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Original Article

Genetic Variation in Bone Mineral Density and Calcaneal Ultrasound: A Study of the Influence of Menopause Using Female Twins

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Abstract. The aim of the study was to determine whether the genetic variance in bone mineral density (BMD) and calcaneal ultrasound differs in pre- and postmenopausal women and to establish whether the genes operating before the menopause are the same as those after the menopause. Twins aged 18–75 years were recruited from the St Thomas' UK Adult Twin Registry. Quantitative model fitting techniques were used to test for differences in genetic influences in pre- and postmenopausal twins of several BMD sites and calcaneal ultrasound measures accounting for age. BMD and heel ultrasound variables were measured in 2490 female twins: 360 monozygotic pairs and 885 dizygotic pairs. The heritability in the group overall ranged from 19% to 76%. A significant increase in total variance was seen for most BMD sites after the menopause. The proportion of total variance explained by genetic influence was higher premenopausally at all sites except the femoral neck. For example, the genetic proportion of total variance for spine BMD was 88% premenopausally and 77% postmenopausally. In contrast there was no significant difference in total variance of ultrasound measures with menopause. There was no indication that traits are influenced by different genes before and after menopause. This study demonstrates that genetic and environmental influences differ significantly in pre- and postmenopausal groups for BMD, but not for calcaneal ultrasound. The total variance in BMD is greater postmenopausally, but there is evidence that the same genes are involved. These data stress the importance of accounting for menopause–gene interactions in the genetic analysis of data on osteoporosis.

Keywords: Bone mineral density; Calcaneal ultrasound; Genetics; Menopause

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Introduction

Bone mineral density (BMD), and to a lesser extent calcaneal ultrasound, are major independent determinants of fracture risk. The risk of fracture increases significantly after the menopause.

The large genetic contribution to BMD and calcaneal ultrasound is well established by both family and twin studies [1–9]. In most of the twin studies the proportion of variance of BMD accounted for by genetic factors was around 65–92% [1–4]. Sibling studies have yielded lower estimates of heritability [5–9]; however, it is clear that variation in BMD between individuals is determined largely by genetic factors. Previous studies have been relatively small, and examined the genetic influence in either pre- or postmenopausal women, and have not compared these groups directly. As genes are the predominant influence on BMD and the menopause is a crucial event in osteoporosis risk, the question of whether genetic mechanisms and influence differ with menopausal status is crucial to our understanding of the pathogenesis of osteoporosis.

It has been proposed that, with increasing age, an individual's environment becomes more influential on bone density and the familial association becomes less evident [10]. If this is the case it is important to quantify

the importance of environmental influence contrasting pre- and postmenopausal women.

The aim of this study was to determine whether the magnitude of the genetic (or environmental) influence on BMD and calcaneal ultrasound is dependent on menopause. A secondary aim was to determine whether these traits are influenced by (partly) different genes before and after menopause.

Materials and Methods

Study Population

The subjects were 1245 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 [11]. None of the study subjects was aware of the hypothesis under test. Ethics approval was obtained from the hospital ethics committee and fully informed written consent was obtained from all subjects at the first visit. Zygosity was determined by standardized questionnaire, and DNA fingerprinting was used for confirmation [12].

Data Ascertainment

Demographic information was obtained by a standardized nurse-administered questionnaire with questions designed to ascertain menopausal status (postmenopausal status was defined as the cessation of menstruation for at least 12 months, as previously described [13]) and medication use. Heights were recorded using a wall-mounted stadiometer and weight with an electronic balance. A validated food-frequency questionnaire was completed to achieve an estimate of calcium intake [14].

Measurements

BMD was measured using dual-energy X-ray absorptiometry (Hologic QDR 2000 plus). Measurements were taken at the lumbar spine (L1–L4), total hip, femoral neck, forearm (ultradistal radius) and whole body skeleton.

Ultrasound of the calcaneus was measured using a McCue Cuba Clinical heel scanner (McCue Ultrasonics, Winchester, Hampshire, UK). The machine produces two output variables – broadband ultrasound attenuation (BUA) and velocity of sound (VOS) – and has a reproducibility error of 2.5% and 0.44% respectively [2].

Statistical Analysis

Background to Twin Analysis. The classical twin study makes use of the fact that monozygotic (MZ) twins share identical genotypes whereas dizygotic (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, therefore, attributed to genetic factors. Structural equation modeling allows 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. Heritability (h^2), can be defined as the ratio of additive genetic variance to total phenotypic variance. Martin et al. [15] give further information regarding the methodology of the classical twin study.

Analytical Approach. Preliminary data analysis was performed and intraclass correlations were estimated using STATA [16].

Aims of our analysis were twofold: (i) to establish the genetic influence overall, and (ii) to examine the effect of menopause on total variance and components of variance. Firstly we estimated the genetic and environmental influences on all variables for the entire group, with age incorporated in the path model [17].

Secondly we investigated the effect of the menopause on changes in: (i) total trait variance and (ii) genetic and environmental sources of variance. We specified a path model in which the total sample was subdivided into six zygosity by menopause groups (i.e. MZ pre/pre, DZ pre/pre, MZ pre/post, DZ pre/post, MZ post/post, DZ post/post). Age was incorporated in the model for all the variables [17].

Finally using a path model with 122 menopause-discordant pairs we examined whether the correlation between the latent genetic factors in these pairs was lower than the normal expected 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 [17].

Model Fitting Procedure. The significance of a, c and d was tested by removing them sequentially in specific submodels. Variance components were dropped from the model if they did not give a significant contribution. Submodels were compared with the full model by hierarchic chi-square tests. In comparing non-nested models, the best model was chosen on the basis of the lowest value of Akaike's information criterion (AIC = χ^2 –2d.f.), which reflects the best balance between goodness of fit and parsimony. Estimates of variance components and their 95% confidence intervals were obtained from the best fitting model. All quantitative genetic model fitting was carried out with Mx [18].

Results

Results for the Entire Group

The data in Table 1 show the general characteristics of the entire group of twin pairs studied (n = 2490).

Table 1. Description of basic characteristics of the study population

	Monozygotic twins	Dizygotic twins
No. of pairs	360	885
Age (years)	50.4 (13.2)	47.6 (11.2)
Weight (kg)	64.0 (10.5)	65.6 (12.4)
Height (cm)	162.2 (6.3)	162.3 (6.0)
BMI (kg/m^2)	24.3 (3.8)	24.9 (4.6)
Postmenopausal (%)	67%	51.5%
Years since menopause[a]	11.2 (7.4)	10.5 (9.3)
Current smokers (%)	16.6	18.8
HRT current user	14.4%	15.8%
OCP current user	0.7%	2.1%
Hysterectomy	16%	10%
Bilateral oophorectomy	4.2%	3.8%
Dietary calcium (mg/day)	703.5 (349.0)	672.5 (303.2)

Values are mean (SD).
HRT, hormone replacement therapy; OCP, oral contraceptive pill.
[a]For postmenopausal subjects.

Comparison of the groups revealed that MZ pairs were, on average, 2.8 years older at the time of ascertainment and a larger proportion were consequently postmenopausal. The DZ pairs were, on average, 1.6 kg heavier than the MZ pairs, reflected in a body mass index (BMI) 0.6 kg/m^2 higher. The mean values for all of the BMD measurements were similar for the two groups and are shown in Table 2.

Table 2. Bone mineral density and calcaneal ultrasound measurements for monozygotic (MZ) and dizygotic (DZ) twins

	MZ	DZ
Total body (g/cm^2)	1.09(.11)	1.13(.11)
Lumbar spine (g/cm^2)	0.96(.14)	1.00 (.15)
Total hip (g/cm^2)	0.90 (.13)	0.92 (.13)
Femoral neck (g/cm^2)	0.78 (.13)	0.80 (.13)
Ultradistal radius (g/cm^2)	0.45 (.07)	0.45 (.06)
BUA (dB/Mz)	77.5 (18.5)	77.7 (18.5)
VOS (m/s)	1651.6 (46.7)	1660.2 (52.8)

Values are mean (SD).
BUA, broadband ultrasound attenuation; VOS, velocity of sound.

The intraclass correlations for the entire group of MZ twins (rMZ) and DZ twins (rDZ) for the different bone density and heel ultrasound measurements are presented in the last column of Table 3. The finding of rMZ greater than rDZ implies an important genetic influence, which was subsequently confirmed by model fitting to the entire group (Table 4). Dominant genetic (D) and shared environmental effects (C) did not contribute significantly to the explanation of the data for BMD sites and BUA and they could be dropped from the model without a significant worsening of the fit. A model specifying additive genetic (a) and unique environmental (e)

Table 3. Intraclass correlation coefficients (no. of twin pairs) for MZ and DZ twins by menopausal status

Measurement		Menopause status grouping			
		Pre/pre	Pre/post	Post/post	Total
Total body	MZ	0.85 (94)	0.90 (12)	0.88 (222)	0.90 (328)
	DZ	0.53 (358)	0.40 (114)	0.50 (388)	0.56 (860)
Lumbar spine	MZ	0.86 (100)	0.82 (12)	0.81 (226)	0.84 (338)
	DZ	0.46 (356)	0.48 (112)	0.42 (389)	0.48 (857)
Total hip	MZ	0.80 (100)	0.83 (12)	0.79 (223)	0.82 (335)
	DZ	0.38 (353)	0.44 (112)	0.39 (385)	0.44 (850)
Femoral neck	MZ	0.80 (100)	0.74 (12)	0.78 (225)	0.82 (337)
	DZ	0.39 (353)	0.44 (112)	0.41 (385)	0.48 (852)
Ultradistal radius	MZ	0.81 (97)	0.91 (12)	0.82 (222)	0.84 (331)
	DZ	0.41 (355)	0.35 (113)	0.43 (386)	0.48 (854)
BUA	MZ	0.51 (81)	0.91 (10)	0.75 (126)	0.67 (219)
	DZ	0.29 (356)	0.23 (111)	0.36 (374)	0.34 (843)
VOS	MZ	0.82 (81)	0.89 (10)	0.78 (126)	0.81 (219)
	DZ	0.63 (356)	0.60 (111)	0.65 (374)	0.65 (843)

Table 4. Variance components estimates (95% confidence intervals) of bone mineral density and calcaneal ultrasound of best fitting models

Sites measured	a^2	c^2	e^2	age^2
Lumbar spine	0.76 (0.72–0.79)	–	0.17 (0.14–0.20)	0.07 (.05–0.10)
Total hip	0.66 (0.62–0.71)	–	0.22 (0.19–0.26)	0.12 (0.09–0.15)
Femoral neck	0.60 (0.55–0.64)	–	0.22 (0.19–0.26)	0.18 (0.15–0.22)
Ultradistal radius	0.68 (0.62–0.72)	–	0.26 (0.22–0.31)	0.06 (0.04–0.09)
Whole body	0.75 (0.71–0.78)	–	0.11 (0.10–0.13)	0.14 (0.11–0.18)
BUA	0.53 (0.45–0.60)	–	0.43 (0.36–0.50)	0.04 (0.03–0.07)
VOS	0.19 (0.03–0.32)	0.49(0.23–0.36)	0.28 (0.23–0.36)	0.04(0.02–0.06)

a^2, additive genetic variance; c^2, common environmental variance; e^2, unique environmental variance; age^2, variance component due to age.

variance components with age incorporated in the model, gave the most parsimonious explanation of the data for all of the variables, with the exception of VOS which demonstrated common environmental influence (49%).

Effect of Menopause

The means of the BMD and calcaneal ultrasound measurements, by menopause status for the (age-identical) menopause-discordant twin pairs, did not

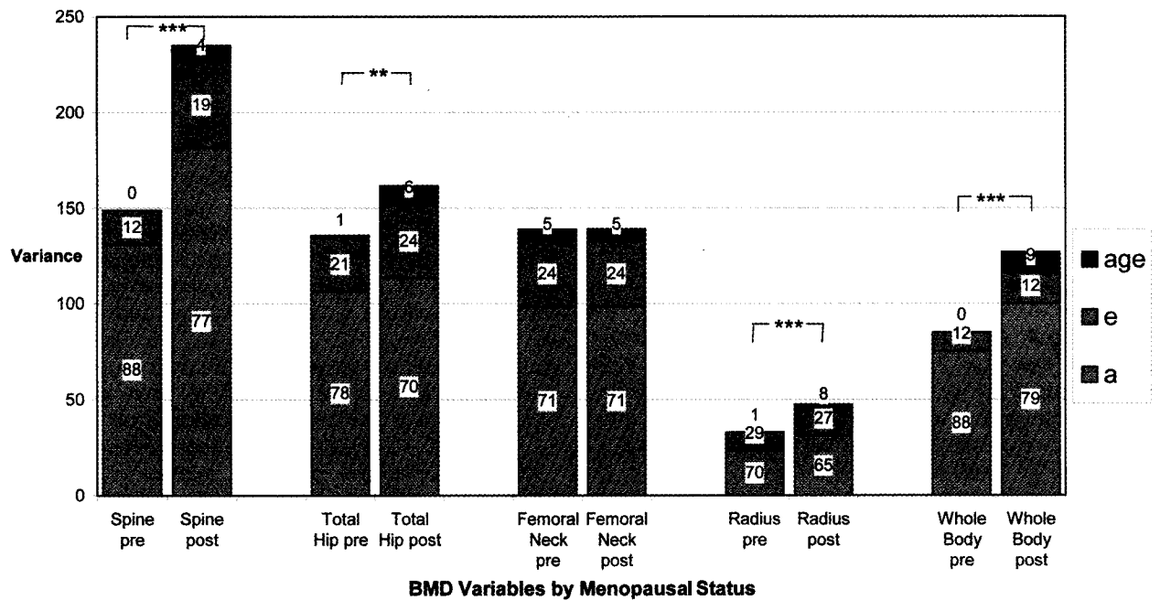

Fig. 1. Total variance and standardized variance components for bone mineral density measures. Variance components (% of total variance) are shown in each bar. Significant differences in total variance by menopausal status are demonstrated by ** for <0.01 and *** for <0.001. Age, variance component due to age; e, unique environmental variance; a, additive genetic variance.

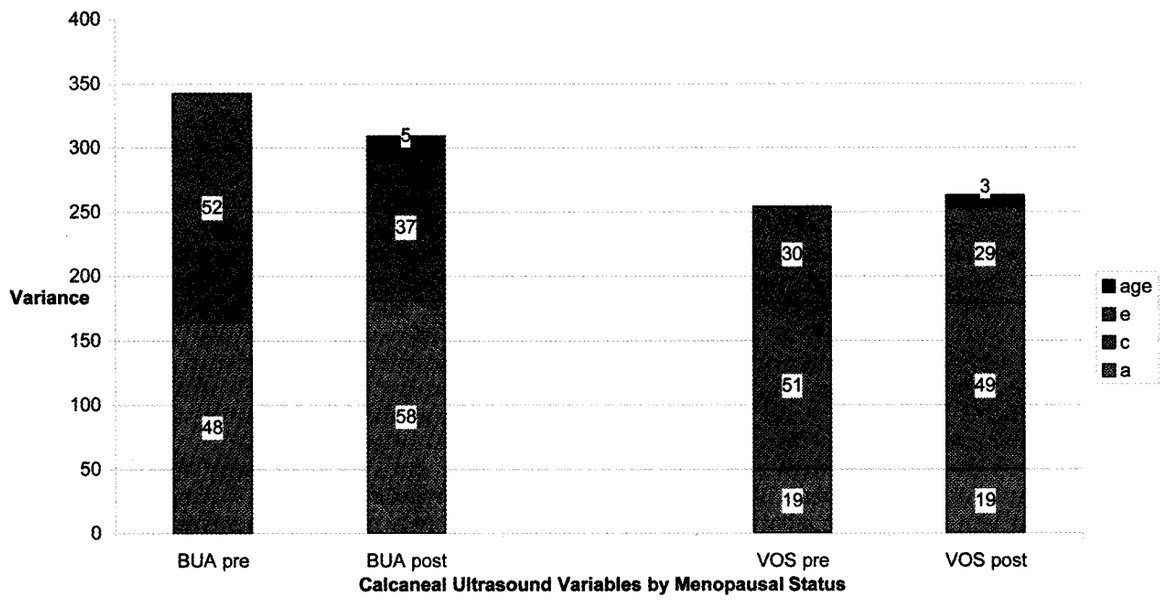

Fig. 2. Total variance and standardized variance components for calcaneal ultrasound measures. Variance components (% of total variance) are shown in each bar. Age, variance component due to age; e, unique environmental variance; c, common environment variance; a, additive genetic variance.

show any significant differences (data not shown). Total variance and its standardized variance components with respect to menopause are shown graphically in Fig. 1 and Fig. 2. There was a significant increase in total variance for all BMD sites with menopause, with the exception of femoral neck BMD which remained the same. This increase was due to increased influence of all three variance components (i.e., genetic, unique environment and age). Whilst the absolute genetic variance increased, the proportion of total variance explained by genetic influence (heritability) decreased by an average of 6.4% for all BMD sites. The total variance remained the same for BUA and VOS after the menopause; however, the proportion of genetic influence for BUA increased after the menopause by 10%.

Different Genes Before and After Menopause

The influence of different genes before and after menopause was also assessed using modeling on 122 pairs discordant for menopause. There was no evidence that different genes were operating as most of the correlations were estimated at or very close to 1 and 0.5 for MZ and DZ twins respectively.

Discussion

This study shows that the large genetic contribution to BMD is maintained after the menopause. We also found an increase in BMD variance for most sites after the menopause, although calcaneal ultrasound total variance did not differ. There was no evidence that the bone-specific genes differed pre- and postmenopausally.

Postmenopausal BMD is determined by the relative contributions of peak bone mass and subsequent bone loss. The strong genetic contribution to peak bone mass is well established; however, the genetic contribution to bone loss and their relative contribution to the total variance in postmenopausal BMD remains unclear [19,20]. Previous research has suggested that at about the age of 70 years, the relative contribution of peak bone mass and bone loss approach equality [21,22]. This implies that postmenopausal bone mass depends increasingly on the rate of bone loss with advancing age.

Although no studies have directly compared young and old subjects, previous family and twin studies have reported heritability estimates are maximal in younger age groups [5,19,23]. Although family studies can provide rough estimates of heritability, they cannot differentiate shared environmental from genetic effects and cannot account for age and cohort effects. Nevertheless, consistent with our findings, family studies have suggested that the degree of familial association for BMD declines with the menopause. Danielson et al. [6] found heritability estimates of 50–63% and 34–48% in pre- and postmenopausal daughters respectively.

This study suggests that although the magnitude of the genetic effect may differ, assuming no gene–environment interaction, the influential genes in premenopausal females are broadly the same as those operating after the menopause. This is suggested by observing the correlations of the menopause-discordant pairs and confirmed by modeling. If the hypothesis of partly different genes were true we would have expected pairs discordant for menopause to show lower correlations (see Table 3) than menopausally-concordant twins, which was not the case. Most of the genes acting on BMD and calcaneal ultrasound are therefore likely to be the same pre- and postmenopause.

Whilst the heritability estimates at all other sites decreased after menopause, that for the femoral neck remained constant. This may reflect the belief that some sites are more responsive to specific environmental influences than others [1]. Conversely it may suggest potential differences in the genetic influence on cortical versus trabecular bone [24–27], the latter being more directly dependent on genetic factors and less affected by the environment or menopause. The reasons for the greater genetic component to BUA than VOS (which had reasonable phenotypic correlation) are unclear. The strong common environment influence on VOS could, in part, be due to standardized measurement errors.

There are several potential sources of bias which could have influenced our results and are worthy of mention. The mean values of general characteristics of MZ and DZ twins in this study were very similar. There was a small difference in age which was accounted for by incorporating this variable in the path model. The mean characteristics of environmental factors known to influence bone turnover, such as hysterectomy, oophorectomy, and either resorptive or antiresorptive agents, were similar in both MZ and DZ twins.

The significant increase in total variance with menopause for the lumbar spine BMD may be contributed to by aortic calcification and/or degenerative spine disease.

The reported results are likely to be representative of singletons in 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 [28].

The study had modest power to detect small amounts of common environmental and dominance variance [29–31]. However, inspection of the intraclass correlation coefficients and models suggests that dominant gene and common environment effects are unlikely to be important for any of the BMD sites. This study had 80% power at the 0.05 level to detect a common environmental influence of 15%.

This is a cross-sectional study making inferences about time-related changes. Without performing a longitudinal study over decades it would not be possible to separate the effects of age and menopause. In an ideal world, these cross-sectional data should be confirmed by large longitudinal twin or family studies following individuals through the menopause.

In conclusion, the total variance of BMD is greater postmenopausally, genetic factors have a proportionally

greater effect on the acquisition of peak bone mass than on its preservation [9], and it is likely that the majority of genes that act pre- and postmenopausally are the same. Realizing the genetic differences that exist and appreciating the potential role of gene–menopause interactions will be critical in unraveling the genetic architecture of osteoporosis.

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