Within- and Across-Sex Inheritance of Bone Microarchitecture

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Context: The maternal heritability of bone microarchitecture according to the sex of the offspring is not known.

Objective: To explore sex difference and influence of mother's menopausal status on the heritability of bone microarchitecture between mothers and their offspring.

Subjects and Methods: In 102 mother-daughter and 161 mother-son pairs, volumetric bone mineral density (BMD) and bone microarchitecture were measured at the distal radius and tibia by high-resolution peripheral quantitative computed tomography. A principal components analysis was applied for the radius and the tibia volumetric BMD and microarchitecture parameters separately. Two components, a trabecular one and a cortical one were identified at the radius and tibia. Half heritability ($\frac{1}{2}h^2$) was estimated as the slope of the regression between offspring and mothers for each bone parameter separately.

Results: The mean age ($\pm$ standard deviation) of mothers and daughters was 50.6 $\pm$ 4.1 years and 20.4 $\pm$ 0.5 years, respectively; that of mothers and sons was 45.8 $\pm$ 3.9 years and 15.2 $\pm$ 0.5 years, respectively. Most trabecular and cortical parameters were inherited in both mother-daughter and mother-son pairs (β = 0.15 to 0.33; P = 0.05 to 0.001). At the tibia, trabecular and cortical principal components were significantly inherited in both sexes, whereas only the trabecular one was inherited at the radius ($\frac{1}{2}h^2$, 21% to 35%). There was no difference in heritability of bone microarchitecture between mother-daughter and mother-son pairs. All heritabilities remained after adjustment for age, weight, height, gonadal status, and areal BMD ($\frac{1}{2}h^2$, 9% to 25%). In the mother-daughter pairs, there was no systematic drop of heritability across menopause.

Conclusions: Volumetric bone density and microarchitecture are highly and similarly inherited between and within sexes. The genetic effects remain predominant across menopause. (*J Clin Endocrinol Metab* **102:** 40–45, 2017)

Sex differences in bone mineral density (BMD), bone microarchitecture, and osteoporosis risk are well documented (1, 2). However, it is unclear whether environmental or genetic factors dissimilarly affect sex-related differences (2, 3). To investigate the proportion of the variance in bone traits explained by genetic factors, known as the heritability (h^2), several studies have shown a high h^2 of the bone densitometric phenotypes, ranging from 60% to 90% (4, 5). Factors that may explain the wide range of h^2 reported so far are age and influence of environmental factors: The mother-daughter pairs analyzed in these studies ranged in age from prepubertal (6, 7) to early adolescent

(8, 9) to adult (10, 11). Moreover, few studies reported differences in the h^2 of areal BMD (aBMD) between mother and young daughter (M-D) pairs as compared with mother and young son (M-S) pairs (7, 12). However, studies in adult parent–offspring pairs have had variable results, with some reporting no difference in h^2 by sex (13–15), whereas others have found approximately 20% higher h^2 estimates in men than in women when considering M-D and father-son pairs (16, 17). Higher h^2 for aBMD has also been reported in M-D and father-son comparisons than across sex (M-S and fathers and daughters) (18).

To explain these differences, not only the age and the sex of the offspring but also the age and the menopausal status of the mothers should be taken into account. Indeed, inheritance of bone traits in premenopausal women is influenced primarily by additive genetic effects on peak bone mass, whereas inheritance of bone traits in postmenopausal women is due to the combined genetic effects on peak bone mass and rate of bone loss (19). In addition to aBMD, trabecular and cortical microarchitecture also plays an important role in the determination of bone strength and fracture risk (20).

Currently, only 3 studies have investigated the h^2 of bone microarchitecture in mothers and offspring, as well as in twins, using high-resolution peripheral quantitative computed tomography (HR-pQCT) (21–23). These studies reported a wide range of the h^2 of volumetric BMD and bone microarchitecture, ranging from 30% to 60%, without assessing the sex-specific h^2 issue, nor the influence of the confounding effects of menopause.

The aim of this study was to investigate the within and across-sex inheritance of bone microarchitecture in M-D and M-S pairs and the influence of the mothers' menopausal status on the apparent h^2 of aBMD, volumetric BMD, and bone microarchitecture.

Methods

Subjects

To establish parent-offspring correlation for the various bone parameters, we analyzed the dual-energy X-ray absorptiometry (DXA) and HR-pQCT results of 161 boys at the mean age of 15.2 years [standard deviation (SD), ±0.5 years] and of 102 girls at the mean age of 20.4 years (SD, ±0.5 years). The boys and girls were recruited in prospective cohorts through the Public Health Youth Service of the Geneva, Switzerland, region at a mean age of 7.4 years (range, 6.5 to 8.5 years) between September 1999 and September 2000, and of 8 years (range, 6.6 to 9.4 years) in 1993, respectively (24, 25). DXA and HR-pQCT results were assessed in their mothers between 2007 and 2008. Exclusion criteria for the children, when they were enrolled, were ratio of weight to height below the third or above the 97th percentile, according to Geneva reference values; presence of physical signs of puberty; chronic disease; gastrointestinal disease with malabsorption; congenital or acquired bone disease; and

regular use of medication. There were no exclusion criteria for their mothers. The protocol was approved by the Ethics Committee of the University Hospitals of Geneva. Written informed consent was obtained from the parents and their descendants.

Clinical assessment

A medical history was obtained from all participants. It included age of menarche and of menopause, and current use of hormone replacement therapy. Weight was measured using a digital scale balance (model 764; SECA®, Hamburg, Germany) to the nearest 0.1 kg; height was measured using a Harpenden stadiometer to the nearest 0.1 cm (Holtain®, Crymych, UK). Pubertal stage was assessed according to Tanner's criteria.

DXA measurements

Areal BMD was evaluated by DXA (Discovery A; Hologic®, Waltham, MA). Five skeletal sites were examined: L2 to L4 lumbar vertebrae, femoral neck, total proximal femur, ultra-distal radius, and one-third of the distal radius in an anteroposterior view, as previously reported (25). The aBMD phantom coefficient of variation was 0.413% for the study DXA measurements.

HR-pQCT measurements

Volumetric bone mineral density (vBMD) and microarchitecture variables were determined at the distal radius and tibia by HR-pQCT using an Xtreme CT instrument (Scanco Medical, Brüttisellen, Switzerland). A stack of 110 slices for computed tomography (CT) were acquired over a 9-mm length with an isotropic voxel size of 82 μm, starting proximally at 9.5 and 22.5 mm from a joint margin reference line for distal radius and distal tibia, respectively. The effective dose was 3 μSv, and the measurement time was 2.8 minutes. Determinations were performed on the nondominant limb, unless a fracture was reported in the region of interest. Recorded variables were as follows: total, cortical, and trabecular vBMD, expressed in milligrams per cubic centimeter of calcium hydroxyapatite; total cross-sectional area and cortical and trabecular areas (in square millimeters); trabecular number (per millimeter), thickness (in millimeters), and spacing (in millimeters); trabecular spacing standard deviation (SD), as an estimate of the heterogeneity of the trabecular structure (in millimeters); and mean cortical thickness (in millimeters) (26). Cortical porosity was calculated as the number of void voxels in each binary cortex image divided by the total number of voxels. The vBMD phantom coefficient of variation was 1.24% for the study period.

Statistical analyses

All data are reported as mean ± SD or as percentages. Between-group differences were assessed by unpaired Student t test. The h^2 estimates by maternal descent (½h^2) was estimated as the slope of the regression (β) between offspring and mothers for each bone parameter separately (7). A principal components analysis (PCA) was conducted for the microstructure parameters measured by HR-pQCT at the radius and the tibia separately (27). PCA is a statistical data reduction technique in which a set of correlated variables is transformed into a smaller set of uncorrelated parameters, defined as the principal components (PCs). Those PCs are linear combinations of the original parameters. The advantage of PCA is that PCs summarize most of the information (or variance) of the original

dataset: The first principal component accounts for as much of the data variability as possible, with the remaining variance being explained decreasingly by the following PCs.

Variables entered in the PCA for radius and tibia were cortical and trabecular vBMD, cortical areas, trabecular number, trabecular spacing SD, and mean cortical thickness (Supplemental Table 1). Trabecular thickness was not included in this analysis because it is strongly affected by partial volume effects. In the PCA, we decided to include normalized parameters that were measured in all samples and with a β value of at least 0.10 in M-D pairs and/or M-S pairs. After optimization by a varimax rotation step, PCA identified 2 uncorrelated PCs at the radius and at the tibia separately (PCs with eigenvalues >1.0), representing, together, 93% and 87% of the variance of HR-pQCT parameters at the radius and tibia, respectively. Based on the initial variables' weight, the first component can be interpreted as the trabecular microarchitecture, the second component as cortical microarchitecture (Supplemental Table 1).

To estimate the h^2 of bone traits independently of the genetic effect of body size, gonadal status of mothers and offspring, aBMD, and the adjusted bone parameter residuals were calculated by multiple regression analysis with 3 models. Model 1 included age, weight, and height. Model 2 included the parameters of model 1 plus pubertal stage for the sons, menarcheal age of the daughters and the mothers, and years since menopause, taking into account years of menopause hormone therapy for the mothers, which means that the years on hormone therapy were considered as being premenopausal. Model 3 included the parameters of model 2 plus femoral neck BMD for HR-pQCT tibia parameters, and ultradistal radius BMD for HR-pQCT radius parameters. The residuals of each model were then regressed between mothers and offspring (27). A P value of <0.05 was considered statistically significant. Statistical analyses were performed using STATA version 14.1 (StataCorp, College Station, TX).

Results

Characteristics of mothers and offspring

The mean age of mothers and daughters was 50.6 ± 4.1 years and 20.4 ± 0.5 years, respectively; and of mothers and sons, 45.8 ± 3.9 years and 15.2 ± 0.5 years, respectively

(Table 1). The parameters of bone trabecular microarchitecture and cortical porosity appeared higher in the mothers than in daughters and sons, with the exception of radial cortical porosity in sons (Supplemental Table 2).

Heritability of areal bone mineral density

The $\frac{1}{2}h^2$ of aBMD ranged in both M-D pairs and M-S pairs from 25% to 46% (Supplemental Table 2) and was not different in both pairs ($\frac{1}{2}h^2$ M-D vs $\frac{1}{2}h^2$ M-S, P values ranged from 0.33 to 0.80).

Heritability of trabecular and cortical microarchitecture: influence of sex

Most parameters of bone microarchitecture were significantly inherited in both M-D and M-S pairs (Supplemental Table 2). We found similar estimates of $\frac{1}{2}h^2$ at the radius and tibia; the highest h^2 values were observed for cross-sectional area, whereas the lowest appeared the cortical vBMD and porosity.

Trabecular and cortical components were significantly inherited at the radius and at the tibia in both sexes ($\frac{1}{2}h^2$, 18% to 35%), with the exception of the cortical component at the radius (Table 2). There was no difference in h^2 of bone microarchitecture between M-D and M-S pairs. All heritabilities remained after adjustment for age, weight, height, gonadal status, and aBMD ($\frac{1}{2}h^2$, 9% to 25%).

Heritability of trabecular and cortical microarchitecture: influence of menopausal status

In the M-D pairs, the group of postmenopausal mothers compared with the premenopausal mothers had a higher mean age, lower body mass index, and lower aBMD values at all sites (Supplemental Tables 3 and 4). Postmenopausal mothers had lower mean cortical vBMD values, cortical thickness at radius and tibia, less radial cortical porosity, and lower trabecular number and

Table 1.   Anthropometric Characteristics of the Subjects

	Daughters (n = 102)	Mothers (n = 102)	Sons (n = 161)	Mothers (n = 161)
Age, mean ± SD, y	20.4 ± 0.5	50.6 ± 4.1	15.2 ± 0.5	45.8 ± 3.9
Height, mean ± SD, cm	165 ± 6.0	164.0 ± 6.0	172.0 ± 9.8	160.0 ± 6.0
Weight mean ± SD, kg	59.4 ± 9.3	64.7 ± 11.6	60.3 ± 13.3	64.4 ± 10.5
Body mass index, mean ± SD, kg/m^2	21.9 ± 3.3	24.1 ± 4.3	20.4 ± 0.6	23.8 ± 3.7
Pubertal stage, no. (%)				
2	0 (0)	—	5 (3.1)	—
3	0 (0)	—	13 (8)	—
4	0 (0)	—	83 (51.5)	—
5	102 (100)	—	60 (37.2)	—
Menarcheal age, mean ± SD, y	13.0 ± 1.2	13.0 ± 1.7	—	13.3 ± 1.5
Premenopause/postmenopause, no. of mothers (%)	—	51 (50)/51 (50)	—	134 (83)/27 (17)
MHT in postmenopause, no. of mothers (%)	—	26 (51)	—	6 (22)
Menopause duration, considering MHT, mean ± SD, y	—	2.4 ± 3.3	—	1.75 ± 2.4

Abbreviations: —, no data; MHT, menopausal hormone therapy; SD, standard deviation.

Table 2. Mother-Daughter and Mother-Son Half Heritability for Principal Components of Microarchitecture

	½h² 95% CI			Model 1ᵃ: Adj ½h² 95% CI			Model 2ᵇ: Adj ½h² 95% CI			Model 3ᶜ: Adj ½h² 95% CI		
	M-D	M-S	P	M-D	M-S	P	M-D	M-S	P	M-D	M-S	P
Radius												
Trab. MA	0.35ᵈ (0.19–0.51)	0.21ᵉ (0.07–0.36)	0.22	0.34ᵈ (0.18–0.50)	0.21ᵉ (0.07–0.35)	0.25	0.30ᵉ (0.14–0.47)	0.23ᵉ (0.09–0.38)	0.51	0.29ᵉ (0.14–0.45)	0.18ᶠ (0.05–0.31)	0.92
Cort. MA	0.19 (−0.02 to 0.47)	0.18 (−0.05 to 0.42)	0.96	0.14 (−0.09 to 0.37)	0.11 (−0.10 to 0.33)	0.80	0.18 (−0.03 to 0.41)	0.14 (−0.06 to 0.35)	0.75	0.16 (−0.12 to 0.45)	0.09 (−0.16 to 0.35)	0.27
Tibia												
Trab. MA	0.32ᵉ (0.17–0.47)	0.23ᵉ (0.07–0.40)	0.44	0.30ᵈ (0.16–0.44)	0.17ᵍ (0.02–0.31)	0.25	0.29ᵉ (0.14–0.44)	0.21ᵈ (0.06–0.30)	0.45	0.16ᵍ (0.02–0.31)	0.17ᵍ (0.03–0.32)	0.90
Cort. MA	0.22ᵍ (0.04–0.40)	0.34ᵉ (0.08–0.60)	0.45	0.25ᵉ (−0.07 to 0.43)	0.30ᵍ (0.06–0.55)	0.83	0.20ᵍ (0.20–0.39)	0.26ᵍ (0.02–0.50)	0.72	0.21ᵍ (0.02–0.39)	0.25ᵍ (0.01–0.49)	0.77

Abbreviations: Adj, adjusted; CI, confidence interval; Cort, cortical; MA, microarchitecture; Trab, trabecular.

[a] Model 1: adjusted ½h² for age, weight, and height.

[b] Model 2: adjusted ½h², parameters as in Model 1, plus pubertal stage in boys, menarcheal age in girls and mothers, years since menopause and hormone replacement therapy in the mothers.

[c] Model 3: adjusted ½h², parameters as in Model 2, plus radius HR-pQCT parameters adjusted also for ultradistal radius areal BMD and tibia parameters for femoral neck areal BMD.

[d] $P \leq 0.0001$.

[e] $P \leq 0.001$.

[f] $P \leq 0.01$.

[g] $P \leq 0.05$ for h² in M-D and M-S separately.

separation at the tibia compared with premenopausal mothers (Supplemental Table 4). The mothers' menopausal status had no influence on ½h² estimates for most aBMD (P values ranged from 0.29 and 0.81; Supplemental Table 4). Trabecular and cortical microarchitecture components remained inherited across menopausal status (Table 3).

Discussion

By analyzing the correlation of bone microarchitecture in mothers-offspring pairs of both sexes and subsets of pre- and postmenopausal mothers, our study led to 2 main findings. First, additive effect of genes on both trabecular and cortical microarchitecture is similar when inherited from mothers by daughters and sons. Second, h² influences on these traits mainly persist across menopausal status.

Our results are in contrast with the hypothesis of a gene-sex interaction in the determination of BMD and microarchitecture in the population, which assumes it could be possible that the genes that influence bone traits in men may not be the same as the genes that influence bone traits in women. Controversy exists because in a

Table 3. Mother-Daughter Half Heritability of Principal Components for Microarchitecture, According to Menopausal Status

Bone	Premenopausal M-D ½h² 95% CI	Postmenopausal M-D ½h² 95% CI	P	Premenopausal M-D Adj ½h² 95% CI: Model 1ᵃ	Postmenopausal M-D Adj ½h² 95% CI: Model 1ᵃ	P
Radius						
Trabecular MA	0.30ᵇ (0.06–0.54)	0.41ᶜ (0.18–0.64)	0.94	0.26ᵇ (0.02–0.50)	0.37ᵈ (0.15–0.59)	0.52
Cortical MA	0.27 (−0.08 to 0.64)	0.14 (−0.14 to 0.44)	0.58	0.23 (−0.11 to 0.57)	0.12 (−0.19 to 0.13)	0.59
Tibia						
Trabecular MA	0.24 (−0.01 to 0.54)	0.43ᵉ (0.23–0.62)	0.24	0.24ᵇ (0.02–0.47)	0.37ᶜ (0.17–0.36)	0.39
Cortical MA	0.28 (−0.06 to 0.64)	0.27ᵇ (0.06–0.48)	0.94	0.27 (−0.02 to 0.57)	0.25ᵇ (0.04–0.46)	0.89

Abbreviations: Adj, adjusted; CI, confidence interval; MA, microarchitecture.

[a] Model 1: Adjusted ½h² for age, weight, and height.

[b] $P \leq 0.05$.

[c] $P \leq 0.001$.

[d] $P \leq 0.01$.

[e] $P \leq 0.0001$.

whole-genome linkage analysis stratified by sex, sex-specific quantitative trait loci were found in the Framingham sample (18) and in some other studies (28, 29), but not in all studies (30).

One explanation of our findings could be that age at menarche and menopause has a h^2 on the order of approximately 50%; therefore, adjusting for these variables in women may possibly reduce some of the genetic effects from the overall h^2 of bone traits (31).

In the M-D and M-S pairs in our study, we found the only principal components of microarchitecture for which h^2 was not statistically significant were the radius cortical variables. It may be possible that radius measurements are more likely affected by the motion artifacts and by the technical challenge of measuring cortical porosity, especially in young people (32, 33).

In particular, because of the young age of the offspring (a mean of 20 years old for the girls and 15 years for the boys), the likelihood of not having reached the peak bone mass is high. Others investigators have reported that there is a transitory increase in intracortical porosity and cortical thinning during the pubertal growth spurt; on the other hand, there were few differences in trabecular structure across the stages of pubertal maturation (34). This might be an additional explanation of the difference we found in the cortical component h^2 compared with the trabecular component in our population.

Indeed, a high h^2 of cortical porosity in twins has been shown with HR-pQCT, using a different type of software (23). The apparent differences in site specificity of h^2 merit further investigation in larger family studies. A recent paper showed that the h^2 of BMD varies across skeletal sites, reflecting the different relative contributions of genetic and environmental influences (35). A possible explanation could be that genetic expression might be influenced by loading; thus, the tibia could be affected differently from the radius.

One strength of our study lies in the evaluation of the maternal h^2, which has been studied separately according to the mothers' menopausal status. We showed that h^2 of bone microarchitecture persists across menopausal status.

Our study suffers from 2 main limitations. The first is noninclusion of fathers in the study because of the difficulty in confirming paternity as compared with maternity. However, it would be desirable to include them and siblings in further studies to determine if the associations we have observed also apply to father-child pairs and sex-concordant and -discordant siblings. The second limitation is that the levels of testosterone and estradiol in the sons were not measured, which could have contributed additional information and could have decreased the adjusted h^2 of bone microarchitecture.

In conclusion, our study showed a high h^2 of bone traits in both sexes, without any sex differences, and that persists across menopausal status. These data suggest that most of the genetic determinants of bone microarchitecture rely on non–X-linked genes not regulated by gonadal status.

Acknowledgments

References

1. Amin S, Khosla S. Sex- and age-related differences in bone microarchitecture in men relative to women assessed by high-resolution peripheral quantitative computed tomography. *J Osteoporos*. 2012; **2012**:129760.
2. Karasik D, Ferrari SL. Contribution of gender-specific genetic factors to osteoporosis risk. *Ann Hum Genet*. 2008;**72**:696–714.
3. Ferrari SL. Human genetics of osteoporosis. *Best Pract Res Clin Endocrinol Metab*. 2008;**22**:723–735.
4. Ralston SH, Uitterlinden AG. Genetics of osteoporosis. *Endocr Rev*. 2010;**31**:629–662.
5. Wu S, Liu Y, Zhang L, Han Y, Lin Y, Deng HW. Genome-wide approaches for identifying genetic risk factors for osteoporosis. *Genome Med*. 2013;**5**:44.
6. Ferrari S, Rizzoli R, Slosman D, Bonjour JP. Familial resemblance for bone mineral mass is expressed before puberty. *J Clin Endocrinol Metab*. 1998;**83**:358–361.
7. Jones G, Nguyen TV. Associations between maternal peak bone mass and bone mass in prepubertal male and female children. *J Bone Miner Res*. 2000;**15**:1998–2004.
8. Runyan SM, Stadler DD, Bainbridge CN, Miller SC, Moyer-Mileur LJ. Familial resemblance of bone mineralization, calcium intake, and physical activity in early-adolescent daughters, their mothers, and maternal grandmothers. *J Am Diet Association*. 2003;**103**: 1320–1325.
9. Kuroda D, Onoe Y, Miyabara Y, Orito S, Ishitani K, Okano H, Ohta H. Influence of maternal genetic and lifestyle factors on bone mineral density in adolescent daughters: a cohort study in 387 Japanese daughter-mother pairs. *J Bone Miner Metab*. 2009;**27**: 379–385.
10. Tylavsky FA, Bortz AD, Hancock RL, Anderson JJB. Familial resemblance of radial bone mass between premenopausal mothers and their college-age daughters. *Calcif Tissue Int*. 1989;**45**: 265–272.
11. Picard D, Imbach A, Couturier M, Lepage R, Picard M. Familial resemblance of bone mineral density between females 18 years and older and their mothers. *Can J Public Health*. 2001;**92**:353–358.
12. Nabulsi M, Mahfoud Z, El-Rassi R, Al-Shaar L, Maalouf J, El-Hajj Fuleihan G. Gender differences in the heritability of musculoskeletal and body composition parameters in mother-daughter and mother-son pairs. *J Clin Densitom*. 2013;**16**:223–230.
13. Guéguen R, Jouanny P, Guillemin F, Kuntz C, Pourel J, Siest G. Segregation analysis and variance components analysis of bone mineral density in healthy families. *J Bone Miner Res*. 1995;**10**: 2017–2022.

14. Brown LB, Streeten EA, Shuldiner AR, Almasy LA, Peyser PA, Mitchell BD. Assessment of sex-specific genetic and environmental effects on bone mineral density. *Genet Epidemiol*. 2004;**27**: 153–161.

15. Kammerer C, Schneider J, Cole S, Hixson JE, Samollow PB, O'Connell JR, Perez R, Dyer TD, Almasy L, Blangero J, Bauer RL, Mitchell BD. Quantitative trait loci on chromosomes 2p, 4p, and 13q influence bone mineral density of the forearm and hip in Mexican Americans. *J Bone Miner Res*. 2003;**18**:2245–2252.

16. Ralston SH, Galwey N, MacKay I, Albagha OM, Cardon L, Compston JE, Cooper C, Duncan E, Keen R, Langdahl B, McLellan A, O'Riordan J, Pols HA, Reid DM, Uitterlinden AG, Wass J, Bennett ST. Loci for regulation of bone mineral density in men and women identified by genome wide linkage scan: the FAMOS study. *Hum Mol Genet*. 2005;**14**:943–951.

17. Jouanny P, Guillemin F, Kuntz C, Jeandel C, Pourel J. Environmental and genetic factors affecting bone mass. *Arthritis Rheum*. 1995;**38**:61–67.

18. Karasik D, Cupples LA, Hannan MT, Kiel DP. Age, gender, and body mass effects on quantitative trait loci for bone mineral density: the Framingham study. *Bone*. 2003;**33**:308–316.

19. Brown LB, Streeten EA, Shapiro JR, McBride D, Shuldiner AR, Peyser PA, Mitchell BD. Genetic and environmental influences on bone mineral density in pre- and post-menopausal women. *Osteoporos Int*. 2005;**16**(12):1849–1856.

20. Boutroy S, Bouxsein ML, Munoz F, Delmas PD. In vivo assessment of trabecular bone microarchitecture by high-resolution peripheral quantitative computed tomography. *J Clin Endocrinol Metab*. 2005;**90**:6508–6515.

21. Bonnet N, Biver E, Durosier C, Chevalley T, Rizzoli R, Ferrari S. Additive genetic effects on circulating periostin contribute to the heritability of bone microarchitecture. *J Clin Endocrinol Metab*. 2015;**100**:E1014–E1021.

22. Nagy H, Sornay-Rendu E, Boutroy S, Vilayphiou N, Szulc P, Chapurlat R. Impaired trabecular and cortical microarchitecture in daughters of women with osteoporotic fracture: the MODAM study. *Osteoporos Int*. 2013;**24**:1881–1889.

23. Bjørnerem Å, Bui M, Wang X, Ghasem-Zadeh A, Hopper JL, Zebaze R, Seeman E. Genetic and environmental variances of bone microarchitecture and bone remodeling markers: a twin study. *J Bone Miner Res*. 2015;**30**:519–527.

24. Chevalley T, Bonjour JP, Ferrari S, Hans D, Rizzoli R. Skeletal site selectivity in the effects of calcium supplementation on areal bone mineral density gain: a randomized, double-blind, placebo-controlled trial in prepubertal boys. *J Clin Endocrinol Metab*. 2005;**90**:3342–3349.

25. Bonjour JP, Carrie AL, Ferrari S, Clavien H, Slosman D, Theintz G, Rizzoli R. Calcium-enriched foods and bone mass growth in pre-pubertal girls: a randomized, double-blind, placebo-controlled trial. *J Clin Invest*. 1997;**99**:1287–1294.

26. Nishiyama KK, Macdonald HM, Buie HR, Hanley DA, Boyd SK. Postmenopausal women with osteopenia have higher cortical porosity and thinner cortices at the distal radius and tibia than women with normal aBMD: an in vivo HR-pQCT study. *J Bone Miner Res*. 2010;**25**:882–890.

27. Vilayphiou N, Boutroy S, Szulc P, van Rietbergen B, Munoz F, Delmas PD, Chapurlat R. Finite element analysis performed on

radius and tibia HR-pQCT images and fragility fractures at all sites in men. *J Bone Miner Res*. 2011;**26**(5):965–973.

28. Ioannidis JPA, Ralston SH, Ng MY, Sham PC, Zintzaras E, Lewis CM, Deng HW, Econs MJ, Karasik D, Devoto M, Kammerer CM, Spector T, Andrew T, Cupples LA, Duncan EL, Foroud T, Kiel DP, Koller D, Langdahl B, Mitchell BD, Peacock M, Recker R, Shen H, Sol-Church K, Spotila LD, Uitterlinden AG, Wilson SG, Kung AWC. Meta-analysis of genome-wide scans provides evidence for sex- and site-specific regulation of bone mass. *J Bone Miner Res*. 2007;**22**:173–183.

29. Peacock M, Koller DL, Lai D, Hui S, Foroud T, Econs MJ. Bone mineral density variation in men is influenced by sex-specific and non sex-specific quantitative trait loci. *Bone*. 2009;**45**:443–448.

30. Liu C-T, Estrada K, Yerges-Armstrong LM, Amin N, Evangelou E, Li G, Minster RL, Carless MA, Kammerer CM, Oei L, Zhou Y, Alonso N, Dailiana Z, Eriksson J, García-Giralt N, Giroux S, Husted LB, Khusainova RI, Koromila T, Kung AW, Lewis JR, Masi L, Mencej-Bedrac S, Nogues X, Patel MS, Prezelj J, Richards JB, Sham PC, Spector T, Vandenput L, Xiao SM, Zheng HF, Zhu K, Balcells S, Brandi ML, Frost M, Goltzman D, González-Macías J, Karlsson M, Khusnutdinova EK, Kollia P, Langdahl BL, Ljunggren O, Lorentzon M, Marc J, Mellström D, Ohlsson C, Olmos JM, Ralston SH, Riancho JA, Rousseau F, Urreizti R, Van Hul W, Zarrabeitia MT, Castano-Betancourt M, Demissie S, Grundberg E, Herrera L, Kwan T, Medina-Gómez C, Pastinen T, Sigurdsson G, Thorleifsson G, Vanmeurs JB, Blangero J, Hofman A, Liu Y, Mitchell BD, O'Connell JR, Oostra BA, Rotter JI, Stefansson K, Streeten EA, Styrkarsdottir U, Thorsteinsdottir U, Tylavsky FA, Uitterlinden A, Cauley JA, Harris TB, Ioannidis JP, Psaty BM, Robbins JA, Zillikens MC, Vanduijn CM, Prince RL, Karasik D, Rivadeneira F, Kiel DP, Cupples LA, Hsu YH. Assessment of gene-by-sex interaction effect on bone mineral density. *J Bone Miner Res*. 2012;**27**:2051–2064.

31. He C, Murabito JM. Genome-wide association studies of age at menarche and age at natural menopause. *Mol Cell Endocrinol*. 2014;**382**(1):767–779.

32. Seeman E. Growth and age-related abnormalities in cortical structure and fracture risk. *Endocrinol Metab (Seoul)*. 2015;**30**(4): 419–428.

33. Zebaze RM, Ghasem-Zadeh A, Bohte A, Iuliano-Burns S, Mirams M, Price RI, Mackie EJ, Seeman E. Intracortical remodelling and porosity in the distal radius and post-mortem femurs of women: a cross-sectional study. *Lancet*. 2010;**375**(9727):1729–1736.

34. Kirmani S, Christen D, van Lenthe GH, Fischer PR, Bouxsein ML. McCready LK, Melton LJ 3rd, Riggs BL, Amin S, Müller R, Khosla S. Bone structure at the distal radius during adolescent growth. *J Bone Miner Res*. 2009;**24**(6):1033–1042.

35. Kemp JP, Medina-Gomez C, Estrada K, St Pourcain B, Heppe DH, Warrington NM, Oei L, Ring SM, Kruithof CJ, Timpson NJ, Wolber LE, Reppe S, Gautvik K, Grundberg E, Ge B, van der Eerden B, van de Peppel J, Hibbs MA, Ackert-Bicknell CL, Choi K, Koller DL, Econs MJ, Williams FM, Foroud T, Zillikens MC, Ohlsson C, Hofman A, Uitterlinden AG, Davey Smith G, Jaddoe VW, Tobias JH, Rivadeneira F, Evans DM. Phenotypic dissection of bone mineral density reveals skeletal site specificity and facilitates the identification of novel loci in the genetic regulation of bone mass attainment. *PLoS Genet*. 2014;**10**(6):e1004423.