by PCR. The PCR product of *GSTM1* was 313 bp, while the β-globin length was 268 bp. Homozygosity of *GSTM1* deletion accounted for 65% of cases and 41% of controls, and the difference was significant ($P < 0.05$). Subjects with homozygosity of deletion had a 2.72-fold increased risk of bone cancer (Figure 2, Table 2).

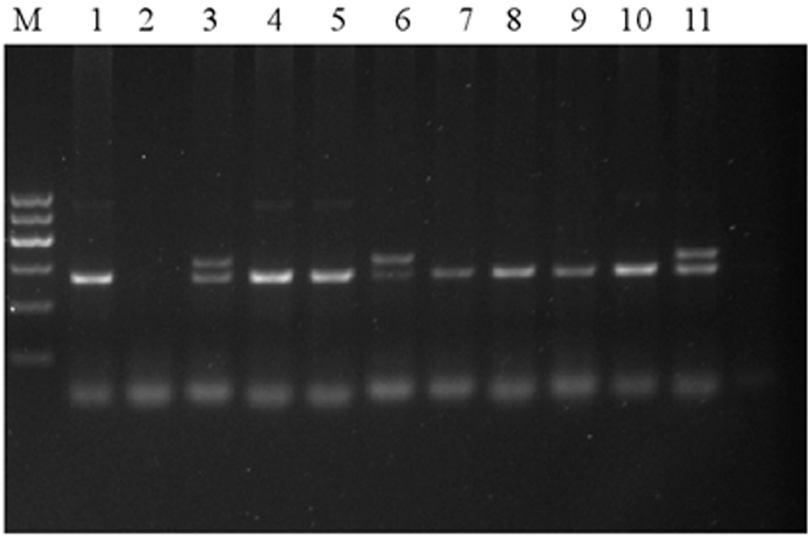

Figure 2. PCR detection of GSTM1 gene in patients with bone cancer. *Lanes 3*, *6*, *11* (GSTM1+/+); *lanes 1*, *4*, *5*, *7*, *8*, *9*, *10* (GSTM1-/-).

Table 2. *GSTM1* polymorphism distribution.

GSTM1 genotype	Case	Control	OR (95%CI)
-	36	42	2.70 (1.32-5.43)
+	16	37	-

Analysis of the combined effect of *CYP1A1* and *GSTM1* polymorphisms

Crossover analysis was applied to calculate ORs, with *CYP1A1* Ile/Ile and *GSTM1* (+) acting as the negative control. As shown in Table 3, the OR of *CYP1A1* Val/Ile combined with *GSTM1* (-) was 6.82 and the OR of *CYP1A1* Va1/Va1 combined with *GSTM1* (-) was 8.55. The two gene mutations exhibited significant synergistic effects (Table 3).

Table 3. Combined effect of *CYP1A1* and *GSTM1*.

CYP1A1 genotype	*GSTM1* genotype	Case	Control	OR (95%CI)
Ile/Ile	+	8	36	1.00
Val/Ile	+	8	20	1.40 (0.50-4.74)
Val/Val	+	3	4	2.85 (0.40-19.85)
Ile/Ile	-	10	26	1.83 (0.66-5.05)
Val/Ile	-	18	12	6.82 (2.23-20.52)
Val/Val	-	5	2	8.55 (1.75-41.50)

DISCUSSION

The cytochrome P450 (CYP) family, which are phase I metabolic enzymes, are important in the metabolism of endogenous and exogenous compounds. They mainly exist in the endoplasmic reticulum of liver and intestine cells, and are monooxygenases that catalyze the metabolism of numerous internal and external substances. The *CYPIA1* gene encodes enzymes that are involved in the activation and metabolism of a variety of environmental carcinogens (Wang et al., 2003) (WangZheng, 2003). The *CYPIA1* gene has a polymorphism, namely, the exon 7 A-G variation, which can change the encoded enzyme (Ile to Val at amino acid 462) and reduce the ability of the body to process and metabolize carcinogens (Amundadottir et al., 2004; Larsen et al., 2005; Masson et al., 2005).

The Msp1 locus has three genotypes: the homozygous type (T/T), the hybrid type (T/C), and the wild type (C/C). Msp1 locus mutation (T→C) of *CYPIA1* gene causes structural change at the 31 flank area. This change not only influences the iron ion electron transfer ability of the *CYPIA1* gene product but also affects protein interactions that leads to the inhibition of transcription of proto-oncogene and tumor suppressor gene, since the *CYPIA1* gene participates in protein and gene transcription. This increases tumor susceptibility. However, the correlation between the effect of the *CYPIA1* gene and tumor susceptibility was inconsistent, which suggests that although tumor susceptibility is closely associated with gene polymorphism, any kind of gene polymorphism can dominate tumor occurrence. Our study also confirmed that the *CYP1A1* gene Msp1 loci polymorphism exists as three genotypes in osteosarcoma. We also found that the A4889G loci allele mutation frequency (0.462) in osteosarcoma was higher than that in the control (0.223), causing an osteosarcoma risk increase of 4.13 fold in subjects carrying the *CYP1A1* Val/Val homozygous genotype. This indicates that *CYP1A1* genetic polymorphism is an osteosarcoma pathogenic factor, and is associated with osteosarcoma susceptibility.

GSTs, which are phase II metabolic enzymes, are composed of soluble dimer proteins, and mainly exist in liver tissue. Their isozymes play an important role in anti-oxidation during cell injury, and they help resist foreign compound invasion (Kiyohara et al., 1998). At present, the different results regarding GST gene polymorphism and cancer susceptibility in China are mainly due to differences in race and region. Based on different races, regions, and cancers, Kidd et al. (2003), Kote-Jarai et al. (2001), Beer et al. (2002), Marchand et al. (1999), and Mitra et al. (2006) suggested the following: the *GSTM1* gene polymorphism is not involved in prostate cancer in Europe; deletion of the *GSTT1* genotype is a risk factor for prostate cancer in Europeans; the *GSTP1* gene polymorphism is correlated with stomach cancer occurrence in Caucasians; and the *GSTM1* gene polymorphism is not related to bladder cancer occurrence in the United States. Our results further confirm that expression of the same genes varies in different races, regions, and tissues. This research focused on *GSTM1* gene polymorphisms in Han patients with osteosarcoma, and found that the *GSTM1* (-) genotype exists in normal subjects, while osteosarcoma patients

carry the *GSTM1* homozygous deletion subtype. The risk of developing osteosarcoma increased by 2.72 times. A combination of the *GSTM1* homozygous deletion subtype and the *CYP1A1* Val/Val genotype may increase risk of osteosarcoma by up to 8.5 times, which proves the existence of a synergy effect. The results also suggest that simultaneous variation in the genes may cause the accumulation of carcinogens in the liver, thus promoting the occurrence of osteosarcoma.

Environmental carcinogens play an important role in carcinogenesis. Polymorphisms in the genes encoding metabolic enzymes GSTM1 and CYP1A1 affect carcinogen activation and deactivation. Our results showed that polymorphisms in the *CYP1A1* and *GSTM1* genes are susceptibility factors for bone tumors, and allelic variation in these polymorphisms increases risk. The results also suggest that *CYP1A1* and *GSTM1* gene polymorphisms may be susceptibility indices for bone tumor, and could be used for screening susceptible people and prevention of osteosarcoma by early intervention.

Conflicts of interest

The authors declare no conflict of interest.

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