Collagen Iα1 Polymorphism is Associated with Bone Characteristics in Caucasian Children and Young Adults

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Abstract. A large proportion of the variation in bone mass can be explained by genetic factors. We analyzed the G to T substitution in the Sp1 binding site in the first intron of the collagen type Iα1 (COLIA1) gene in relation to bone mass. The genotypes GG, GT, and TT were determined in 148 Caucasian children and young adults. We performed dual energy X-ray absorptiometry twice (mean follow-up time 4.4 years), and speed of sound (SOS) was assessed by tibial ultrasonometry at follow-up. Genotype distribution was 104 (70%) GG, 40 (27%) GT and 4 (3%) TT. Carriers of the T-allele had a 0.5 SDS (standard deviation score) decreased bone mineral content (BMC) of total body ($P = 0.001$), and a 0.4 SDS decreased bone mineral density (BMD) for both lumbar spine ($P = 0.04$) and total body ($P = 0.05$). The genotype effect on BMD and BMC decreased after adjustment for height or body mass index. When we calculated apparent BMD, these differences diminished to 0.1 SDS and were no longer significant. T-allele carriers had shorter stature (0.4 SDS; $P = 0.04$) and smaller bones (0.5 SDS lower width of the lumbar vertebral body; $P = 0.01$). The T-allele was also associated with lower SOS ($P = 0.03$), independent of BMD and BMC, and lower lean body mass. Similar associations were found at follow-up. The change in BMD and BMC SDS between the first and second measurement did not differ between the GG and GT&TT group. In conclusion, the COLIA1 polymorphism in children and young adults is associated with several bone characteristics. However, at least a part of the COLIA1 effect on bone mass may be related to differences in frame size.

Key words: Bone density — Collagen Iα1 polymorphism — Children — Ultrasonometry — Dual energy X-ray absorptiometry

Osteoporosis is a common disorder in the elderly with an increasing incidence world-wide. It is characterized by reduced bone mineral density (BMD), deterioration of the micro-architecture of bone tissue, and increased fracture risk [1]. Variation in the attainment of peak bone mass plays an important role in the development of osteoporosis in later life [2].

Over the past decade, observed differences in BMD between races [3] and twin and family studies [4] strongly suggest that genetic factors play a major role in the pathogenesis of osteoporosis. It has been estimated that up to 80% of the variation in BMD is (poly)genetically determined [4, 5]. Therefore, genetic research on osteoporosis is worthwhile for identifying subjects with an increased risk of developing osteoporosis at an early stage.

Polymorphisms in several candidate genes have been investigated to analyze their contribution to aspects of osteoporosis, including polymorphism at the 3’ end of the vitamin D receptor gene [6, 7] and a G to T substitution in the Sp1 binding site at the collagen type Iα1 (COLIA1) gene [8–10]. Most studies have been performed in elderly women who had undergone substantial bone loss. However, candidate gene polymorphisms may play a role in the attainment of peak bone mass as well. Children also have had shorter exposure to lifestyle and environmental factors which can influence the overall effect of genetic factors on bone mass. A few studies in pediatric populations have been performed, however, with conflicting results. For example, Sainz et al. [11, 12] found an association of bone density with COLIA1 and the VDR gene in prepubertal girls. Berg et al. [13], however, could not confirm this association for the COLIA1 polymorphism in a group of healthy children and young adults.

We hypothesize that COLIA1 genotype might affect anthropometric or bone characteristics in children and young adults. Therefore, we investigated the association between COLIA1 polymorphism and several bone characteristics in a group of healthy Dutch children and
young adults. We chose to recruit the same population as was used for our reference study on bone density and body composition, which was performed approximately 4 years ago. Thus, we were able to evaluate bone gain as well as cross-sectional data.

Material and Methods

Subjects

For this study we recruited 176 Dutch children and young adults (65 boys and 111 girls) from the Rotterdam Region in the Netherlands. All but 6 subjects participated in our previous study (1994–1995) to assess normative values for bone density and body composition measured by dual energy X-ray absorbometry (DXA). The cross-sectional results of this first study have been presented previously [14, 15]. The mean follow-up time was 4.4 years (range 3.2–6.7 years). The ethnicity was Caucasian for 142 children (55 males), Black for 7 children (3 males), Hispanic for 9 children (3 males), Asian for 6 children (1 male), and mixed ethnicity 6 (1 male). The 6 children who participated only at follow-up were all Caucasian (4 males). This study was approved by the medical ethics committee of the University Hospital Rotterdam, and written informed consent was obtained from the parents or guardians and from all children aged 12 years and over.

Anthropometry

Height was assessed using a fixed stadiometer and expressed as standard deviation scores (SDS) [16]. Weight was measured without shoes on a standard clinical balance. The questionnaire to determine calcium intake, physical activity, medical history, menarche, and use of oral contraceptives was identical to the one used in the previous study [14]. Body mass index was calculated (kg/m²) and lean body mass (LBm, g) and percentage body fat (% fat) were measured by total body DXA (Lunar DPX-L, Madison, WI), all expressed as SDS [15, 17]. As validated previously [18], pubertal development was evaluated by self-assessment of breast and pubic hair stage in girls and genitalia and pubic hair stage in boys, according to Tanner [19].

Bone Characteristics

Bone mineral density (BMD, g/cm²) was determined by DXA of the lumbar spine (L2-L4) and total body. DXA measurements were performed twice (in 1994–1995 and in 1998–1999). For children with weight below 30 kg, pediatric software was used. To account for differences in bone size we calculated apparent BMD of the lumbar spine (Ls) with the model BMAD\(_{Ls}\) = BMAD\(_{Ls}\) \(\times 4 (\pi \times \text{width})\), in which width is the mean width of the second to fourth lumbar vertebral body. This model was validated by in vivo volumetric data obtained from magnetic resonance imaging of the lumbar vertebrae [20]. The coefficient of variation has been reported to be 1.04% for lumbar spine BMD and 0.64% for total body (TB) BMD [21]. Total body DXA also measures bone mineral content (BMC, g), BMD, BMAD, BMC, and width of the lumbar vertebrae were compared to our age- and sex-matched Dutch reference values and expressed as SDS [14, 15].

In 102 subjects we also performed tibial ultrasonometry once (in 1998–1999), using the SoundScan® Compact (Myriad Ultrasound Systems LTD, Rehovot, Israel). Following standard operating procedures, all bone assessments were done on the right tibia at the mid-tibial point. The results [speed of sound (SOS in m/s)] were compared with healthy age-matched Dutch controls and expressed as SDS [22].

Genotyping

DNA was isolated from blood according to standard procedures. The Sp1 polymorphism of the COLIA1 gene was detected by the polymerase chain reaction (PCR) with a mismatched primer that introduces a di-allelic restriction site, as described previously [8, 9]. This test discriminates between the two alleles represented as G and T, previously described as ‘S’ and ‘s’, respectively. G represents guanine and T thymidine as the first bases in the Sp1-binding site in the first intron of the gene for COLIA1. The polymorphism results in three genotype groups, GG, GT, and TT.

Statistical Analysis

Because of the known difference in allele frequency and BMD between different races [23], we limited the analysis to the Caucasian children. Allele dose was defined as the number of copies of a certain allele in the genotype group. To quantify the strength of association, we performed linear regression analysis in which genotype groups were designated as 0, 1, or 2, corresponding with the number of T-alleles. We used regression analysis to adjust for possible confounders. Independent samples t-tests were performed to test for differences in bone density, body composition, and SOS between the COLIA1 genotypes. Firstly, we analyzed the results of the 1994–1995 study. Secondly, the same analyses were performed on the data of the 1998–1999 study. Thirdly, the absolute change between follow-up and baseline measurements and the association with genotype was analyzed.

In childhood, and especially during puberty, bone mineral density and body size change markedly. We therefore analyzed separately the allele-dose effects before, during, and after puberty. To correct for the age differences between the genotype groups, bone characteristics were expressed in age and sex-matched standard deviation scores (SDS). P-values of less than 0.05 (two-tailed) were considered significant.

Results

Subjects

The genotype distribution was 104 (70.3%) GG, 40 (27.0%) GT, and 4 (2.7%) TT. There was no difference in genotype distribution between boys and girls. The allelic frequencies were G = 0.84 and T = 0.16. The distribution of genotypes was in Hardy-Weinberg equilibrium (P = 0.95). Mean age at follow-up was 15.6 years (range: 7.6–25.3). Baseline characteristics of all Caucasian subjects and according to COLIA1 genotype are reported in Table 1. The three genotype groups did not differ significantly in age, calcium intake, or physical activity. Because of the small numbers in the TT group we pooled the GT and TT groups and compared them with the GG group. Lean body mass (LBM) SDS and height SDS were significantly higher in the GG group compared with the combined GT&TT groups (P = 0.02 and P = 0.04, respectively). No genotype effect on % fat was observed. The change in height SDS, BMI SDS, and LBM SDS between the first and second measurement did not differ between the GG and GT&TT groups.

The mean age at menarche was 13.1 years (11–16 yrs), similar to normal age of menarche in the Nether-