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The Effect of *LRP5* Polymorphisms on Bone Mineral Density Is Apparent in Childhood

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Abstract

Bone mass acquired during childhood is the primary determinant of adult bone mineral density (BMD) and osteoporosis risk. Bone accrual is subject to genetic influences. Activating and inactivating *LRP5* gene mutations elicit extreme bone phenotypes, while more common *LRP5* polymorphisms are associated with normal variation of BMD. Our aim was to test the hypothesis that *LRP5* gene polymorphisms influence bone mass acquisition during childhood. The association between *LRP5* gene polymorphisms and bone size and mineralization was examined in 819 unrelated British Caucasian children ($n = 429$ boys) aged 9 years. Height, weight, pubertal status (where available), total-body and spinal bone area, bone mineral content (BMC), BMD, and area-adjusted BMC (aBMC) were assessed. Dual-energy X-ray absorptiometry (DXA)-gene associations were assessed by linear regression, with adjustment for age, gender, pubertal status, and body size parameters. There were 140, 79, 12, and 2 girls who achieved Tanner stages I-IV, respectively, and 179 and 32 boys who achieved Tanner stages I and II, respectively. The rs2306862 (N740N) coding polymorphism in exon 10 of the *LRP5* gene was associated with spinal BMD and aBMC (each $P = 0.01$) and total-body BMD and aBMC ($P = 0.04$ and 0.03 , respectively). Adjusting for pubertal stage strengthened associations between this polymorphism and spinal BMD and aBMC ($P = 0.01$ and 0.002 , respectively). Individuals homozygous for the *T* allele had greater spinal BMD and aBMC scores than those homozygous for the *C* allele. A dose effect was apparent as the mean spinal BMD and aBMC of heterozygous *TC* individuals were intermediate between those of their *TT* and *CC* counterparts. The N740N polymorphism in exon

10 of *LRP5* was associated with spinal BMD and aBMC in pre- and early pubertal children. These results indicate that *LRP5* influences volumetric bone density in childhood, possibly through effects on trabecular bone formation.

Keywords

LRP5; Association; Growth; Height; Bone mineral density

Introduction

Bone accrual during childhood and adolescence is the primary determinant of adult bone mass and has been estimated to account for more than 60% of adult bone mineral density (BMD) [1]. Thus, peak bone mass achieved in adolescence is a key predictor of osteoporosis risk in later life. Bone mass acquisition in childhood reflects two distinct processes, which are likely to be regulated independently: growth in skeletal size (achieved through a combination of longitudinal and appositional growth) and alterations in the mass of bone tissue per unit volume (largely reflecting the extent of endosteal expansion and trabecular bone formation). The relative contribution of these processes to bone mass varies according to site, and although skeletal growth is the primary determinant of bone mass accrual in childhood, trabecular bone formation contributes significantly to the process, particularly at the spine [2].

Environmental factors such as vitamin D and calcium intake [3, 4], weight-bearing exercise [3, 5-7], cigarette smoking, endocrine status [8], protein intake, and carbonated soft drink consumption [9] have been reported to influence skeletal growth and attainment of peak bone mass. However, these factors alone do not account for the bulk of the observed variance of peak BMD.

There is increasing evidence from family and twin studies that genetic influences modulate attainment of peak bone mass [4, 10]. Indeed, familial resemblance of BMD is greater in adolescence and early adulthood than in later life [11, 12]. Furthermore, these influences upon BMD are expressed as early as prepuberty [13]. To date, studies of the genetic influences upon peak BMD have focused upon a variety of genes already implicated in the determination of adult BMD. Thus, associations between peak BMD and polymorphisms in genes encoding the calcium-sensing receptor [14], collagen type 1 α 1 [3], collagen type 1 α 2 [15], estrogen receptor [3, 14], osteocalcin [15], parathyroid hormone [14], and vitamin D receptor [3, 5, 6, 14] have been demonstrated.

Given the central role of osteoblasts in bone formation, genes influencing osteoblast function, such as *LRP5*, are more likely than others to influence skeletal development. Activating *LRP5* gene mutations cause the high bone mass phenotype [16, 17]. Missense mutations of the *LRP5* gene are associated with the osteopetroses [18, 19], while inactivating *LRP5* gene mutations are associated with the osteoporosis-pseudoglioma syndrome [20, 21]. Additionally, common *LRP5* gene polymorphisms are pertinent to BMD determination in normal adult populations. Association of *LRP5* gene polymorphisms to BMD has been reported in European [22, 23], North American [24], and Australian Caucasians [25] as well as Japanese [26] and Korean [27] populations. These genetic findings are supported by *in vitro* and *in vivo* studies which indicate that *LRP5* mediates bone formation. This transmembrane receptor is expressed by osteoblasts and transduces canonical Wnt signaling. Mice overexpressing wild-type or constitutively active *LRP5* [28] have excessive bone accrual. In contrast, receptor truncations which abrogate Wnt signaling impair bone accrual by murine calvarial explants [21]. Furthermore, the altered BMD

phenotype of mice with dysregulated *LRP5* function is evident early in development [28, 29]. Together, these human and murine studies show that common *LRP5* gene allelic variants may determine bone mass and that its influence is evident during bone mass acquisition.

This report examines the hypothesis that *LRP5* polymorphisms influence bone mass accrual in childhood. To test this hypothesis, association between *LRP5* polymorphisms and bone mass indices (measured by dual-energy X-ray absorptiometry [DXA]) was tested in 819 9-year-old children from the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort. This population-based collection comprised of children and their mothers is representative of the population in Great Britain [30-32]. We demonstrate association between one coding *LRP5* polymorphism and spinal bone mass indices.

Methods

Study Cohort

The study population is a subgroup of the ALSPAC cohort. This is a geographically based population longitudinal study which recruited women resident in Avon, United Kingdom, with an expected delivery date between April 1, 1991, and December 31, 1992 [30]. There were 15,541 mothers, approximately 85% of those eligible, enrolled into the study, yielding 14,062 children. Subjects have been extensively characterized by self-reported questionnaires, medical records, and physical examinations. Biological samples including DNA were collected. Ethical approval was obtained from the ALSPAC Law and Ethics Committee and relevant local ethics committees, and written informed consent was provided by all parents.

Ten percent of the cohort of births between June and December 1992 were randomly selected to form the Children in Focus (CIF) subgroup and studied in greater detail than the rest of the cohort over the first 5 years of life. Twin pregnancies and non-Caucasians were excluded, but this cohort was otherwise a random subset of the ALSPAC population. DXA was performed in this cohort when they reached 9 years of age. In all, 6,160 children had DXA data, 1,157 had genotype data, and 819 had both.

Measurement of BMD Variables

Whole-body BMD of 7,336 children was measured by DXA (Lunar Prodigy, Madison, WI). Sitting and standing height were measured using a Harpenden stadiometer (Holtain, Crymych, GB). Weight was quantified using a Tanita Body Fat Analyzer (Tanita, Arlington Heights, Illinois, USA). Total body less head (TBLH) and spinal values were expressed as BMD, bone mineral content (BMC), and bone area. Conventional BMD measures, obtained by dividing BMC by bone area, represent an “areal” density that is only partially corrected for skeletal size. To provide a more accurate estimate for volumetric BMD, we derived a further parameter from BMC, termed “area-adjusted BMC” (aBMC), by linear regression of BMC on bone area. Adjusting BMC for bone area by linear regression analysis in this way generates a variable which, unlike areal BMD, is fully independent of skeletal size [33]. Based upon comparisons performed on 122 children who had two BMD scans performed on a single day, the coefficient of variation for total body BMD was 0.8. In addition, spinal BMC, BMD, area, and aBMC were obtained by subregional analysis of total-body scans in the subset of children whose scans showed no evidence of spinal curvature, as previously described [34]. DXA-derived estimates for volumetric bone density were also obtained by calculation of bone mineral apparent density (BMAD), defined as $(\text{BMC}/\text{area})^{1.5}$ [35].

Measurement of Pubertal Status

Pubertal staging was performed using a self-reported Tanner stage questionnaire [36]. Pubertal data were used only if collected within 16 weeks of the DXA scans. As puberty has a profound effect upon bone development, DXA variables were corrected for pubertal stage where this information was available.

Genotyping for LRP5 Gene Polymorphisms

Peripheral blood DNA from the CIF subgroup was chosen for *LRP5* genotyping. Genomic amplification using a GenomiPhi DNA amplification kit (Amersham Biosciences, Aylesbury, UK) was performed per the manufacturer's instructions.

The intronic rs4988319, rs4988320, and rs2291466 polymorphisms following exons 6, 7, and 21, respectively, and the exonic rs545382 (F549F), rs2277268 (E644E), rs2306862 (N740N), rs556442 (V1119V), and rs53736228 (A1330V) polymorphisms in exons 8, 9, 10, 15, and 18, respectively, were genotyped by polymerase chain reaction/restriction fragment-length polymorphism. Details of the primers and restriction endonucleases used were previously described [23]. Duplication of samples and addition of DNA-free water blanks permitted testing for genotyping errors. Concerns regarding the fidelity of the genotyping of the intronic rs4988319 polymorphism resulted in exclusion of this single-nucleotide polymorphism (SNP) from further analysis.

Statistical Analysis

Age, height, weight, and the DXA variables were normally distributed; comparisons were made between pubertal stages using *t*-tests for the boys and girls separately. Genotype frequencies were calculated and comparisons between genders performed using Fisher's exact test. Associations between each *LRP5* polymorphism and each of the DXA outcomes (TBLH and spinal BMC, BMD, area, and aBMC) were examined using linear regression, with tests on two degrees of freedom to assess differences in the outcomes between genotypes. Analyses were adjusted for age and gender (minimal adjustment) and were repeated with additional adjustment for height and weight (full adjustment). In further analyses, data were restricted to pre- and early pubertal children and then adjusted for pubertal stage by including those in stage I and stage II on the basis of matching Tanner stage questionnaire data, thus restricting analysis to 429 children with TBLH indices and 239 children with spinal bone mass indices.

Haplotypes were derived from genotypic data and compiled using Phase [37]. Haplotypes were composed of pairs of two consecutive SNPs, and a sliding window approach was taken. Associations between haplotypes and the DXA outcomes were examined using linear regression after minimal and full adjustment, with *P* values for tests on two degrees of freedom. A trend test was used to determine the association between common homozygote/heterozygote/rare homozygote and DXA outcomes. For this computation, data from both boys and girls were pooled following adjustment for age, sex, pubertal stage (in girls), height, and weight.

Uncorrected *P* values are presented throughout. The number of independent genetic and phenotypic measures was assessed by principal components analysis [38].

Results

Study Cohort

There were 1,157 children genotyped for *LRP5* gene polymorphisms. Both DXA and *LRP5* genotypic data were available in 819 children (429 boys). Bone densitometric findings in

these children are similar to those from the entire ALSPAC cohort [34]. There were 179 and 32 boys in Tanner stages I and II of puberty, respectively. There were 139, 79, 12, and 3 girls in Tanner stages I, II, III, and IV, respectively. (Pubertal data were not available at age 9 from 217 boys and 157 girls.) Where gene-bone mass associations were adjusted for pubertal status, analysis was restricted to 429 children (211 boys, 218 girls) in Tanner stages I and II. Within the latter cohort, total body (less head) bone mass indices were available in all 429 children but spinal indices were available in only 239 children.

Clinical and demographic data on the cohort are summarized in Table 1.

Association of LRP5 Polymorphisms and Spinal Bone Mass

The genotype frequencies for each polymorphism are described in Table 2. No difference in genotype frequencies was seen between genders (data not shown), and all genotype frequencies were in Hardy-Weinberg equilibrium. Linkage disequilibrium between markers was low ($D' < 0.8$ for all marker combinations), which is appropriate as the markers were selected to tag the genetic variation in *LRP5* with minimal redundancy.

The rs2306862 (N740N) polymorphism in exon 10 was associated with various bone mass parameters. In the overall cohort of 819 children, this polymorphism was associated with spinal BMD ($P = 0.01$, adjusted for age, gender, height, and weight) (Table 3a). Following pubertal adjustment, the association of spinal BMD and the N740N polymorphism persisted ($P = 0.01$, adjusted for age, gender, puberty, height, and weight) (Table 3b).

To explore the relationship between *LRP5* polymorphisms and volumetric bone density, we analyzed these in relation to aBMC, which provides a more accurate estimate for volumetric bone density than BMD. Analysis of the overall cohort without regard for pubertal status demonstrated association of the rs2306862 polymorphism with spinal aBMC ($P = 0.01$, adjusted for age, gender, height, and weight) (Table 3a). When analysis was limited to children in Tanner stages I and II, the association between the rs2306862 polymorphism and spinal aBMC was strengthened ($P = 0.002$, adjusted for age, gender, pubertal status, height, and weight; $P = 0.02$, adjusted for age, gender, and pubertal status) (Table 3b). In contrast, no association was noted between *LRP5* polymorphisms and spinal bone area or BMC.

Individuals homozygous for the *T* allele had greater spinal BMD and aBMC scores than those homozygous for the *C* allele (Table 4). A dose effect was apparent as bone mass indices of heterozygous *TC* individuals were intermediate between those of their *TT* and *CC* counterparts. This observation persisted when analysis was limited to a smaller cohort of 239 children in Tanner stages I and II.

A second coding polymorphism, rs556442 (V1119V) in exon 15, was also associated with spinal BMD and aBMC ($P = 0.05$ and 0.01 , respectively, adjusted for age, gender, height, and weight) in the overall cohort of 819 children (Table 3a). Homozygous individuals for the *G* allele had a greater aBMC than homozygotes for the *A* allele (Table 4). No such association was observed in the smaller subgroup of children with pubertal stage information (Table 3b). No association was observed between the exon 15 polymorphism and BMC or bone area in either the whole group or the subgroup defined by Tanner stage

Association of LRP5 Polymorphisms and Total-Body Bone Mass

In the overall cohort of 819 children, the rs2306862 polymorphism was associated with TBLH BMD and aBMC ($P = 0.04$ and 0.03 , respectively, adjusted for age, gender, height, and weight) (Table 3a). Adjustment for pubertal status attenuated the strength of the association between the polymorphism and TBLH BMD ($P = 0.06$, adjusted for age, gender, puberty, height, and weight). These associations were lost, however, upon restriction of

analyses to the much smaller subset of children in Tanner stages I and II (Table 3b). No other *LRP5* gene SNPs demonstrated association with TBLH bone mass indices. In addition, no association was noted between any *LRP5* gene polymorphisms and either height or weight.

Haplotyping Analysis

Haplotyping analysis confirmed the association of skeletal mass phenotypes to the *LRP5* gene. Haplotypes composed of the polymorphisms within exon 15 and following exon 21 were associated with spinal adjusted BMC and spinal volumetric BMD following adjustments for age, height, weight, and pubertal status ($P = 0.03$ and 0.04 , respectively; Table 5).

Statistical Correction

Principal components analysis suggested that the six SNPs genotyped were equivalent to 5.68 independent markers. Phenotypes assessed were also correlated and were equivalent to two independent factors. Therefore, an unadjusted P value of <0.005 is equivalent to a corrected P value of <0.05 , assuming that this is a discovery rather than a confirmation study.

Discussion

LRP5 gene polymorphisms have previously been shown to modulate the normal population range of adult BMD [22, 23, 25, 39-43]. To determine whether the influence of *LRP5* gene polymorphisms is exerted during growth, association between *LRP5* gene polymorphisms and pediatric BMD was examined in 819 Caucasian children aged 9 years. Association of two *LRP5* gene polymorphisms to spinal and total-body aBMC and BMD supports the hypothesis that *LRP5* gene polymorphisms influence bone accrual during growth. Specifically, the rs2306862 (N740N) polymorphism in exon 10 was associated with spinal and total-body BMD and aBMC (respectively, $P = 0.04$ and 0.03 uncorrected for pubertal stage, $P = 0.01$ and 0.002 corrected for pubertal stage), while the rs556442 (V1119V) polymorphism in exon 15 was associated with spinal BMD and aBMC ($P = 0.05$ and 0.01 , respectively). The association of the rs2306862 (N740N) polymorphism in exon 10 with aBMC is significant even allowing for strict Bonferroni correction. These results suggest that the influence of *LRP5* gene polymorphisms upon BMD is expressed by early puberty. Our findings are consistent with those of heritability studies, suggesting that the genetic influences modulating BMD are already evident in childhood [13]. *In vitro* and murine studies have demonstrated that *LRP5* mediates bone accrual; however, a contribution of the *LRP5* gene toward the modulation of childhood BMD has not previously been demonstrated.

The influence of specific *LRP5* variants on the phenotypes measured could not be determined with certainty in this linkage disequilibrium mapping study. The haplotype composed of the SNPs in exon 15 and following exon 21 was associated with BMD in our cohort. Significantly, the rs53736228 (A1330V) polymorphism in exon 18, which has repeatedly been associated with adult hip and lumbar BMD [22, 23, 26, 44], is within the physical boundaries of the exon 15–21 haplotype associated with BMD in our cohort. In this study, we were unable to specifically analyze this polymorphism due to genotyping difficulties. However, linkage disequilibrium studies performed in British Caucasian [23, 45] and European Caucasian [22] cohorts have demonstrated the presence of a single haplotypic block encompassing the SNPs in exons 15 and 18 and that following exon 21. It cannot be excluded that this haplotypic association may belie true association between the

rs53736228 polymorphism, or another variant within this haplotype block, and bone mass in children.

Skeletal growth in childhood reflects both changes in skeletal size and the density of bone tissue within. In the present study, there was clear evidence for a distinct influence of *LRP5* gene polymorphisms on volumetric density rather than skeletal size since associations were only observed with DXA measures that were adjusted for skeletal size (i.e., BMD and aBMC). Indeed, associations were stronger with aBMC compared to BMD, of which the former is more rigorously adjusted for skeletal size. In further analyses, we found that *LRP5* gene polymorphisms showed similar associations with spinal BMAD compared to aBMC (results not shown).

The preservation of associations between *LRP5* gene polymorphisms and area-adjusted bone mass indices highlights a potential role for *LRP5* in the modulation of trabecular volume. Correlations between bone or body size and genetic variants have been observed in children and adolescents [46, 47]. Murine models of altered LRP5 function suggest a role for LRP5 in both the modulation of bone size, which was not observed in the current study, and trabecular volume. Both volumetric BMD and bone volume measures were increased in mice expressing the mutant *LRP5*^{G171V} receptor (reported in individuals bearing the high bone mass trait) [28]. Similarly, bones derived from *LRP5*^{-/-} mice are reduced in volume and thickness in comparison to those of wild-type littermates [29, 48]. The mechanism by which LRP5 regulates bone size is uncertain. Reduced osteoblast number, leading to reduced bone formation and matrix accrual, has been described in *LRP5*^{-/-} mice [29]. Decreased trabecular bone volume has been reported in mice heterozygous for an *LRP5*-null mutation in comparison to wild-type littermates [49]. In contrast, increments of both the trabecular and cortical bone compartments are observed in mutant mice expressing the *LRP5*^{G171V} receptor [28]. This is in contrast to the increased bone mass described in *sFRP1*^{-/-} mice, which is almost solely attributed to increased trabecular bone [50]. Whether the potential differential influence of *LRP5* gene polymorphisms upon cortical and trabecular bone compartments also contributes to gender-specific differences in bone size arising from enhanced (age-related) periosteal expansion in men remains uncertain.

In this study, no association between *LRP5* gene polymorphisms and height was noted. Certainly, differences in the length of bones derived from mice expressing wild-type and mutant forms of the LRP5 receptor are not consistently noted. However, Ferrari et al. [22] described association between *LRP5* gene polymorphisms and height. It is uncertain whether the time point measured in our study was premature for meaningful assessment of height.

Despite the repeated associations reported in our study, the associations we have observed are not highly statistically significant nor of large magnitude. Indeed, many findings are not significant if fully corrected *P* values are used. However, given the previous data documenting the role of *LRP5* in bone, this would be significantly over-conservative. It is possible that *LRP5* may play a more significant role in pubertal growth than is evident in our largely prepubertal cohort. Femoral neck width and BMD plateaus between the ages of 14.5 and 16.5 years in girls and boys, while lumbar spine BMD plateaus between the ages of 15 and 17 years in girls and boys [51]. Thus, the time point studied is too early to permit observation of puberty-related skeletal growth, with most subjects in this study within Tanner's puberty stages I-II. It is possible that the strength of these associations would be increased at later stages of puberty and with peak bone mass.

Studies in adults have shown association of *LRP5* gene polymorphisms with BMD, BMC, or size in men and women, adults, and adolescents of various ethnicities. A previous study of

prepubertal children analyzed the rs2277268 exon 9 and rs53736228 exon 18 *LRP5* variants and reported association in boys only with the rs2277268 SNP and haplotype of these two SNPs with change in lumbar spine BMC and area over 1 year [22]. Change in pubertal status during the study was not adjusted for. No association was seen at baseline in this longitudinal study with these haplotypes, but no individual marker data were presented. We could not reliably genotype the rs53736228 SNP, and our study is cross-sectional, limiting our ability to compare findings with the previous study. Nonetheless, these findings are consistent with our conclusion that *LRP5* polymorphisms affect skeletal development in prepubertal children.

A limitation of the current study is that pubertal status was self-reported as clinical assessment of pubertal stage was not considered ethical. It is likely that this will have introduced some error into the data, reducing the study power. Also, the study would have been strengthened if densitometric data had been available from the whole cohort, although the subset we studied was clearly representative of the overall ALSPAC cohort. When that data become available, the study will also have adequate power to investigate the effect of potential environmental covariates of the effect of *LRP5* polymorphisms on the skeleton.

Here, we confirm that the influence of *LRP5* gene polymorphisms upon skeletal development, and trabecular volume in particular, is also evident in prepubertal and early pubertal children. Study of these SNPs in young adulthood, reflecting peak bone mass attainment, would be equally pertinent and is eagerly awaited.

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Table 1

Age, height, weight, and DXA results in 429 boys (179 stage I and 32 stage II) and 390 girls (139 stage I and 79 stage II) (maximum numbers) at age 9

	Stage I	Stage II	P	All
Boys				
Age (months)	117.2 (1.4)	117.6 (1.2)	0.1	118.2 (2.1)
Height (cm)	139.4 (5.5)	140.7 (7.2)	0.2	139.5 (5.7)
Weight (kg)	34.3 (7.0)	36.3 (8.9)	0.2	34.7 (7.4)
TBLH				
BMC (g)	890.9 (156.0)	964.5 (209.3)	0.02	904.2 (171.4)
BMD (g/cm ³)	0.78 (0.05)	0.79 (0.05)	0.1	0.78 (0.05)
Area (cm ²)	1,140.1 (140.9)	1,208.1 (196.5)	0.02	1,150.4 (153.3)
aBMC (g)	891.1 (38.8)	890.1 (38.4)	0.9	893.2 (38.4)
Spine				
BMC (g)	77.4 (14.0)	83.0 (18.1)	0.1	78.6 (16.4)
BMD (g/cm ³)	0.77 (0.07)	0.79 (0.10)	0.2	0.77 (0.08)
Area (cm ²)	99.8 (10.7)	103.8 (11.7)	0.1	100.9 (11.8)
aBMC (g)	77.7 (6.1)	78.6 (8.8)	0.6	77.7 (7.0)
Girls				
Age (months)	117.0 (1.0)	117.1 (0.9)	0.4	118.0 (2.0)
Height (cm)	137.0 (5.4)	140.5 (6.3)	<0.001	139.0 (6.0)
Weight (kg)	32.0 (5.8)	38.1 (7.9)	<0.001	34.8 (7.3)
TBLH				
BMC (g)	812.0 (143.2)	943.4 (197.5)	<0.001	880.0 (183.1)
BMD (g/cm ²)	0.76 (0.05)	0.79 (0.06)	<0.001	0.77 (0.05)
Area (units)	1,067.6 (128.9)	1,187.9 (174.7)	<0.001	1,129.3 (162.9)
aBMC (g)	891.5 (33.7)	891.2 (39.5)	0.95	891.8 (37.6)
Spine				
BMC (g)	71.9 (12.1)	84.7 (17.2)	<0.001	78.5 (16.5)
BMD (g/cm ²)	0.75 (0.07)	0.82 (0.10)	<0.001	0.78 (0.09)
Area (units)	95.4 (9.6)	103.0 (11.8)	<0.001	99.5 (11.7)
aBMC (g)	77.5 (6.1)	81.3 (9.7)	0.01	79.2 (8.0)

Values are means (standard deviations). *P* values are for comparisons of stage I and stage II boys and stage I and stage II girls (*t*-tests)

Table 2

Genotype counts and frequencies for each polymorphism

SNP	Genotype	n (%)
rs4988320 (intron 7)	<i>AA</i>	535 (71)
	<i>AG</i>	212 (28)
	<i>GG</i>	11 (1)
rs545382 (exon 8)	<i>CC</i>	628 (84)
	<i>CT</i>	107 (14)
	<i>TT</i>	16 (2)
rs2277268 (exon 9)	<i>GG</i>	664 (88)
	<i>GA</i>	84 (11)
	<i>AA</i>	8 (1)
rs2306862 (exon 10)	<i>CC</i>	21 (3)
	<i>CT</i>	211 (27)
	<i>TT</i>	557 (71)
rs556442 (exon 15)	<i>AA</i>	81 (12)
	<i>AG</i>	275 (40)
	<i>GG</i>	339 (49)
rs2291466 (intron post-exon 21)	<i>CC</i>	641 (87)
	<i>CA</i>	81 (11)
	<i>AA</i>	17 (2)

Table 3a

P values for differences in mean DXA values between common homozygotes, heterozygotes, and rare homozygotes (two degrees of freedom tests): 819 children had TBLH data and 479 had spine data

	BMC		BMD		Area		aBMC	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Spine								
rs4988320	0.6	0.8	0.3	0.3	0.8	0.7	0.3	0.2
rs545382	0.6	0.4	0.3	0.2	0.8	0.96	0.1	0.2
rs2277268	0.3	0.2	0.4	0.3	0.2	0.2	0.3	0.2
rs2306862	0.3	0.07	0.1	0.01	0.6	0.5	0.2	0.01
rs556442	0.5	0.3	0.3	0.05	0.4	0.3	0.09	0.01
rs2291466	0.7	0.1	0.5	0.2	0.8	0.3	0.6	0.6
TBLH								
rs4988320	0.2	0.1	0.3	0.3	0.3	0.1	0.4	0.3
rs545382	0.7	0.4	0.7	0.4	0.6	0.6	0.4	0.5
rs2277268	0.3	0.3	0.3	0.4	0.5	0.4	0.5	0.4
rs2306862	0.6	0.1	0.1	0.04	0.9	0.5	0.05	0.03
rs556442	0.9	0.5	0.7	0.4	0.8	0.6	0.1	0.1
rs2291466	0.5	0.6	0.5	0.5	0.4	0.5	0.5	0.6

Table 3b

P values for differences in mean DXA values between common homozygotes, heterozygotes, and rare homozygotes (two degrees of freedom tests): results are based on stage I and stage II boys and girls only, so 429 had TBLH data and 239 had spine data

	BMC		BMD		Area		ABMC	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Spine								
rs4988320	0.6	0.5	0.6	0.6	0.8	0.8	0.6	0.7
rs545382	0.9	0.4	0.4	0.08	0.5	0.9	0.08	0.07
rs2277268	0.8	0.5	0.99	0.7	0.4	0.2	0.6	0.4
rs2306862	0.3	0.08	0.06	0.01	0.96	0.7	0.02	0.002
rs556442	0.8	0.6	0.6	0.4	0.8	0.7	0.3	0.1
rs2291466	0.8	0.3	0.6	0.4	0.99	0.8	0.5	0.5
TBLH								
rs4988320	0.4	0.09	0.6	0.5	0.4	0.05	0.8	0.8
rs545382	0.5	0.8	0.7	0.6	0.4	0.9	0.3	0.4
rs2277268	0.3	0.4	0.3	0.4	0.4	0.6	0.4	0.4
rs2306862	0.4	0.09	0.1	0.06	0.7	0.3	0.1	0.08
rs556442	0.8	0.4	0.5	0.2	0.9	0.8	0.1	0.1
rs2291466	0.6	0.6	0.6	0.4	0.6	0.6	0.8	0.8

Model 1, adjusted for age at time of DXA scan and gender; model 2, adjusted for age, gender, height, and weight

Table 4

Mean (95% confidence interval) BMD and aBMC values for individuals segregated by genotype for the N740N (rs2306862) and V1119V (rs556442) polymorphisms in exons 10 and 15, respectively

Spine	SNP	Genotype	Model 1	Model 2
BMD	N740N	TT	0.78 (0.77–0.79)	0.78 (0.77–0.79)
		TC	0.77 (0.76–0.78)	0.76 (0.75–0.77)
		CC	0.75 (0.73–0.78)	0.74 (0.71–0.76)
aBMC	N740N	TT	78.7 (77.9–79.5)	79.0 (77.9–80.0)
		TC	77.7 (76.7–78.8)	76.6 (75.2–77.9)
		CC	76.7 (74.5–78.9)	74.2 (71.5–76.9)
aBMC	V1119V	GG	79.2 (78.2–80.2)	79.1 (77.9–80.4)
		GA	78.3 (77.5–79.2)	77.9 (76.9–79.0)
		AA	77.4 (75.8–79.1)	76.7 (74.7–78.7)

Values shown are adjusted for age at time of DXA scan and gender ($n = 479$, model 1) or adjusted for age, gender, pubertal status, height, and weight ($n = 239$ stage I and stage II only, model 2)

Table 5

Mean spine adjusted BMC and BMAD for haplotypes of the rs556442 and rs2291466 polymorphisms in exons 15 and 21

rs556442/rs2291466 (<i>n</i>)	Spine adjusted BMC	Spine BMAD
<i>AC</i> (97)	77.0	0.077
<i>AA</i> (9)	77.6	0.078
<i>GC</i> (150)	79.0	0.078
<i>GA</i> (2)	80.3	0.080

Values shown are adjusted for age, gender, pubertal status, height, and weight (*n* = 239 stage I and stage II only)