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The Heritability of Bone Mineral Density, Ultrasound of the Calcaneus and Hip Axis Length: A Study of Postmenopausal Twins

N.K. ARDEN, J. BAKER, C. HOGG,* K. BAAN, and T.D. SPECTOR

ABSTRACT

Population based studies have demonstrated that having a first degree relative with a hip fracture is predictive of future hip fractures. Postmenopausal bone mineral density (BMD), ultrasound of calcaneus and hip axis length are associated with hip fracture, with the association for ultrasound and hip axis length being independent of BMD. The aim of this study was to determine the genetic component of these three important risk factors. We performed a classical twin study using 500 normal female twins, 128 identical and 122 non-identical pairs, aged 50 to 70 years. We measured bone mineral density at multiple sites, hip axis length (distance from the inner rim of the acetabulum to the greater trochanter), broadband ultrasound attenuation and velocity of sound of the calcaneus. Bone density had a strong genetic component at all sites with estimates of heritability ranging from 0.46 to 0.84. Hip axis length and velocity of sound had major genetic components with estimates of 0.62 and 0.61 respectively, which remained virtually unchanged after adjustment for bone mineral density. Broadband ultrasound attenuation had a moderate genetic component with an estimate of 0.53, which was reduced further to 0.45 after adjustment for BMD. In summary, all three bone measurements, which are independently associated with hip fracture, are independently heritable. This study suggests that a combination of different genetic factors acting on the structure, dimensions and density of bone may explain the importance of family history as a risk factor for hip fracture. (J Bone Miner Res 1996;11:530–534)

INTRODUCTION

OSTEOPOROSIS IS A MAJOR HEALTH problem in western societies and is associated with significant morbidity and mortality. It is characterised by a low bone mineral density (BMD) and an increased fracture rate, with each standard deviation reduction in BMD increasing the risk of fracture by a factor of 2–3.(1,2) Previous family and twin studies have shown that peak bone mass has a strong genetic component with heritability estimates (the proportion of variance attributed to genetic factors) of 0.42–0.98 according to site,(3–6) whereas the genetic contribution of postmenopausal BMD, which take bone loss into consideration, has been estimated as 0.66 to 0.75.(7)

Changes in BMD, however, only account for approximately 60% of the increased risk of fracture in osteoporosis.(8) Ultrasound measurement of the calcaneus has been used in osteoporosis in the hope that by measuring structural changes in bone, it may explain the remaining 40% of fracture risk. In general, broadband ultrasound attenuation (BUA), a measure of the frequency dependence of ultrasound attenuation through bone, and velocity of sound (VOS), which reflects the transmission velocity of ultrasound through soft tissue and bone, correlate moderately with BMD with correlation coefficients of approximately $r = 0.4-0.7$.[9] Both BUA and VOS have been shown to be associated with fractures at most sites with odds ratios of 1.5–2.7 per standard deviation change in BUA, depending on site of fracture. Furthermore this association appears to be independent of the association of ultrasound with BMD,

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Department of Rheumatology and Twin Research Unit, St. Thomas' Hospital, London, United Kingdom.
*London School of Hygiene and Tropical Medicine, London, United Kingdom.

as it is still associated with fractures after correction for BMD, with odds ratios of 1.3–2.2.(10,11)

Hip geometry, in particular an increase in hip axis length (HAL), is associated with an increased risk of hip fracture, with an odds ratio of 1.7 per standard deviation change in HAL, even after adjustments for BMD have been made.(1) Some authors believe that differences in HAL may partially explain the racial differences in susceptibility to hip fracture.(12) A recent large population-based cohort study has demonstrated that having a first degree relative with a history of hip fracture, but not other fractures, is predictive of hip fracture even after adjusting for BMD, suggesting that other risk factors for hip fracture may have a genetic component.(13)

In this study, using the traditional twin model on a large population of postmenopausal female twins, we estimate the genetic component of BUA, VOS, HAL and postmenopausal BMD including sites not previously measured.

METHODS

250 pairs of female twins aged 50 to 70 years, 128 monozygous (MZ) and 122 dizygous (DZ), were recruited from 2 sources: 46% from a twin register at the Institute of Psychiatry, London, where they were initially asked to participate in research into an unspecified range of fields, the other 54% via a national media campaign asking for female twins to take part in a research project on bone and joint problems.

The zygosity of the twins was determined using a validated questionnaire and confirmed with multiplex DNA fingerprinting using variable tandem repeats.(14) All subjects completed a general osteoporosis risk factor questionnaire, and those with serious medical illnesses affecting mobility or bone (2 cases of cancer, 1 multiple sclerosis and 1 morbid obesity) were excluded from the study. Bone mineral density was measured using a Hologic QDR-2000 DXA scanner (Hologic, Inc. Waltham, MA, U.S.A.). The sites measured included the lumbar spine (L1–L4), femoral neck, Ward's triangle, total hip, mid-forearm, distal forearm (ultradistal radius) and whole body. Reproducibility was assessed by performing duplicate scans of 40 normal volunteers and elderly patients and was between 0.6% and 1.6%, depending on the site scanned. This software also gave an automated measurement of hip axis length defined as the distance from the inner rim of the acetabulum to the greater trochanter. Twenty patients underwent duplicate scans, getting off and on the scanner table in-between scans, to assess reproducibility. The automated measurement for HAL had a reproducibility error of 1.19%. Ultrasound of the calcaneus was measured using a McCue Cuba Clinical heel scanner (McCue Ultrasonics, Winchester, Hampshire, U.K.). The machine produced two output variables, BUA and velocity of sound (VOS) and has a reproducibility error, assessed by duplicate readings on 30 subjects, of 2.5% and 0.44% respectively.

Statistical analysis

Twin analysis was performed using the fortran based TWINAN90 software package.(15) Intraclass correlations (r) were calculated for each zygosity. For MZ twins: rMZ = (AMZ − WMZ)/(AMZ + WMZ) where AMZ = among mean squares and WMZ the within mean squares for the MZ twins. The Falconer estimate of heritability (h^2) was calculated from the formula $h^2 = 2(\mathrm{rMZ} - \mathrm{rDZ})$.(16) This estimate requires three assumptions to be met: first, that any genetic variance is additive, i.e. there are no gene interactions; second, that the environmental covariance of MZ twins is equal to that of DZ twins; and third, that total MZ and DZ variances are equal. Deviations from these assumptions can bias the estimate; for example, if there is a gene dominance effect—if DZ variance is greater than MZ variance or if MZ environmental covariance is greater than DZ—then this effect will tend to exaggerate the heritability estimate. To allow for the effect of potentially confounding variables, multiple linear regression modelling was performed using forward stepwise analysis with a limit for entry into the model of 0.05. The intraclass correlation coefficients and heritability estimates were then calculated as previously described using the residuals produced from the regression.

RESULTS

The mean age of the MZ twins was 60.8 years (SD 4.7), and for the DZ twins 60.2 (SD 5.3). The mean weight for MZ twins was 62.5 kg (SD 9), and for the DZ twins 64.6 kg (SD 9.4). 200 pairs (108 MZ and 92 DZ) were postmenopausal. Information on the duration of menopause was available on 84 MZ and 64 DZ pairs with mean values of 11.7 years (SD 6.5) for MZ and 11.4 years (SD 7.1) for DZ twins. 76 (35%) MZ and 75 (40%) DZ twins had ever used hormone replacement therapy (HRT), with mean duration of usage being of short and similar duration in both groups: MZ 14.2 months (SD 34), DZ 14.8 months (SD 33). To estimate the similarity of environmental covariance between MZ and DZ twins, the concordance for smoking and alcohol intake and the intrapair differences in total current activity, duration of menopause and duration of HRT use were compared between the 2 zygosities. The intrapair difference of duration of menopause was more similar in MZ twins (2.92 years (SD 3.58)) than DZ twins (4.54 years (SD 4.36)) (p = 0.014), but there were no statistically significant differences between the two zygosities for any of the other parameters measured.

The intraclass correlation coefficient for identical twins (rMZ) for BMD in all postmenopausal twins, ranged from 0.67 to 0.87 for different sites, with an equivalent rDZ of 0.27 to 0.45. This produced heritability estimates ranging from 0.76 to 1.05, which were all significantly different from zero. Table 1 shows the same results after adjustment for weight, HRT use, HRT duration and years since menopause. In general, these adjustments modestly reduced the values of rMZ, rDZ and heritability, and for all sites except Wards triangle the 95% confidence intervals still excluded

TABLE 1. INTRACLASS CORRELATION COEFFICIENTS FOR MONOZYGOUS AND DIZYGOUS TWINS AND HERITABILITY ESTIMATES FOR BONE MINERAL DENSITY ADJUSTED FOR AGE, WEIGHT, HRT USE AND DURATION AND YEARS SINCE MENOPAUSE

Site	*MZ variance*	*rMZ (SE)*	*DZ variance*	*rDZ (SE)*	*Heritability*	*(95% CI)*
Lumbar spine	0.021	0.68 (0.06)	0.029	0.29 (0.11)	0.78	(0.28–1.28)
Total hip	0.027	0.60 (0.07)	0.028	0.26 (0.12)	0.67	(0.14–1.20)
Femoral neck	0.022	0.61 (0.07)	0.021	0.19 (0.12)	0.84	(0.30–1.38)
Wards triangle	0.20	0.47 (0.09)	0.18	0.22 (0.12)	0.51	(−0.06–1.08)
Distal forearm	0.0045	0.63 (0.07)	0.0053	0.32 (0.11)	0.61	(0.10–1.12)
Mid forearm	0.0037	0.70 (0.06)	0.0045	0.47 (0.10)	0.46	(0.02–0.90)
Whole body	0.0086	0.63 (0.07)	0.01	0.25 (0.12)	0.76	(0.23–1.29)
BUA	0.47	0.62 (0.07)	0.63	0.36 (0.10)	0.53	(0.05–1.01)
BUA (adjusted for BMD femoral neck)	281.5	0.40 (0.10)	308.4	0.18 (0.11)	0.45	(−0.13–1.03)
VOS	0.29	0.65 (0.07)	0.44	0.35 (0.10)	0.61	(0.14–1.08)
VOS (adjusted for BMD femoral neck)	0.011	0.63 (0.07)	0.017	0.34 (0.10)	0.58	(0.10–1.06)
Hip axis length	1.01	0.74 (0.04)	0.96	0.43 (0.09)	0.62	(0.22–1.02)
Hip axis length (adjusted for height)	0.10	0.70 (0.05)	0.09	0.40 (0.10)	0.60	(0.18–1.02)

Ultrasound and hip axis length results are presented before and after adjustment for femoral neck BMD or height.

zero. The heritability estimates for BMD remained virtually unchanged after adjustments for BUA and VOS (data not shown).

Ultrasound data were available on 68 MZ and 81 DZ postmenopausal pairs. The correlation of BUA with BMD at the various sites ranged from $r = 0.47$ to $r = 0.60$, with the strongest correlation being with total hip BMD. BUA was significantly correlated with age ($r = -0.35$), weight ($r = 0.34$) and duration of menopause ($r = -0.35$). The intra-class correlations of rMZ = 0.62 and rDZ = 0.36 gave a heritability estimate of $h^2 = 0.53$ (95% CI 0.05–1.01). Adjusting for femoral neck BMD gave a heritability estimate of $h^2 = 0.45$ (95% CI −0.13–1.03) which just fails to achieve statistical significance, the addition of weight and age into the model had little effect on the estimate.

VOS had a weaker correlation with BMD than BUA with correlation coefficients of $r = 0.30$ to $r = 0.42$, the strongest correlation again being with total hip BMD. The correlations with other variables were also weaker than for BUA with values of age ($r = -0.14$), menopause duration ($r = -0.13$) and HRT duration ($r = 0.12$). The rMZ of 0.65 and rDZ of 0.35 gave a heritability estimate of 0.61 (95% CI 0.14–1.08) and adjusting for femoral neck BMD gave an estimate of h^2 of 0.58 (95% CI 0.10–1.06). After adjusting for BMD, the addition of weight and age into the model had little effect on the estimate.

Hip axis length data was available on 108 MZ and 78 DZ pairs. The only significant correlation was with height with a coefficient of $r = 0.42$. The rMZ of 0.74 and rDZ of 0.43 gave a heritability estimate of h^2 0.62 (95% CI 0.22–1.02). Adjustment for height did not significantly alter the estimate of $h^2 = 0.60$ (95% CI 0.18–1.02).

DISCUSSION

Our data demonstrate a strong genetic component to the three known risk factors for hip fracture measured in this study. Furthermore, for each of the risk factors (with the exception of BUA), adjustments for the other risk factors still yielded a heritability estimate that was significantly greater than 0, implying that the genetic component was independent of the other risk factors. Several studies have shown that there is a strong genetic component to peak bone mass, with the size of this component varying according to the site measured. In a study of 38 MZ and 27 DZ premenopausal pairs using dual photon absorptiometry, lumbar spine had a heritability estimate of 0.92 whereas the estimate for femoral neck in the same population was only 0.42.(3,4) There are less data available on postmenopausal bone density, which is determined by a combination of peak bone mass and subsequent bone loss. The only previous study to specifically examine postmenopausal BMD(7) had similar lumbar spine estimates to our study (0.73 vs 0.78), both of which are lower than premenopausal estimates. They reported a femoral neck heritability estimate of 0.66, marginally lower than our estimate of 0.84. There is little information on the genetics of bone loss, although one study in 48 middle aged male twin pairs has shown that rates of loss at the midshaft of the radius are predominantly determined by environmental factors.(17) A small study of 40 twin pairs, of mixed age and sex, has examined the heritability of bone loss at other sites over a period of 3 years and found a strong genetic component at the lumbar spine ($h^2 = 0.76$) but not at the femoral neck ($h^2 = 0.2$).(18) Unfortunately they had insufficient numbers of postmenopausal twins to look specifically at postmenopausal bone loss. Several additional important confounding variables must be taken into account when measuring postmenopausal bone density, the most important being HRT use and menopausal status. After adjusting for these factors in our population, the heritability estimates for postmenopausal bone density remain high, but are lower at most sites compared to estimates for peak bone mass. This observation implies an increasing role of environmental risk factors in determining postmenopausal BMD.

For both BUA and VOS the rMZ was greater than the rDZ giving estimates of heritability within the same range as BMD. VOS showed only weak associations with BMD or any other of the known risk factors for osteoporotic fractures, and not surprisingly the estimates of heritability were virtually unchanged after adjusting for these factors. BUA, however, was strongly associated with BMD, menopause duration and weight, but adjustments for these factors did reduce the heritability estimates, and in most cases the estimates remained significantly different from zero.

Population studies have demonstrated that a subject having a first degree relative with a fracture has an increased risk of fracture at the same site, independent of BMD, with relative risks of 1.4–3.3.(13) Ultrasound of the calcaneus has been shown to predict hip fractures independently of BMD.(10) In vitro studies have shown that ultrasound of the calcaneus reflects structural changes in bone with BUA reflecting both trabecular separation and connectivity, and VOS reflecting mainly trabecular separation and elasticity.(8) These studies taken together would suggest that ultrasound is measuring an aspect of bone structure that is related to its strength or resistance to fracture independent of BMD and that is determined genetically. Our data are consistent with this hypothesis as we have shown that there is a strong genetic component to both BUA and VOS independent of BMD.

The twin model makes three main assumptions as mentioned previously. The assumptions about gene interactions and equal variance were tested statistically by the TWINAN90 program and were generally satisfied in this study. However, it is difficult in twin studies to exclude an unequal sharing of common environment between MZ and DZ twins, which may bias the heritability estimate. We have examined our data on smoking, alcohol, exercise, duration of menopause, and HRT duration and found that with the exception of a small difference menopause duration, which is known to have a genetic component, there was no significant difference within pair concordance for environmental covariates between the two zygosities. Although this is reassuring, we cannot exclude unequal sharing of other important parameters, such as calcium intake. Even HRT usage in the twins, although of short duration (14 months on average), was relatively high at 35 to 40% which may affect BMD estimates, but seemed to have little association with either BUA, VOS or HAL. However, analyses performed after adjusting for HRT usage had little effect on heritability estimates, as did analysing only the HRT-free cohort.

We have confirmed that hip axis length is strongly correlated with height, a trait which is known to be heritable. We have also shown that HAL has a strong genetic component, and that this genetic effect is independent of height. This observation, along with the heritability of ultrasound, may explain the racial and familial differences in susceptibility to hip fractures. A recent article has suggested that the age adjusted increase in hip fracture rates in developed countries over the last 3 decades may be due to an increase in HAL due to better nutrition in the population.(19) Although showing a high heritability for HAL, our data are not inconsistent with this hypothesis, as a heritability of 0.60 still leaves up to 40% of variance to be explained by environmental factors and environmental-genetic interactions. In summary, this study has confirmed a genetic contribution to postmenopausal bone mass at all sites measured. Although it is still strong, the size of this component is modestly but not significantly reduced when compared to studies of peak bone density and when taken together with the limited data available of the genetics of bone loss. This implies a greater potential for lifestyle intervention in reducing postmenopausal bone loss and hence the incidence of osteoporotic fractures. We have also shown that two other independent predictors of fracture, ultrasound and HAL, have a strong genetic contribution and furthermore that the effect is independent of BMD.

These data demonstrate a major genetic contribution to three independent predictors of hip fracture, suggesting bone architecture as well as density is under genetic control. This finding should stimulate the search for genes controlling bone structure and density.

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Address reprint requests to:
TD Spector
Department of Rheumatology
St. Thomas' Hospital
Lambeth Palace Road
London
SE1 7EH U.K.

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