

An Investigation of Unique and Shared Gene Effects on Speed of Sound and Bone Density Using Axial Transmission Quantitative Ultrasound and DXA in Twins

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ABSTRACT

The genetic influences of speed of sound (SOS) and BMD were evaluated using 215 pairs of healthy female twins. Genetic influences were found for all SOS and BMD measurements. A combination of shared and unique genetic influences was found to control BMD and SOS at the radius.

Introduction: The aim of this study was to investigate to what extent axial transmission speed of sound (SOS) measurements in cortical bone at multiple, peripheral skeletal sites will be influenced by genetic factors and to estimate the proportion of shared and unique genetic influences controlling bone mineral density (BMD) and SOS at a single site, the radius.

Materials and Methods: The study population consisted of 215 pairs of healthy female twins. Of these, 85 pairs were monozygotic (MZ) and 130 pairs were dizygotic (DZ). The twins had measurements of the nondominant third proximal phalanx, one-third radius, midshaft tibia, and fifth metatarsal using the Sunlight Omnisense and DXA measurements of the lumbar spine, nondominant proximal femur, nondominant radius, and whole body using Hologic QDR-4500W densitometers. Calcaneal quantitative ultrasound (QUS) measurements were performed using the McCue CUBA clinical. Intraclass correlations were calculated, and heritability was estimated using multiple regression analysis. Bivariate analysis of site-matched SOS and BMD measurements at the radius was performed using a variance components analysis program.

Results: Age- and body mass index-adjusted heritability estimates ranged from 0.51 (95% CI, 0.32–0.70) to 0.56 (0.37–0.76) for SOS measurements, 0.58 (0.41–0.75) for broadband ultrasound attenuation (BUA), 0.72 (0.58–0.86) to 0.77 (0.63–0.91) for axial BMD, and 0.53 (0.16–0.90) to 0.63 (0.26–1.00) for radius and whole body BMD. The correlation between SOS and DXA at the radius was $r = 0.34$ (0.29–0.47). Thirty-eight percent (16–57%) of the genetic variance explained by SOS at the radius was also explained by BMD (one-third radius region of interest), with 62% being unique.

Conclusion: In conclusion, genetic influences were demonstrated for SOS measurements in cortical bone at multiple sites, axial BMD, calcaneal BUA, radius, and whole body BMD. At the radius, up to 38% of the genetic influence is shared by genes controlling BMD and SOS. Clarifying the site specificity and pleiotropic effects of bone genes should help our understanding of these complex pathways.

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Key words: twin study, bone densitometry, genetic effect, bone ultrasound

INTRODUCTION

A FAMILY HISTORY of osteoporosis or osteoporotic fracture is a well-recognized risk factor for osteoporosis.⁽¹⁾ Family studies have shown reduced bone mass in daughters

of women with osteoporosis^(2,3) and in daughters or sisters of patients with osteoporotic fractures.⁽⁴⁾ Similar bone density has been found between family members,⁽⁵⁾ accounting for 46–62% of the variance in bone mineral density (BMD) in one study by Krall et al.⁽⁶⁾ Family studies offer strong evidence of the heritability of bone density. However, family studies are confounded by age differences between par-

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ents and their children and by possible differences in lifestyle factors during the development of peak bone mass.^(3,5) In addition, families share a common environment that may contribute to the heritable appearance of bone density.⁽⁶⁾

Twin studies offer an ideal tool for evaluating the heritability of a trait because monozygotic (MZ) twins share 100% of their genes, whereas dizygotic (DZ) twins share on average 50% of their genes. In addition, twin studies are not confounded by age differences or cohort effects and are less likely to be confounded by differences in lifestyle factors than family studies. Quantitative ultrasound (QUS) has been used in twin studies to evaluate the contributions of genetic and environmental factors to the variation and covariation of QUS measurements and their relationship to BMD.⁽⁷⁾ Arden et al. performed the first classical twin study investigating hip axis length, BMD at multiple skeletal sites, and QUS of the calcaneus. All three measurements were known to be independently associated with hip fracture and were found to be independently heritable. This suggests that a combination of different genetic factors act on the structure, dimensions, and density of bone.⁽⁸⁾ Howard et al., in a similar study, confirmed QUS and BMD to be highly heritable traits and that bone parameters measured by calcaneal QUS share some common genetic factors with those assessed by bone density.⁽⁷⁾ Other studies have found a strong genetic determinant of bone mass^(6,9,10) that persists with age and after menopause.⁽¹¹⁾ Most twin studies to date have been undertaken using DXA measurements at multiple sites. QUS measurements have also been taken at a single site (typically the calcaneus), usually of predominantly trabecular bone, which is not matched to that of the DXA measurements. However, none have investigated site-matched speed of sound (SOS) and BMD measurements to estimate whether these are distinct or related phenotypes. The factors affecting SOS in bone using the Omnisense have been evaluated *in vitro* by Njeh et al., who found SOS to be dependent on sample thickness if the thickness is smaller than the wavelength of the ultrasound.⁽¹²⁾ Sievanen et al., in an *in vivo* study, found cortical density as measured by peripheral quantitative computed tomography (pQCT) to account for 34% of the variability in radial SOS and 29% in tibial SOS,⁽¹³⁾ suggesting that there are other factors in addition to density affecting SOS in bone as measured by axial transmission quantitative ultrasound.

The aim of this study was first to investigate to what extent axial-transmission SOS measurements in cortical bone at multiple sites will be influenced by genetic factors, and second, to estimate the proportion of shared and unique genetic influences controlling BMD and SOS at the same site, the radius.

MATERIALS AND METHODS

The Omnisense

The Omnisense (Sunlight Ltd., Tel-Aviv, Israel) is the first QUS system with the ability to perform SOS measurements at multiple skeletal sites. To accomplish this, it uses a number of hand held probes designed for specific sites. The probes contain an array of transducers, some acting as

transmitters and others as receivers, and they measure the path of the sound wave taking the shortest propagation time between the transmitting and receiving transducers. The time taken for the signal to travel between the transmitting and receiving transducers is used to infer the SOS in bone.⁽¹⁴⁾ The Omnisense also corrects for soft tissue thickness, giving a true SOS measurement of bone.⁽¹⁵⁾

Subjects

The study population consisted of 215 pairs of healthy female twins recruited from twin volunteers to the Twin Research and Genetic Epidemiology Unit at St Thomas' Hospital. Of these, 85 pairs were MZ and 130 pairs were DZ. The zygosity of the twins was tested using a validated questionnaire⁽¹⁶⁾ with an accuracy of 96%.⁽¹⁷⁾ However, if this proved inconclusive or there were conflicting results between the twins, blood samples were used to perform multiplex DNA fingerprinting using AmpFLSTR identifier polymerase chain reaction (PCR) amplification kit (Applied Biosystems, Warrington, Cheshire, UK).

Measurements

The twins had SOS measurements taken of the nondominant third proximal phalanx, one-third radius, midshaft tibia, and fifth metatarsal using the Sunlight Omnisense. The short-term precision (CV%) for the Omnisense was measured by duplicate scans in 37 subjects, who had a mean age of 42 ± 13.2 years. Each subject had duplicate scans performed on the same day, with repositioning between measurements. The areas for acquisition at each site were measured and marked using a skin marker as instructed in the manufacturer's user manual.⁽¹⁵⁾ The root mean square SD (RMSSD; CV%) was 22.8 m/s (0.55%) for the radius, 17.7 m/s (0.45%) for the tibia, 44.8 m/s (1.11%) for the phalanx, and 27.8 m/s (0.76%) for the metatarsal. When expressed as T-score units, they became 0.21, 0.16, 0.28, and 0.13 for the radius, tibia, phalanx, and metatarsal, respectively. Each twin also had DXA measurements of the lumbar spine, nondominant proximal femur, nondominant radius, and whole body using Hologic QDR-4500W densitometers (Hologic, Bedford, MA, USA). Calcaneal QUS measurements were also performed using the McCue CUBA clinical (McCue, Winchester, UK).

Axial-transmission SOS and BMD may measure different aspects of bone. If this is the case, it is possible these differing properties of bone may be controlled by shared or unique genetic influences. The site-matched measurements at the radius provide an ideal tool to investigate this. However, a SOS measurement is independent of the diameter of the radius, while BMD is directly dependent on bone size. To allow for this, the radius BMD measurements were corrected to derive an equivalent of volumetric BMD assuming that the radius is a cylinder, using Eq. A1 (see Appendix). The estimated volumetric (vBMD) was then compared with SOS at the radius.

Statistical analysis

The mean and SD for the MZ and DZ twin groups were calculated for age, body mass index (BMI), weight, height, menopause age, years since menopause, and all SOS and BMD

TABLE 1. TWIN CHARACTERISTICS

	MZ		DZ	
	Mean	SD	Mean	SD
Number of twin pairs	85		130	
Number premenopausal	32		55	
Number postmenopausal	53		70	
Number perimenopausal	—		5	
Age	51.4	11.7	50.2	12.9
BMI (kg/m ²)	25.6	5.1	25.9	4.7
Weight (kg)	67.3	14.1	67.9	13.2
Height (cm)	162.2	6.4	162.2	6.2
Men age (yrs)	47.1	6.2	47.0	6.5
YSM (yrs)	11.3	7.8	12.3	9.2
SOS				
Radius (m/s)	4107	130	4111	128
Tibia (m/s)	3892	150	3866	145
Phalanx (m/s)	3960	184	3979	207
Metatarsal (m/s)	3608	226	3618	229
BUA (dB/MHz)	72	17	74	18
Axial BMD				
L spine (g/cm ²)	0.982	0.144	0.982	0.134
NOF (g/cm ²)	0.802	0.123	0.807	0.120
T hip (g/cm ²)	0.933	0.131	0.929	0.124
Peripheral BMD				
One-third radius (g/cm ²)	0.684	0.073	0.676	0.065
T radius (g/cm ²)	0.555	0.060	0.549	0.058
Other BMD				
W/B BMD (g/cm ²)	1.162	0.110	1.146	0.106

TABLE 2. INTRACLAS CORRELATIONS

	MZ (n = 170)		DZ (n = 260)	
	r		r	
SOS				
Radius	0.77		0.25	
Tibia	0.64		0.28	
Phalanx	0.77		0.46	
Metatarsal	0.70		0.36	
BUA	0.69		0.37	
Axial BMD				
Lumbar spine	0.86		0.46	
Femoral neck	0.85		0.55	
Total hip	0.87		0.49	
Peripheral BMD				
One-third radius	0.87		0.56	
Total radius	0.87		0.59	
Other BMD				
Whole body	0.87		0.53	

measurements. Differences between the MZ and DZ twin group means and variances were tested controlling for the non-independence of sib-pairs. The intraclass correlations were calculated for the MZ and DZ twin groups to obtain an initial estimate of genetic effect. Model fitting enables a more extensive separation of the observed phenotypic variance into its genetic and environmental components of additive genetic variance (a^2), dominance genetic variance (d^2), shared or common environmental variance (c^2), and unique environmental variance (e^2); the latter also contained the measurement error.⁽¹⁸⁾ Univariate analysis was performed using linear regression to separate the variance components using the method of DeFries and Fulker.⁽¹⁹⁾ Age has a strong association with both BMD and SOS measurements and therefore was included in the regression equation as a confounder. Additionally, a significant correlation was found between BMI and some of the SOS and BMD variables, and this was therefore also included in the regression analysis.

The correlation between the SOS and BMD measurements at this site was calculated using linear regression. Bivariate analysis was performed using a Cholesky decomposition⁽²⁰⁾ implemented in Mx to test the existence of genetic factors that are shared by the BMD and SOS measurements at the radius.

RESULTS

Table 1 shows the characteristics of the two twin groups. No significant differences were found between the MZ and DZ twin groups. Table 2 shows the intraclass correlations

for the MZ and DZ groups. All intraclass correlations were significantly different from zero at a level of $p < 0.01$. All MZ intraclass correlations were greater than the DZ correlations, suggesting a genetic influence, with the MZ correlations for the BMD measurements being greater than those for the SOS and broadband ultrasound attenuation (BUA) measurements. Both age and BMI were found to have a significant effect on BMD and SOS. The variance within the population associated with age ranged from 4% to 30%, and with BMI, ranged from 0% to 7%. The influence of menopause was also explored by dividing the data into pre- and postmenopausal groups. The groups did not show any consistent difference in heritability, although the smaller size of the groups meant confidence limits were wider (data not shown).

Table 3 shows the age and BMI adjusted heritability estimates calculated using the Defries Fulker multiple regression analysis. The additive genetic influence (a^2) was as follows: SOS measurements, 0.51 (95% CI, 0.32,0.70) to 0.56 (0.37,0.76); BUA, 0.58 (0.41,0.75); axial BMD, 0.72 (0.58,0.86) to 0.77 (0.63,0.91); radius BMD, 0.53 (0.16,0.90) to 0.63 (0.26,1.00); and whole body BMD, 0.63 (0.23,1.03). No common environmental influences were found for the SOS, BUA, or axial BMD measurements. However, although nonsignificant, a suggestion of common environmental influences, ranging from 0.13 (0.00,0.42) to 0.22 (0.00,0.49), were found for radius BMD and 0.13 (0.00,0.43) for whole body BMD.

The unique environmental influences ranged from 0.19 to 0.37 for the SOS measurements, 0.32 for BUA, 0.12 to 0.14 for axial BMD, and 0.04 to 0.15 for radius and whole body BMD.

When the phenotypic correlation between SOS and BMD at the radius was calculated using linear regression, the correlation coefficient was $r = 0.34$ (95% CI, 0.29–0.47) for the one-third region of interest (ROI) and $r = 0.32$ (0.25–0.43) for the total radius ROI. The correlation between the one-third radius ROI and radius SOS yielded the

TABLE 3. HERITABILITY ESTIMATES

	a^2	c^2	e^{2*}	Age*	BMI*
SOS					
Radius	0.54 (0.33–0.71)		0.29	0.16	0.01
Tibia	0.52 (0.36–0.68)		0.37	0.04	0.07
Phalanx	0.51 (0.32–0.70)		0.19	0.30	0.00
Metatarsal	0.56 (0.37–0.76)		0.30	0.14	0.00
BUA	0.58 (0.41–0.75)		0.32	0.05	0.05
Axial BMD					
Lumbar spine	0.77 (0.63–0.91)		0.14	0.06	0.03
Femoral neck	0.72 (0.58–0.86)		0.12	0.10	0.06
Total hip	0.73 (0.59–0.87)		0.14	0.06	0.07
Peripheral BMD					
One-third radius	0.63 (0.26–1.00)	0.13 (0.00–0.42)	0.04	0.19	0.01
Total radius	0.53 (0.16–0.90)	0.22 (0.00–0.49)	0.13	0.10	0.02
Other BMD					
Whole body	0.63 (0.23–1.03)	0.13 (0.00–0.43)	0.15	0.07	0.02

Age and BMI adjusted heritability estimates for the whole twin group including pre- and postmenopausal twins.

a^2 is the additive genetic variance, c^2 is the common or shared environmental variance, and e^2 is the unique environmental variance, which also contains measurement error.

* e , age, and BMI were not directly estimated using DeFries Fulker regression analysis. Variance components attributable to covariates were based on the observed difference between the heritability model with and without covariates.

largest correlation coefficient and also gave the closest overlap of all the radius ROIs. Therefore, this was chosen as the site for the bivariate analysis. The vBMD correlation between the one-third radius and the radius SOS was $r = 0.32$ (0.21–0.42).

The genetic correlation for the radius SOS and the radius one-third vBMD ROI led to the estimate that 38% (16–57%) of the genetic variance explained by SOS at the radius was also explained by genes influencing BMD of the radius. These results suggest a combination of shared and unique genetic influences for SOS and BMD measurements at the radius.

DISCUSSION

Heritabilities

This is the first time that the heritability of SOS measurements at multiple peripheral sites in cortical bone has been evaluated and contrasted with BMD. A number of previous studies have reported the strong heritability of cortical BMD, especially in the axial skeleton.^(6–10,21) Addressing the possible pitfalls of the twin method; the MZ and DZ twin groups were well matched for age, BMI, weight, height, menopause age, and years since menopause. Both SOS and BMD are strongly related to age, and therefore age adjustment of the data were important. BMI was also found to account for a proportion of the variance for some of the parameters, and this was therefore also accounted for in the analysis. There is often concern about the generalization of results from twins. However, we have recently shown very similar values of means and variances of environmental bone and musculoskeletal parameters in our twin cohort compared with an age-matched cohort of singletons from the population.⁽²²⁾

DeFries and Fulker regression analysis results confirm SOS measurements in cortical bone to be a heritable trait, with the variance explained by additive genetic influence

ranging from 51% to 56%. The additive genetic influence for BUA at the calcaneus was 58%. The additive genetic influence for BMD was similar to previous studies in the axial skeleton and ranged from 72% to 77%, whereas it was lower at the radius, ranging from 53% to 63%. The greater heritability of BMD in the axial skeleton is consistent with a previous study conducted by Dequeker et al.⁽¹⁰⁾

Confounders

Both age and BMI were found to account for a significant proportion of the variance within the population, with age accounting for 4–30% of the variance in multisite SOS measurements, 5% for calcaneal BUA, and 6–19% of the variance in BMD. Previous twin studies have also adjusted for age because BMD and SOS are highly age-dependent. The greatest proportion of the variance accounted for by age was at the phalanx, which is explained by the greatest postmenopausal age-related bone loss also demonstrated at this site.⁽²³⁾ The BUA heritability estimate is consistent with that reported by Arden et al. in a similar study⁽⁸⁾ on the same cohort, whereas it is lower than the results of a study by Howard et al.⁽⁷⁾ Phalangeal SOS using the DBM sonic (IGEA, Italy) was also investigated by Howard et al., with an age- and weight-adjusted, but not a BMI-adjusted heritability estimate of 0.82 in 49 MZ and 44 DZ twins,⁽⁷⁾ which was somewhat greater than found in this study using the Omnisense. The axial BMD heritability estimates are similar to those reported in other studies.^(8–10) The age adjustment in this study was performed using quadratic rather than a linear age terms, because the relationship between SOS and BMD and age ranging from 20 to 79 is curvi-linear.

Correlations

Weak correlations between site-matched measurements of SOS and BMD at the radius were found. This is consis-

tent with a previous study using the Omnisense,⁽²³⁾ suggesting that SOS may be measuring different aspects of bone to BMD. Our bivariate analysis showed that approximately 40% of the genetic influences of SOS were also explained by BMD at this site, suggesting a combination of shared and unique genetic influences controlling BMD and the aspects of bone measured by SOS. This also showed that SOS has an effect independent of BMD. Howard et al. also reported that modeling suggested a common set of genes were found to have a greater effect than shared genes on each trait,⁽⁷⁾ although the BMD and QUS measurements were at different body sites. Despite the site-matched SOS and BMD measurements compared in this study, the results should be treated with a degree of caution. The radius measurements compare the areal BMD of the cortical bone and the medullary cavity, with a SOS measurement that relates to the density of the cortical bone and the thickness of the cortex.⁽²³⁾ Ultrasound measurements of bone have been demonstrated to reflect aspects of trabecular structure and are dependent on the direction in which the ultrasound measurement is made.⁽²⁴⁾ It is therefore possible that the same is true for ultrasound measurements of cortical bone. To reduce any errors as a result of this, all SOS scans were made in the longitudinal axis along the radius, with measurements marked on the skin to aid accurate acquisition. Previous in vitro and in vivo studies have suggested that SOS as measured by axial transmission SOS measures the cortical density but is also affected by cortical thickness if this is less than the wavelength. Cortical bone density, however, does not correlate strongly with SOS, suggesting that there are other factors influencing the SOS in cortical bone.^(12,13) The results in this study suggest that SOS and BMD measure different phenotypes to an extent and that a combination of shared and genetic influences affect these phenotypes.

In conclusion, this study has demonstrated genetic influences on SOS measurements in cortical bone, axial BMD, and calcaneal BUA, and weaker genetic influences on radius and whole body BMD. Additionally, a combination of both shared and unique genes have been found to contribute at the same site to BMD and SOS. Further research should focus on searching for the shared genes that may have a more influential effect on fracture risk.

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APPENDIX 1

vBMD for the one-third ROI in the distal radius was estimated from the conventional areal BMD measured on the radius DXA scans by

modeling the radius as a cylinder with outer cortical diameter D and inner cortical diameter d . Let L be the length of the one-third ROI on the scan. $vBMD$ is related in the following way to the bone mineral content (BMC), areal BMD (BMD), and projected area ($Area$) of the one-third ROI:

$$vBMD = \frac{BMC}{\frac{\pi}{4}(D^2 - d^2)L} = \frac{BMD}{\frac{\pi}{4}D(1 - d^2/D^2)} = \frac{BMD \times L}{\frac{\pi}{4}Area(1 - d^2/D^2)}$$

$$= \left(\frac{BMD \times L}{Area} \right) \times \frac{4}{\pi(1 - d^2/D^2)}. \quad (A1)$$

$vBMD$ was calculated assuming that the ratio of the inner to the outer cortical diameter was the same in all subjects. The exact value of the

term $(1 - d^2/D^2)$ in Eq. A1 is immaterial for the purposes of the statistical calculations because it represents a constant scaling factor.

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