Decreased Bone Mineral Density during GnRH Analog Therapy and Polymorphism of Estrogen Receptor Gene in Precocious Puberty

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**ESTROGEN** plays an important role in bone maturation and bone mineralization during puberty [1]. We previously reported that bone mineral accretion was not adversely affected by short time period of GnRH analog (GnRHa) therapy [2]. In the present study, we followed changes in bone mineral density (BMD) in precocious puberty for 15-27 months to assess the long-term effect of GnRHa. And we also determined whether the estrogen receptor (ER) genotype is related to BMD gain during exposure to excess estrogen in patients.

**Subjects and Methods**

**Patients**

Twenty-one girls with central precocious puberty aged 5–12 years (mean 8.88 ± 1.78 years) who had a history of breast development before the age of 8 were studied. One of the patients had been receiving treatment with GnRHa administered subcutaneously, three with GnRHa administered nasally, two with cyproterone acetate, and the remaining 15 patients were untreated. Ten patients (including 3 who had been receiving another treatment) started to receive GnRHa administration subcutaneously and 3 started to receive GnRHa nasally just after the first BMD analyses.

**Methods**

Height and weight were measured, and sexual maturity was assessed by one investigator with the criteria of Tanner in breast. Bone age (BA) was analysed by the TW2 RUS method for Japanese. The patients were assigned to a treatment with depot leuprorelin acetate (30–60 μg/kg) subcutaneously every 4 weeks, or a treatment with buserelin acetate (1200 μg daily) intranasally.

BMD (g/cm²) was assessed at the baseline, 7–15 months (second measurement) and 15–27 months (third measurement) after the start of GnRHa treatment by dual energy X-ray absorptiometry (Hologic QDR-2000), at the lumbar spine (L2–4).

ER genotype was analysed using polymorphism in intron 1 by the polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method. After the amplification of genomic DNA by PCR, the PCR product was digested with restriction endonuclease (PvuII or XbaI), and electrophorased in an agarose gel. The RFLPs were coded either P or p (when digested with PvuII) and X or x (XbaI), where capital letters signify the absence of restriction sites and small letters the presence of restriction sites.

**Result**

Before treatment, all of the patients except two
had accelerated BA (BA/CA: 1.11–1.62), and the pubertal stage ranged from Tanner stage II to IV.

BMD values at the baseline were approximately equal to those for chronological age (0.06 ± 0.98 SD, but BMD values adjusted for BA were lower than the normal mean even though within ± 2 SD except in one case. The second or third measurement showed decreased lumbar BMD at 13–27 months after the start of therapy in one patient with nasal GnRHa administration (Fig. 1), and 4 of them receiving subcutaneous GnRHa administration (Fig. 2). The subcutaneous GnRHa administration caused a significant decrease in the SD score of BMD compared to no treatment or another treatment (subcutaneous GnRHa administration: −0.320 ± 0.362 SD, others: 0.285 ± 0.309 SD, P=0.05). Neither Pvull nor Xbal ER gene polymorphism was related to the SD score for BMD at the baseline (Fig. 3) and BMD gain during GnRHa treatment in patients with precocious puberty (data not shown).

**Discussion**

There is abundant evidence suggesting that estrogen is essential for normal pubertal epiphyseal maturation and skeletal mineralization in both sexes.
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[1]. Estrogen deprivation due to GnRHa treatment might affect bone mineral accretion, and the GnRHa treatment of endometriosis and other disorders in adult women has resulted in rapid loss of BMD [3]. In patients with central precocious puberty, some reports showed both loss of BMD [4] and normal BMD gain [5] during GnRHa administration. Recently, Bertelloni et al. reported that peak bone mass is not impaired by GnRHa therapy [6].

The present data showed that bone maturation was not associated with BA-matched mineralization in untreated precocious puberty, and long-term treatment with GnRHa may affect bone mineral accretion and result in loss of BMD in precocious puberty. It will be necessary to evaluate the peak bone mass of these patients.

Although a few studies on adult Japanese women have reported an association of BMD with polymorphism of the ER gene [7–9], it is still unclear how intronic polymorphism in the ER gene can affect peak bone mass. However, elucidation of the linkage between ER polymorphism and bone metabolism would provide a useful assessment to prevent from reduction of BMD during GnRHa therapy. Further examination will be needed to determine whether the ER genotype affects bone mineral accretion during exposure to excess estrogen in patients.

References