Association of estrogen receptor-α gene polymorphisms with bone mineral density in postmenopausal Korean women

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Abstract We examined the potential associations between PvuII and Xbal polymorphisms in the first intron of the estrogen receptor alpha (ER-α) gene and bone mineral density (BMD) in a population-based study of 174 postmenopausal Korean women. BMD was measured at the lumbar spine (L2–L4), right femoral neck, right trochanter, and right Ward’s triangle. ER-α gene polymorphisms were detected by PvuII and Xbal restriction endonuclease digestion of polymerase chain reaction products. Differences in BMD values between the ER-α genotypes were analyzed in a general linear model, with adjustments for age, height, weight, and smoking status. The following genotype frequencies were noted: PP, 14.9%; Pp, 46.0%; pp, 39.1%; XX, 3.5%; Xx, 29.3%; and xx, 67.2%. Both the femoral neck and Ward’s triangle BMD values in women with the Pp genotype were significantly (P < 0.05) higher than those in women with the pp genotype. No significant effect of the Xbal genotype on BMD was found at any site. Carriers of the pX haplotype were more likely to have lower BMD values at the trochanter than noncarriers, after adjustment for potentially confounding factors. Women with the pp genotype had more previous hip or spine fractures than those with other genotypes (P < 0.05). These results suggest that the PvuII polymorphism and the ER-α haplotype may be associated with the BMD at several femur sites in postmenopausal Korean women.

Key words Bone mineral density (BMD) · Estrogen receptor · Polymorphism · Population-based study

Introduction

Bone mineral density (BMD) late in life is a function of the previous level of peak bone mass and the amount of bone that has been lost. Genetic factors play an important role in the regulation of BMD. It has been estimated that 60%–85% of BMD variations are genetically determined [1–4]. Following the report of an association between vitamin D receptor (VDR) genotypes and BMD [5], several candidate gene association studies have been performed [6–13]. One candidate gene is the estrogen receptor (ER) gene. Because estrogens have important effects on bone mass and bone remodeling, many investigators have evaluated the role of ER gene polymorphisms in the genetic regulation of BMD [14].

A previous report of an osteoporotic phenotype in a man with a disruptive mutation of the ER alpha (ER-α) gene, as well as reports of decreased BMD values in mice that lack functional ER-α, but not in those that lack ER-β, strongly support the involvement of the ER-α gene in the development of osteoporosis [15]. For this reason, many studies of osteoporosis have targeted polymorphisms in the ER-α gene.

The PvuII and Xbal polymorphisms of the ER-α gene have been associated with BMD in some studies [6,14,16–18], but not in others [19–21]. The variable results that have been obtained in previous studies of Korean subjects [17,19,20] demand clarification. The subjects of the previous Korean studies were hospital-based volunteers rather than population-based women. To date, a population-based study of BMD in women of Korean ethnicity has not been carried out.

We performed a population-based study to determine whether PvuII and Xbal polymorphisms in the first intron of the ER-α gene, alone or in combination, were associated with BMD changes in postmenopausal Korean women.
Subjects and methods

Subjects

The study included 174 postmenopausal Korean women, aged 45 to 84 years, who were selected from participants in The Jansung Epidemiologic Study of Osteoporosis, which was a population-based epidemiologic study that was carried out in Jansung County, Korea, in August 2000. The Korean people constitute a highly homogeneous ethnic group. All of the subjects were questioned regarding their age, education, smoking status, and medical history during home visits. Exclusion criteria included previous bilateral oophorectomy, estrogen replacement therapy, and any medication for thyroid disease, parathyroid disease, rheumatoid arthritis, or epilepsy. The study was reviewed and approved by the Institutional Review Board of Chonnam National University Hospital. Informed, written consent was obtained from each subject before entering the study.

Clinical measurements

After completion of the initial survey, each subject visited one community hospital for measurements of height, weight, and BMD. BMI was calculated by dividing body weight (kg) by the square of an individual’s height (m²). BMD measurements were carried out using the Lunar DPX-L dual-energy X-ray absorptiometer (Lunar Radiation, Madison, WI, USA). BMD was measured, in grams per square centimeter, at the lumbar spine (L2-L4), right femoral neck, right trochanter, and right Ward’s triangle.

Genotyping

Genomic DNA was extracted from ethylene-diamine tetraacetic acid (EDTA)-treated blood, using the AccuPrep Genomic DNA Extraction Kit (Bioneer, Seoul, Korea). ER-α gene polymorphisms were determined by PvuII and XbaI (TaKaRa, Kyoto, Japan) restriction endonuclease digestion of polymerase chain reaction (PCR) products, as described by Kobayashi et al. [6]. The PCR products that contained a part of intron 1 and exon 2 of the ER-α gene were digested at 37°C for 4h with PvuII and XbaI, thereby producing fragments of 1300bp (P allele) or 850bp and 450bp (p allele), and of 1300bp (X allele) or 900bp and 400bp (x allele). By convention, the presence of the endonuclease restriction site is indicated with uppercase letters (P and X, for PvuII and XbaI, respectively) while the absence of the restriction site is indicated with uppercase letters (p and x, for PvuII and XbaI, respectively).

Statistical analysis

The genotype frequencies for the two markers were tested against the Hardy-Weinberg ratios, using the χ² test. Linkage disequilibrium that resulted from nonrandom associations of genotypes from the two different loci was also assessed by the χ² test. Tests for associations between BMD values and genotypes or haplotypes were performed using analysis of covariance (ANCOVA), followed by the Tukey method. In all the statistical analyses, the raw BMD values were adjusted by regression for covariates of age, height, weight, and smoking. Qualitative data were analyzed by the χ² test. A P value of less than 0.05 was considered to be statistically significant. Haplotype frequencies were estimated using the SNPAnalyzer ver. 1.0 program (Istech, Gyeonggi, Korea).

Results

The genotype frequencies of the PvuII (P = 0.70) and XbaI (P = 0.52) ER gene RFLPs were as follows: PP, 14.9%; Pp, 46.0%; pp, 39.1%; XX, 3.5%; Xx, 29.3%; and xx, 67.2%. The genotype distributions of these RFLPs were compatible with a population in Hardy-Weinberg equilibrium (P = 0.70 for ER PvuII, and P = 0.52 for XbaI). The clinical characteristics of the study groups are presented in Table 1. There were no statistically significant differences in terms of subject age, years since menopause, height, weight, or smoking between the genotypes for each RFLP. The proportions of no-schooling among the subjects were not significantly different between the genotypes for each

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Table 1. General characteristics of the study subjects according to ER-α genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>PvuII</th>
<th>XbaI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PP (n = 26)</td>
<td>Pp (n = 80)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>63.7 ± 6.3</td>
<td>64.9 ± 7.7</td>
</tr>
<tr>
<td>Years since menopause</td>
<td>14.1 ± 7.5</td>
<td>17.4 ± 10.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>149.7 ± 3.8</td>
<td>149.6 ± 5.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>54.6 ± 8.2</td>
<td>54.8 ± 9.7</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>15.4</td>
<td>10.0</td>
</tr>
</tbody>
</table>

Values are means ± SD except where indicated.