Genetic Determinants of Bone Mass in Adults

A Twin Study

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Abstract

The relative importance of genetic factors in determining bone mass in different parts of the skeleton is poorly understood. Lumbar spine and proximal femur bone mineral density and forearm bone mineral content were measured by photon absorptiometry in 38 monozygotic and 27 dizygotic twin pairs. Bone mineral density was significantly more highly correlated in monozygotic than in dizygotic twins for the spine and proximal femur and in the forearm of premenopausal twin pairs, which is consistent with significant genetic contributions to bone mass at all these sites. The lesser genetic contribution to proximal femur and distal forearm bone mass compared with the spine suggests that environmental factors are of greater importance in the aetiology of osteopenia of the hip and wrist. This is the first demonstration of a genetic contribution to bone mass of the spine and proximal femur in adults and confirms similar findings of the forearm. Furthermore, bivariate analysis suggested that a single gene or set of genes determines bone mass at all sites.

Introduction

Osteoporosis is a major health problem of Western societies that affects up to half of the elderly female population (1). Osteoporosis-related fractures are an increasing problem in aging men and women. In women, the sudden decline in estrogen production at the menopause is an important aetiological factor in the subsequent accelerated rate of bone mineral loss (2, 3). However, the significant prevalence of the disease in aging men and its absence in some postmenopausal women indicate that other factors are also important in determining bone mass and hence the risk of osteoporotic fractures. Environmental factors such as tobacco and alcohol use, physical activity, and body weight have all been reported to influence bone mass (4–10). A genetic contribution to bone mass has previously been reported for the bones of the upper limb (11–13). One study found a genetic component of spinal bone mass in individuals less than 25 years old, but not in subjects older than 25 (11). To our knowledge, no data have been published in relation to a possible genetic contribution to bone mass in the clinically important site of the proximal femur.

The study of twins provides a unique method to investigate the importance of genetic and environmental factors in determining bone mass. We report here the results of a twin study examining heritability of bone mass in the lumbar spine, proximal femur, and distal forearm.

Methods

The twin pairs studied were volunteers obtained through the Australian National Health and Medical Research Council Twin Registry and from appeals through the media. Only adult twins of the same sex were studied, and informed written consent was obtained from all participants. Twins were only excluded from analysis on the basis of disease, such as rheumatoid arthritis, or use of medications, such as corticosteroids, which may have affected bone density in one or both twins. 65 twin pairs were studied in full.

The zygosity of the twins was determined from their own classification. This has been shown to be accurate to within 5% and is comparable with classification by more sophisticated and extensive investigation (14).

The study group comprised 32 female and 6 male monozygotic (MZ)[1] twin pairs, as well as 26 female and 1 male dizygotic (DZ) twin pairs. 13 of the MZ and 4 of the DZ female twin pairs were postmenopausal. Two other DZ twin pairs were discordant for menopausal status; one twin of each pair was premenopausal and the other postmenopausal (four years in each case).

Bone mineral density (BMD, grams per square centimeter) was measured in the lumbar spine (L2–L4) and right proximal femur using a DP3 dual photon absorptiometer (Lunar Radiation, Madison, WI). Dual photon absorptiometry utilizes the relative transmission of photons of two energies (44 and 100 keV), emitted from a Gadolinium 153 radiation source, to determine bone mineral content (BMC) (15). BMD was derived by dividing the BMC of each region by the projected bone area. In the proximal femur three sites were measured: the femoral neck at a trans-cervical position, the trochanteric region, and Ward's triangle within the femoral neck. All femoral scans were checked, without knowledge of zygosity or bone density estimate, to ensure there was no difference in positioning of region of interest between twin pairs. The radiation dose to the skin and gonads was < 200 and 100 μGy, respectively (10–20 mrad). As we have described previously the coefficient of variation was 1.4% over 36 determinations (weekly) with cadaveric vertebrae and 2.6% on repeated determinations in five normal volunteers (16), consistent with published values (17).

Forearm BMC (units per centimeter) of the distal radius and ulna was measured using a single photon densitometer (Molsgaard Instruments, Copenhagen, Denmark). Scanning was commenced at a site corresponding to 8 mm separation between the radius and the ulna; five subsequent scans were performed, each 4 mm more proximal. Forearm BMC was calculated from the mean of the six scans. At the 8-mm site the radius and ulna are comprised of ~ 20 and 12% trabecu-

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1. *Abbreviations used in this paper:* BMC, bone mineral content; BMD, bone mineral density; DZ, dizygotic; MZ, monozygotic; VO_2max, maximum oxygen uptake.

lar bone, respectively, while at the most proximal site the radius and ulna are both ~ 5% trabecular bone (18). The coefficient of variation on repeated measurements in normal volunteers was 1.5%. Measurements of BMD were made without knowledge as to the zygosity of the twins.

Lumbar spine radiographs were obtained in all subjects older than 40 years. Scans of each twin were analyzed with reference to relevant X rays without knowledge of the sibling's results. The lumbar BMD estimates were excluded from analysis for six twin pairs because of the presence of spinal osteoarthritis, which may falsely elevate estimates of spinal bone density. Vascular calcification was not a problem in any subject. No subject in the study had a prior history of renal disease and all had normal renal function as assessed by creatinine clearance (available in 100 subjects) and/or a normal serum creatinine. Weight (kilogram) and height (meter) were measured in all subjects and body mass index (kilogram per squared meter) was calculated. Physical fitness was estimated in 26 MZ and 23 DZ twin pairs by measurement of predicted maximal oxygen uptake (VO_2max, liters per minute) according to the criteria of Astrand and Ryhming (19). Subjects were exercised at a known work load on a bicycle ergometer. The plateau pulse rate, steady for at least 2 min, was used in conjunction with the load to estimate the VO_2max according to the nomogram of Astrand and Ryhming (19).

Statistical Methods

Analyses of twin studies assume that intrapair variance of MZ and hence genetically identical twins is due to environmental factors and measurement error, while intrapair variance in DZ twins is additionally affected by genetic factors. It is also assumed that common environmental factors are shared to a similar extent between MZ and DZ twins. Comparison of the correlation of BMD in MZ twin pairs with that in DZ twin pairs can therefore provide a means of determining the genetic contribution to observed variation in BMD. Thus, at any particular location: $BMD = \mu + G + C + E$, where μ is the mean BMD for that location (and may itself be a function of measured variables such as age, sex, years postmenopause, etc.), G represents genetic determinants of bone mass, C represents common environmental factors affecting bone mass, and E represents environmental factors particular to an individual including measurement error. Furthermore, for each twin, G, C, and E are assumed to be independent, normally distributed random variables with means equal to zero and non-negative variances σ_g^2, σ_c^2, and σ_e^2, respectively. The relationship of BMD in twin pairs (BMD_1,BMD_2) as measured by covariance (Cov) can therefore be expressed as: $Cov(BMD_1,BMD_2) = Cov(G_1,G_2) + Cov(C_1,C_2)$. For MZ pairs the correlation of G_1 and G_2 = 1, while for DZ pairs the correlation of G_1 and G_2 = ½ (20). The classical twin model assumes the correlation of C_1 and C_2 = 1 independent of zygosity. Hence for MZ pairs: $Cov(BMD_1,BMD_2) = Cov(MZ) = \sigma_g^2 + \sigma_c^2$, while for DZ pairs

$$Cov(BMD_1,BMD_2) = Cov(DZ) = \tfrac{1}{2}\sigma_g^2 + \sigma_c^2,$$

and therefore

$$Cov(MZ) = Cov(DZ) \quad \text{if and only if} \quad \sigma_g^2 = 0.$$

In summary, for any trait (e.g., BMD), demonstration of a significant difference in covariance, and hence in the correlation, between MZ twin pairs and DZ twin pairs is consistent with a significant genetic determinant of variation in that trait.

Estimation of parameters. The correlations of BMD and BMC at different sites in MZ and DZ twin pairs were calculated by maximum likelihood as outlined below. In the analyses the mean was fitted independently of zygosity and as a linear function of age for males and premenopausal females, or a linear function of years postmenopause for postmenopausal females. In all analyses the mean was allowed to differ between locations. Tests of fit for outliers were performed after Hopper and Mathews (21): In no instance was there evidence of a poor fit. For each twin pair, at each location, it is assumed that BMD has a bivariate normal distribution with covariances that can be expressed as above in terms of σ_g^2 and σ_c^2. Under the restriction that all are non-negative, the variance components σ_g^2, σ_c^2, and σ_e^2 are estimated by maximum likelihood (22) using the algorithm Fisher (23, 24). After convention we define heritability to be $h = \sigma_g^2/\sigma^2$, and similarly define $c = \sigma_g^2/\sigma^2$, and $e = \sigma_g^2/\sigma^2$, where $\sigma^2 = \sigma_g^2 + \sigma_c^2 + \sigma_e^2$. That is, h, c, and e are the proportions of total variation explained by genetic, common environmental, and other factors, respectively. The correlations between twins were estimated by maximum likelihood as $r_{MZ} = Cov(MZ)/\sigma^2$, and $r_{DZ} = Cov(DZ)/\sigma^2$, with both Cov(MZ) and Cov(DZ) not constrained.

The likelihood ratio criteria is used to test the null hypothesis $\sigma_g^2 = 0$, i.e., heritability = 0. The maximum log likelihood was calculated under two models with $\sigma_g^2 = 0$ and $\sigma_g^2 \geq 0$. Under the null hypothesis, twice the absolute difference in log likelihood between these models will be asymptotically distributed as a 50:50 mixture of a χ^2 variate and a point mass at the origin (21).

Bivariate analyses. The similarity between the factors G, C, and E determining BMD at different skeletal sites was investigated. This statistical analysis was carried out in the premenopausal female twin pairs who constituted the largest subgroup. All possible pairs of skeletal sites were analyzed using bivariate analysis. Thus, for each location (k) and twin (i) (i = 1, 2), $BMD_{ki} = \mu_k + G_{ki} + C_{ki} + E_{ki}$, with corresponding variance components σ_{gk}^2, σ_{ck}^2, and σ_{ek}^2 as above. For each pair of skeletal sites k and m, we calculated ρ_g, ρ_c, and ρ_e, the correlations between the G, C, and E components, respectively, at different locations in the same individual. That is, $\rho_g = Corr(G_{ki},G_{mi})$, independent of i, so that, for two individuals, i and j, if $i \neq j$ for MZ pairs, $Cov(G_{ki},G_{mj}) = \rho_g\sigma_{gk}\sigma_{gm}$, while for DZ pairs $Cov(G_{ki},G_{mj}) = \frac{1}{2}\rho_g\sigma_{gk}\sigma_{gm}$. Also, $Cov(C_{ki}C_{mj}) = \rho_c\sigma_{ck}\sigma_{cm}$, and $Cov(E_{ki}E_{mj}) = \rho_e\sigma_{ek}\sigma_{em}$ independent of zygosity. The correlations $\rho = Cor(Y_{ki},Y_{mi})$, between BMD at different sites in the same individual were also calculated by maximum likelihood as outlined above.

Results

The mean age of the MZ twins was 47 yr (range, 24 to 75 yr), and for the DZ twins 40 yr (range, 24 to 65 yr). The mean body mass index of the MZ twins was 23 kg/m^2 (range, 17 to 31 kg/m^2) and for the DZ twins, 23 kg/m^2 (range, 18 to 36 kg/m^2). There was no significant difference between the correlation of body mass index in the MZ twins and in the DZ twins (0.39 and 0.25, respectively). The mean VO_2max of the female MZ twins was 34 ml/kg per min (range, 18 to 57 ml/kg per min) and for the DZ twins, 36 ml/kg per min (range, 21 to 62 ml/kg per min). There was no significant difference between the intrapair correlation of VO_2max, in the MZ twins (r = 0.88) and in the DZ twins (r = 0.81). There was no significant difference between the mean BMD or BMC at any site in the twins compared with values from age-matched controls. Also, for no skeletal site were the means or interpair variances different between MZ and DZ pairs.

The correlations of BMD at the different skeletal sites are shown in Table I (top) for all MZ and DZ twin pairs. At all sites the correlation between MZ twins was greater than that between DZ twins. Estimates of heritability derived from maximum likelihood analysis are also listed. From this analysis we found that heritability was significantly greater than zero at all locations except the distal forearm. Analysis excluding the two twin pairs discordant for years postmenopause did not alter the results. However, analysis of the correlations of BMD at the different skeletal sites confined to the premenopausal female twin pairs (Table I, bottom) resulted in somewhat different estimates of heritability. In this subgroup at all sites the correlation between MZ twins was greater than between DZ twins and heritability was significant for the spine, forearm,

Table I. Twin Correlation and Heritability of Bone Mass

Parameter	rMZ	rDZ	Heritability
All twin pairs			
Lumbar BMD	0.92	0.36	0.92 ($P < 0.001$)
Proximal femur BMD			
Femoral neck	0.73	0.33	0.73 ($P < 0.005$)
Ward's triangle	0.85	0.36	0.85 ($P < 0.001$)
Trochanteric	0.75	0.47	0.57 ($P < 0.02$)
Forearm BMC	0.71	0.50	0.42 ($P < 0.08$)
Premenopausal twin pairs			
Lumbar BMD	0.92	0.36	0.92 ($P < 0.001$)
Proximal femur BMD			
Femoral neck	0.77	0.56	0.46 ($P < 0.08$)
Ward's triangle	0.88	0.48	0.87 ($P < 0.001$)
Trochanteric	0.88	0.56	0.66 ($P < 0.005$)
Forearm BMC	0.88	0.42	0.88 ($P < 0.001$)

Intrapair correlation coefficients for MZ (rMZ) and DZ (rDZ) twins and heritability for the five skeletal sites, determined from analysis of all twin pairs (top) and from analysis of only premenopausal twin pairs (bottom). Heritability was calculated using maximum log likelihood with the constraint that σ^2, σ_c^2, and σ_e^2 were all greater than zero.

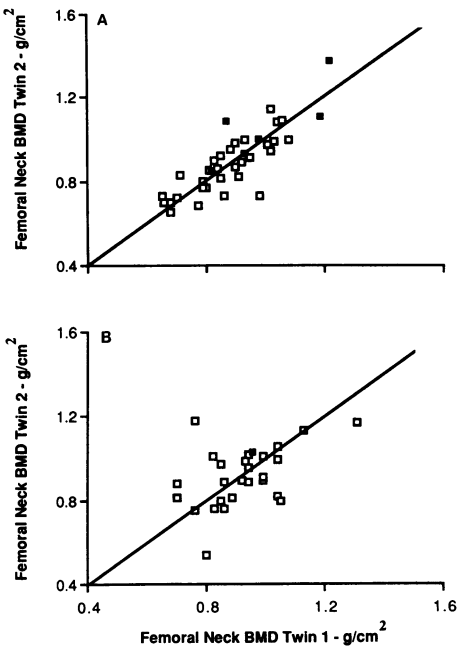

Figure 2. Correlation of femoral neck BMD between (*A*) MZ twins and (*B*) DZ twins showing male (■) and female (□) twin pairs. The line of identity is shown.

and two sites in the proximal femur (i.e., Ward's triangle and the trochanteric region). However, heritability did not reach significance at the femoral neck. Forearm BMC, on the other hand, showed significant heritability in the premenopausal women. The correlations of lumbar spine and femoral neck BMD as well as forearm BMC in all the MZ and DZ twins are shown in Figs. 1–3, respectively. The range of BMD observed in the twins studied was the same as we have observed in a normal population. The close correlation between the twin pairs occurs throughout the range of BMD.

Table II shows the cross-correlations of BMD in premenopausal women at different sites in the same individual and the estimated cross correlations ρ_g, ρ_c, and ρ_e for each pair of locations. The estimates of ρ_g are positive between all sites but

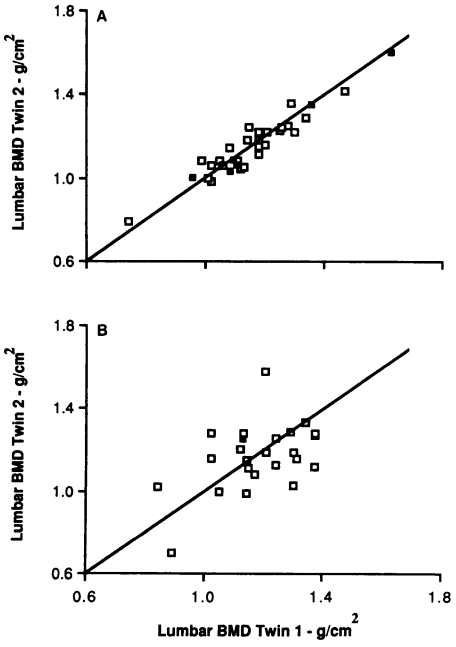

Figure 1. Correlation of lumbar spine BMD between (*A*) MZ twins and (*B*) DZ twins showing male (■) and female (□) twin pairs. The line of identity is shown.

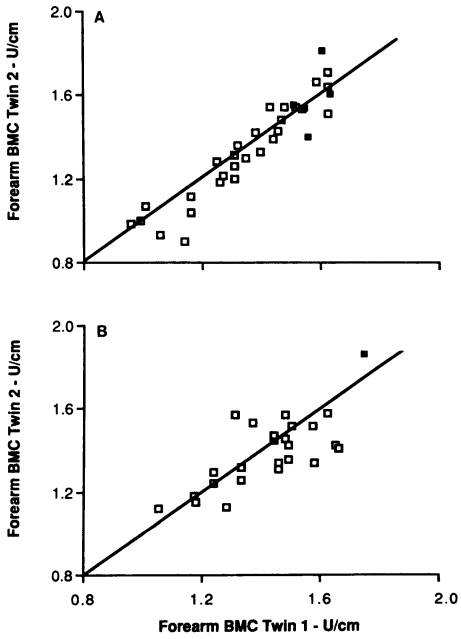

Figure 3. Correlation of forearm BMC between (*A*) MZ twins and (*B*) DZ twins showing male (■) and female (□) twin pairs. The line of identity is shown.

Table II. Correlations between Determinants of Skeletal Mass at Different Skeletal Sites

	Femoral neck	Ward's triangle	Trochanteric	Forearm
Lumbar				
ρ	0.59	0.57	0.52	0.58
ρ_g	0.80	0.77	0.62	0.41
ρ_c	0.82	0.64	0.60	0.53
ρ_e	−0.55	−0.50	−0.27	0.56
Femoral neck				
ρ		0.91	0.84	0.43
ρ_g		0.98	0.97	0.69
ρ_c		0.98	0.95	0.55
ρ_e		0.64	0.36	−0.47
Ward's triangle				
ρ			0.80	0.38
ρ_g			0.80	0.64
ρ_c			0.91	0.31
ρ_e			0.58	−0.37
Trochanteric				
ρ				0.44
ρ_g				0.48
ρ_c				0.55
ρ_e				−0.21

Correlations (ρ) between BMD at different skeletal sites. The correlations between genetic (ρ_g), common environmental (ρ_c), and individual environmental (ρ_e) components of bone mass at different skeletal sites in the same individual are shown.

are greater between the three proximal femur sites than between these sites and the lumbar spine and forearm. The estimates of ρe are positive between femoral neck, Ward's triangle, and trochanteric, and between lumbar and forearm, but are negative between the former three locations and the latter two. Estimates of heritability from bivariate analyses, which impose a greater structure on the cross-covariances, are lower than those estimated from univariate analysis but nevertheless are consistently between 0.4 and 0.6.

Discussion

These data demonstrate for the first time in adults a strong genetic component to the determination of bone mass in the spine and proximal femur. They also confirm the previously reported genetic contribution to bone mass in the appendicular skeleton of the upper limb (11, 13).

Analysis of all twin pairs studied, as well as separate analysis of the premenopausal twins, suggests a greater genetic determinant of BMD in the lumbar spine than in either the proximal femur or the distal forearm. The apparent greater contribution of genetic factors in determining forearm bone mass in premenopausal women compared with the group as a whole suggests that environmental factors are of increasing importance after the menopause and/or with advancing age at this site.

The bivariate analysis of BMD in the premenopausal women, demonstrating a significant correlation between the genetic components of BMD at different locations, is consistent with one gene or a single set of genes determining bone mass at all skeletal sites measured. There were also strong correlations between common environmental components across the different locations. However, there was a negative association between individual environmental components of the sites in the proximal femur and both the lumbar and the forearm locations. This suggests that although there are genetic and environmental components common for bone density at all locations, there are some environmental factors specific for bone density at the hip compared with the lumbar spine or forearm.

A genetic contribution to bone mass in the upper limb has been previously suggested by single photon absorptiometry (11, 13) and by metacarpal morphometry (12). Dequeker et al. (11) have shown, by absorptiometry, a genetic contribution to spinal bone mass in individuals younger than 25 yr, as well as to distal radius BMC in individuals > 25 yr. The values for heritability reported by that study, i.e., 0.88 and 0.75, respectively, were calculated as described by Holzinger (25): H = (DZ intrapair variance − MZ intrapair variance)/DZ intrapair variance. Similar values derived from our data are 0.85 for the lumbar spine, and in the premenopausal females, 0.78 for the distal forearm, which is virtually identical to those reported by Dequeker et al. (11).

To our knowledge, there are no previous studies that have examined the role of genetic factors in determining bone mass of the proximal femur, the site of the most clinically severe osteoporosis-related fractures. That our data suggest a smaller genetic contribution to bone mass of the proximal femur and forearm compared with the lumbar spine imply that environmental factors play a more dominant role in determining variation of bone mass at these sites. This is consistent with the observation that skeletal sites in the forearm and proximal femur are exposed to large individual variations in mechanical loading; e.g., the load on the upper and lower limbs are highly variable between occupations. However, the spine bears the weight of the upper half of the body with relatively little variation during waking hours except in occupations involving heavy labor. Even the transition from a sitting position to a walk does not result in a large change in mechanical load on the spine, but results in a major change in mechanical stress exerted on the hip. We have previously demonstrated (9) that physical fitness (VO_2max), and by implication habitual physical activity (26, 27), is important in determining BMD in the femoral neck. The high correlation of VO_2max between MZ twins was similar to that between DZ twins. This suggests that both MZ and DZ twin pairs are concordant for habitual physical activity. This concordance could contribute to the relative similarity of intrapair correlation of proximal femur BMD in MZ and DZ twins.

In summary, this study has demonstrated for the first time a significant genetic contribution to bone mass in the spine and proximal femur in adults and confirms the previously reported genetic contribution to upper limb bone mass. The smaller genetic determinant of bone density in the hip and forearm compared with the lumbar spine, and by implication the importance of environmental factors, supports the findings of our previous study (9) and suggests a greater potential for lifestyle intervention in achieving a reduction in the incidence of hip and forearm fractures. The data emphasize the importance of a family history and suggest the potential for DNA

studies (e.g., restriction fragment length polymorphism studies of appropriate genes) to identify individuals at risk. Identification of such individuals would allow early life style (e.g., exercise, diet) and other therapeutic interventions and possibly prevent the development of clinical disease.

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