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Original Article

Lack of association of glutathione s-transferase (*GSTM1* and *GSTT1*) polymorphisms with susceptibility of osteoporosis

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Abstract: Osteoporosis is a skeletal disorder characterized by low bone mineral density (BMD). Several studies have been suggested that some of the genetic factors may be contributed to osteoporosis by specific polymorphisms. The aim of the present study was to evaluate relationship between the glutathione s-transferase (*GSTM1* and *GSTT1*) gene polymorphisms and osteoporosis. To explore this possible association, polymorphisms of *GSTM1* and *GSTT1* genes were tested in in 79 osteoporosis patients and 53 healthy control subjects. The distributions of polymorphisms of *GSTM1* and *GSTT1* genes between the osteoporosis group and the control group were similar. The difference in distributions of polymorphisms of *GSTM1* and *GSTT1* genes did not show any association with susceptibility of osteoporosis (*GSTM1*, OR=0.858, 95% CI=0.403-1.827, p=0.691; *GSTT1*, OR=1.491, 95% CI=0.706-3.145, p=0.395). Our results suggested that *GSTM1* and *GSTT1* polymorphisms are not associated with susceptibility of osteoporosis in Korean population.

Keywords: Glutathione s-transferase, polymorphism, osteoporosis

Introduction

Osteoporosis is common bone diseases, which is characterized by lowered bone mineral density and susceptibility to fractures [1]. Bone mineral metabolism is a dynamic process of balance between bone forming and bone resorption [2], and oxidative stress may play a role in the process of bone metabolism [3, 4]. Osteoblast and osteoclast actions may include the production of free radicals [5, 6], and it may also involve in the bone remodeling process [6, 7]. Moreover, osteoporosis is common in postmenopausal women [4, 8], and estrogen deficiency may lead to increased oxidative damage in mesenchymal stem cells in bone tissue [9].

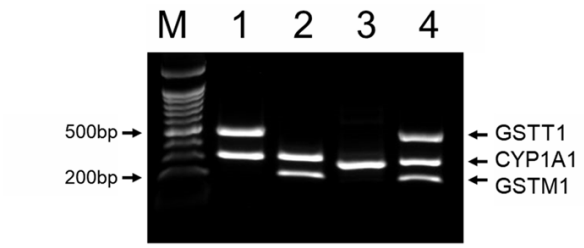
Glutathione-S-Transferases (GSTs) are a family of enzymes responsible for detoxification of toxic metabolites or xenobiotic substrates. The family is composed of many subclass genes and they are widespread throughout human body organs [10], however, their actions are similar, that is, bring the substrate into glutathione (GSH) and activate GSH to induce the process of making hydrophilic conjugates [10]. However, polymorphic GST genes were reported to be associated with various diseases [11-18], and also regarding osteoporosis or decreased bone mineral density [19-22].

These studies suggest that the GST polymorphisms may be associated with osteoporosis. And because *GSTT1* and *GSTM1* are known to be frequently polymorphic in major population [23], therefore we investigated the relationship between polymorphisms of *GSTT1* and *GSTM1* genes and osteoporosis in a selected Korean population consisted of controls and osteoporosis case.

Materials and methods

Subjects

79 Korean adult patients with osteoporosis (61.9 ± 8.6 years in age, mean ± standard deviation) and 53 Korean healthy adult controls



Lane: M-100bp marker
1- GSTM1 (-) / GSTT1 (+)
2-GSTM1 (+) / GSTT1 (-)
3- GSTM1 (-) / GSTT1 (-)
4- GSTM1 (+) / GSTT1 (+)
The CYP1A1 gene was used as a positive marker to determine whether the PCR was successful.

Figure 1. Results of electrophoresis.

Table 1. Demographic and clinical characteristics of osteoporosis patients and the control subjects

	Osteoporosis	Control	p
Number of subjects	79	53	
Age (mean ± SD)	61.9 ± 8.6	57.8 ± 8.6	0.010
BMD in L1-L4 (g/cm^2)	0.677 ± 0.068	1.019 ± 0.803	<0.000

N, number of subjects; BMD, bone mineral density; L, lumbar vertebrae; SD, standard deviation. The *p* value between the control group and the osteoporosis was calculated using t-test.

(57.8 ± 8.6 years) were recruited for this study (**Table 1**). Osteoporosis was diagnosed by bone mineral density in lumbar spine. The bone mineral density in each subject was measured using dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy, GE Healthcare, Madison, WI, USA) to assess bone status. The T-score was used to diagnose osteoporosis. When the measured T-score was less than -2.5, it was diagnosed as osteoporosis. The control group was selected for subjects with a T-score of -1.0 or higher. Written informed consent was obtained from each patient or the legal guardian. The protocol for this study was approved by the ethics review committee of School of Medicine, Kyung Hee University, Seoul and School of Medicine, Eulji University, Daejeon, Republic of Korea.

DNA extraction and genotyping of GSTM1 and GSTT1 gene polymorphisms

Subjects DNA were prepared from peripheral blood using a genomic DNA isolation reagent kit (High Pure PCR template preparation kit, Roche, USA). The multiplex polymerase chain reaction (PCR) was used for amplification with the specific primer pairs for the *GSTM1* (215 base pair), *GSTT1* (480 bp), and *CYP1A1* (312 bp) genes [24]. The primers used in this experiment are as follows: GSTM1 primers of (sense) 5-GAACTCCCTGAAAAGCTAAA-GC-3 and (antisense) 5-GTTG-GGCTCAAATATACGGTGG-3, GSTT1 primers of (sense) 5-TT-CCTTACTGGTCCTCACATCTC-3 and (antisense) 5-TCACGGGA-TCATGGCCAGCA-3, and CYP1-A1 (sense) 5-GAACTGCCACTT-CAGCTGTCT-3 and (antisense) 5-CAGCTGCATTTGGAAGTGC-TC-3. *CYP1A1* was used to obtain internal positive control to distinguish the null genotype obtained PCR products from the aborted PCR products. The PCR products were loaded onto 1.8% agarose gels and electrophoresis was done. Next, the gels were stained with ethidium bromide and photographed under UV light (**Figure 1**).

Statistical analysis

The distribution of each genotype in the osteoporosis group was compared with that of the control group. The logistic regression analysis was performed to calculate odds ratio (OR), 95% confidence interval (CI), and *p* value. And *p* value was calculated using logistic regression and adjusted for age. All data were analyzed using the statistical analysis system software (SPSS 23.0). For all statistical tests, the significant level was set at 0.05.

Results

Table 1 shows the clinical information of the subjects in this study. They were older than 50 years of age and showed significant differences in BMD.

The PCR end-products of *GSTM1* (215 bp), *GSTT1* (480 bp), and *CYP1A1* (312 bp) genes on 1.8% agarose gel are shown (**Figure 1**). **Table 2** shows single analysis of distributions of *GSTM1* and *GSTT1* gene polymorphisms in the control group and the osteoporosis group. The frequencies of *GSTM1* null genotype in control group and the osteoporosis group were 54.7% (29/53) and 55.7% (44/79), respectively. The frequencies of GSTT1 null genotype in control

Table 2. Genotype frequency and single analysis of GSTM1 or GSTT1 polymorphisms between control and osteoporosis

Polymorphism	Control N (%)	Osteoporosis N (%)	OR	95% CI	p
GSTM1					
Null	29 (54.7)	44 (55.7)	0.858	0.403-1.827	0.691
Positive	24 (45.3)	35 (44.3)		1	
GSTT1					
Null	28 (52.8)	37 (46.8)	1.491	0.706-3.145	0.295
Positive	25 (47.2)	42 (53.2)		1	

OR, odds ratio; CI, confidence interval; N, number; p, *p* value. The odds ratio (OR), 95% confidence interval (CI), and *p* value was calculated using logistic regression and adjusted for age.

Table 3. Genotype frequency and combination analysis of GSTM1 and GSTT1 polymorphisms between control and osteoporosis

GST genotype		Control N (%)	Osteoporosis N (%)	OR	95% CI	p
GSTM1	GSTT1					
Positive	Positive	15 (28.3)	21 (26.6)	1		
Null	Null	19 (35.8)	23 (29.1)	0.652	0.246-1.729	0.390
Null	Positive	10 (18.9)	21 (26.6)	1.388	0.455-4.230	0.564
Positive	Null	9 (17.0)	14 (17.7)	0.980	0.321-2.990	0.972

OR, odds ratio; CI, confidence interval; N, number; p, *p* value. The odds ratio (OR), 95% confidence interval (CI), and *p* value was calculated using logistic regression and adjusted for age.

group and osteoporosis group were 52.8% (28/53) and 46.8% (37/79), respectively. The null genotype distributions of the *GSTT1* and *GSTM1* gene polymorphisms did not show any association with osteoporosis (OR=0.858, 95% CI=0.403-1.827, p=0.691 in *GSTM1*; OR=1.491, 95% CI=0.706-3.145, p=0.395 in *GSTT1*, **Table 2**).

Table 3 shows combination analysis of *GSTM1* and *GSTT1* gene polymorphisms between control and osteoporosis. In combination analysis, the distributions of both *GSTM1* and *GSTT1* null genotypes, *GSTM1* positive/*GSTT1* null genotype, and *GSTM1* null/*GSTT1* positive genotype in the control group and the osteoporosis group were 35.8% *vs*. 29.1%, 17.0% *vs*. 14.7%, and 18.9% *vs*. 26.6%, respectively. However, all combinations (*GSTM1* null/*GSTT1* null genotype, *GSTM1* positive/*GSTT1* null genotype, and *GSTM1* null/*GSTT1* positive genotype) did not also show any significant association with osteoporosis between the control group and the osteoporosis group (*GSTM1* null/*GSTT1* null genotype, OR=0.652, 95% CI=0.246-1.729, p=0.390; *GSTM1* positive/*GSTT1* null genotype, OR=1.388, 95% CI=0.455-4.230, p=0.564; *GSTM1* null/*GSTT1* positive genotype, OR=0.980, 95% CI=0.321-2.990, p=0.972).

Discussion

The *GSTM1* gene is on the chromosomal location of 1p13.3, and *GSTM1* is deletion of the whole *GSTM1* gene is a frequent polymorphism [23, 25-27]. The *GSTT1* gene is on the chromosomal location of 22q11.2, and deletion in *GSTT1* has also been reported to be frequent [23, 25-27]. They have been designated as null polymorphism or *0 genotype, and they were lack of enzyme activities.

There have been many studies with disagreeing results of *GSTT1* and *GSTM1*. Mlakar et al. reported that *GSTT1* deletion is associated with higher BMDs in femoral neck, lumbar spine, and total hip measured by DEXA in Slovenian elderly. And they suggested that *GSTM1* deletion may show opposite tendency [22]. Ramalhinho et al. suggested that *GSTM1* and *GSTT1* null genotype alone, or *GSTT1* and *GSTM1* combined, or combined with polymorphic *GSTP1* alleles, are associated with higher susceptibility to breast cancer development [28]. Martin et al. suggested that smokers with *GSTM1* null types may tend to have more coronary heart diseases [29].

However, our observation was diverse from them. The genotype frequencies of *GSTM1* and *GSTT1* were not significantly different, and none of each of *GSTM1* or *GSTT1* were significantly associated with osteoporosis.

Coughlin et al. reported that results of epidemiologic studies do not confirm associations between *GSTM1*, *GSTT1*, and *GSTP1* and epithelial ovarian cancer [30]. Kadouri et al. concluded that *GSTM1* or *GSTT1* deletions were not risk effective regardless of BRCA1/2 types [31]. And Li et al., performed a meta-analysis of GST polymorphism and thyroid cancer risk in

12 studies of *GSTM1* null polymorphism (1569 cases and 2907 controls), 11 studies concerning *GSTT1* null polymorphism (1515 cases and 2863 controls), and 8 studies on *GSTP1* Ile105Val, but they concluded no association [32]. The results might indicate that *GSTM1* and *GSTT1* are not related to the risk of cancer development despite their important function.

GST families may be associated with detoxification of various substrates and their substrates may encompass between different isoforms and subclasses [23, 33]. Additionally, there was no report of firmly linked gene polymorphism with *GSTT1* or *GSTM1* null polymorphism in Korean population. Besides, GSTT1 and GSTM1 may be only small part to determine the fate of bone cells, because calcium homeostasis hormones are important modulators of them [2]. Most of all, number of our study subject were small.

In conclusion, our results do not support that *GSTT1* or *GSTM1* null polymorphism may be associated with susceptibility to osteoporosis. However, this study has a limitation about sample size and age. It is known that the older the age, the higher the prevalence of osteoporosis. Therefore, further study is needed to investigate the relationship with GST in the normal group without osteoporosis over 70 years of age.

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Disclosure of conflict of interest

None.

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