

Genetic Contribution to Bone Metabolism, Calcium Excretion, and Vitamin D and Parathyroid Hormone Regulation

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ABSTRACT

A classical twin study was performed to assess the relative contribution of genetic and environmental factors to bone metabolism, calcium homeostasis, and the hormones regulating them. It was examined further whether the genetic effect is menopause dependent. The subjects were 2136 adult twins (98.3% female): 384 monozygotic (MZ) and 684 dizygotic (DZ) twin pairs. The intraclass correlations were calculated, and maximum likelihood model fitting was used to estimate genetic and environmental variance components. The intraclass correlations for all of the variables assessed were higher in MZ twin pairs. The heritabilities (95% CIs) obtained from model fitting for hormones regulating bone metabolism and calcium homeostasis were parathyroid hormone (PTH), 60% (54–65%); 25-hydroxyvitamin D [25(OH)D], 43% (28–57%), 1,25-hydroxyvitamin D [1,25(OH)₂D], 65% (53–74%); and vitamin D binding protein 62% (56–66%). The heritabilities (95% CIs) for markers of bone formation also were assessed; bone-specific alkaline phosphatase (BSAP), 74% (67–80%), and osteocalcin, 29% (14–44%); marker of bone resorption deoxypyridinoline (DPD), 58% (52–64%); and measure of calcium homeostasis 24 h urine calcium, creatinine (Cr), 52% (41–61%). The magnitude of genetic influence differed with menopause for most variables. This study provides evidence for the importance of genetic factors in determining bone resorption and formation, calcium excretion, and the hormones regulating these processes. It shows for the first time a clear genetic effect on bone resorption in premenopausal women and the regulation of PTH, vitamin D metabolism, and calcium excretion. The genes controlling bone hormones and markers are likely to be useful therapeutic and diagnostic targets. (*J Bone Miner Res* 2001;16:371–378)

Key words: bone, formation, resorption, hormones, genetics

INTRODUCTION

OSTEOPOROSIS IS a disease characterized by low bone mass and architectural deterioration of bone tissue; both are related to abnormalities in bone turnover. In addition, bone turnover has been shown to be a risk factor for fracture, independent of bone mineral density (BMD). Our understand-

ing of these findings has been improved by the recent development of specific and sensitive biochemical markers reflecting the overall rate of bone formation (bone-specific alkaline phosphatase [BSAP] and osteocalcin) and bone resorption (urinary excretion of pyridinoline cross-links).

Although the search for genes controlling BMD is ongoing, to date, little attention has been focused on the genes

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influencing bone turnover, calcium homeostasis, and the hormones regulating these processes. Similarly, despite the strong association of fracture risk with menopause,^(1,2) there has been little attention on whether the genetic influence on bone metabolism is menopause dependent.

The previous literature on this subject includes a study by Kelly et al.⁽³⁾ of 70 female twin pairs that reported that approximately 80% of serum osteocalcin variance is explained by genetic factors. Tokita et al.,⁽⁴⁾ in a study of 82 female twin pairs, suggested that the synthesis and degradation of type I collagen, the major protein of bone matrix, is determined genetically and is related to the genetic regulation of bone density. In a study by our group of 120 postmenopausal twin pairs the genetic influence of various markers of bone formation and resorption were shown to have heritabilities ranging from 37% to 99%.⁽⁵⁾ These earlier studies had wide CIs and had insufficient power⁽⁶⁾ to estimate precisely genetic or environmental influence. The St. Thomas' UK Adult Twin Registry has a large subject population with data on a wide selection of biochemical variables and confounders. A genetic influence on bone resorption in premenopausal women has not, to our knowledge, been shown previously, nor has a genetic influence on vitamin D metabolites or the heritabilities of parathyroid hormone (PTH) and calcium excretion.

The aims of this study were first to determine the proportion of the variance in bone metabolism, calcium homeostasis, and their controlling hormones, explained by genetic factors. The second aim was to determine whether the magnitude of the genetic (or environmental) influence on these phenotypes was dependent on menopause.

MATERIALS AND METHODS

Study population

The subjects were 1068 twin pairs (age range, 18–71 years) from the St. Thomas' UK Adult Twin Registry, a volunteer sample recruited through a national media campaign in the United Kingdom.⁽⁷⁾ The subject sample was 1.75% male. Zygosity was determined by a standardized questionnaire, and DNA fingerprinting was used for confirmation.⁽⁸⁾

Data ascertainment

Demographic information was obtained by a standardized nurse-administered questionnaire with questions designed to ascertain their menopausal history (defined as the cessation of menstruation for at least 12 months, as previously described⁽⁹⁾) and medication use. Any subjects who were identified as being on hormone replacement therapy (HRT) or another antiresorptive or bone therapy were excluded from the analysis (384 subjects, 104 monozygotic [MZ] and 280 dizygotic [DZ]). Height and weight were measured and body mass index (BMI) (kg/m^2) was calculated. Blood and second-void urine samples were collected in the early morning (at the same time and place for both twins of the pair) after an overnight fast and stored at -20°C until assayed. Twenty-four-hour urine collection was com-

menced on the same day and returned the following day. All twin pair samples were measured in duplicate in the same assay.

Assays

Serum intact PTH was measured using a two-site chemiluminometric immunoassay (MagicLite Intact PTH; Chiron Diagnostics, South Notwood, MA, USA). The sensitivity is 1.4 ng/liter, and the intra- and interassay CV at 40 ng/liter is <10%.

Serum 25-hydroxyvitamin D [25(OH)D] was measured by specific radioimmunoassay (INCSTAR 25-Hydroxyvitamin D RIA kit, Incstar Corp., Stillwater, MN, USA) after extraction of serum with acetonitrile. The sensitivity is 3 $\mu\text{g}/\text{liter}$, and the intra- and interassay precision at 30 ng/ml was 6.1% and 15.6%, respectively. 1,25-Hydroxyvitamin D [1,25(OH)D] was measured using a quantitative radioimmunoassay (Nichols Institute Diagnostics Radioceptor Assay; Nichols Institute, San Juan Capistrano, CA, USA). The sensitivity is 2.1 ng/liter, and the intra- and interassay precision at 50 ng/liter is 8% and 10%, respectively.

Serum vitamin D binding protein (vit D BP) was measured by an immunonephelometric assay.⁽¹⁰⁾ The detection limit was 50 mg/liter and the intra and interassay CV at 250 mg/liter was 2.0% and 3.8%, respectively.

Serum total osteocalcin was measured with a competitive immunoassay (NovoCalcin; Metra Biosystems, Mountain View, CA, USA). This monoclonal antibody is believed to recognize only intact osteocalcin. The sensitivity is 0.45 $\mu\text{g}/\text{liter}$, and the intra- and interassay CV were 8% and 10% respectively at 8 $\mu\text{g}/\text{ml}$.^(11,12)

Serum total alkaline phosphatase (ALP) was measured using an automated analyzer (Vitros ALKP Slides, Johnson and Johnson). The sensitivity is 20 U/liter and the intra- and interassay precision was below 5.2% and 7.4%, respectively.

BSAP was measured with a monoclonal antibody that shows specificity for BSAP (Alkphase-B; Metra Biosystems), thus providing a quantitative measure of BSAP activity in serum as a measure of osteoblastic activity. The sensitivity is 0.7 U/liter, and the intra- and interassay CV at 28 U/liter were 3.3% and 7.9%, respectively.⁽¹³⁾

Urinary deoxypyridinoline (DPD) cross-links were measured on nonhydrolyzed urine samples using a competitive enzyme immunoassay (Pyrilinks-D; Metra Biosystems) and corrected for urinary creatinine (Cr) concentration. The monoclonal antibody has <1% cross-reactivity with free pyridinoline and no significant interaction with cross-linked peptides. The minimum detection limit is 1.1 nmol/liter, and the intra- and interassay CVs at 30 nmol/liter and 80 nmol/liter are less than 10%.

Twenty-four-hour urinary calcium and Cr were measured using a standard assay (Vitros; Johnson and Johnson Diagnostics, Rochester, NY, USA). The sensitivity of the calcium assay is 0.25 mmol/liter, while the intra- and interassay precisions are below 3% and 4.3%, respectively. The sensitivity of the Cr assay was 0.04 μM , and the intra- and interassay precisions were below 2.6% and 4%, respectively.

*Statistical analysis**Background to twin analysis*

The classical twin study makes use of the fact that MZ twins share identical genotypes, whereas DZ twins are no more alike genetically than siblings, sharing on average, 50% of their segregating genes. If MZ twins show a larger resemblance for a specific trait than DZ twins, this is likely to be caused by genetic factors. A higher MZ than DZ intraclass correlation (ICC) provides a first impression of the magnitude of genetic influence. Structural modeling allows a more extensive separation of the observed phenotypic variance into its genetic and environmental components: additive genetic variance (A), dominance genetic variance (D), shared (or common) environmental variance (C), and specific (or unique) environmental variance (E), which also contains measurement error.

Analytical approach

Preliminary data analysis was performed and ICCs were estimated using STATA.⁽¹⁴⁾ Data were skewed and therefore log transformed to obtain a normal distribution for all variables. Aims of our analyses were 2-fold: (i) to establish the genetic influence overall and (ii) to examine menopause dependency. First, we estimated genetic and environmental influences on all variables for the entire group, both before and after adjustment for age and BMI, respectively. Adjusted estimates were obtained by model fitting to trait residuals after removal of the effect of age or BMI by linear regression.⁽¹⁵⁾ Second, we investigated the effect of the menopause on changes in (i) mean trait values (ii) total trait variance, and (iii) genetic and environmental sources of variance. We specified a path model in which the total sample was subdivided into six menopause by zygosity groups (i.e., MZ pre/pre, DZ pre/pre, MZ pre/post, DZ pre/post, MZ post/post, and DZ post/post). Age was incorporated in the model for all of the variables. Additionally, using menopause discordant pairs, we tested whether the correlation between the latent genetic factors in these pairs was lower than the normal correlation of 1 in MZ pairs and 0.5 in DZ pairs, which would indicate that (partly) different genes influence these measured variables in pre- and postmenopausal women (Fig. 1).⁽¹⁶⁾

Model fitting procedure

The significance of A, C, and D was tested by removing them sequentially in specific submodels, eventually leading to a model that gives the most parsimonious fit to the data, that is, a model in which the pattern of variances and covariance is explained by as few parameters as possible. Submodels were compared with the full model by hierarchical χ^2 tests. The difference between a submodel and that of the full model itself is distributed as χ^2 , with degrees of freedom equal to the difference of the number of estimated parameters in the full model and the number of estimated parameters in the submodel. Akaike's information criterion ($AIC = \chi^2 - 2$ degrees of freedom [df]) also was used to

TABLE 1. CHARACTERISTICS OF MZ ($n = 384$) AND DZ ($n = 684$) TWIN PAIRS AND MEAN VALUES (SD) OF VARIABLES

	MZ twins	DZ twins
Age (years)	47.1 (13.9)	45.2 (12.1)
Height (cm)	162.7 (6.2)	162.8 (6.2)
Weight (kg)	63.9 (10.4)	66.4 (12.9)
BMI (kg/m ²)	24.1 (3.9)	25.0 (4.7)
Pre-menopausal (%)	45	55
Current smokers (%)	16	18
PTH (ng/liter)	30.9 (12.1)	32.3 (13.7)
25-Vitamin D (μ g/liter)	37 (14.0)	35 (15.4)
1,25-Vitamin D (ng/liter)	33 (15.4)	29 (14.2)
Vitamin D binding protein (mg/liter)	290 (45)	295 (46)
BSAP (U/liter)	18.8 (11.5)	18.1 (7.8)
Serum total ALP (U/liter)	71 (21)	70 (21)
Osteocalcin (μ g/liter)	8.0 (3.3)	8.2 (3.7)
DPD/Cr (nmol/mmol)	5.4 (2.2)	5.3 (2.7)
C/Cr (mmol/mol)	0.51 (0.25)	0.50 (0.26)

evaluate the fit of the genetic models. The model with the lowest AIC reflects the best balance between goodness of fit and parsimony. Estimates of variance components and their 95% CIs were obtained from the best fitting model. All quantitative genetic model fitting was carried out with the software package Mx (Commonwealth University of Virginia, Richmond, VA, USA).⁽¹⁷⁾

RESULTS

Heritabilities

The data in Table 1 show the general characteristics of the entire group of twin pairs studied ($n = 1068$). For most variables (except DPD) biochemical data were not available in the entire group but the twin characteristics of these subgroups did not differ significantly from the entire group. The MZ pairs were, on the average, 2 years older at the time of ascertainment. This is reflected in a larger proportion of the MZ pairs being postmenopausal. The DZ pairs were, on average, 2.5 kg heavier than the MZ pairs reflected in a BMI 0.9 kg/m² higher. However, the mean values for all of the markers and hormones did not differ significantly between the MZ and DZ groups.

The ICC for MZ twins (rMZ) and DZ twins (rDZ) for the calcium regulating hormones, measure of calcium homeostasis, and markers of bone metabolism are presented in Table 2. The finding of rMZ greater than rDZ implies an important genetic influence, which was subsequently confirmed by model fitting to the entire group (Table 3). Dominant genetic effects (D) did not contribute significantly to the explanation of the data and they could be dropped from the model without a significant worsening of fit. Those in which there was evidence of shared environmental effect included 25(OH)D and osteocalcin. A model specifying additive genetic (A) and unique environmental (E) variance

TABLE 2. ICC (NUMBER OF TWIN PAIRS) FOR CALCIUM REGULATING HORMONES AND MARKERS OF BONE METABOLISM

Menopause status grouping		Pre/pre	Pre/post	Post/post	Total
PTH	MZ	0.66 (141)	0.45 (11)	0.58 (223)	0.61 (375)
	DZ	0.33 (308)	0.22 (76)	0.32 (279)	0.34 (663)
25-Vitamin D	MZ	0.84 (105)	0.80 (10)	0.63 (215)	0.85 (330)
	DZ	0.58 (264)	0.51 (58)	0.48 (253)	0.58 (575)
1,25-Vitamin D	MZ	— (1)	— (0)	0.59 (88)	0.52 (89)
	DZ	0.94 (8)	0.92 (9)	0.53 (51)	0.01 (68)
vit D BP	MZ	0.63 (149)	0.61 (14)	0.83 (216)	0.62 (379)
	DZ	0.33 (312)	0.37 (66)	0.45 (287)	0.31 (665)
BSAP	MZ	0.37 (12)	0.46 (3)	0.79 (152)	0.71 (167)
	DZ	0.67 (15)	0.59 (11)	0.45 (80)	0.55 (106)
Serum ALP	MZ	0.78 (134)	0.87 (10)	0.67 (213)	0.75 (357)
	DZ	0.42 (306)	0.40 (65)	0.41 (298)	0.47 (669)
Osteocalcin	MZ	0.65 (146)	0.23 (11)	0.66 (225)	0.64 (382)
	DZ	0.49 (311)	0.53 (64)	0.42 (281)	0.47 (656)
DPD	MZ	0.65 (164)	0.40 (15)	0.56 (205)	0.60 (384)
	DZ	0.25 (341)	0.35 (75)	0.23 (268)	0.25 (684)
Ca/Cr	MZ	0.25 (11)	— (2)	0.54 (156)	0.52 (169)
	DZ	0.35 (28)	0.33 (19)	0.33 (113)	0.35 (161)

TABLE 3. HERITABILITY (95% CIs) OF CALCIUM REGULATING HORMONES AND BIOCHEMICAL MARKERS OF BONE TURNOVER

	Crude heritability			Age-adjusted heritability		
	a^2	c^2	e^2	a^2	c^2	e^2
PTH	0.63 (0.57–0.68)	—	0.38 (0.32–0.43)	0.60 (0.54–0.65)	—	0.40 (0.35–0.46)
25-Vitamin D	0.43 (0.28–0.57)	0.27 (0.23–0.30)	0.30 (0.25–0.35)	0.43 (0.28–0.57)	0.27 (0.22–0.31)	0.30 (0.26–0.35)
1,25-Vitamin D	0.37 (0.19–0.52)	—	0.63 (0.48–0.81)	0.65 (0.53–0.74)	—	0.35 (0.26–0.47)
vit D BP	0.63 (0.56–0.66)	—	0.37 (0.33–0.43)	0.62 (0.56–0.66)	—	0.38 (0.34–0.44)
BSAP	0.77 (0.70–0.82)	—	0.23 (0.18–0.30)	0.74 (0.67–0.80)	—	0.26 (0.20–0.33)
ALP	0.60 (0.47–0.60)	0.17 (0.12–0.22)	0.23 (0.20–0.27)	0.75 (0.71–0.79)	—	0.25 (0.21–0.29)
Osteocalcin	0.29 (0.14–0.44)	0.34 (0.28–0.40)	0.37 (0.32–0.43)	0.29 (0.14–0.44)	0.34 (0.28–0.40)	0.37 (0.32–0.43)
DPD	0.59 (0.53–0.64)	—	0.41 (0.36–0.48)	0.58 (0.52–0.64)	—	0.42 (0.36–0.48)
Ca/Cr	0.52 (0.41–0.61)	—	0.48 (0.39–0.59)	0.52 (0.41–0.61)	—	0.48 (0.39–0.59)

a^2 , additive genetic variance; c^2 , common environmental variance; e^2 , unique environmental variance.

components gave the most parsimonious explanation of the data for the remainder of variables. Heritability estimates ranged from 0.37 for 1,25(OH)D to 0.74 for BSAP. The model and the genetic and environmental estimates for all of the variables remained the same after adjusting for age and BMI (data not shown) with the exception of total alkaline phosphatase (ALP) and 1,25(OH)D.

Effect of menopause

The means of the variables measured by menopause status are shown in Table 4. There was a significant increase in the mean levels of PTH, BSAP, and osteocalcin after menopause. Total variance and its standardized variance components with respect to menopause status are shown in Figs. 2 and 3. The total variance was the same pre- and postmenopause for PTH, BSAP, and total ALP, whereas for the other variables measured [25(OH)D, vitamin D binding protein, Ca/Cr, osteocalcin, and DPD] the total variance differed significantly with menopause. There was a large

decrease in total variance for 25(OH)D because of a reduction in shared environmental and genetic influence and a small increase in unique environmental influence after menopause. Total variance of vitamin D binding protein also was reduced significantly largely because of a reduction in unique environmental influence and age effect. Total variance of Ca/Cr increased after menopause because of increased genetic and unique environmental influence. The total variance of osteocalcin increased after menopause largely because of an increased genetic effect. There was a small reduction in total variance for DPD predominantly because of the effect of age. Unfortunately, there were insufficient premenopausal MZ pairs for subanalysis of 1,25-vitamin D.

Different genes before and after menopause

In addition to estimating the magnitude of genetic influences on all the variables in pre- and postmenopausal women, the influence of different genes before and after

TABLE 4. VARIABLE MEANS (SD)—DIFFERENCE WITH MENOPAUSE

Menopause status	Means		p Value
	Pre	Post	
PTH (ng/liter)	27.5 (11.9)	33.2 (14.0)	<0.001
25-Vitamin D (μ g/liter)	33 (17.0)	32 (13.8)	NS
vit D BP (mg/liter)	298 (54)	288 (39)	NS
BSAP (U/liter)	14 (3.7)	19 (10.8)	<0.001
ALP (U/liter)	61 (19)	73 (22)	NS
Osteocalcin (μ g/liter)	7.1 (3.1)	8.7 (3.9)	<0.001
DPD (nmol/mmol)	4.8 (2.4)	5.2 (2.7)	NS
Ca/Cr (mmol/mol)	0.48 (0.19)	0.51 (0.26)	NS

menopause also was assessed. None of the variables reached statistical significance but the small numbers of pairs discordant for menopause gave us low power to detect a difference. We could not therefore tell whether the genes influencing these traits were different pre- or postmenopause.

DISCUSSION

In this classical twin study we found that bone formation, bone resorption, calcium homeostasis, and the hormones regulating calcium homeostasis are under strong genetic control. Specifically, up to 74% of bone formation, 58% of bone resorption, 52% of calcium excretion, 60% of PTH, and up to 65% of vitamin D variance is accounted for by genetic influence. The total variance differed with menopause in five of the eight variables measured. It shows for the first time a genetic effect on bone resorption in premenopausal women and the regulation of PTH, vitamin D metabolism, and calcium excretion.

PTH and vitamin D

A genetic influence of a large magnitude (~60% of variance) influences PTH concentration. Current evidence suggests that the extracellular fluid ionized calcium concentration is the major determinant of PTH secretion, while 1,25(OH)D and phosphate also appear to be important regulators.⁽¹⁸⁻²⁰⁾ This presumably is in large part a result of their control of PTH gene expression and resultant effects on PTH secretion.⁽²¹⁾ Estrogens, magnesium, catecholamines, and other agents also influence PTH secretion, but their relevance to normal physiology is uncertain.

Our data show for the first time (to our knowledge) a genetic influence on the production of vitamin D metabolites. Vitamin D is a secosteroid that is made in the skin by the action of sunlight and also can be obtained from the diet. The first step in the metabolic activation of vitamin D is hydroxylation of carbon 25, which occurs primarily in the liver. 25(OH)D is the major circulating vitamin D metabolite, and hepatic production increases in proportion to vitamin D intake. There also are important seasonal variations in the levels of this metabolite because of different degrees

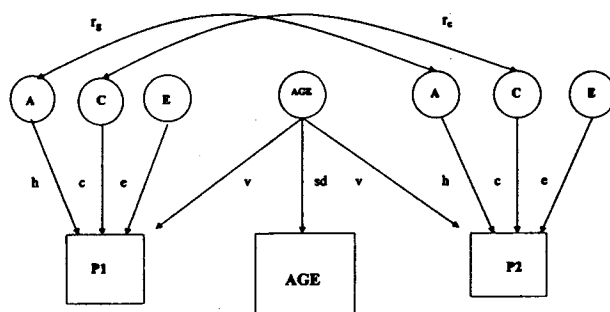


FIG. 1. Path diagram for twin model including age regression. Observed variables for twin 1 and twin 2 are shown in the squares. Latent variables (or factors) are shown in circles. A single-headed arrow indicates a direct influence of one variable on another, its value represented by a path coefficient. Double-headed arrows indicate a correlation without any assumed direct relationship. A, additive genetic factor; C, common environmental factor; E, unique environmental factor; h, additive genetic factor loading; c, common environmental factor loading; e, unique environmental factor loading; P1 (P2), phenotypic value of twin 1 (twin 2); r_s , genetic correlation (1 for MZ and 0.5 for DZ twins and estimated for twin pairs discordant for menopause); r_c , common environmental correlation (1 for both MZ and DZ twins); v, factor loading on age; sd, standard deviation of age.

of exposure to sunlight. It has a strong genetic component of ~43%, despite known common environmental factors (effects of sunlight and diet) that contribute ~27%. The total variance decreased after menopause because of a reduction in genetic and common environmental influence.

1,25(OH)D is the most biologically active metabolite of vitamin D. Because of its potent effect on calcium homeostasis, its circulating levels are controlled tightly.^(22,23) Interestingly, 65% of the variance of this metabolite is explained by genetic influence, presumably largely the control of hydroxylation enzymes involved in both formation and degradation, which may have important therapeutic implications.

Within the limitations of our current understanding of vitamin D receptor function, some studies (but not all) have suggested that certain polymorphisms explain some of the variance in peak bone mass.⁽²⁴⁾ The strong genetic effect on peak bone mass in part may be mediated by the genetic regulation of the hormones interacting with these receptors.

The vitamin D binding protein, also called group-specific component (Gc), is recognized to be a member of a gene family that includes albumin and α -fetoprotein. It plays the major role in the egress of endogenously synthesized vitamin D, from skin and appears to restrain D-sterols from too rapid/excessive cell entry. It also facilitates actin removal, has been shown to behave as a chemotaxin, and has a role in macrophage activation.⁽²⁵⁾ Gc messenger RNA (mRNA) synthesis is influenced by interleukin-6 (IL-6), transforming growth factor β (TGF- β), and steroids,⁽²⁶⁾ and this study shows that approximately 60% of the variance is explained by genetic influence, and that the variance reduces after menopause largely because of a reduction in unique environmental influence.

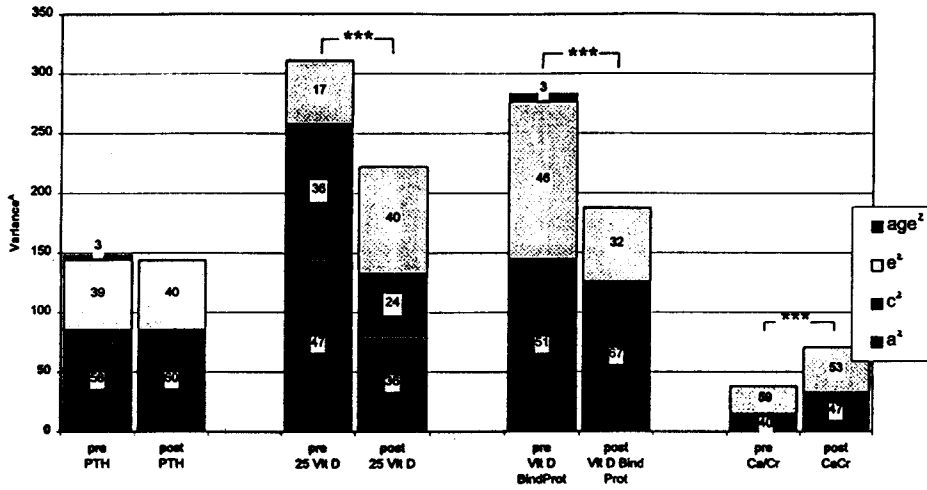


FIG. 2. Total variance and standardized variance components for hormones and calcium excretion. Variance components (% of total variance) are shown in each bar. Significant differences in total variance by menopausal status are shown by * $p < 0.05$ and *** $p < 0.001$. Age, variance component caused by age; e, unique environmental variance; c, common environmental variance; a, additive genetic variance. ^AUnits of variance are square of units in Table 1.

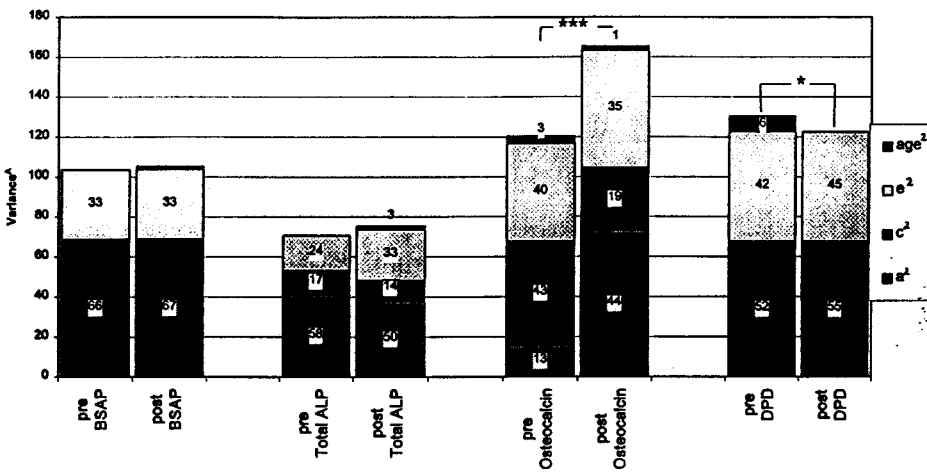


FIG. 3. Total variance and standardized variance components for bone markers. Variance components (% of total variance) are shown in each bar. Significant differences in total variance by menopausal status are shown by * $p < 0.05$ and *** $p < 0.001$. Age, variance component caused by age; e, unique environmental variance; c, common environmental variance; a, additive genetic variance. ^AUnits of variance are square of units in Table 1.

Bone formation

BSAP is a prominent product of osteoblasts and osteoblast precursors, and it plays a key role, as yet poorly defined, in mineralization. The measure of bone formation provided is indirect, depending presumably on a spillover of excess or spent enzyme from active osteoblasts and preosteoblasts.

Osteocalcin is a polypeptide expressed under 1,25(OH)D control by osteoblasts as they actively deposit bone. Serum osteocalcin seems to reflect primarily a spillover of osteoblast synthetic activity rather than degradation products of resorbed bone matrix.⁽²⁷⁾

For the markers of bone formation, 74% of the observed variation in BSAP and 44% of the variation in osteocalcin in postmenopausal females were explained by genetic factors. Menopause is associated with an increase in bone formation, which has been well documented by histomorphometric data comparing normal women in their 30s with women in their late 60s.⁽²⁸⁾ It has been shown in some studies that serum osteocalcin increases after menopause and that it is correlated closely with histomorphometric parameters of

bone formation in postmenopausal osteoporosis.^(29,30) There is an increase in both the mean and the total variance of osteocalcin after menopause, and this increase in total variance is largely caused by genetic influence. Although BSAP and osteocalcin are emerging as reasonable indicators of bone formation activity, they do not always show parallel responses.⁽³¹⁾

Bone resorption

Urinary levels of pyridinoline have been the most studied markers of osteoclastic activity in collagen breakdown. A genetic regulation of bone resorption was shown with a heritability of 59% for DPD. The heritability was largely unchanged after menopause, suggesting the magnitude of genetic and environmental regulation of bone resorption after menopause is as great as it is before menopause.

Calcium excretion

Renal calcium absorption is mediated by both active and passive processes. Absorption in both proximal tubule and

thick ascending limb mainly is coupled indirectly to sodium absorption and is a passive process through the paracellular pathway. In the distal convoluted tubule, Ca absorption is regulated independently of sodium absorption, this is, the principal site of action of PTH, calcitonin, and 1,25(OH)₂D. Renal tubular calcium reabsorption is dependent on the calcium sensing receptor for regulation of extracellular calcium homeostasis.⁽³²⁾ A genetic influence on calcium excretion was shown with heritability of 52% for Ca/Cr. The total variance increased after menopause, supporting the role of estrogen in regulation of calcium excretion.⁽³³⁾

Limitations

There are several potential sources of bias, which could influence our results and are worthy of mention. Medication use (HRT and other antiresorptive bone therapy) is unlikely to have had an effect on model fitting because those subjects were excluded before analysis. The mean values of general characteristics of MZ and DZ twins in this study were very similar. Only age, weight, and BMI showed slight difference between zygosity. Adjusting for age and other potential confounders made little difference to the estimates of heritability in our analysis and provides reassurance that these estimates are a valid reflection of the genetic contribution to these hormones and biochemical markers.

The reported results are likely to be representative of the general population, because basic and bone-related characteristics of the twins were similar to a population-based sample of 1003 women participating in the Chingford Cohort Study, London.⁽³⁴⁾

Using the twin model to assess the genetic contribution may give only limited power to detect the influence of common environmental and dominance variance.⁽³⁵⁻³⁷⁾ To have sufficient statistical power to exclude confidently a common environmental influence or dominant genetic effects would require a study with larger sample sizes. However, inspection of the ICC coefficients and models suggests that dominant gene effects are unlikely to be important for any of the variables except perhaps for 1,25(OH)₂D.

Given the small number of menopause discordant pairs, which is probably a consequence of the fact that timing of the menopause itself is under genetic influence,⁽⁹⁾ this study had limited power to detect and distinguish between different genes operating in pre- and postmenopausal women. Ideally, this information could be obtained by large longitudinal twin or family studies.

Our results confirm and extend previous data published in this area. The heritability estimates from our much smaller previous study of postmenopausal twins⁽⁵⁾ for osteocalcin and DPD are very similar to the current study.

The heritability for BSAP is very similar to that found by Harris et al. based on 194 subjects,⁽³⁸⁾ while the heritability for vitamin D binding protein is similar to the 70% found by Diager et al. based on 88 subjects.⁽³⁹⁾

The results of this study for osteocalcin and Ca/Cr are different from the earlier but much smaller study by Kelly et al. of 140 subjects.⁽³⁾ They found the heritability of osteocalcin to be approximately 82% while despite a higher rMZ than rDZ for Ca/Cr this did not reach statistical significance.

The rMZ for their study for osteocalcin was very high, possibly a manifestation of smaller numbers or the different assay, and their Ca/Cr assay was a single 2-h urine specimen, whereas we used a more reliable 24-h measurement.

The acquisition of peak bone mass results from bone remodeling during skeletal development and linear growth, whereas its maintenance in adults results from the coupling mechanism between the activities of bone formation and resorption. A number of prospective studies have shown that increased bone turnover is a risk factor for subsequent fracture, independent of BMD and mobility status.⁽⁴⁰⁾ In support of a genetic effect on net bone turnover, our data show a strong genetic influence on the key indices of formation and resorption and the hormones regulating both bone metabolism and calcium homeostasis.

The genetic regulation of PTH synthesis and the large genetic influence on vitamin D metabolites and their binding protein suggest the possibility of novel therapeutic avenues.

Thus, although a genetic influence on osteoporotic risk is now well established, the number of genes involved, their chromosomal location, the magnitude of their effects, and the way they interact with each other and with other risk factors are not well defined. As to whether BMD, bone turnover, and calcium homeostasis are under common genetic or environmental control needs further clarification. These findings should encourage further work on bone metabolism, calcium homeostasis, and regulating hormones and the search for genes controlling these variables.

ACKNOWLEDGMENTS

We thank the staff of the Twin Research Unit, the technical staff of ChemPath, and the twins themselves for their participation. This study has been funded in part by grants from the Wellcome Trust, Chronic Diseases Research Foundation, Medical Research Council, and Gemini Genomics Ltd. D.H. is sponsored by the Arthritis Foundation of Australia and Roger Vanderfield Traveling Fellowship. M.D.L. and H.S. are sponsored by the British Heart Foundation, and A.J.M. is sponsored by the Arthritis Research Council.

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Received in original form November 2, 1999; in revised form January 31, 2000; accepted March 9, 2000.