

# Genetic and environmental determinants on bone loss in postmenopausal Caucasian women: a 14-year longitudinal twin study

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## Abstract

**Summary** This longitudinal twin study documented that genetic factors explain 44–56% of the between-individual variance in bone loss at femoral neck, lumbar spine, and forearm in postmenopausal Caucasian women, providing a rationale for identifying the specific genes involved.

**Introduction** Although there is a significant genetic effect on peak BMD, until recently, no substantive studies on heritability of bone loss in human were available. The aim of the study was to estimate the heritability of the bone loss at multiple sites in postmenopausal Caucasian women.

**Methods** Postmenopausal female monozygotic (MZ) and dizygotic (DZ) twins aged 40 or above at baseline were selected from the TwinsUK registry and followed up for an average of 8 years (range 5–14 years). All twins were noncurrent hormone replacement therapy users and not on any osteoporosis treatment. They had dual-energy X-ray absorptiometry (DXA) scans of their hip, lumbar spine, and forearm several times (range 2–9) during the follow-up period. Individual bone losses at femoral neck, lumbar spine, and forearm were estimated by linear regression modeling. Structural equation modeling was utilized to estimate the heritability of the bone loss.

**Results** A total of 712 postmenopausal Caucasian female twins (152 MZ and 204 DZ pairs) were included. MZ twins

were older and had slightly lower BMD at all sites than DZ twins. DZ twins had slightly higher bone loss at lumbar spine, but similar at femoral neck and forearm compared to MZ twins. Intraclass correlation coefficients (ICC) for the bone loss at all sites were significantly higher in MZ than DZ twin pairs ( $p=0.0045$ ,  $0.0003$ , and  $0.0007$  for femoral neck, lumbar spine, and forearm, respectively), indicating a significant genetic influence on bone loss at these sites. After adjustment for age at baseline and weight change during the follow-up, the heritability estimate was 47% (95% CI 27–63%) for bone loss at femoral neck, 44% (95% CI 27–58%) for lumbar spine, and 56% (95% CI 44–65%) for forearm.

**Conclusions** Our data suggest that up to 56% of the between-individual variance in bone loss is due to genes, providing a rationale to identify specific genetic factors for bone loss.

**Keywords** BMD · Bone loss · Genetics · Longitudinal · Twin study

## Introduction

Longitudinal studies [1, 2] have reported a significant bone loss with age at both lumbar spine and femoral neck in peri- and postmenopausal women. Rates of bone loss between women vary widely with mean levels after menopause estimated to be 0.5–3% per annum [2–5].

Although it is generally assumed that bone loss in humans is heritable, until recently, no substantive or conclusive studies on heritability of bone loss in human were available. The findings from three previous twin studies [6–8] yielded conflicting results possibly due to very small sample size. A larger family-based study of 388

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healthy young sisters with a mean age of 35 from 178 families [9] reported a significant genetic component to the bone loss at femoral neck. But a recent study [10] of older 724 female twins with a mean age of 56 followed for on average 4.9 years did not confirm a genetic effect at the hip, although they did find a role in bone loss at lumbar spine and forearm. We, therefore, undertook this longitudinal twin study to estimate the heritability of bone loss at lumbar spine, forearm, and particularly the hip to justify continued pursuit of genes for these phenotypes.

## Methods and subjects

### Subjects

The study participants were Caucasian of North European origin monozygotic (MZ) and dizygotic (DZ) twin pairs from the TwinsUK adult twin registry, a group ascertained to study the heritability and genetics of age-related diseases (<http://www.twinsUK.ac.uk>). These unselected twins were recruited from the general population through national media campaigns in the UK and shown to be comparable to age-matched population singletons in terms of disease-related and lifestyle characteristics [11]. The study was approved by St. Thomas' Hospital Research Ethics Committee, and all twins provided informed written consent.

As part of our project of ageing, all twins have been invited to come back to the clinical center biannually since the study started in 1992. During each visit, various clinical examinations were performed including DXA scan and weight measurement. All twins completed three comprehensive nurse-administered questionnaires providing details in their lifestyles, medical, drug, and obstetric and gynecological histories during the follow-up period up to 2008. For the purpose of this study, postmenopausal twins aged 40 or above at baseline and with at least 5 years follow-up in terms of DXA scan were selected. Twins who were using HRT use or any osteoporosis treatment based on the data from the three questionnaires were excluded.

### BMD measurements

BMD was measured at the lumbar spine (L1–4), left total forearm, and left hip using dual energy X-ray absorptiometry (DXA; Hologic QDR2000 (1992 to 1996); Hologic QDR4500W (1996 to 2004); Hologic Discovery W (2004 to present; Hologic, Bedford, MA, USA). At installation, the manufacturer's engineer calibrated each instrument using a spine phantom. An *in vivo* cross-calibration study showed close agreement between the QDR2000 and the QDR4500W with an intraclass correlation coefficient of 0.968–0.995. The Discovery system has the same X-ray

generator as the QDR4500 and is mechanically identical. Daily quality control scans were performed using the spine phantom. Intrascanner reproducibility, expressed as a coefficient of variation from duplicate measurements in healthy volunteers 1 week apart, was 0.8% at the lumbar spine and 1.6% at the femoral neck. All BMD measurements were performed using a standardized protocol of measurement. Twins within each pair were always scanned at the same time.

The average follow-up time was 8.45 years (range 5–14 years), and all twins had their BMD measured a median of three times (range 2–9). There was no difference in the follow-up time and frequency of BMD measurements between MZs and DZs.

### Statistics

Because we had multiple time point measurements of BMD, the annual percent change in BMD at each site studied (e.g., lumbar spine, forearm, and femoral neck) for each individual was calculated using linear regression models as utilized by others [12]. In this approach, a linear regression model was fitted to each individual's data, from which the intercept and slope for the individual were obtained. The percentage of change was estimated as the ratio of slope over the intercept and used in subsequent genetic analyses. Weight change for each individual during the follow-up was also calculated using the same method.

Intraclass correlation coefficient (ICC) was calculated for bone loss at each site studied and compared between MZ and DZ twin pairs. Any significantly higher ICC in MZs than in DZs would indicate a significant genetic influence on the bone loss. Structural equation modeling, which is a statistical technique allowing separation of the observed phenotypic variance into its genetic and environmental components, implemented in a software called Mx [13] was then utilized to estimate the heritability of the bone loss at each site studied. Heritability is defined as the proportion of total variance due to genetic effects, assuming that variance in bone loss is due to additive genetic factors (A), shared environmental factors (C), and non-shared environmental factors (E).

The analyses were performed in STATA (StatCorp LP, College Station, TX, USA) except for structural equation modeling, and a  $p$  value  $\leq 0.05$  was considered as statistically significant.

## Results

A total of 712 postmenopausal Caucasian female twins (152 MZ and 204 DZ pairs) aged 40 or above were included. None were current HRT users or on any osteoporosis treatment. The characteristics of the sample

**Table 1** Characteristics of the studied twins

	MZ ( <i>n</i> =304)	DZ ( <i>n</i> =408)	<i>P</i> value
Age at baseline (year)	58.075 (6.051)	55.516 (7.233)	<0.0001
FN BMD at baseline (g/cm <sup>2</sup> )	0.743 (0.115)	0.772 (0.127)	0.01
LS BMD at baseline (g/cm <sup>2</sup> )	0.920 (0.142)	0.962 (0.157)	0.002
Forearm BMD at baseline (g/cm <sup>2</sup> )	0.525 (0.059)	0.539 (0.063)	0.009
Height at baseline (cm)	161.178 (5.889)	163.018 (6.235)	<0.0001
Weight at baseline (kg)	64.595 (10.451)	67.190 (11.732)	0.01
Weight change (% per year)	0.428 (0.985)	0.430 (1.011)	0.99
ΔFN BMD (% per year)	-0.447 (0.057)	-0.593 (0.053)	0.06
ΔLS BMD (% per year)	-0.212 (0.076)	-0.509 (0.049)	0.0006
Δforearm BMD (% per year)	-0.714 (0.964)	-0.548 (0.076)	0.17

Values are mean for continuous variables (SD).

are presented in Table 1. MZ twin pairs were older (mean age 58.1) than DZ pairs (mean age 55.5). Weight change was +0.43% per year in the whole sample, and there was no difference between MZs and DZs (Table 1). The baseline BMD at all three sites (e.g., femoral neck, lumbar spine, and forearm) were all slightly lower in MZ pairs than DZ pairs (Table 1). After adjustment for age at baseline, the difference in baseline BMD between MZs and DZs became nonsignificant for femoral neck and forearm but not lumbar spine. On average, bone losses for the whole sample were -0.52%, -0.36%, and -0.63% per year at femoral neck, lumbar spine, and forearm, respectively. There was no difference in bone loss at femoral neck and forearm between MZs and DZs, but bone loss at lumbar spine was higher in DZs than in MZs (Table 1). Of the sample, 77%, 67%, and 67% experienced bone loss at femoral neck, lumbar spine, and forearm, respectively, during the follow-up.

Age at baseline and weight change during the follow-up were significantly associated with bone loss at both lumbar spine and femoral neck (all  $p < 0.001$ ). Bone loss at forearm was significantly associated with weight change ( $p = 0.03$ ) but not with age at baseline.

Table 2 presents the results of ICC comparison between MZ and DZ twin pairs. The ICCs for bone loss at all three sites were statistically significantly higher in MZ than in DZ twin pairs (Table 2), indicating a significant genetic control on bone loss at all sites.

The full model is the ACE model containing additive genetic effects (A), shared environmental effects (C), and unique environmental effects (E). Nested models (namely, AE which contained additive genetic effects and unique environmental effects, CE which contained shared environmental effects and unique environmental effects, and E

which contained only unique environmental effects) were then compared to the full ACE model. The most parsimonious model was AE model based on the likelihood ratio test, and the parameter estimates were obtained from the most parsimonious model.

The heritability estimates were 42%, 56%, and 57% for bone loss at femoral neck, lumbar spine, and forearm, respectively (Table 3). After adjustment for age at baseline and weight change, heritability estimates were similar for bone loss at femoral neck and forearm but reduced by 12% at lumbar spine (Table 3). When we restricted the analysis only for those who had at least three BMD measures, which included 116 MZ and 128 DZ twin pairs with slightly longer average follow-up time of 8.93 years, the estimates were the same for bone loss at lumbar spine and forearm but increased to 53% (95% CI 34–67%) for femoral neck.

## Discussion

Although genetic effects on cross-sectional BMD are pronounced with heritability estimates of up to 90% depending on site [14–18], it is difficult to interpret the genetic effects on bone loss from those studies. This is because cross-sectional BMD variation more likely reflects peak bone mass than bone loss. In this study, we demonstrated that 44% to 56% of between-individual variance in bone loss in postmenopausal Caucasian women is due to genetic factors. A particular strength of this study is that twins had BMD measured at multiple time-points, which allowed us to get a more accurate estimate for the bone loss. In addition, we used the same acquisition protocol throughout the follow-up period, and twins within

**Table 2** Correlation coefficients in MZ and DZ twin pairs for bone loss

	MZ twins	DZ twins	<i>P</i> value (one sided)
ΔFN BMD	0.42 (0.23–0.60)	0.19 (0.03–0.34)	0.0045
ΔLS BMD	0.61 (0.46–0.73)	0.36 (0.24–0.48)	0.0003
ΔForearm BMD	0.59 (0.34–0.80)	0.35 (0.09–0.62)	0.0007

**Table 3** Heritability estimate by structural equation modeling in Mx

	Unadjusted		Adjusted for age and weight change	
	<i>A</i> (95% CI)	<i>E</i> (95% CI)	<i>A</i> (95% CI)	<i>E</i> (95% CI)
ΔFN BMD	0.42 (0.24–0.57)	0.58 (0.43–0.76)	0.47 (0.27–0.63)	0.52 (0.37–0.73)
ΔLS BMD	0.56 (0.45–0.66)	0.44 (0.34–0.55)	0.44 (0.27–0.58)	0.56 (0.42–0.73)
ΔForearm BMD	0.57 (0.45–0.66)	0.43 (0.34–0.55)	0.56 (0.44–0.65)	0.45 (0.35–0.56)

*A* additive genetic effect, *E* unique environmental effect

each pair were always scanned at the same time, thus reducing any potential confounding related to acquisition. As the twin model does not allow the separation of unique environment and measurement error—these heritability estimates are likely to be conservative—as environmental effects may be inflated by the inherent errors in DXA measures over time. Biomarkers for bone formation and resorption have been reported to have significant genetic components [19–21], which, as they have a clear role in predicting bone loss to some extent, could be one potential mechanism for the genetic effects seen on BMD loss.

In a study of mainly premenopausal sisters aged 20–47, Hui et al. [9] reported that BMD at femoral neck decreased at a rate of  $-0.0026 \text{ g/cm}^2$  over 5.7 years, and the heritability estimate for the bone loss at femoral neck was 35%. Our results extend their findings to postmenopausal women. We demonstrated that genetic effects accounted for 47% of the between-individual variance in bone loss at femoral neck. A previous twin study [8] did not find any significant genetic influence on bone loss at femoral neck. However, the sample size was small (only 21 MZ and 19 DZ pairs), the period of the study was short, ranging from only 1–5 years, the subjects were a mixture of men and premenopausal and postmenopausal women, and the age range was wide, from 25 to 65 years. In a recent study of 177 MZ and 185 DZ Australian female twins aged between 45 and 82 years, Makovey et al. [10] also did not find any significant genetic effects on bone loss at hip over an average 4.9 years. The reason for this discrepancy is unclear, possibly having less repeat measures than our study might have introduced more measurement errors, thus reducing their power to detect a significant change in BMD at hip. Indeed, when we restricted our analysis to those who only had two BMD measures, the significance was reduced (data not shown).

However, Makovey et al. [10] reported that the bone loss at forearm and lumbar spine were under genetic regulation with a heritability estimate of up to 40%. Consistent with their findings, we confirmed that genetic factors played a significant role in bone loss at both forearm and lumbar spine. The heritability estimate on bone loss at lumbar spine in the current study was higher than the report of Makovey et al. [10]. The bone loss at lumbar spine in MZs was

different from DZs in the current study, which may potentially have biased the heritability estimates. However, we accounted for this by incorporating the different mean rate of bone loss for MZs and DZs separately in the modeling.

The heritability for bone loss found in the current study as well as in the previous reports [9, 10] is consistently lower than that for cross-sectional BMD measurement [14–18]. This apparently lower heritability for the bone loss is probably in part explained by the larger measurement error. The effect of measurement error on heritability estimates is usually negligible in cross-sectional BMD studies because the error variance of the DXA measurement is small relative to the total variance in the population [9]. However, this is not the case when one considers bone loss as the change in BMD with age is small relative to the measurement error. We minimized the measurement errors by increasing follow-up period and the number of the repeated BMD measurements, thus the effect of measurement error on heritability estimates while still present is likely to be reduced in the current study. Indeed, the heritability estimates in the current study are higher than that in the previous reports [9, 10] as the 95% CIs did not overlap between the current study and the previous reports.

There are some further caveats to the current study. Firstly, the sample was a Caucasian female population; therefore, the results may not be generalizable to men and other populations. Secondly, we did not have data on osteophytes, which is a confounding factor for BMD measurement, particularly for lumbar spine [3]. Age-related formation of osteophytes at the lumbar spine increases the BMD at lumbar spine artificially. In our sample, MZs were older and showed slightly less bone loss than DZ pairs, which may have reduced the heritability estimates for these data. Thirdly, environmental factors also play a role in bone loss [3, 22–25]. Our study sample did not include any women who were on HRT or any osteoporosis treatment; however baseline age, weight change, and change in menopausal status during the follow-up may have influence on estimates of bone loss and consequently heritability estimates. Although the estimates changes, the significance of the results were not altered after adjustment for all these confounders (Table 3). Apart from age, weight change and

menopause timing are all under genetic influence, so it is not surprising that the estimates generally reduce when these factors are removed from the models.

In conclusion, our data suggests that approximately half of individual variance in bone loss is due to genetic factors, providing a clear rationale for identifying susceptibility genes via linkage or association studies.

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**Conflicts of interest** None.

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