Bone Maturation and Bone Mineralization in Precocious Puberty: Relation to Estrogen Receptor Gene Polymorphisms

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Abstract. Estrogen plays an important role in bone maturation and bone mineralization. To elucidate the physiological roles of estrogen in the regulation of bone growth, we investigated the relationship between bone mineral status and the estrogen receptor (ER) gene polymorphism in subjects with precocious puberty, where excess estrogen is exposed to the bone. Thirty-six patients with central precocious puberty or early puberty were enrolled in the study. The relationships between PvuII and XbaI restriction fragment length polymorphisms of the ER gene and the lumbar spine bone mineral density (BMD), BMD SD score adjusted for either chronological age or bone age, were evaluated. The mean BMD value for bone age (–0.52 ± 0.83 SD) was significantly lower than the BMD value for chronological age (0.30 ± 0.96 SD) (p<0.01), indicating that bone maturation (advancement of bone age) was not associated with bone age-matched mineralization (increase in BMD). Furthermore, the PP genotype for the PvuII polymorphism appeared to be associated with an increased BMD (p<0.05), suggesting that the ER gene genotype may be related to bone mineral accretion during estrogen exposure. ER gene polymorphisms might thus account for the varying degrees of increase in BMD after estrogen exposure, leading to the dissociation of bone maturation and bone mineralization.

Key words: estrogen receptor, polymorphism, bone age, bone mineral density, precocious puberty

Introduction

Sex steroids, androgen and estrogen, play an important role in bone maturation and bone mineralization (1–6). Two recent case studies have demonstrated that estrogen plays a more critical role than androgen in bone maturation and bone mineralization (7, 8). The estrogen receptor α (ER) gene mutation appears to cause a delay in bone maturation and osteoporosis in male patients (7). This raises the possibility that ER gene polymorphisms may play a role in bone metabolism; in fact, ER gene polymorphism has been reported to be related to genetic variations in peak bone mineral density (BMD) (9–13). Understanding the possible physiological roles of estrogen in the regulation of bone mineral status is important for the clinical practice of pediatric endocrinology.

Precocious puberty, where premature exposure to estrogen occurs, is considered to be a model for investigating the physiological roles of estrogen exposure on bone mineral status. In one study, the lumbar spine BMD of untreated patients with precocious puberty was elevated when
evaluated according to chronological age but appropriate when evaluated according to bone age (14). However, subsequent measurements in a small group of girls with precocious puberty did not confirm this finding (15).

The present study examined bone mineral status and the relationship between bone maturation and bone mineralization during exposure to excess estrogen in patients with precocious puberty and early puberty. Moreover, we determined whether the ER gene polymorphism was related to the degree of increase in BMD during exposure to estrogen. This is the first study to examine the relation between ER genotypes and bone mineral status in subjects with precocious puberty.

Subjects and Methods

Subjects

Thirty-six girls between the ages of 4.1 and 12.0 years (mean age 8.8 ± 1.7 (SD) years) with a history of breast development before the age of 8 years were enrolled in the study. The stage of breast development at the time of first examination was Tanner stage II through IV for all patients. Plasma estradiol concentrations were 10–40 pg/ml (prepubertal level <10) and testosterone concentrations were below 20 ng/dl. None of the patients had received treatment for the symptoms of precocious puberty. The diagnosis of central precocious puberty (CPP) was made based on the criteria including early occurrence of breast development before the age of 7 years and the progression of bone age and pubertal secretion of gonadotropin. Twenty-six patients were diagnosed as having CPP, and ten patients, who experienced breast development between the ages of 7 and 8 years (and who did not meet the CPP criteria), were diagnosed as having early puberty or early adolescent growth spurt. Of the CPP patients, 20 subjects had idiopathic CPP and 6 subjects had organic CPP (hypothalamic hamartoma, 1; optic glioma, 1; ectopic pinealoma, 1; hydrocephalus, 3). Informed consent was obtained from the parents of the patients before inclusion in this study (16).

Bone mineral density assessment

Dual-energy x-ray absorptiometry was performed using a Hologic QDR 2000W densitometer (Hologic Inc., Waltham, Mass). BMD (measured in grams per square centimeter) was calculated for the second, third, and fourth lumbar vertebrae. The precision errors for children are 1% and 2%. The BMD values were compared with normal Japanese control data (17), and the standard deviation scores for chronological age and bone age were calculated respectively.

Bone age according to the Tanner Whitehouse-2 (RUS) method for Japanese children was determined by the same observer. Bone age is of great importance in evaluating growth disorders in children because the chronological age does not necessarily correspond with the bone age of these children. Discrepancies between bone age advancement and growth are particularly likely to occur in children with elevated levels of sex steroid hormones (18). Therefore, BMD values adjusted to bone age, and not to chronological age, are considered to be appropriate for evaluating altered bone mineral status in subjects with CPP or early puberty.

Analysis of estrogen receptor gene polymorphism

Genomic DNA was extracted from 200 µl of peripheral blood using a QIAamp Blood Kit (QIAGEN, Tokyo, Japan). PCR primers (forward, 5'-CTGCCACCCCTATCTGTATCTTTTCCTATTC-3' and reverse, 5'-TCTTTCTCTGCCACCCCTGGCGTCGATTATCTGA-3' (9)) were used for the reaction. The PCR reaction was performed using the Expand High-Fidelity PCR System (Roche, Mannheim, Germany) in a 50 µl solution for 35 cycles of denaturation at 94°C for 30 sec., primer annealing at 61°C for 40 seconds, and primer extension at 72°C for 90 seconds (GeneAmp PC System 9700, California, USA). The resulting
product contained a part of intron 1 and exon 2 of the ER gene. After amplification, the PCR products were digested with restriction endonuclease (XbaI or PvuII, Takara, Tokyo, Japan) and electrophoresed in a 1% agarose gel (19). The genotypic polymorphisms were defined as XX or PP (absence of a restriction site on both alleles), xx or pp (presence of a restriction site on both alleles), and Xx or Pp (heterozygous). The restriction fragment length polymorphism (RFLP) analysis was performed by the same investigator.

Statistical analysis

Data were expressed as the mean ± standard deviation (SD). The Mann-Whitney U-test was used to compare the means of the groups. A p value of less than 0.05 was considered to be statistically significant.

Results

Bone maturation and bone mineralization

The BMD and bone age values (SD score) for each of the 36 patients are shown in Fig. 1. The BMD values of the lumbar spine exceeded the normal mean for the same chronological age in 27 out of 36 cases; when adjusted for bone age, however the BMD values were lower than the normal mean in all but 6 cases. The mean lumbar spine BMD SD score for bone age (–0.52 ± 0.83 SD) was significantly lower than the BMD SD score for chronological age (0.30 ± 0.96 SD) (p<0.01).

Estrogen receptor polymorphisms and bone mineral density

The frequencies of the PvuII and XbaI polymorphisms in 27 patients are shown in Table 1. These genotype distributions are almost similar to those previously reported for Japanese women (9). More specifically, Kobayashi et al. reported finding the following distribution of ER polymorphisms in 238 healthy postmenopausal women aged 45–91 years in Japan: PP 46 (19.3%), Pp 122 (51.3%), pp 70 (29.4%), and XX 7 (3.0%), Xx 77 (32.4%), xx 154 (64.7%), and they were not statistically significantly different from the data in our report. The allele effect of the ER genotype for the lumbar spine BMD (SD score for chronological

Fig. 1 Relation between bone age and lumbar spine BMD in 36 patients with precocious puberty or early puberty.
Table 1  Frequencies of RFLP genotypes

<table>
<thead>
<tr>
<th>PvuII genotype</th>
<th>Number of patients (n=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP</td>
<td>6 (22.2%)</td>
</tr>
<tr>
<td>Pp</td>
<td>10 (37.0%)</td>
</tr>
<tr>
<td>pp</td>
<td>11 (40.7%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>XbaI genotype</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>XX</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Xx</td>
<td>6 (22.2%)</td>
</tr>
<tr>
<td>xx</td>
<td>21 (77.8%)</td>
</tr>
</tbody>
</table>

No significant differences in BMD were observed between patients with Xx or xx genotypes. By combining the two RFLPs with XbaI and PvuII, the subjects could be grouped into five genotypes: PPXx 2 (7.4%), PPxx 4 (14.8%), PpXx 4 (14.8%), Ppxx 6 (22.2%), and ppxx 11 (40.7%). No significant differences in BMD were observed among these 5 genotypes.

Discussion

Rapid gains in bone mass are observed during the periods of maximal bone growth in infancy and adolescence. Although much of the gain during puberty is influenced by sex steroids, growth hormone, height, body mass, calcium intake, and exercise (20–22), the sex steroid estrogen is thought to be essential for normal pubertal epiphyseal maturation and bone mineralization in both sexes; the effect of androgen may be mediated via aromatization to estrogen (23).

In the present study, we analyzed the effect of estrogen exposure on bone maturation and age and bone age) is shown in Figs. 2 and 3. With regard to the PvuII RFLP, patients with the PP genotype exhibited an elevated BMD for their chronological age, compared to those with the Pp genotype (p<0.05). No statistical differences were observed between the PP and pp or the Pp and pp genotypes; and no statistical differences in BMD for bone age were observed among the ER genotypes.

No significant differences in BMD were observed between patients with Xx or xx genotypes. By combining the two RFLPs with XbaI and PvuII, the subjects could be grouped into five genotypes: PPXx 2 (7.4%), PPxx 4 (14.8%), PpXx 4 (14.8%), Ppxx 6 (22.2%), and ppxx 11 (40.7%). No significant differences in BMD were observed among these 5 genotypes.

Fig. 2a  Lumbar spine BMD values for chronological age and estrogen receptor (ER) PvuII and XbaI genotypes. Values are expressed as medians ± standard error (SE). Patients with the PP genotype showed an increased BMD for their chronological age, compared to the Pp genotype (p<0.05). No statistical significant differences were observed between the PP and pp or the Pp and pp genotypes.

Fig. 2b  Lumbar spine BMD values for bone age and ER PvuII and XbaI genotypes. Values are expressed as medians ± SE. No significant statistical differences were observed among the ER genotypes.
mineralization in girls with precocious puberty or early puberty. The lumbar spine BMD of untreated patients with CPP has been reported to be elevated for their chronological age but appropriate for their bone age (14). Contrary to expectation, however, the present study revealed a dissociation between the degree of BMD increase and advanced bone age. In other words, bone maturation was not associated with bone age-matched mineralization in subjects with precocious puberty or early puberty. In 30 out of 36 cases, bone mineral accretion was not at the levels that reflected the bone age, and the BMD value adjusted for bone age was variable, indicating that bone maturation and bone mineralization do not advance synchronously. Variations in estrogen concentration and the duration of bone exposure to estrogen may be related to the observed variability of bone mineralization.

ER gene polymorphism has been variously reported to be related to bone mass (9–13). Two studies have reported that Pvu II and Xba I RFLPs at the ER gene locus have a significant effect on bone growth. The study reported by Kobayashi et al. (9) showed that the PPxx genotype of the two combined RFLPs was associated with low BMD, whereas the report by Qi et al. (10) revealed that the pp genotype of the Pvu II RFLP was related to a low BMD. Another study has suggested that ER polymorphism may have different effects on bone metabolism depending on menopausal status; the ER gene polymorphism association with BMD seems to differ in postmenopausal and premenopausal women (11, 12). Moreover, since physique has an effect on BMD in precocious puberty, we investigated the SD scores for BMD in relation to height age for each ER polymorphism, but the data were very widely distributed, and there were no statistically significant differences. Therefore, we hypothesized that variants in the ER gene may account for the varying response of bone growth to premature estrogen exposure in patients with CPP or early puberty. The present results suggest that the PP genotype for the Pvu II RFLP appears to be involved in the accretion of BMD. The mechanism by which these polymorphisms...
are associated with BMD remains unclear. ER gene polymorphism may have a modulatory effect on calcium and bone metabolism or it might influence transcriptional regulation (13). Moreover, we speculate that the different dynamics of circulating estrogen concentration, the decrease of estrogen level during the menopausal phase and the increase of estrogen level during the pubertal phase, may differently affect ER, resulting in no consistent relationship between ER polymorphisms and BMD. Further studies are required to identify other sites of the ER gene that may affect BMD. Patients with CPP are usually treated with agonistic analogs of gonadotropin-releasing hormone (GnRH) that suppress the estrogen secretion from the gonads; this therapy can have an adverse effect on bone mineralization, which may increase the contribution to osteoporosis (24–26). This concern must be carefully considered because bone maturation is probably not associated with bone age-matched mineralization in untreated CPP. Thus, ER genotype analysis may be useful for identifying children at risk of experiencing a reduction in BMD when treated with GnRH analogs.

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References


