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Body Composition and Bone Mineral Density in Children with Premature Adrenarche and the Association of *LRP5* Gene Polymorphisms with Bone Mineral Density

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Context: Precocious increase in adrenal androgen production is the hallmark of premature adrenarche (PA). Adrenal androgens have anabolic properties.

Objective: The objective of the study was to test whether body composition and bone mineral density (BMD) are altered in PA and study whether genetic variation in low-density lipoprotein receptor-related protein 5 (*LRP5*) affects BMD in PA.

Design: This was a cross-sectional study.

Setting: The study was conducted at a university hospital.

Subjects and Measures: The study included 126 prepubertal children (64 with PA, 10 boys; 62 non-PA controls, 10 boys). Femoral neck and lumbar spine areal and calculated volumetric BMD (dual energy X-ray absorptiometry), body composition (bioimpedance), serum 25-hydroxyvitamin D, and markers of bone turnover and calcium homeostasis were compared between the PA and control groups. Single-nucleotide polymorphisms of *LRP5* were determined and associated with BMD.

Results: Children with PA had higher femoral neck and lumbar spine BMD_{areal} than the controls (Z-score 0.56 *vs.* −0.09, $P < 0.001$, and 0.20 *vs.* −0.31, $P = 0.009$, respectively). However, the mean BMDs did not differ significantly between the groups when adjusted for height or bone size. BMD_{areal} correlated strongly with height SD score in both groups. Among the PA children, *LRP5* single-nucleotide polymorphism E644E minor variant was associated with lower and F549F minor variant with higher BMD. Total body fat mass, fat percent, serum PTH, and alkaline phosphatase concentrations were higher and 25-hydroxyvitamin D lower in the PA group.

Conclusions: Prepubertal children with PA had higher BMD_{areal} compared with healthy controls. This was mainly explained by their increased height. *LRP5* polymorphisms may contribute to bone mass accrual in prepubertal PA children. **(*J Clin Endocrinol Metab* 94: 4144–4151, 2009)**

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Abbreviations: ALP, Alkaline phosphatase; BMC, bone mineral content; BMD, mineral density; BMI, body mass index; CV, coefficient of variation; DHEAS, dehydroepiandrosterone sulfate; FN, femoral neck; ICTP, telopeptide of type I collagen; LRP5, low-density lipoprotein receptor-related protein 5; LS, lumbar spine; OGTT, oral glucose tolerance test; 25-OHD, 25-hydroxyvitamin D; PA, premature adrenarche; PP, premature pubarche; SDS, SD score; SNP, single-nucleotide polymorphism.

Premature adrenarche (PA) refers to earlier than normal rise in adrenocortical production of androgens (1–3). PA is diagnosed when adrenarcheal signs, pubic or axillary hair, acne, adult type body odor and/or oily hair, appear before the age of 8 yr in a girl or 9 yr in a boy, and steroid-producing tumors, steroidogenic enzyme defects and central puberty have been excluded (4–6). At diagnosis, PA children typically present with slightly accelerated growth in height and advanced skeletal maturation compared with peers (4, 7).

Dehydroepiandrosterone, dehydroepiandrosterone sulfate (DHEAS), and androstenedione are weak androgens, but they are peripherally converted to more potent androgen receptor ligands, testosterone and dihydrotestosterone, and to estrogens. Estrogens, especially 17-β-estradiol, promote linear bone growth (8) and may influence bone mass accrual (9, 10). There is also evidence for a positive effect of androgens on bone mineral content (BMC) (10, 11). One could thus assume that prepubertal children with PA have greater BMC than their peers. Furthermore, children with PA are often overweight (12–14), which may also contribute to their bone mass (15). Hitherto, only one small study has compared bone mineral density (BMD) between premature pubarche (PP) and control subjects; that study on Hispanic girls suggested that PA associates with increased BMD (16).

Low-density lipoprotein receptor-related protein 5 (LRP5) is a transmembrane receptor of the low-density lipoprotein receptor family. Since its characterization, *LRP5* has been linked to several metabolic and bone mass phenotypes. Homozygous loss-of-function mutations in *LRP5* cause the autosomal recessive osteoporosis-pseudoglioma syndrome (17), and heterozygous mutations predispose to primary osteoporosis in children (18). On the other hand, gain-of-function mutations lead to high bone mass phenotypes (19, 20). Several common polymorphic variants in *LRP5* contribute to normal bone mass variation (21–23). The effect of *LRP5* genotype on bone mass is evident already in childhood and young adulthood, suggesting that *LRP5* influences bone mass accrual (24, 25). Furthermore, stronger association between *LRP5* variants and BMC in men than women indicates that sex hormones may modulate the effect of *LRP5* on bone (23).

The determinants of bone mass accrual in children are incompletely characterized. The aims of the present study were to evaluate the effect of PA on BMC, BMD, markers of bone metabolism and body composition at prepubertal age and to test the hypothesis that *LRP5* genotype contributes to bone mass accrual in prepubertal children with PA.

Subjects and Methods

Subjects

The PA group comprised 64 children with any clinical sign(s) of adrenarche (pubic/axillary hair, acne, comedones, adult type body odor, or oily hair) before the age of 8 yr in girls and 9 yr in boys. The age at examination had to be less than 9 yr in girls and 10 yr in boys. All children meeting the clinical criteria between October 2004 and January 2006 in Northern Savo, a region in eastern Finland with a population of 250,000, were invited to participate in our Premature Adrenarche study (26). Study subjects were recruited from patients admitted to the pediatric outpatient clinic at Kuopio University Hospital due to hyperandrogenic symptoms. In addition, information letters were sent through health care centers to local well-baby and school clinics. Announcements were also published annually in main local newspapers. For the present study, 64 subjects (10 boys) with biochemically confirmed adrenarche (serum DHEAS $\geq$1 μmol/liter) of the 73 originally recruited prepubertal children with signs of androgen action were included.

The control group comprised 62 healthy prepubertal children (10 boys) without any signs of androgen action and with serum DHEAS less than 1 μmol/liter. They were recruited as controls for the Premature Adrenarche study according to a list of a random sample of child citizens obtained from Finland's population register and living in the same area. Invitation letters were sent to the families of the children selected from the list by order sampling in each age and gender group, to yield a matched control group for the original group of children with signs of PA (26).

Children with long-term medication (including oral or inhaled corticosteroids) were excluded from both study groups. Central puberty was excluded by Tanner staging and a GnRH test; all subjects had Tanner B/G stage 1 and prepubertal GnRH response (peak LH/FSH ratio $<$1). Adrenal virilizing tumors and congenital adrenal hyperplasia were excluded by abdominal ultrasonography (performed for those with pubic/axillary hair) and an iv ACTH test (in all children, 1 μg per 1.73 m^2 Synacthen; Novartis Pharma GmbH, Nürnberg, Germany) with cortisol, 17-hydroxyprogesterone, dehydroepiandrosterone, and androstenedione measurements.

The study protocol was approved by the Research Ethics Committee of the Kuopio University Hospital. An informed written consent was obtained from the parents and an approval from the children themselves.

Clinical assessment

All children and their parents were interviewed, and the consumption of dairy products (yes/no) and appearance of the adrenarcheal signs (oily hair, adult type body odor, comedones, acne, axillary and pubic hair) were recorded. Regarding the physical activity, children were divided into three groups: 1) children with no or only occasional physical activity in addition to school-based physical education, 2) children with regular after-school physical activity, and 3) children participating in competitive sports or exercising daily. Complete physical examination with thorough evaluation of adrenarcheal signs was performed to each subject. Height was measured with a calibrated Harpenden stadiometer (Holtain Ltd., Crymych, UK), recorded to the nearest 0.1 cm as the mean of three repeated measurements and converted to SD score (SDS) according to the current Finnish growth charts (27). Weight was measured after an overnight fast with a

digital scale and recorded to the nearest 0.1 kg. Body mass index (BMI; [weight (kilograms)/height^2 (m)] was calculated and BMI SDS determined by British reference values (28). Retrospective growth data and current anthropometrics of the girl subjects have been reported recently (29).

Biochemical analyses and genotyping

Venous blood samples for basal biochemistry and DNA isolation were drawn between 0900 and 1000 h after an overnight fast. Due to consumption of all basal serum samples for previous analyses, we had to use the samples collected during an oral glucose tolerance test (14) for serum C-terminal telopeptide of type I collagen (ICTP; a marker of bone resorption), osteocalcin (a marker of bone formation; 60 min time point for both, between 1000 1100 h), calcium, phosphate, and PTH measurements (120 min, between 1100 and 1200 h). Serum alkaline phosphatase (ALP) concentrations were measured with a kinetic colorimetric method (Konelab 60i clinical chemistry analyzer; Labsystems, CLD, Vantaa, Finland). Serum concentration of 25-hydroxyvitamin D (25-OHD) was analyzed by a specific competitive RIA (DiaSorin, Stillwater, MN) with intraassay coefficient of variation (CV) of 8.6–12.5% and total CV of 8.2–11.0% in the concentration range of 22–123 nmol/liter. Serum DHEAS, insulin, estradiol, and testosterone concentrations were analyzed as previously described (14, 26). Serum ICTP and osteocalcin concentrations were measured with specific EIA and ELISA (Orion Diagnostica Oy, Espoo, Finland, and Immunodiagnostics Systems Ltd., Boldon, UK, respectively). Serum PTH concentration was determined by electrochemiluminescence immunoassay technique using Cobas e601 analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Total CV was 1.6–3.4% and intraassay CV 1.1–2.8%. Serum phosphate and calcium were analyzed with routine automated methods in the laboratory of Kuopio University Hospital. All serum samples were separated within an hour of sampling, immediately frozen, and stored at −80 C until assayed.

DNA was isolated from peripheral blood samples using the Wizard genomic DNA purification kit (Promega, Madison, WI). The DNA sample was obtained in all but one control subject. Genotyping of the *LRP5* gene was performed by sequencing all 23 coding exons and the flanking intronic, 5′, and 3′ untranslated regions as described previously (30).

Determination of BMD and body composition

BMC and areal density (BMD_{areal}; grams per square centimeter) of lumbar spine (LS; L2–4) and femoral neck (FN) were measured by dual-energy x-ray absorptiometry using Lunar DPX (Lunar Radiation Corp., Madison, WI). All subjects were measured with the same scanner by trained personnel in Kuopio University Hospital. Quality assurance tests with the Lunar DPX scanner showed an interassay variation of 0.8% for the LS and 2.3% for the FN measurements in children (31). To minimize the effect of bone size, surrogates of volumetric BMD (BMD_{vol}; grams per cubic centimeter) were calculated as follows: 1) LS BMD_{vol} (grams per cubic centimeter) = LS BMD_{areal} (grams per square centimeter) $\times$ 4/[$\pi \times$ width of measurement area in LS (centimeters)] and 2) FN BMD_{vol} (grams per cubic centimeter) = FN BMD_{areal} (grams per square centimeter) $\times$ 4/$\pi \times$ [height (centimeters) of the measurement area/measurement area (square centimeter) in FN] (31). Body composition was assessed by eight-tactile bioelectrical, multifrequency and segmental impedance analysis with Inbody 3.0 (Biospace, Seoul, Korea). This device measures volume of water based on the body's resistance to electric current and thereby derives fat-free mass and fat mass and calculates various indirect measures of body composition for a given height of the subject.

Statistical analyses

All parameters were first tested for normality. Differences in continuous parameters between the PA and control group were analyzed with independent-samples *t* test or univariate linear model with gender, age, BMI SDS, and height SDS as covariates when appropriate. When comparing the polymorphism subgroups or the PA and control boys, the nonparametric Mann-Whitney test was used because of the small number of subjects in each group. Correlations between BMD and other parameters were tested with the Pearson or Spearman correlation test as appropriate. Independent associations between BMD and other parameters were assessed by linear regression. $P < 0.05$ was regarded significant in all statistical analyses. Values are presented as mean ± 95% confidence interval unless otherwise mentioned.

Results

Body composition and BMD

The children with PA had higher fat-free, soft lean, and fat mass and body fat percentage than their controls (Table 1). They also had higher FN and LS BMC and BMD_{areal} compared with the controls, but the mean calculated FN and LS BMD_{vol} did not differ between the study groups (Table 2). Both in the PA and control group, FN and LS BMD_{areal} correlated strongly with height SDS (r = 0.43–0.45 and r = 0.62–0.63, respectively, $P < 0.001$ for all) and moderately with BMI SDS (r = 0.29–0.39, $P < 0.03$ for all). FN BMD_{vol} correlated with height SDS in the controls (r = 0.55, $P < 0.001$) but not in the children with PA (r = 0.17, $P = 0.18$). When FN and LS BMD_{areal} Z-scores were adjusted for height SDS, no significant differences were found between the PA and control groups ($P = 0.43$ and $P = 0.52$, respectively). The difference in the mean FN BMD_{areal} Z-scores remained significant after adjustment for BMI SDS (PA *vs.* control subjects; $P = 0.01$). There was no significant difference in the physical activity between the PA and control groups (32-50-18 *vs.* 34-46-20% in the activity groups 1-2-3, respectively, $P = 0.083$).

Biochemical parameters and their association with BMD

The mean serum ALP concentration was higher in the PA than control subjects (Table 1). This difference was significant in both girls (641 *vs.* 560 U/liter, $P = 0.008$) and boys (655 *vs.* 511 U/liter, $P = 0.02$). The mean serum 25-OHD concentration was lower in the PA than control group (Table 1); the difference remained significant when

TABLE 1. Biochemical parameters and body composition in children with PA and their controls

	PA (n = 64; 54 girls/10 boys)	Control (n = 62; 52 girls/10 boys)[a]	P
Age (yr)	7.5 ± 0.22	7.3 ± 0.23	0.11[1]
S-DHEAS (μmol/liter)[b]	2.3 ± 0.32	0.60 ± 0.05	<0.001
S-testosterone (nmol/liter)[c]	0.42 (<0.35, 0.57)	0.35 (<0.35, 0.38)	<0.001[2]
S-estradiol (nmol/liter) (girls)[c]	0.03 (0.03, 0.03)	0.02 (0.02, 0.03)	<0.001[2]
S-ALP (U/liter)	643 ± 39	549 ± 36	0.001
S-25-OHD (nmol/liter)	53 ± 3.7	59 ± 4.0	0.024[2]
S-ICTP (μg/liter)	11.9 ± 1.02	11.7 ± 0.69	0.71
S-osteocalcin (μg/liter)	43.3 ± 3.1	41.1 ± 2.6	0.36
S-calcium (mmol/liter)	2.37 ± 0.01	2.37 ± 0.02	0.99
S-phosphate (mmol/liter)	1.36 ± 0.03	1.38 ± 0.04	0.42
S-PTH (ng/liter)	34.6 ± 2.6	31.1 ± 2.02	0.06
Body composition			
BMI SDS	+1.09 ± 0.31	+0.26 ± 0.29	<0.001[2]
Height SDS	+1.19 ± 0.29	+0.07 ± 0.24	<0.001
Fat-free mass (girls/boys) (kg)	25.0 ± 1.0 (24.7 ± 1.0/26.7 ± 3.6)	21.3 ± 0.7 (21.0 ± 0.7/22.7 ± 1.7)	<0.001 (<0.001[3]/0.08[2])
Soft lean mass (girls/boys) (kg)	23.4 ± 0.9 (23.1 ± 0.9/25.0 ± 3.5)	19.8 ± 0.6 (19.6 ± 0.7/21.2 ± 1.6)	<0.001 (<0.001[3]/0.08[2])
Soft lean mass percent (girls/boys) (%)	72.1 ± 2.1 (71.6 ± 2.4/74.8 ± 5.7)	77.2 ± 1.5 (77.1 ± 1.6/77.3 ± 4.6)	0.001[2] (0.001[2]/0.45[2])
Fat mass (girls/boys) (kg)	8.1 ± 1.2 (8.3 ± 1.3/7.4 ± 4.1)	4.6 ± 0.6 (4.6 ± 0.6/4.8 ± 1.8)	<0.001[2] (<0.001[2]/0.13[2])
Fat percent (girls/boys) (%)	22.7 ± 2.3 (23.2 ± 2.6/20.0 ± 6.2)	17.0 ± 1.5 (17.1 ± 1.7/17.0 ± 4.9)	0.001[2] (0.001[2]/0.45[2])

Means ± 95% confidence intervals are shown unless otherwise stated. *P* values taken from univariate linear model adjusted for gender and age unless otherwise marked: [1] Univariate linear model adjusted for gender; [2] Mann-Whitney test or [3] Univariate linear model adjusted for age and BMI SDS. S, Serum.

[a] Blood sample was not obtained in one control girl (n = 51 in serum parameters); [b] ln-transformed prior to analyses to yield normal distribution; [c] Median (25th, 75th percentile).

the comparison was adjusted for sex and the time of sampling (winter/summer season) ($P = 0.02$) but not when adjusted also for fat mass ($P = 0.08$). In gender-specific analyses, this difference was significant only in boys (PA *vs.* controls 50 *vs.* 65 nmol/liter; $P = 0.02$).

Serum PTH concentrations were higher in the PA than control group ($P = 0.04$), but the difference was not significant when adjusted for age and gender (Table 1). Serum calcium, phosphate, osteocalcin, or ICTP concentrations did not differ between the study groups. Serum PTH and 25-OHD concentrations were intercorrelated in the PA (r = −0.36, $P = 0.004$) but not in the control group ($P = 0.87$). Serum ALP, ICTP, calcium, or phosphate concentration did not correlate significantly with BMD parameters in either study group, whereas serum PTH and osteocalcin concentrations correlated positively with FN BMD_{areal} in the PA subjects (r = 0.35, $P = 0.004$ and r = 0.25, $P = 0.05$, respectively) and serum 25-OHD concentration with LS BMD_{areal} in the controls (r = 0.33, $P = 0.009$).

Serum mean insulin during oral glucose tolerance test (OGTT) was not correlated with FN/LS BMD_{areal} in either study group ($P \geq 0.09$ for all). Testosterone but not DHEAS concentrations correlated positively with LS BMD_{areal} only in the PA subjects (r = 0.40, $P = 0.001$). Serum estradiol concentration (measured only in girls) did not correlate significantly with BMD values in either study group.

LRP5 polymorphisms and BMD

We recently reported the sequence variants of the *LRP5* gene and its flanking regions found in this study population (30). The *LRP5* single-nucleotide polymorphisms (SNPs) with minor allele carrier frequency of at least 5% in either of the present study groups are listed in Table 3.

In the PA group, the minor variant of SNP E644E and c.2318 + 6T > C were coinherited in seven individuals. The PA children carrying these variants had lower mean FN BMC, BMD_{areal}, and BMD_{vol} (2.45 *vs.* 2.91 g, $P = 0.015$; 0.66 *vs.* 0.76 g/cm^2, $P = 0.008$; 0.34 *vs.* 0.38 g/cm^3, $P = 0.021$, respectively), and LS BMC and BMD_{areal} (14.0 *vs.*

TABLE 2. BMDs in children with PA and their controls

BMD parameters	PA (n = 64; 54 girls/10 boys)	Control (n = 62; 52 girls/10 boys)	*P*
All subjects (Z-scores)			
BMD areal FN	+0.56 ± 0.25	−0.09 ± 0.23	<0.001
BMD volumetric FN	+0.28 ± 0.25	+0.08 ± 0.24	0.26
BMD areal L2-4	+0.20 ± 0.26	−0.31 ± 0.29	0.009
BMD volumetric L2-4	+0.07 ± 0.25	+0.14 ± 0.28	0.73
Girls (n = 106)	n = 54	n = 52	
BMC FN (g)	2.83 ± 0.12	2.42 ± 0.10	<0.001
BMD areal FN (g/cm^2)	0.74 ± 0.02	0.67 ± 0.02	<0.001
BMD volumetric FN (g/cm^3)	0.38 ± 0.01	0.36 ± 0.01	0.07
BMC L2-4 (g)	17.3 ± 0.94	13.4 ± 0.87	<0.001
BMD areal L2-4 (g/cm^2)	0.74 ± 0.02	0.66 ± 0.02	<0.001
BMD volumetric L2-4 (g/cm^3)	0.30 ± 0.01	0.29 ± 0.01	0.30
Boys (n = 20)	n = 10	n = 10	
BMC FN (g)	3.05 ± 0.39	2.7 ± 0.24	0.17[a]
BMD areal FN (g/cm^2)	0.78 ± 0.08	0.76 ± 0.05	0.57[a]
BMD volumetric FN (g/cm^3)	0.38 ± 0.03	0.40 ± 0.02	0.29[a]
BMC L2-4 (g)	16.9 ± 2.34	15.6 ± 1.80	0.23[a]
BMD areal L2-4 (g/cm^2)	0.70 ± 0.02	0.70 ± 0.04	1.00[a]
BMD volumetric L2-4 (g/cm^3)	0.28 ± 0.02	0.29 ± 0.02	0.23[a]

Means ± 95% confidence intervals are shown.

[a] *P* values taken from independent-samples *t* test unless otherwise marked: [a]Mann-Whitney test.

17.7 g, $P = 0.013$, and 0.64 *vs.* 0.74 g/cm^2, $P = 0.010$, respectively) than those with the more common variant at both sites. The corresponding FN and LS BMD Z-scores were also lower in the PA subjects with the SNP E644E minor variant ($P \leq 0.05$ for all). The SNP F549F minor allele was associated with higher FN BMD_{areal} (A/a *vs.* A/A: 0.80 *vs.* 0.74 g/m^2, $P = 0.047$; Z-score +1.30 *vs.* +0.43, $P = 0.019$). The PA subjects with the SNP A1330V carried also the SNP N740N minor allele, and they had higher LS BMC ($P = 0.046$) but not FN BMD_{areal}. In the control group, no significant differences in the BMD parameters were observed between the minor and major allele carriers of the SNP F549F, E644E, N740N, V1119V, A1330V, or D1363V.

In the PA group, FN BMD_{areal} was independently associated with age, BMI SDS, height SDS, and *LRP5* SNPs E644E and F549F; LS BMD_{areal} was associated with age, sex, BMI SDS, height SDS, soft lean mass percent, and *LRP5* SNP E644E in linear regression models (Table 4). In similar models in the control group, height SDS ($P = 0.002$) and physical activity ($P = 0.045$) were the only parameters associating independently with LS BMD_{areal}, whereas height SDS ($P < 0.001$), age ($P < 0.001$), and male sex ($P = 0.036$) were positively associated with FN BMD_{areal}.

Discussion

In the present study, prepubertal children with PA had higher mean FN and LS BMC and BMD_{areal} compared with the controls. However, bone size adjusted BMD_{vol} or height adjusted BMD_{areal} did not differ significantly between the PA and control subjects. Children with PA also had higher fat percentage and their mean serum ALP and PTH concentrations were higher and 25-OHD concentration lower compared with the controls.

Our finding of higher BMD_{areal} in the PA subjects is partly in accordance with a previous study that reported increased mean total body BMD in 14 PA girls compared with that in 16 controls (16). However, whereas the previous study showed significantly higher BMDs in PA subjects even after adjustment for the size of the child, in our study the higher mean BMD_{areal} was mostly explained by larger bone sizes in prepubertal PA children. This controversy may partly be explained by different methods applied for BMD measurements and/or different selection criteria of the PA subjects. We included prepubertal children with various adrenarcheal signs and biochemically confirmed adrenarche (DHEAS ≥1 μmol/liter), whereas the previous study (16) included only girls with PP without biochemical confirmation of adrenarche. Moreover, genetic and other ethnic differences between the Hispanic and Finnish populations may modulate the association between adrenarche and bone mass. Another study on Spanish PP girls at variable pubertal stages also suggested that BMD is increased in PA, but this study had no control group (32). In that study, circulating insulin concentration was the best predictor of BMD in the nonobese PP girls. We did not find such correlation in our PA subjects, although our PA subjects also had increased insulin concentrations (14). Our PA children were heavier and taller than

TABLE 3. *LRP5* polymorphisms found by sequencing of the *LRP5* gene and its flanking regions in children with PA in control children and the whole prepubertal study population (30)

SNP	Position	Genotype	Amino acid change	Minor allele carrier frequencies (%)[a]	
				PA (n = 64)	Controls (n = 61)[b]
rs314776	Intron 4	c.844−4C > T		51.6	45.9
rs4988319	Intron 6	c.1412 + 8G > A		18.8	23.0
rs545382	Exon 8	c.1647C > T	F549F	15.6	8.2
rs2277268	Exon 9	c.1932G > A	E644E	10.9	13.1
rs2306862	Exon 10	c.2220T > C	N740N	14.1	4.9
rs4988322	Intron 11	c.2318 + 6T > C		10.9	13.1
rs556442	Exon 15	c.3357A > G	V1119V	39.1	23.0
rs3736228	Exon 18	c.3989C > T	A1330V	14.1	4.9
rs3736229	Exon 19	c.4089C > T	D1363D	1.6	11.5
rs11574426	Exon 21	c.4380C > T	S1460S	6.3	1.6

Polymorphisms with at least 5% carrier frequency in either group are depicted.

[a] Minor allele carrier frequencies, subjects either homozygous or heterozygous for the minor allele; no significant differences in the carrier frequencies between the PA and control group.

[b] DNA sample was not obtained in one control subject.

their controls (29). High body weight, especially increased lean body mass, may promote bone mass accrual (15, 33). Taken together, the differences in the mean FN and LS BMD_{areal} between our study groups seemed to reflect the enhanced growth in height and weight in the PA subjects.

The role of estrogens in BMD phenotypes in adult women is well established (34), and the hormonal milieu during puberty influences the magnitude of peak bone mass (33, 35). Androgens have been suggested to be responsible for the sexual dimorphism in bone mass after puberty (11). In contrast, only a few studies investigated the effect of circulating estrogens and androgens on bone mass acquisition in children. In line with a previous study on prepubertal children (10), we found no independent association between DHEAS concentrations and BMD in our prepubertal PA subjects. On the other hand, a recent study showed that testosterone is an important predictor of BMD in boys at the age of 10–18 yr with different pubertal stages (36). Another study showed that prepubertal adrenal androgen production predicts diaphyseal BMC and bone strength at pubertal age (37). Thus, the influence of prepubertal adrenal androgens on bone strength may not become detectable before late puberty. As opposed to some previous findings (9, 33), estradiol did not correlate significantly with BMD parameters in our prepubertal girls. This is presumably due to the low estradiol concentrations in our prepubertal subjects in general and the relatively low sensitivity of the assays available in this study.

Interestingly, our PA subjects had lower serum 25-OHD and higher PTH concentrations compared with their controls. This is probably explained by their higher

TABLE 4. A linear regression model depicting body composition and biochemical parameters associating independently with FN and LS BMD in prepubertal children with premature adrenarche (n = 64)

	FN BMD areal			LS BMD areal		
	Stand. coeff.	Regression coeff. (95% CI)	*P*	Stand. coeff.	Regression coeff. (95% CI)	*P*
Age	0.54	0.057 (0.04, 0.08)	<0.001	0.22	0.023 (0.00, 0.05)	0.04
Gender (boys *vs.* girls)	0.029	0.007 (−0.05, 0.06)	0.79	−0.33	−0.076 (−0.14, 0.01)	0.02
DHEAS (μmol/liter)	0.051	0.004 (−0.01, 0.02)	0.54	0.065	0.004 (−0.01, 0.02)	0.53
S-25-OHD (nmol/liter)	−0.027	0.000 (−0.00, 0.00)	0.75	−0.18	−0.001 (−0.00, 0.00)	0.11
BMI SDS	0.56	0.040 (0.00, 0.08)	0.04	0.79	0.055 (0.01, 0.11)	0.02
Height SDS	0.36	0.027 (0.01, 0.04)	0.001	0.30	0.022 (0.00, 0.04)	0.02
Soft lean mass (%)	0.48	0.510 (−0.02, 1.04)	0.06	0.63	0.650 (0.00, 1.29)	0.05
LRP5 SNP E644E	−0.26	−0.072 (−0.12, −0.03)	0.006	−0.33	−0.087 (−0.14, 0.03)	0.003
LRP5 SNP F549F	0.26	0.060 (0.02, 0.10)	0.003	−0.13	−0.030 (−0.08, 0.02)	0.25
Physical activity	0.08	0.009 (−0.02, 0.03)	0.43	−0.18	−0.022 (−0.05, 0.01)	0.14
S-mean insulin (IU/liter)	0.13	0.000 (0.00, 0.00)	0.18	−0.04	0.000 (−0.00,0.00)	0.73
		R^2 of the model[a]	0.62		R^2 of the model[a]	0.41

Stand. coeff., Standardized coefficient; S, serum; mean insulin, serum mean insulin during 2-h OGTT.

[a] Coefficient of determination, estimated size of effect of all variables in the model.

fat mass; the difference in the 25-OHD levels between the study groups was not significant when adjusted for fat mass. Hypovitaminosis D has recently been connected with overweight in both adults and children, presumably because vitamin D is sequestered in sc fat (38, 39). Slightly higher PTH concentration in our PA than control group was probably due to the lower 25-OHD concentrations. This view is supported by the finding of inverse correlation between 25-OHD and PTH concentrations in the PA group. Slightly lower availability of 25-OHD may attenuate the possible positive effect of higher weight and/or androgens on BMD in PA children. The higher circulating ALP concentrations in our PA than control subjects presumably reflect accelerated bone growth. Another possible explanation for the higher ALP concentrations in the PA than control children could be slightly increased parathyroid activity due to relative vitamin D deficiency in the PA subjects.

There is clear evidence for the association between genetic variation of *LRP5* and BMD in the general population (21–23, 25). In particular, the SNP A1330V has been repeatedly linked to lower BMD, as recently confirmed in two metaanalyses (40, 41). We were not able to show such association in the present study. This may be explained by the relatively small study population or the young age of our subjects. Our finding of a negative association between the minor variant of SNP E644E and BMD_{areal} in the PA subjects is in line with a previous study linking this variant to low BMD in a British adult cohort (22). Instead, there was a positive association between the minor variant of SNP F549F and FN BMD_{areal} in our PA children, which disagrees with previous findings in adult subjects (22).

A limitation in the present study is the lack of detailed data concerning consumption of dairy products which have a positive effect on BMC in childhood (42). All our study subjects used dairy products as part of typical Finnish diet; there were no vegan or milk protein allergic children in either study group. Thus, it is unlikely that there were significant differences in dairy product consumption between the PA and control subjects. Most dairy products are fortified with vitamin D in Finland. Another limitation is the lack of bone age measurements. They were not included in the study protocol because of the radiation exposure. Due to the shortage of fasting serum samples, we measured ICTP, osteocalcin, PTH, phosphate and calcium concentrations in samples obtained during OGTT, which is not optimal. However, sampling and OGTT were performed at the same time and with the same protocol in all subjects. Thus, the comparisons between the study groups should not be biased. The amount and quality of physical activity are known to influence BMD, also in children (43). Because we found no significant difference in the physical activity between the study groups, it should not have caused any significant bias in this study.

In conclusion, prepubertal children with PA had higher mean BMC and BMD_{areal} than their peers, which was mainly explained by their larger bone size. Our results indicate that growth in height and bone size, and to lesser extent body weight, are more important determinants of bone mass accrual than increased circulating adrenal androgen concentrations in prepubertal children. Genetic variation in *LRP5* may slightly contribute to BMD in prepubertal PA children.

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