Interaction of interleukin-6 and estrogen receptor gene polymorphisms on bone mass accrual in Chinese adolescent girls

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Abstract We assessed the main and interaction effects of interleukin-6 and estrogen receptor gene polymorphisms on bone mass accrual in Chinese adolescent girls. A total of 228 premenarche Chinese girls (9–11.5 years old) were recruited for a 2-year follow-up study. Bone mineral density (BMD) at the total body, lumbar spine (L1–L4), and total left hip were measured by dual-energy X-ray absorptiometry at baseline and follow-up. The −174G/C and −634C/G polymorphism of IL-6 gene, and PvuII and XbaI polymorphisms of the estrogen receptor (ER)-α gene, were determined. The −634C/G polymorphism of the IL-6 gene and PvuII polymorphism of ER-α gene were significantly associated with bone mass accrual after adjusting the potential confounding factors. Girls with pp genotype of ER-α gene had greater percentage accrual in BMD of total body (P = 0.010) and femoral intertrochanter (P = 0.038) than their PP and Pp counterparts. Girls with CC genotype of IL-6 −634G/C gene had higher percentage accrual in BMD of total body (P = 0.032) and femoral trochanter (P = 0.048) than their CG + GG counterparts. Significant interaction effects of IL-6 −634C/G polymorphism and ER-α PvuII polymorphism were observed on percentage change in BMD of total left hip (P = 0.009) and femoral intertrochanter (P = 0.007). The genotype CC (IL-6 −634C/G) × pp (ER-α PvuII) was associated with greater BMD accrual than other genotype combination in Chinese adolescent girls. We found that the IL-6 −634C/G and ER-α PvuII polymorphism were significantly associated with BMD accrual and that they have an interactional effect on BMD accrual in Chinese adolescent girls.

Key words interleukin-6 (IL-6) · estrogen receptor · polymorphism · bone mineral density · association

Introduction

Osteoporosis is a major public health problem in both Caucasian and Asian populations. The archived peak bone mass in adolescence and young adulthood is probably a major determinant of the risk of contracting osteoporosis and resulting fracture later in life [1]. Bone mineral density (BMD) is a combined result of multiple genetic and environmental factors, with heritability estimates ranging from 0.5 to 0.9 [2]. So far, a long list of candidate genes have been identified in genetic epidemiology studies [3].

Interleukin-6 (IL-6) is a pleiotropic cytokine playing a central role in the regulation of bone metabolism and has been suggested to be a key factor responsible for increasing bone resorption caused by loss of gonadal function [4,5]. Several allelic variants have been identified in the IL-6 gene promoter region. Among them, G > C polymorphisms at the position −174 and −634 have been associated with BMD in some populations [6,7]. The estrogen receptor (ER)-α gene plays a major role in bone metabolism in human. It regulates the production of a number of growth factors and cytokines, has direct effects on osteoblast differentiation and apoptosis, and also regulates osteoclast development, activity, and apoptosis [8]. Some studies have observed significant associations between polymorphism of the ER-α gene and BMD in postmenopausal women [9–11]. However, little is known about their effects on BMD accrual in adolescence.

Extensive functional research has addressed the individual effect of these genes on bone metabolism. Furthermore, increasing evidence has been observed that the effect of a particular gene on the BMD variation may depend on the context defined by other genes, and that there exist effects of interaction among bone metabolism-related genes [12–16]. The true effects of a single genetic factor on BMD may be masked by such interactions. Interaction studies are needed to clarify their true effects on BMD. The present study aims to investigate the main and interaction effects of IL-6 and ER-α gene polymorphisms on bone mass accrual in Chinese adolescent girls in a 2-year cohort study.
Materials and methods

Subjects

We recruited 228 premenarche girls aged 8 to 11 years for this 2-year cohort study from four elementary schools in Guangzhou City, China. They all belong to the Chinese Han ethnic group, which comprises more than 90% of the total population of China. Girls with the following conditions were excluded: vegetarian, taking part in professional sport exercise, taking calcium supplement or other medicines that affect bone metabolism, past or current history of deformity, hereditary disease, psychosis, cancer, thyroid and parathyroid diseases, renal failure, or autoimmune diseases. The study has been approved by the Ethics Committee of Sun Yat-sen University, and informed consent was obtained from each girl’s parents or legal guardians. During the study period, 30 subjects lost to contact due to moving to another city or another school, 22 subjects withdrew for personal reasons, and 176 subjects completed the follow-up study. There were no differences in BMD, age, height, and weight at the baseline between girls who completed and did not complete the follow-up study (data not shown).

Bone mineral density measurements

BMD at total body, lumbar spine (L1–L4), and left hip (including femoral neck, trochanter, intertrochanter, and Ward’s triangle area) were measured by a dual-energy X-ray absorptiometry (DXA) scanner (Hologic QDR 4500A; Hologic Corporation, Waltham, USA). All bone mass tests were done and analyzed by the same technician. The machine was calibrated daily; the coefficient of variation (CV) for a phantom BMD tests was 1% in the daily calibration. BMD was measured at baseline and 2 years later, and percentage change in BMD was calculated.

Questionnaire interview

Dietary calcium intake was assessed using the food frequency questionnaire (FFQ) and the 3-day (2 weekdays and 1 weekend day) food record, and calculated using the Calculating Software of Nutrients (Edition V1.6, China CDC, Beijing, China). The FFQ was designed at Sun Yat-sen University and contained 49 items and 9 subquestions. All foods were amplified according to the primers described previously [18,19] (forward: 5’-TGACTTCACTTACTTCTGT-3’ and reverse: 5’-CTGATTGGAAACCTTATAAAG-3’ for -174G/C; forward: 5’-CACGCCACCCCTCTCTTA-3’ and reverse: 5’-CCAAGCCTGGATTGAA-3’ for -634C/G). The PCR was performed in a 50-μl reaction volume containing 100 ng genomic DNA, 15 pmol of each primer, and 2 units Taq polymerase (Dingguo, Biotechnology Co., Beijing, China), 1× PCR buffer, 1.0 mmol dNTP (Dingguo, China), and 2 mmol MgCl2 for -174G/C or 1 mmol MgCl2 for -634C/G. PCR was performed by the following steps: denaturation at 94°C for 4 min, then 30 cycles of denaturation at 94°C for 30 s, annealing at 52°C (for -174G/C) or 50°C (for -634C/G) for 30 s, and extension at 72°C for 30 s, ending with a final extension at 72°C for 7 min. After amplification, the PCR products were digested with the restriction endonuclease (NlaIII for -174G/C and BsrI for -634C/G; New England Biolabs, Hellerup, Denmark) overnight at 37°C and electrophoresed in 2% agarose gel.

Statistical analyses

All data shown are expressed as means and standard deviations. Statistical analyses were performed using SPSS.

Serum estradiol measurements

Fasting blood was collected and stored at −80°C until analyzed. Serum estradiol was measured by enzyme-linked immunoassay using the specific kit (Biocheck, Foster City, CA, USA). The intra- and interassay coefficients of variation (CVs) were 6.6% and 10.3%, respectively.