Original Article

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

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Received April 19, 2017; Accepted February 16, 2018; Epub June 15, 2018; Published June 30, 2018

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 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 post-menopausal 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
GST polymorphism and osteoporosis

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

<table>
<thead>
<tr>
<th></th>
<th>Osteoporosis</th>
<th>Control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>79</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>Age (mean ± SD)</td>
<td>61.9 ± 8.6</td>
<td>57.8 ± 8.6</td>
<td>0.010</td>
</tr>
<tr>
<td>BMD in L1-L4 (g/cm²)</td>
<td>0.677 ± 0.068</td>
<td>1.019 ± 0.803</td>
<td>&lt;0.000</td>
</tr>
</tbody>
</table>

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.

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 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.

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-GAATCCCTGGAAAGCTAAAGC-3 and (antisense) 5-GTTGGGCTCAATATACGGTG-3, GSTT1 primers of (sense) 5-TCACGGAAATATACGGTGAGAGCAGCA-3, and CYP1A1 (sense) 5-GAATCTGGCCACTTCAGCTGTC-3 and (antisense) 5-CAGCTGCATTGGAGATGCTTC-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 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 group and the osteoporosis group were 54.7% (29/53) and 55.7% (44/79), respectively.

(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.

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 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 group and the osteoporosis group were 54.7% (29/53) and 55.7% (44/79), respectively.
Table 2. Genotype frequency and single analysis of GSTM1 or GSTT1 polymorphisms between control and osteoporosis

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

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

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

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.

Discussion

The GST1 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 GST1 gene is on the chromosomal location of 22q11.2, and deletion in GSTT1 has also been reported to be frequent [23, 25-27]. It has 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. Coughlin 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]. Ralmainho 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.

Acknowledgements
This study was supported by the Traditional Korean Medicine R&D program funded by the Ministry of Health & Welfare through the Korean Health Industry Development Institute (KHIDI) (HI15C0133).

Disclosure of conflict of interest
None.

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References
GST polymorphism and osteoporosis


