

## CLINICAL STUDY

# The Sp1 binding site polymorphism in the collagen type I $\alpha$ 1 (COLIA1) gene is not associated with bone mineral density in healthy children, adolescents, and young adults

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## Abstract

Up to 85% of the variance in bone mineral density (BMD) is genetically determined. A putative candidate gene involved in the regulation of bone mass is the *COLIA1* gene encoding type I collagen, which is the major protein of bone. We examined possible allelic influences of a G to T *COLIA1* gene polymorphism in a recognition site for the transcription factor Sp1 on: (i) gain of forearm BMD using single photon absorptiometry (SPA); and (ii), BMD of the forearm, spine, hip, and whole body with dual X-ray absorptiometry (DXA). At baseline, 269 healthy boys and girls aged 8.2–16.5 years were eligible for the study. Forearm BMD measurements obtained at baseline and after  $3.8 \pm 0.1$  years ( $\pm$  S.D.) were used to calculate the annual percentage change in BMD. Calcium intake and physical activity were determined by a detailed questionnaire at baseline and after 1 year. Essentially no significant differences in forearm BMD gain or in BMD assessed at the forearm, spine, and whole body were observed among the three *COLIA1* genotypes.

In conclusion, the data indicate that the polymorphism at the Sp1 site in the *COLIA1* gene is not associated with BMD or gain of forearm BMD in healthy boys and girls.

*European Journal of Endocrinology* 143 261–265

## Introduction

Bone growth and accretion of bone mass for a limited period of time during puberty are essential for the resulting peak bone mass and to reduce the subsequent risk of having osteoporotic fractures in later life. Based on family and twin studies, peak bone mass has been estimated to be up to 85% genetically determined (1–4). However, bone mineral density (BMD) is the result of a complex interaction between multiple genes and environmental factors. Several polymorphisms in candidate genes have been proposed as genetic factors that could influence BMD (5).

Grant *et al.* (6) described a polymorphism (G to T base change) in a regulatory region that forms a recognition site for the transcription factor Sp1 in the gene *COLIA1*. The *COLIA1* and *COLIA2* genes encode type I collagen, which is the major protein in bone. The T allele (denoted 's') was associated with reduced hip and spine bone density in two British populations of mostly postmenopausal women. The allele was also over-represented in patients with osteoporotic vertebral fractures (6). Several studies have supported these

observations (7–13), whereas others have failed to show any association between the 's' allele and BMD or bone markers (14–16).

Between 11 and 16 years of age, 30–40% of peak bone mass is attained (17, 18), but the influence of the polymorphism in the *COLIA1* gene on bone gain has not been studied. In this longitudinal population-based study, the increase in forearm BMD was determined over a  $3.8 \pm 0.1$  ( $\pm$  S.D.)-year period and correlated to the *COLIA1* genotypes in children, adolescents, and young adults aged 8.2–16.5 years at the start of the study. At the end of the study BMD was also assessed at the spine, hip, and whole body.

## Subjects and methods

Data from this population-based study, initiated by Dr M Gunnes, Haugesund, Norway, have been previously presented as cross-sectional results, 1-year follow-up data, and results from vitamin D receptor genotyping (18–21). Briefly, 494 healthy boys and girls were enrolled in a prospective study of determinants of bone

**Table 1** Clinical characteristics, calcium intake, and weight-bearing physical activity in boys and girls in relation to *COLIA1* gene alleles.

Boys	SS	Ss	ss	p <sup>a</sup>
n(sum) = 126	92	28	6	
Age baseline (years)	12.0 ± 0.24	12.6 ± 0.43	12.2 ± 0.93	0.44
Height baseline (cm)	152.9 ± 1.53	158.9 ± 2.78	153.9 ± 6.00	0.17
Weight baseline (kg)	44.8 ± 1.21	47.7 ± 2.19	47.1 ± 4.73	0.47
Height gain (cm) <sup>b</sup>	18.9 ± 0.85	15.0 ± 1.53	21.7 ± 3.31	0.05
Weight gain (kg) <sup>b</sup>	19.0 ± 0.70	17.8 ± 1.27	18.7 ± 2.74	0.71
Body fat mass (%)	18.2 ± 0.78	19.0 ± 1.41	13.2 ± 3.04	0.22
Body lean mass (%)	78.1 ± 0.74	77.2 ± 1.34	82.7 ± 2.89	0.23
Calcium (mg/d) <sup>c</sup>	1131 ± 41.1	1148 ± 72.6	1137 ± 165.5	0.98
WPA (h/week) <sup>c</sup>	8.3 ± 0.56	7.7 ± 0.97	7.2 ± 2.18	0.78

  

Girls	SS	Ss	ss	p <sup>a</sup>
n(sum) = 143	105	33	5	
Age baseline (year)	12.6 ± 0.23	12.3 ± 0.41	12.8 ± 1.06	0.72
Height baseline (cm)	154.4 ± 1.34	152.5 ± 2.39	156.7 ± 6.13	0.71
Weight baseline (kg)	47.7 ± 1.22	45.8 ± 2.18	54.7 ± 5.60	0.33
Height gain (cm) <sup>b</sup>	10.8 ± 0.88	13.0 ± 1.57	9.8 ± 4.03	0.46
Weight gain (kg) <sup>b</sup>	12.9 ± 0.78	13.9 ± 1.39	17.1 ± 3.58	0.46
Body fat mass (%)	30.6 ± 0.63	29.7 ± 1.13	37.2 ± 2.90	0.06
Body lean mass (%)	65.8 ± 0.60	65.7 ± 1.07	59.6 ± 2.76	0.06
Calcium (mg/d) <sup>c</sup>	882 ± 40.2	985 ± 68.3	837 ± 175.4	0.40
WPA (h/week) <sup>c</sup>	5.9 ± 0.39	7.0 ± 0.68	9.6 ± 2.20	0.10

Values are the means ± S.E.M.; n, number of subjects in the three *COLIA1* genotype groups.

<sup>a</sup> Significance of difference among the three *COLIA1* genotypes by one-way ANOVA.

<sup>b</sup> Weight and height gain between baseline and 3.8 ± 0.1 years follow-up.

<sup>c</sup> Mean values of calcium intake and weight-bearing physical activity assessed at baseline (1992) and at 1 year follow-up (1993).

mass in 1992. In 1996, 3.8 ± 0.1 (± S.D.) years after the baseline examination, 273 subjects from the original cohort agreed to a second follow-up. This included assessment of forearm BMD gain with single photon absorptiometry (SPA), and measurement of BMD with dual x-ray absorptiometry (DXA) at the forearm, spine, hip, and whole body. Blood samples were taken from 269 of the subjects. The age range of the study population was 8.2–6.5 years at baseline and 12.2–20.3 years at the last follow-up examination.

The protocol was approved by the regional ethical committee, and written informed parental consent was obtained.

### Analysis of BMD

BMD was measured as previously described (21) at baseline in 1992, in 1993, and in 1996 at the nondominant distal and ultradistal forearm by SPA using an Osteometer DT 100. In 1996, at the second follow-up, BMD was additionally measured at the anteroposterior spine (L1–L4), proximal femur, radius, and whole body by DXA with a QDR 4500 device (Hologic QDR 4500 A, Waltham, MA, USA), which was also used to analyse body composition. Details on short-term precision and correlation between SPA and DXA measurements have been presented previously (21).

### *COLIA1* genotyping

The G to T polymorphism in the Sp1 binding site in the *COLIA1* gene was detected by a polymerase chain reaction-based method with primers and reaction conditions as described by Grant *et al.* (6) using DNA extracted from whole blood. The reaction products were digested with the restriction enzyme *BalI* (Promega, Madison, WI, USA) and analysed by agarose gel electrophoresis. The absence or presence of the restriction site represents a G or T nucleotide and is indicated by 'S' or 's' respectively.

### Statistics

SPA data from baseline and after 3.8 ± 0.1 years (± S.D.) were used to calculate BMD accretion rates assessed as the annual percentage change from the baseline BMD. Comparison of variables between genotypes was assessed by ANOVA, and all statistical analyses were performed using Systat program version 5 (Systat, Evanston, IL, USA). Data are presented as the means ± S.E.M.

### Results

Genotyping of the Sp1 polymorphism in the *COLIA1* gene of the 269 persons eligible for the study showed that the distribution between 'SS' (n=197), 'Ss' (n=61), and 'ss' (n=11) genotypes was in Hardy–Weinberg

**Table 2** Forearm BMD gain measured by single photon absorptiometry and BMD of the forearm, spine, hip, and whole body measured by dual x-ray absorptiometry in relation to COLIA1 gene alleles.

Boys	SS	Ss	ss	p <sup>a</sup>
n(sum) = 126	92	28	6	
BMDd gain/year (%) <sup>b</sup>	6.4 ± 0.48	6.3 ± 0.88	6.8 ± 1.89	0.98
BMDud gain/year (%) <sup>b</sup>	5.3 ± 0.45	3.6 ± 0.81	5.9 ± 1.76	0.17
BMD total forearm (g/cm <sup>2</sup> )	0.472 ± 0.0079	0.493 ± 0.0143	0.488 ± 0.0310	0.41
BMD spine (L1-L4) (g/cm <sup>2</sup> )	0.922 ± 0.0159	0.946 ± 0.0287	0.968 ± 0.0621	0.62
BMD total hip (g/cm <sup>2</sup> )	0.989 ± 0.0158	0.998 ± 0.0287	1.155 ± 0.0620	0.04
BMD whole body (g/cm <sup>2</sup> )	1.083 ± 0.0136	1.099 ± 0.0246	1.166 ± 0.0532	0.30
Girls	SS	Ss	ss	p <sup>a</sup>
n(sum) = 143	105	33	5	
BMDd gain/year (%) <sup>b</sup>	5.8 ± 0.44	5.6 ± 0.78	6.6 ± 2.00	0.90
BMDud gain/year (%) <sup>b</sup>	3.5 ± 0.37	3.6 ± 0.66	5.0 ± 1.69	0.68
BMD total forearm (g/cm <sup>2</sup> )	0.463 ± 0.0065	0.457 ± 0.0115	0.477 ± 0.0296	0.81
BMD spine (L1-L4) (g/cm <sup>2</sup> )	0.990 ± 0.0131	0.998 ± 0.0233	1.014 ± 0.0599	0.90
BMD total hip (g/cm <sup>2</sup> )	0.969 ± 0.0118	0.973 ± 0.0211	0.951 ± 0.0541	0.93
BMD whole body (g/cm <sup>2</sup> )	1.072 ± 0.0103	1.064 ± 0.0183	1.044 ± 0.0470	0.80

Values are the means ± s.e.m.; n, number of subjects in the three COLIA1 genotype groups. BMDd, BMDud, bone mineral density measured at the distal (d) and ultradistal (ud) forearm respectively with SPA.

<sup>a</sup>Significance of difference among the three COLIA1 genotypes by one-way ANOVA.

<sup>b</sup>Average annual bone gain (percentage) between baseline (1992) and 3.8 ± 0.1 years follow-up (1996).

equilibrium. Age, height, weight, mean calcium intake, and weight-bearing physical activity were similar among the three genotypes at baseline as shown in Table 1. Weight gain from baseline to 3.8 ± 0.1 years (± s.d.) follow-up, and body fat mass and lean mass after follow-up were also similar. Height gain during the study period was significantly higher among the small group of boys with the 'ss' genotype (n = 6) when compared with boys with the 'Ss' and 'SS' genotypes.

Table 2 shows that the calculated gain of BMD per year was similar among the COLIA1 genotypes. DXA measurements of BMD at various sites showed essentially no differences in BMD among the COLIA1 genotypes, although the small group of boys with the 'ss' genotype had significantly higher total hip BMD. When the mean BMD values were adjusted for age, height, weight, and BMD at baseline, weight gain, height gain, body fat, lean mass, dietary calcium intake, and physical activity, the P value for femoral neck comparisons in boys changed from 0.033 to 0.007 (data not shown).

## Discussion

In this study, no association was found between forearm BMD gain in puberty and genotypes based on the polymorphism in the Sp1 binding site in the COLIA1 gene. In the small group of boys with the 'ss' genotype (n = 6), total hip BMD was significantly higher than in boys with 'Ss' and 'SS' genotypes. This difference was not observed in girls, and BMD in both boys and girls measured at the forearm, spine, and whole body was not associated with the polymorphism. Adjusting the

mean BMD values for relevant anthropometric variables, weight-bearing physical activity, and calcium intake essentially did not change the results, but the association between the 'ss' genotype and increased total hip BMD in boys was strengthened. This apparent association should be interpreted cautiously because the group of boys with the 'ss' genotype is small, it appeared only in boys, and there was no association between this genotype and BMD measured at other sites.

A power calculation indicated that true BMD differences of about 0.7 s.d., when comparing the larger groups, and 1.5 to 1.7 s.d. where the smaller groups are involved, would lead to results classified as statistically significant (assuming two-sided Student's t-test with  $\alpha = 0.05$  and power = 0.9). The s.d. values of the BMD measurements ranged typically from 0.05 to 0.08 g/cm<sup>2</sup>; hence 'true' differences lower than 0.08–0.13 g/cm<sup>2</sup> may have escaped the present investigation.

Bone measurements in this study were performed with SPA and DXA, which are based on absorptiometry. These projection techniques have limitations for the assessment of bone mass. BMD values reported in this study reflect bone mass as area density and do not allow direct comparisons with three-dimensional measurements of bone mass.

Type I collagen is the major protein of bone, and mutations in the coding regions of the COLIA1 and COLIA2 genes are found in osteogenesis imperfecta, which is associated with severe osteoporosis (22). The polymorphism detected by Grant *et al.* (6) is in the first intron of the COLIA1 gene. It involves a binding site for the transcription factor Sp1, which is important for the regulation of gene expression and type I procollagen

synthesis, but *COLIA1* gene expression is regulated by several transcription factors (23).

Studies of adults have shown that the *COLIA1* 's' allele may have a negative effect on BMD in hip and spine (6). In a study of 1778 postmenopausal women, this allele was associated with a modest, but significant reduction of BMD in the femoral neck and lumbar spine and was over-represented among women who had incident nonvertebral fractures (10). The small reduction in BMD associated with the 's' allele among French premenopausal women was not significant after adjustment for height (7). Hampson *et al.* (9) demonstrated an association between this allele and reduced BMD at the femoral neck only, whereas Keen *et al.* (12) found a reduction in lumbar spine BMD, increased fracture risk and elevated urinary pyridinoline levels in women with the allele. The 's' allele was also associated with more severe osteoporosis of the spine and hip in men with thalassaemia major when compared with women with the disease (11).

Ethnic differences in the frequency of the *COLIA1* 's' allele have been correlated to the incidence of hip fracture (24). In a low incidence area, such as Korea, the Sp1 polymorphism was not detected among 200 postmenopausal women (25). In Scandinavia both the incidence of hip fracture and the frequency of the 's' allele are high. Langdahl *et al.* (8) showed that in Denmark 31% of subjects with osteoporosis had the 's' allele compared with 18% of the controls. The allele was also associated with an increased fracture risk. In Sweden Lidén *et al.* (14) did not observe any difference in genotype frequency between patients with primary osteoporosis and healthy controls. In this Norwegian study the 's' allele frequency was similar to the levels in Denmark and Sweden, but there was no association to BMD or increase in BMD levels. In a review (5), Ralston indicated that the *COLIA1* polymorphism may be a marker for increased bone loss rather than peak bone mass.

During a few years of rapid bone growth a large proportion of the resulting peak bone mass is attained, but few studies of potential genetic markers of bone mass have been performed in children during puberty. Reports of vitamin D receptor genotypes and bone gain in this age group have been contradictory (21, 26). Pre-pubertal Mexican-American girls with the *COLIA1* 'SS' genotype were recently shown to have 0.5 s.d. higher cancellous vertebral bone density assessed with computed tomography compared with individuals with the 'Ss' genotype (13). Our study had a power of 0.65 to detect a 0.5 s.d. difference in BMD levels between these two groups; i.e. the chance of detecting a real difference at this level was approximately twice as high as to reject it. Differences in ethnic background, age of the children participating in the studies, and methods used for the measurement of bone mass or BMD may explain some of the discrepancies between the two studies.

In conclusion, the polymorphism in the Sp1 binding site in the *COLIA1* gene cannot be used to identify

young individuals with a low BMD or reduced increase in BMD during puberty.

## Acknowledgements

This study is the result of a project initiated by Dr M Gunnes to identify factors influencing bone accretion in children. The authors thank AM Aasbu, AL Robins, A Nærby, and N Gjerlaugsen for excellent technical assistance. The study was supported by the Norwegian Research Council.

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Received 20 July 1999  
Accepted 26 April 2000