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Genetic and environmental influences on bone mineral density in pre- and post-menopausal women

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Abstract

Genetic factors influencing acquisition of peak bone mass account for a substantial proportion of the variation in bone mineral density (BMD), although the extent to which genes also contribute to variation in bone loss is debatable. Few prospective studies of related individuals have been carried out to address this issue. To gain insights into the nature of the genetic factors contributing to variation in BMD, we studied 570 women from large Amish families. We evaluated and compared the genetic contributions to BMD in pre- and post-menopausal women, with the rationale that genetic variation in pre-menopausal women is due primarily to genetic determinants

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of peak bone mass, while genetic variation in post-menopausal women is due to the combined genetic effects of peak bone mass and bone loss. Bone mineral density was measured at one point in time at the hip and spine by dual energy X-ray absorptiometry (DXA). We used variance decomposition procedures to partition variation in BMD into genetic and environmental effects common to both groups and to pre- and post-menopausal women separately. Total variation in BMD was higher in post- compared to pre-menopausal women. Genes accounted for 58–88% of the total variation in BMD in pre-menopausal women compared to 37–54% of the total variation in post-menopausal women. In absolute terms, however, the genetic variance was approximately similar between the two groups because the environmental variance was 3 1/2- to 4-fold larger in the post-menopausal group. The genetic correlation in total hip BMD was 0.81 between pre- and post-menopausal women and differed significantly from one, consistent with the presence of at least some non-overlapping genetic effects in the two groups for BMD at this site. Overall, these analyses suggest that many, but not all, of the genetic factors influencing variation in BMD are common to both pre- and post-menopausal women.

Keywords

Bone loss; Bone mineral density; Genetics; Heritability; Peak bone mass; Variance

Introducton

Osteoporosis is a major public health problem that is associated with significant morbidity and mortality. According to the Third National Health and Nutrition Examination Survey (NHANES III), 50% of U.S. women over age 50 have low bone mass and 20% of white postmenopausal women have osteoporosis at the femoral neck (hip) [1]. Osteoporotic fractures are one of the most common causes of disability and contributors to medical care costs in many regions of the world [2]. Large prospective studies have shown that almost all types of fracture are increased in adults with low bone mineral density (BMD) [3]. Fractures result in functional impairment, including impaired basic activities of daily living, subsequent nursing home care, the loss of ambulatory ability and loss of the ability to live independently [2, 4].

Risk of fracture is particularly acute in women because of the sharp decline in BMD that begins at about the time of the menopause. On average, women lose 1 to 3% of their BMD per year during the first 3 years after menopause, and this rate of bone loss is faster in the spine than other skeletal sites [5, 6]. After this period of accelerated bone loss, the rate of bone loss slows down until age 70, when bone loss begins to accelerate again [7]. Despite this overall trend, there appears to be considerable variability among women in the rate of bone loss, with up to 35% of women losing at least 3% per year for more than 2 years [8]. At least one study has shown that with over 12–15 years of follow up, the risk of fracture was increased to a similar degree for women starting out with low bone mass (defined as a T score <–1 SD) and for women starting out with normal bone mass, but experiencing a rapid rate of bone loss, with each group experiencing a doubling of the fracture risk. When baseline bone mass was low and bone loss was rapid, however, the risk of fracture was higher still (odds ratio =3.0) [9].

From studies of young adults, it is well established that peak bone mass is under substantial genetic control [10, 11, 12, 13, 14, 15, 16, 17, 18, 19]. Additionally, women with a maternal history of hip fracture have lower BMD than women without a history of such fracture [20] and are themselves twice as likely to suffer a hip fracture [3, 21]. There are very few published data, however, on the heritability of bone loss. Kelly et al. estimated genetic effects on changes in spine and hip BMD measured 3 years apart from a sample of 21 monozygotic (MZ) and 19 dizygotic (DZ) twin pairs. These investigators observed a significantly higher correlation in spine BMD change among MZ (r =0.93) compared to DZ (r =0.51) twin pairs, with differences consistent with a heritability in spine BMD change as high as 80% [22]. Correlations in BMD changes at several hip sites were also higher among MZ compared to DZ twin pairs, although these differences did not achieve statistical significance in this small sample. In contrast, Christian et al. reported no difference in 16-year changes in forearm BMD between 25 and 21 DZ twin pairs [11]. These results may indicate that BMD change is heritable in some sites (e.g., the spine) more than others (e.g., the forearm), or alternatively, that genetic influences on BMD changes at the forearm (and perhaps other sites) are not detectable over a 16-year period.

In a previous study, we used a variance decomposition approach to evaluate the relative contributions of genetic and environmental effects in accounting for variation in male and female differences in BMD [23]. These analyses did not provide evidence for sex-specific genetic effects, suggesting that genes influencing variation in BMD should be detectable, and in many cases common, in both men and women. In the current study, to begin to dissect genetic components of peak bone mass and bone loss, we evaluated the genetic and environmental contributions to BMD in women before and after menopause. The rationale for this approach is that genetic variation in pre-menopausal women is due primarily to genetic determinants of peak bone mass, while genetic variation in post-menopausal women is due to the combined genetic effects of peak bone mass and bone loss. Specifically, we considered the following questions: (1) is the magnitude of the genetic variation larger in pre-menopausal women than in post-menopausal women?; (2) does the magnitude of the environmental variation differ between pre-menopausal women and post-menopausal women?; (3) is there evidence for menopausal-status-specific genetic effects on BMD (i.e., is there a subset of genes that influences variation in BMD in all women and another subset of genes that influences variation in pre- and post-menopausal women separately)? Our findings suggest that there are common genetic contributions to BMD in both pre- and post-menopausal women (presumably determinants of peak bone mass), but in addition a separate genetic contribution, albeit modest, to BMD in post-menopausal women (presumably determinants of bone loss).

Materials and methods

Subjects and measurements

The Amish Family Osteoporosis Study (AFOS) began in 1997 with the goal of identifying the genetic determinants of osteoporosis. Details of ascertainment, pheno-typing and clinical characteristics of AFOS participants were reported previously [23, 24]. Briefly, individuals believed to be at risk for osteoporosis by virtue of their fracture history or prior bone density

measurements were recruited into the study as index cases. These individuals were recruited by word-of-mouth, a community-wide mailing, advertisements in an Amish newspaper or by referral from local physicians. The diagnosis of osteoporosis in these individuals was verified by the measurement of BMD using dual energy X-ray absorptiometry (DXA). Individuals found to have a T score of −2.5 or less in either the hip or spine were designated as probands. We then invited the probands' spouses and all first-degree relatives aged 20 years and over to participate in the study. In addition, we recruited into the study the first-degree relatives of any other examined individual (e.g., spouses) having a T score of −2.5 or lower at the spine or hip on our bone densitometry test.

Between the initiation of recruitment in 1997 and February 2002, a total of 1,002 individuals (617 women and 385 men) were enrolled into the AFOS, including 57 probands and their relatives. Complete information on DXA phenotypes and menopausal status was obtained from 570 of the 617 women enrolled. Using the extensive genealogical records maintained by the Amish [25, 26,27], these individuals could be combined into a single 14-generation pedigree. Study participants were evaluated by qualified nurses known to the participants at the Amish Research Clinic in Strasburg, PA. A medical interview included past medical history, family history of medical problems including fractures and specific details regarding previous fractures, history of medication use and menstrual and reproductive history for women. Height was measured using a stadiometer, and weight was recorded with the participant in standard Amish clothing, but without shoes. Women were considered to be post-menopausal if they reported fewer than two menstrual cycles over the previous 12 months. Thirty-three women reported a history of oophorectemy/hysterectomy and were considered for purposes of these analyses to be post-menopausal.

The mineral content at the lumbar spine and hip was measured by dual energy X-ray absorptiometry (DEXA) by a registered nurse certified in bone densitometry (Hologic 4500 W, Hologic, Inc., Bedford, Mass.). BMD was determined by dividing the total bone mineral content (g) by the projected area of the region scanned (cm^2). For this report, we have restricted analysis of BMD to measures obtained at the spine, femoral neck and total hip. Total hip BMD was defined as the sum of the bone mineral content at the femoral neck, trochanter and intertrochanter sites divided by the total area of these three sites.

The protocol for the AFOS was approved by the Institutional Review Board at the University of Maryland. Informed consent was obtained from all subjects prior to participation.

Analytical methods

We carried out a series of statistical analyses using a full pedigree-based variance component approach for the purpose of partitioning variation in BMD into selected components [28]. Initially, we modeled variation in BMD as a function of measured environmental covariates [e.g., age, age^2, height and body mass index (BMI)], additive genetic effects (or heritability) and a residual error component. Maximum likelihood methods were used to estimate the covariate and genetic effects simultaneously. The covariates selected were included because they were each independently associated with one or more BMD measures in preliminary analyses on pre- and post-menopausal women

separately. The significance of particular components can be assessed by comparing the likelihood of a model with the component of interest estimated to the likelihood of a model in which the component effect is constrained to a prespecified value (e.g., zero). The full and restricted models are then compared by likelihood ratio test, which produces a test statistic that is asymptotically distributed as a χ^2 distribution.

In an initial variance component analysis, we estimated the proportion of the total phenotypic variation in BMD (σ_P^2) that could be attributable to the additive genetic effects in pre- and post-menopausal women separately (σ_G^2/σ_P^2). This effect corresponds to 'narrow' sense heritability since it reflects the degree of additive genetic variance only. We tested whether the heritabilities in BMD differed between pre- and post-menopausal women (i.e., $h^2_{pre} = h^2_{post}$) by comparing the difference between the heritability estimates in the two groups with the estimated variance of the difference.

Following the approach of Blangero, [23, 29, 30], we then expanded the basic variance component model to allow the genetic variances in pre-menopausal and post-menopausal BMD to differ. Briefly, we constructed a general model that partitioned variance in BMD into the following 13 terms: an overall mean, a coefficient corresponding to the effect of menopausal status ($\beta_{menostat}$), coefficients for age and menopausal status*age (β_{age} and $\beta_{age*menostat}$), coefficients for age^2 and menopausal sta tus*age^2 (β_{age}^2 and $\beta_{age}^2{}_{*menostat}$), coefficients for height (β_{height}), and BMI (β_{BMI}), pre- and post-menopausal genetic standard deviations ($\sigma_{G\text{-}pre}$ and $\sigma_{G\text{-}post}$), pre and post-menopausal environmental standard deviations ($\sigma_{E\text{-}pre}$ and $\sigma_{E\text{-}post}$) and the genetic correlation between pre- and post-menopausal women (ρG). Menopausal status was coded as 1 if post-menopausal and 0 if not (i.e., pre- or peri-menopausal). The genetic correlation reflects the degree to which the genetic effect on BMD in pre-menopausal women correlates with the genetic effect of BMD in post-menopausal women [31]. Interaction terms of menopausal status with age and age^2 were included because the relationship between age and BMD differs according to menopausal status. A more complete description of the statistical model has been previously published in a study where components of variance could vary by sex [23].

This expanded model allowed us to test several explicit hypotheses related to menopausal status by gene interactions. First, we considered if the magnitude of the genetic effect was similar between the groups by testing whether the genetic standard deviations were similar between pre- and post-menopausal women (i.e., H_0: $\sigma_{G\text{-}pre}=\sigma_{G\text{-}post}$). Rejection of this hypothesis implies that genes account for a larger proportion of the variance in one menopausal status group than in the other. A second hypothesis that we tested is whether the magnitude of the genetic correlation was significantly less than one (i.e., H_0: $\rho G_{(pre,post)}=1$). A genetic correlation between pre- and post-menopausal women that is significantly less than one implies that a different gene or suite of genes contributes to variance in BMD in preand post-menopausal women.

As before, significance testing was conducted using the likelihood ratio test. Specifically, we compared likelihoods between models in which values of $\sigma_{G\text{-}pre}$ and $\sigma_{G\text{-}post}$ were allowed to differ (full model) and in which they were constrained to be the same (restricted model). Similarly, we compared the likelihood between a model in which $\rho G_{(pre,post)}$ was estimated

(full model) to that in which its value was constrained to be one (restricted model). The degrees of freedom for the likelihood ratio test depend on whether the parameter of interest in the nested model is constrained to a boundary value (e.g., $h^2=0$, where the possible range is 0 to 1.0) or not [e.g., beta (covariate) = 0, where the possible range is $-\infty$ to $+\infty$]. If the parameter constraint is not set to a boundary, then the degrees of freedom are equal to the difference in the number of parameters between the two models. If the parameter constraint is set to a boundary, then the degrees of freedom are based on a 1/2:1/2 mixture distribution with a point mass of zero (in which case the *P* -value is obtained by dividing the *P* -value of the one degree of freedom test by two) [32].

Results

Basic characteristics of the study population are shown in Table 1. Pre-menopausal women (*n*=318) ranged in age from 20 to 57 years, while the age of post-menopausal women (*n*=252) ranged from 42 to 79. In addition to being older, post-menopausal women had significantly shorter height (*P*<0.0001) and higher BMI (*P*=0.04) compared to pre-menopausal women. Mean parity in the two groups was 5.7±3.2 and 6.2±3.9 births among pre- and post-menopausal women, respectively (*P*=0.09). BMD measurements at the spine (L1-L4), total hip and femoral neck were significantly higher in pre-menopausal women compared to post-menopausal women (*P*<0.0001). The overall (phenotypic) variance in BMD was significantly greater in post-menopausal women than in pre-menopausal women at all three BMD sites.

The numbers of pre- and post-menopausal relative pairs who were phenotyped and included in the analysis are shown in Table 2. The sample included 813 premenopausal pairs (17 mother-daughter, 267 sister-sister, 79 aunt-niece and 450 first cousin pairs) and 318 post-menopausal pairs (24 mother-daughter, 185 sister-sister, 80 aunt-niece and 29 first cousin pairs). Overall, there were 2,027 pairs of female relatives in the sample (217 mother-daughter, 547 sister-sister, 662 aunt-niece and 601 first cousin pairs).

To gain insights into the factors contributing to variation in BMD in pre- and post-menopausal women, we partitioned the total variance in BMD into components attributable to measured covariates (e.g., age, age^2, height and BMI), the additive effects of genes and to unmeasured, or residual, environmental factors. Results of these analyses are shown in Table 3. In premenopausal women, measured covariates accounted for 11% of the total variation in spine BMD, and from 37 to 38% of the variation in hip BMD. The additive effects of genes accounted for 58 to 63% of the total variation in hip BMD and 88% of the variation in spine BMD. Thus, very little of the total variation in BMD in premenopausal women could not be accounted for by genes or measured covariates. Since relatively little bone loss occurs in healthy adults prior to menopause, the variation in BMD due to genes and environmental factors in this premenopausal group is likely to reflect the variation in peak bone mass, typically achieved in the 2nd to 3rd decades of life. In post-menopausal women, the measured covariates accounted for 26% of the total variation in spine BMD and 42 to 50% of the total variation in hip BMD. The additive effects of genes accounted for an additional 54% of the variation in spine BMD and 38 to 41% of the total variation in hip BMD. An additional 20% of variation in spine BMD and femoral neck BMD and 10% of total hip

BMD could not be accounted for by genes and/or measured covariates in the post-menopausal women. The determinants of BMD variation in post-menopausal women are likely to be genes and environmental factors that influence both peak bone mass and bone loss.

Another way to interpret the genetic effects described in Table 3 is in terms of the residual heritability, or the proportion of the unexplained phenotypic variance in BMD after accounting for the effects of the measured covariates age, age^2, height and BMI. The residual heritability of BMD ranged from 0.94 [femoral neck, computed as (0.578/(1.0–0.383)] to 1.0 (total hip) in premenopausal women and from 0.64 (femoral neck) to 0.82 (total hip) in post-menopausal women. At each site, the estimated residual heritability in BMD was significantly larger in pre-menopausal women than in post-menopausal women (P<0.001 at all sites) (data not shown).

We then tested several additional hypotheses, including whether the magnitude of the genetic and environmental variances differed between women before and after menopause and whether the genetic correlation in BMD differed between pre- and post-menopausal women. To accomplish this goal, we performed a more complete partitioning of BMD into its constituent genetic and environmental components. In these analyses, we allowed the genetic and environmental variances in BMD between pre- and post-menopausal women to differ, and also estimated the genetic correlations in BMD between pre- and post-menopausal women. Results from the full model, in which all parameters were estimated, are shown in Table 4. Following estimation of the full set of model parameters, we performed a series of nested tests in which we constrained values of selected parameters, which enabled us to test, first, whether the magnitude of the genetic variance in BMD differed between pre- and post-menopausal women; second, whether the magnitude of the environmental variance in BMD differed between pre- and post-menopausal women; third, whether the genetic correlation in BMD between pre- and post-menopausal relative pairs differed from one. With respect to the first hypothesis, we observed that the genetic SD did not differ significantly between pre- and post-menopausal women ($\sigma_{G\text{-}post}$ vs. $\sigma_{G\text{-}pre}$: spine: 12.61 vs. 10.82; femoral neck: 9.19 vs. 9.63; total hip: 10.78 vs. 9.48; P >0.30 for all). In contrast, the environmental SD was greater than three-fold higher at each site in post-menopausal compared to pre-menopausal women (spine: 5.13 vs. 1.34; total hip: 4.20 vs. 0; femoral neck: 5.58 vs. 1.48), although in no case did these differences achieve statistical significance, perhaps because of the relatively small magnitude of the environmental variances in this population. With respect to the third hypothesis, we observed that the genetic correlation between pre- and post-menopausal women did not differ significantly from one for the spine (ρG=0.82, P =0.11) and femoral neck (ρG=0.95, P =0.32), although the genetic correlation did differ significantly between the two groups for total hip (ρG=0.81, P =0.025), suggesting the possibility that different sets of genes may influence total hip BMD in pre- vs. post-menopausal women.

In total, these analyses reveal total variance in BMD to be larger in post- compared to pre-menopausal women, with both measured and unmeasured environmental factors contributing to much of the excess variability in the post-menopausal group. In contrast, there was little evidence for meaningful differences in the overall contribution of genetic

factors to the variance in BMD between pre- and post-menopausal women, indicating that the magnitude of genetic influences on BMD in the two groups was approximately similar. The very high genetic correlations in BMD between pre- and post-menopausal women suggest that common genes, or sets of genes, influence BMD variation in both groups, although at the total hip there was modest evidence for unique or non-overlapping sets of genes that also influence BMD in the two groups.

Discussion

From large family samples, it has been estimated that genes account for 60–80% of the total variation in BMD [10, 18, 19, 23, 33]. However, this estimate incorporates genetic effects that occur at different ages. For example, a very large genetic contribution to acquisition of peak bone mass is well established [10], although in later years another major source of variation, especially in women, is the rate of bone loss, much of which occurs during the peri- to post-menopausal period. The overall contribution of genes to variation in bone loss is much less clear. Understanding the factors influencing bone loss is very important from a therapeutic point of view since slowing the rate of bone loss presents a potentially valuable target for prevention of age-related osteoporotic fracture.

The optimal approach for understanding the genetics of bone loss would be to follow a cohort of related individuals prospectively. However, only a few such studies have been published, and results have been inconclusive, with some reporting strong genetic effects on bone loss [22] and others relatively modest effects [11]. In the absence of more such studies, we have considered an indirect approach using a cross-sectional family sample in which we compared genetic variation in BMD in pre- and post-menopausal women ranging in age from 20 to 79 years. The variance decomposition approach we used enabled us to address whether the same genes control variation in BMD variation in the two groups of women. One would expect there to be some overlap because BMD in older women is influenced by genes affecting both peak bone mass and bone loss, while BMD in younger women is influenced by genes affecting peak bone mass only.

As expected, we observed significantly greater total variation in BMD in post- compared to pre-menopausal women. We further observed that after accounting for the effects of age, height and BMI on BMD, genes accounted for a larger proportion of the residual variation in BMD in pre- compared to post-menopausal women. These results are probably related to the fact that variation in BMD in the post-menopausal group is influenced both by factors affecting peak bone mass and factors influencing the rate of bone loss.

Our partitioning of the variance revealed little difference in the amount of genetic variance between pre- and post-menopausal women, but a 3 1/2- to 4-fold higher environmental variance in post-menopausal women. Although this difference did not achieve statistical significance, it does nonetheless suggest that environmental factors contribute largely to the greater variability observed in the post-menopausal group. In contrast, there was very little evidence for large differences in the genetic variances between the pre- and post-menopausal groups. Furthermore, for spine and femoral neck BMD, the genetic correlation between pre- vs. post-menopausal women did not differ significantly from one, consistent

with the view that the same genes, or sets of genes, act jointly on both groups of women. For total hip BMD, however, the genetic correlation, although high, was significantly less than one, thereby providing modest evidence for incomplete pleiotropy for genes influencing BMD in pre- and post-menopausal women at this site, or for the existence of some distinct genetic effects that do not act jointly on both groups of women. Such genes might presumably influence variation in rates of bone loss in the post-menopausal group. The fact that the genetic correlations were substantially greater than zero across all three sites indicates that there are at least some sets of genes that influence BMD at these sites in both sets of women jointly.

The unique attributes of the Old Order Amish make this population an attractive one for attempting to dissect out the genetic contributions to phenotypic variation. Amish families typically tend to be very large, so that there are a large number of sibling relative pairs available for analysis. Moreover, the Amish have a strong interest in their genealogies, and accurate record-keeping dating back many generations allows the large Amish families to be linked into a single pedigree. Finally, the relatively homogenous environment of this population and their reluctance to use prescription medication may allow more clear elucidation of the genetic factors contributing to BMD.

In summary, our results support the hypothesis that the same sets of genes influence BMD at the spine and femoral neck in both pre- and post-menopausal women, presumably by influencing acquisition of peak bone mass. However, we also observed some evidence for additional genetic effects acting on one group independently of the other group for total hip BMD. Such genes might play a role in bone loss. Future studies involving longitudinal follow-up of women as they lose bone will be required to elucidate the nature of these effects.

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Table 1

Characteristics (mean ± SD) of female Amish Family Osteoporosis Study participants

Variable	Pre-menopausal women (*n* = 318)	Post-menopausal women (*n* = 252)	*P* value	Equality of variance (*P* value)
Age (years)	38.0±8.1	63.8±8.6	<0.0001	0.27
Height (in)	63.5±2.0	61.8±2.6	<0.0001	<0.0001
Weight (lb)	156.8±33.5	156.8±36.1	0.95	0.20
BMI (kg/m^2)	27.4±5.6	28.9±6.4	0.04	0.02
Parity	5.7±3.2	6.2±3.9	0.09	0.0003
BMD (g/cm^2)				
Spine (L1-L4)	0.974±0.115	0.832±0.154	<0.0001	<0.0001
Total hip	0.951±0.118	0.835±0.161	<0.0001	<0.0001
Femoral neck	0.880±0.121	0.734±0.139	<0.0001	0.009

Table 2

Number of relative pairs included in the sample of 570 Amish female subjects

Relative pair class	Number
Parent-offspring (mother-daughter)	
Pre-menopausal women	17
Post-menopausal women	24
All pairs	217
Sibling-sibling (sister-sister)	
Pre-menopausal women	267
Post-menopausal women	185
All pairs	547
Avuncular (aunt-niece)	
Pre-menopausal women	79
Post-menopausal women	80
All pairs	662
Cousin-cousin (1st cousin only)	
Pre-menopausal women	450
Post-menopausal women	29
All pairs	601
Total numbers of female pairs*	
Pre-menopausal pairs	813
Post-menopausal pairs	318
Total	2,027

* Does not include grandparent-grandchild, grand avuncular, 2nd cousins, and more distantly related pairs

Table 3

Components of variance for BMD

	Pre-menopausal women (*n* = 318)			**Post-menopausal women (*n* = 252)**		
BMD site:	Measured covariates	Genetic	Residual environment	Measured covariates	Genetic	Residual environment
Spine	0.111	0.880	0.010	0.261	0.536	0.202
Total hip	0.368	0.632	0.000	0.500	0.408	0.092
Femoral neck	0.383	0.578	0.039	0.424	0.367	0.210

Measured covariates include age, age^2, height and BMI

Table 4

Model parameters estimated from variance partitioning of bone mineral density

Parameter	Spine BMD (×100)	Total hip BMD (×100)	Femoral neck BMD (×100)
μ	100.86	98.11	89.24
β(age)	−1.01	−1.35	−1.18
β(being post-menopausal)	−8.57	−14.31	−13.11
β(age* post-menopausal)	−0.41	3.40	2.08
β(age^2)	0.01	0.01	0.01
β(age^2* post-menopausal)	−0.003	−0.03	−0.02
β(height)	0.75	0.90	0.92
β(bmi)	0.84	1.34	1.18
$\sigma_{G\text{-post}}$	12.61	10.78	9.19
$\sigma_{G\text{-pre}}$	10.82	9.48	9.63
$\sigma_{E\text{-post}}$	5.13	4.20	5.58
$\sigma_{E\text{-pre}}$	1.34	[0][a]	1.48
ρG	0.82	0.81	0.95
LL(full model)	−331.70	−247.07	−241.25
Hypothesis 1:	H_0: genetic variance equal between pre-menopausal and post-menopausal women		
	Parameterization: $\sigma_{G\text{-pre}} = \sigma_{G\text{-post}}$		
LL (nested model):	−332.19	−247.58	−241.30
$\chi_{2\,1}$	0.98	1.02	0.09
P	0.32	0.31	0.76
Conclusion:	Accept H_0	Accept H_0	Accept H_0
Hypothesis 2:	H_0: environmental variance equal between pre-menopausal and post-menopausal women		
	Parameterization: $\sigma_{E\text{-pre}} = \sigma_{E\text{-post}}$		
LL (nested model):	−331.97	−247.87	−242.20
χ^2_1	0.54	1.60	1.90
P	0.46	0.20	0.17
Conclusion:	Accept H_0	Accept H_0	Accept H_0
Hypothesis 3:	H_0: genetic correlation similar between pre-menopausal and post-menopausal women		
	Parameterization: ρG = 1		
LL (nested model):	−332.43	−248.99	−241.36
χ^2_1	1.46	3.84	0.22
P *	0.11	0.025	0.32
Conclusion:	Accept H_0	Reject H_0	Accept H_0

Model parameters: μ= mean BDM; β= regression coefficients (for age, age*sex, age2, age2*sex, height and body mass index); $\sigma_{G\text{-pre}}$ = genetic SD in pre-menopausal women; $\sigma_{G\text{-post}}$= genetic SD in post-menopausal women; $\sigma_{E\text{-pre}}$= environmental SD in pre-menopausal women; $\sigma_{E\text{-post}}$= environmental SD in post-menopausal women; ρG= genetic correlation in BMD between pre-menopausal and postmenopausal women. *LL* log likelihood.

[a]Maximum likelihood estimate converged at estimate at lower boundary.

* *P* values for hypothesis 3 based on a 1/2:1/2 mixture of a χ^2_1 and a point mass of zero