Original Article

Osteocalcin Gene Polymorphism is Related to Bone Density in Healthy Adolescent Females

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Abstract. Recently a polymorphism was found in the human osteocalcin gene, and its association with bone mass was investigated in healthy postmenopausal Japanese women. The osteocalcin gene allelic variant HH was found to be overrepresented in women with osteopenia. The purpose of this study was to investigate whether the previously demonstrated polymorphism of the osteocalcin gene was related to bone mineral density (BMD; g/cm²) or osteopenia in a group of 97 healthy Caucasian adolescent females (aged 16.9 ± 1.2 years, mean ± SD). BMD of the left humerus, right femoral neck, lumbar spine and total body was measured using dual-energy X-ray absorptiometry. The relation between the allelic variants and bone density was analyzed as presence or absence of the H allele. Presence of the H allele was found to be an independent predictor of humerus BMD (β = −0.21, p<0.05) and femoral neck BMD (β = −0.23, p<0.01). Using logistic regression, presence of the H allele was also independently associated with a 4.5 times increased risk of osteopenia (p = 0.03) in the whole group. Osteopenia was defined as at least 1 SD lower bone density than the mean for the whole group of at least one of the BMD sites measured. We have demonstrated that the osteocalcin HindIII genotype is independently related to bone density in healthy adolescent females. The present study also suggests that presence of the H allele is predictive of osteopenia at an early age.

Keywords: Bone density; Osteocalcin; Osteopenia; Physical activity; Polymorphism; Women

Introduction

Osteocalcin, also called bone GLA protein, is a biochemical marker that is often used in the assessment of bone turnover in patients with osteoporosis [1,2]. Osteocalcin is the most abundant noncollagenous protein in bone produced exclusively by mature osteoblasts and is used as a marker for osteoblast specific activity. It has been assumed to be involved in the mineralization of bone due to the capacity of the GLA residues to bind calcium and hydroxyapatite [1]. Osteocalcin has also been suggested to act as a chemoattractant for osteoclasts and their precursor cells, as well as to be involved in the adhesion and spreading of osteoclasts [3,4]. In a recent study, it was shown that osteocalcin-deficient mice developed a phenotype with a higher bone mass and bones of improved functional quality [5]. Thus, osteocalcin may be a negative regulator of bone formation, at least in mice. Osteocalcin may also be involved in human bone metabolism. Dohi et al. [6] recently demonstrated that a polymorphism in the human osteocalcin gene was associated with osteopenia in healthy postmenopausal Japanese women.
Osteoporosis is a multifactorial disease where both genetic and environmental factors affect the outcome of the disease. Genetic factors are important to investigate since studies have demonstrated that at least 70% of the variance in bone mass is related to genetic factors [7–9]. Peak bone mass is attained at the end of the second decade of life, with a progressive loss of bone thereafter [10,11]. Thus, peak bone mass may influence the later risk of contracting osteoporosis [12]. Identification of genetic factors influencing peak bone mass may increase the possibilities for prevention of osteoporosis.

In the present study, we investigated whether the previously demonstrated polymorphism of the osteocalcin gene [6] is related to bone density in healthy adolescent females.

Materials and Methods

Subjects

This study was performed in Umeå, in the northeastern part of Sweden. From advertisements and information in schools and local sports clubs 97 healthy Caucasian girls (aged 16.9 ± 1.2 years, mean ± SD) were recruited and included in the present study. The girls were at least 2 years postmenarche. None of the girls had any disease or medication known to affect bone metabolism. Using a standardized questionnaire, the amount of weight-bearing physical activity per week during the previous year was assessed. Informed written consent was given by all the participants and the study protocol was approved by the Ethics Committee of the Medical Faculty, Umeå University.

Bone Mineral Density

Bone mineral density (BMD, g/cm²) of the right femoral neck, lumbar spine and total body was measured using a Lunar DPX-L (Lunar, Madison, WI) dual-energy X-ray absorptiometer, software version 1.3y. BMD of the left humerus was estimated from the total body scan using the region of interest option. The accuracy and precision of this method has been discussed in detail by others [13,14]. In our laboratory the CV value (standard deviation/mean) is 0.7–2.0%, depending on the application [15].

Biochemical Analysis

Blood samples were collected after an overnight fast. Osteocalcin was analyzed in plasma samples from 92 girls by a commercially available radioimmunoassay kit (DiaSorin, Stillwater, MI). All samples were analyzed in duplicate. The sensitivity of this assay is 0.8 ng/ml. Intact parathyroid hormone (PTH) was measured in all 97 girls, using Imulite intact PTH, solid-phase sandwich chemiluminescent immunological assay (DPC, CA). The detection level of this assay is 0.1–2.63 pmol/l. Total calcium was measured in plasma samples from 94 of the subjects using atomic absorption spectroscopy [16] with 1.2% intra-assay precision. Albumin was measured with complexocolorimetric dry chemistry by Bromresol Green on Vitros 950 (Ortho-Clinical Diagnostics, NY). All samples were analyzed in duplicate.

Genomic DNA Analysis

Determination of the osteocalcin genotypes was performed as previously described [6], with some modifications. Genomic DNA was extracted from EDTA-stabilized blood of 97 girls using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI). Genomic DNA (30 ng) was amplified in a 50 μl reaction mixture consisting of 0.4 μM forward primer (5’-CCG CAG CTC CCA ACC ACA ATA AGC T-3’), 0.4 μM reverse primer (5’-CAA TAG GGC GAG GAG T-3’), 0.2 mM each of dATP, dCTP, dGTP, dTTP, 1 × PCR buffer and 2.5 U of Taq polymerase (Roche Biochemicals, Stockholm, Sweden). Polymerase chain reaction (PCR) was carried out in 35 cycles of 30 s denaturation at 94 °C, 30 s annealing at 59 °C and 30 s elongation at 72 °C (Peltier Thermocycler, MJ Research, Watertown, MA). The PCR products were cleaved over night with HindIII restriction endonuclease (Roche Biochemicals, Stockholm, Sweden), electrophoresed and analyzed on 2% agarose gels. The allelic variants were termed HHI, Hh and hh. Uppercase letters represent absence, and lowercase letters represent presence, of the restriction site. To validate the accuracy of the genotyping, 10 random patients (of the total 97 patients) had their samples redetermined for the polymorphic site and no discrepancies were found.

Statistical Analysis

Differences between the two groups were investigated using an independent samples t-test. Bivariate correlations were measured using Pearson’s coefficient of correlation. A multiple linear regression was used to analyze the independent significant predictors of BMD at the different sites and a binary logistic regression was used to analyze the independent predictors of osteopenia in the whole group. The SPSS statistical package for PC was used for the analyses. A p value less than 0.05 was considered significant.

Results

The 97 girls studied were 16.9 ± 1.2 years old (mean ± SD). The genotypes were coded as H or h (HindIII; presence (H) or absence (h) of the restriction site). The frequencies of the different genotypes were 5.2% (HH), 29.9% (Hh) and 64.9% (hh). Since there were only five subjects defined by the HH genotype the subjects were categorized as presence or absence of the H allele. Physical characteristics, levels of PTH, osteocalcin, calcium and bone density, for all subjects, and according to presence or absence of the H allele, are presented in