The Role of Gene–Environment Interaction in Determining Bone Mineral Density in a Twin Population

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The possibility that specific environmental factors such as smoking and estrogen use modify the genetic influences (gene–environment interaction) on bone mineral density (BMD) has not been explored in genetic epidemiological studies such as twin studies. The aim of this study was to look for evidence of gene–environment interaction in BMD determination by analyzing data collected on a large number of healthy female twins. BMD of the hip, distal forearm and lumbar spine were measured by dual-energy X-ray absorptiometry on 287 identical and 265 nonidentical volunteer female twin pairs. The environmental factors examined were hormone replacement therapy (HRT) and smoking. In genetic modeling analysis using path analysis, there was evidence of 'HRT-specific' genetic component of BMD variance at the forearm (50% of total variance) but not at the hip. At the lumbar spine the magnitude of the genetic component of variance in HRT users (> 60-month HRT use) was less than the genetic component of variance for little or no exposure to HRT (48% vs. 84%). There was no evidence of gene–environment interaction for smoking. The main evidence for gene–environment interaction was the finding that forearm BMD variance was influenced by a significant HRT-specific genetic component. There was also evidence that in HRT users, the genetic component of total variance for lumbar BMD was lower.

Twin studies have shown that genetic influences play a large role in determining the population variance of bone mineral density (BMD; Arden & Spector, 1997; Hopper et al., 1998; Naganathan et al., 2002; Pocock et al., 1987). Bone mineral density is also influenced by environmental factors such as hormone replacement therapy (HRT; Anonymous, 1996; Ensrud et al., 1995) and smoking (Bauer et al., 1993; Burger et al., 1998). When assessing the role of genetic influences on a particular phenotype, it is also important to consider the possible impact of interactions between these genetic and environmental influences (gene–environment interaction).

The possibility that specific important environmental factors such as estrogen use or smoking could modify the genetic influences on BMD and vice versa have not been explored in genetic epidemiological studies such as twin studies. The classic twin design provides one approach to identify the existence of gene–environment interaction (G × E).

The aims of this study were to look for evidence of G × E between estrogen use/smoking and BMD using the classical twin design by exploring the possibility that:

1. a proportion of the total variance of BMD could be explained by an environment-specific genetic component of variance
2. the magnitude of the genetic component of variance was different between twins exposed versus unexposed to HRT or smoking.

Materials and Methods

Subjects

The study cohort consisted of 552 female like-sex, identical (MZ) and nonidentical (DZ) twin pairs. The twins were recruited through the Australian NHMRC Twin Registry and from local media campaigns. Healthy volunteer twins aged between 18 and 80 were invited to participate in an investigation into the genetics of various diseases including osteoarthritis, cardiovascular disease, asthma and osteoporosis. The hospital's Human Research Ethics Committee approved the study.

Questions on HRT use and amount smoked were part of the standard questionnaire on demographic details and risk factors for osteoporosis administered to all twins. Smoking profile (number of cigarettes a

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day and the smoking period) was used to calculate pack-years of smoking for current and ex-smokers. A pack-year is defined as smoking 20 cigarettes a day for a year. Twins who used medications or who had medical conditions that could interfere with bone metabolism were excluded from the analysis.

Zygosity was determined from the twins' self-report using questions from a validated questionnaire (Sarna et al., 1978). DNA fingerprinting was used to determine zygosity in twin pairs where their zygosity was either unknown or disputed.

Measurement of Bone Mineral Density and Quantitative Ultrasound

BMD at the left hip, forearm and lumbar spine (L1-L4) was measured on the same dual-energy X-ray absorptiometry machine (*Hologic — QDR 4500*). BMD measures were obtained of two regions of the proximal femur: total hip and neck of femur (NOF).

Statistical Analysis

To determine if genetic and environmental components of BMD variance were influenced by either HRT use or smoking, intraclass correlations were calculated and compared in twins concordant for exposure, concordant for nonexposure and discordant for exposure to the environmental factor of interest (HRT use or smoking).

Estimates of genetic and environmental effects based on comparison of intraclass correlations have limitations; therefore, a genetic model-fitting technique (twin path analysis; Lange & Boehnke, 1983; Naganathan et al., 2002; Neale & Cardon, 1992; Snieder et al., 1997; Spector et al., 2000) was also used to determine if the relative influence of genetic and environmental factors on total BMD variance differs under disparate environmental conditions. Using path analysis it is possible to quantify the genetic and environmental components of total BMD variance and, with the study aims in mind, determine if any of these components of variance are unique to HRT or smoking. In this method of analysis, a test of goodness-of-fit of the model is determined which allows comparison of alternative models. Figure 1 shows a variation on the classic twin model that allows for differences in the genetic and environmental effects on trait variance based on exposure or nonexposure to an

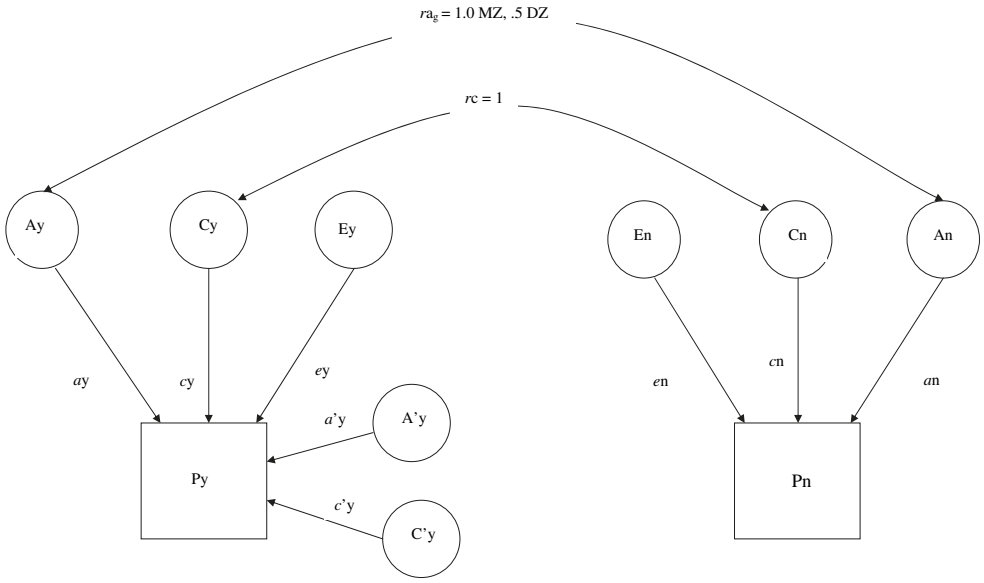

Figure 1
General G × E ACE model.

Note: General genotype × environment interaction model. Path diagram is shown for MZ and DZ twins discordant for environmental exposure. Observed variables for Twin 1 and Twin 2 are shown in the squares. Latent variables (or factors) are shown in circles. A single-headed arrow indicates a direct influence of one variable on another, its value represented by a path coefficient. Double-headed arrows indicate a correlation without any assumed direct relationship.

Explanation of symbols: y = exposed to environmental factor, n = nonexposed, A = additive genetic factor, C = common environmental factor, E = unique environmental factor, A'y = environment-specific additive genetic factor, C'y = environment-specific common environmental factor, Py (Pn) = phenotypic value of exposed and nonexposed , *a* = additive genetic factor loading, *c* = common environmental factor loading, *e* = unique environmental factor loading, *a*'y = environment-specific additive genetic factor loading in exposed, *c*'y = environment-specific common environment factor loading in exposed, ra_g = additive genetic correlation, *rc* = common environment correlation.

ra_g = 1 for MZ and .5 for DZ twins, *rc* = 1 for MZ and DZ twins.

In the analysis the data is fitted to a number of different models by varying assumptions of the model. Some examples are:

Full Model: estimate *a*'y, estimate *c*'y – *ay* ≠ *an*, *cy* ≠ *cn*, *ey* ≠ *en* (assumes presence of environment-specific additive genetic factor and common environment factor plus differences between exposed and unexposed twins in magnitude of genetic and environmental variance components).

Submodel: Fix *a*'y = 0, Fix *c*'y = 0 – *ay* ≠ *an*, *cy* ≠ *cn*, *ey* ≠ *en* (assumes no environment-specific additive genetic or common environment factors but assumes differences between exposed and unexposed twins in magnitude of genetic and environmental variance components).

Submodel: Fix *a*'y = 0, Fix *c*'y = 1 – *ay* = *an*, *cy* = *cn*, *ey* = *en* (assumes no environment-specific additive genetic or common environment factors and no differences between exposed and unexposed twins in magnitude of genetic and environmental variance components).

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Table 1

Characteristics of Postmenopausal Twins When Categorized by Zygosity and HRT Exposure

	MZ (No–No) *n* = 82 pairs	DZ (No–No) *n* = 60 pairs	MZ (Yes–Yes) *n* = 22 pairs	DZ (Yes–Yes) *n* = 11 pairs	MZ (Yes–No) *n* = 28 pairs		DZ (Yes–No) *n* = 49 pairs	
					Yes *n* = 28	No *n* = 28	Yes *n* = 49	No *n* = 49
Mean age	62.0	58.8	58.9	59.0	59.4	59.4	60.4	60.4
(*SD*)	(9.7)	(7.7)	(6.9)	(6.5)	(7.3)	(7.3)	(6.2)	(6.2)
Mean weight (kg)	65.0	67.4	63.8	68.6	66.0	61.7	69.1	70.2
(*SD*)	(11.2)	(13.0)	(8.7)	(12.2)	(11.2)	(11.2)	(6.2)	(13.1)
Mean height (cm)	159.1	160.2	160.1	161.6	158.9	159.2	160.8	160.7
(*SD*)	(7.5)	(6.1)	(4.6)	(3.8)	(4.5)	(5.6)	(6.3)	(6.2)
Median HRT duration (months)	0	0	114	144	90	0	84	1
(range)	(0–50)	(0–55)	(0–240)	(0–372)	(0–350)	(0–48)	(0–372)	(0–48)
Median pack-year (range)	0 (0–95)	0 (0–107)	0 (0–42)	0 (0–56)	0 (0–23)	0 (0–48)	0 (0–86)	0 (0–116)

Note: Yes = > 60 months of exposure to HRT, No = ≤ 60 months of exposure to HRT

SD = standard deviation

environmental factor. The raw data are analyzed by maximum likelihood as various models are compared to arrive at a model that provides the best explanation of the data. The footnote for Figure 1 provides a detailed explanation of these models. The model shown is for the twins discordant for the environmental exposure of interest, although in the analysis all twin groups were analyzed simultaneously.

MZ and DZ covariances matrices were computed for twins concordant for exposure, concordant for nonexposure and discordant for exposure for the environmental factor of interest (HRT use or smoking). Structural models were then fitted to the resulting six zygosity–environment groups. A number of different models were compared to see which model best explained the data (Figure 1). The choice of submodels for testing was determined by the parameter estimates and model-fitting statistics of the initial 'full model'. By comparing models with and without an environment-specific genetic factor (A'y) it was possible to test whether there was a significant unique environment-specific genetic variance component. By comparing models where A (additive genetic), C (shared/common environment) and E (nonshared/unique environment) effects were the same ($ay = an$, $cy = cn$ and $ey = en$) to models where they were different between exposed and unexposed twins ($ay \neq an$, $cy \neq cn$, $ey \neq en$), the magnitude of genetic components of variance could be compared under different environmental conditions.

Using this method it was possible to 'test' a number of explanations for the observed covariances for BMD between twin pairs. In deciding the model with the best fit, submodels were compared with the full model by hierarchical χ^2 tests, and the decision was also guided by the minimum value of the Akaike Information Criterion, which is equal to $\chi^2 - 2$ degrees of freedom (*df*). The magnitude of A, C, and E components of variance were expressed as a standardized ratio of a^2, c^2 and e^2 respectively over total phenotypic variance.

Data handling and preliminary analysis were done with STATA and quantitative genetic modeling was performed with Mx (Neale et al., 1998).

Results

G × E (HRT Duration)

Only postmenopausal females (132 MZ and 120 DZ pairs) were included in these analyses. Individuals with more than 60 months of exposure to HRT were considered as having a positive exposure to HRT (Yes). No HRT use or less than 60 months was considered nonexposure (No). The study sample was not large enough to consider nonexposure to HRT as only those who had never taken HRT. In the groups categorized as 'no exposure to HRT', 55% to 65% had never used HRT. Table 1 shows the characteristics of the group studied when divided by zygosity and HRT use. There was no significant difference in mean age between any of the groups. There were some weight differences between groups but BMD values were adjusted for weight along with height, age and age^2. There was no significant difference in pack-years smoked between the Twin 1 group and Twin 2 group for the six zygosity–HRT exposure groups (Wilcoxon's signed rank test).

Correlations

The differences in correlation between MZ and DZ twins for concordant nonexposed, concordant exposed and discordant groups are similar for total hip and femoral neck BMD (see Table 2). At the forearm and lumbar spine, the difference in correlation between MZ and DZ discordant pairs, however, was lower than the

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Table 2

Intraclass Correlation Coefficients for BMD (G × HRT) Categorized by Zygosity and HRT Exposure

	MZ (No–No) *n* = 82 pairs	DZ (No–No) *n* = 60 pairs	MZ (Yes–Yes) *n* = 22 pairs	DZ (Yes–Yes) *n* = 11 pairs	MZ (Yes–No) *n* = 28 pairs	DZ (Yes–No) *n* = 49 pairs
Hip	.77 (.69–.86)	.33 (.10–.55)	.72 (.52–.92)	.47 (.0–.94)	.69 (.49–.89)	.37 (.12–.61)
NOF	.82 (.75–.89)	.33 (.10–.56)	.77 (.60–.94)	.28 (.0–.84)	.81 (.68–.94)	.39 (.15–.63)
Arm	.79 (.71–.87)	.35 (.13–.57)	.80 (.65–.95)	.37 (.0–.89)	.41 (.10–.72)	.27 (.01–.53)
Lumbar	.82 (.74–.89)	.36 (.14–.58)	.53 (.23–.84)	.42 (.0–.92)	.58 (.33–.83)	.37 (.13–.61)

Note: BMD adjusted for age, age^2, weight and height

95% confidence intervals are shown in brackets

difference between MZ and DZ concordant pairs, which may indicate differences in the genetic components of BMD variance as a result of HRT use. It is important to note that the number of twin pairs in each group was small. As a result the confidence intervals for correlation estimates were wide.

Model-Fitting Analysis

A summary of the best fitting models for all BMD sites are shown in Table 3. (Details of all models compared at each BMD site are available from authors on request.) Total hip BMD and NOF BMD were best explained by the AE models that assumed no environment-specific (HRT) additive genetic or common environment-specific effects. In addition the magnitude of genetic variance components were the same for exposed (more than 60 months HRT duration) and nonexposed (60 months or less HRT duration) groups.

At the forearm, the data were best explained by the model which assumed a significant environment-specific additive genetic effect. A large proportion (64%) of the genetic component of variance for HRT users of more than 60 months was unique to this group and not shared by the nonexposed group. The data could not be explained by HRT-related differences in the specific environment component of variance. The proportion of total variance explained by genetic factors (either unique or common) was similar between the two groups (heritability .78 for exposed vs. .83 for nonexposed).

At the lumbar spine there was no evidence of environment-specific genetic effects but the proportion of total variance explained by genetic factors (heritability) was lower in the HRT group. A common environmental component was significant in only the exposed group (see Table 3).

The main conclusions based on the results of genetic modeling as described above (summarized by the best fitting models shown in Table 3) are (1) HRT-specific genetic component was present only at the forearm, (2) the magnitude of the genetic component of variance in HRT users of more than 60 months was less compared to users of 60 months or less at the lumbar spine, and (3) HRT did not influence the genetic components of variance of hip BMD.

There was no evidence of a significant HRT-specific common environmental factor. Adjusting the BMD values for HRT duration in addition to age, age^2, weight and height made no difference to these conclusions.

G × E (Smoking — Pack-Years)

In this analysis, only female twin pairs aged 40 or older who were concordant for menopausal statuses were included. Individuals with a 10 pack-year history or more of smoking were considered to have a positive exposure to smoking (Yes). Twins who never smoked or had a less than 10 pack-years of exposure were categorized as nonexposure (No). Table 4 shows the number of twin pairs in each group when divided by zygosity and smoking exposure. There was no significant difference in mean age between any of the groups (data not shown). There was no significant difference in HRT exposure between the Twin 1 group and Twin 2 group for the six zygosity–HRT exposure groups (Wilcoxon's signed rank test). The intraclass correlations for BMD are shown in Table 4. The main finding is that the differences in correlation between MZ and DZ pairs were highest for twins concordant for exposure to more than 10 pack-years. This would be consistent with an increased genetic variance component in smokers but may also be related to sampling problems as there were only 23 MZ and DZ pairs who were concordant for a positive exposure to smoking. In the model-fitting analysis (data not shown), the data for all BMD sites was best explained by an AE model that had no smoking-specific genetic or shared environmental component. Any differences in magnitude of genetic variance components between more than 10 pack-years of exposure and nonexposure were not

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Table 3

Standardized Parameter Estimates and Model Fit Statistics of Best Fitting Models for BMD for G × E (HRT) Models

BMD site	Description best model	Squared standardized coefficients				Fit statistics			
		A'y estimate (95% confidence interval)	A estimate (95% confidence interval)	C estimate (95% confidence interval)	E estimate (95% confidence interval)	*df*	χ^2	*p* value	AIC
Total hip	AE model	*ns*	.79 (.72–.83)	—	.21 (.16–.28)	16	22.0	.1	−10.0
	No significant A'y		Yes and No		Yes and No				
	A & E equal for Y & N								
NOF	AE model	*ns*	.85 (.79–.88)	—	.15 (.12–.21)	16	19.1	.27	−12.9
	No significant A'y		Yes and No		Yes and No				
	A & E equal for Y & N								
Forearm	AE model	.50 (.25–.72)	.28 (.08–.52) Yes	—	.22 (.15–.30) Yes	16	19.1	.27	−15.5
	Significant A'y		.83 (.76–.87) No		.17 (.12–.23) No				
	Heritability for Y = .50 + .28 = .78								
Lumbar	ACE (Y)/AE (N) model	*ns*	.48 (.22–.71) Yes	.33 (.08–.58) Yes	.19 (.13–.28) Yes	14	17.1	.25	−10.9
	No significant A'y		.84 (.77–.87) No	Fixed (= 0) No	.16 (.12–.22) No				
	A lower in Y + significant C for Y								

Note: A = additive genetic factors, C = shared environmental factors, E = unique environmental factors, A'y = environment-specific additive genetic factors, *ns* = not significant, *df* = degrees of freedom, AIC = Akaike's information criterion, Y = > 60 months of exposure to HRT, N = ≤ 60 months of exposure to HRT

significant. These findings remained unchanged when BMD was adjusted for duration of HRT use or restricted to postmenopausal twins.

Discussion

This study looked for a number of possible manifestations of gene–environment interaction in data from a population-based twin study. The main findings were that for total forearm BMD variance there was a significant HRT-specific genetic variance component. There was also evidence that in HRT users the genetic component of total variance for lumbar BMD was lower.

One explanation for the finding of a HRT-specific genetic variance component for forearm BMD may be that such 'HRT genes' have a different influence on forearm BMD variance than the genes common to both 'exposed' and 'unexposed' groups. At the forearm, it could be that the common genetic component between exposed and nonexposed twins reflects the genetic influence on peak bone mass variance. Some of these common genes may also influence bone loss as it has been shown in a twin study, using similar methodology to our study, that the majority of genes that act pre- and postmenopausally on BMD variance are the same (Hunter et al., 2001). The influence of the unique HRT-specific genetic component, on the other hand, is likely to be predominantly on estrogen-dependent postmenopausal bone loss. The mechanisms behind how HRT prevents bone loss are not clear but this study would suggest it might involve genetic mechanisms that are different from the mechanisms involved in the attainment of peak bone mass and subsequent bone loss.

The finding that the genetic component of variance was lower for lumbar BMD for the group of women with a longer exposure to HRT is not unexpected since HRT does prevent postmenopausal bone loss, particularly at trabecular bone sites (forearm and lumbar spine). The significant common environment influence on BMD variance in twins concordant for HRT exposure (ACE model) is largely due to the fact that both twins shared a long exposure to HRT, as the shared environmental component was not significant (AE model) in twins concordant for nonexposure or discordant for HRT use.

It is interesting that in concordant HRT users at the forearm, unlike the lumbar spine, an HRT-specific genetic component in the genetic model, rather than a greater shared environmental component, better explained the data. This suggests that HRT use was more strongly associated with lumbar spine BMD than at other sites. In this context it is unlikely that in a study of this size, a smaller genetic-specific component of variance in lumbar spine BMD would be significant, especially when the overall genetic component of variance is less. The finding of a significant common environmental component for lumbar spine BMD also suggests that HRT use may be associated with confounders that were not measured in this study

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Table 4

Intraclass Correlation Coefficients for BMD (G × Smoking)

	MZ (No–No) *n* = 141 pairs	DZ (No–No) *n* = 119 pairs	MZ (Yes–Yes) *n* = 23 pairs	DZ (Yes–Yes) *n* = 23 pairs	MZ (Yes–No) *n* = 30 pairs	DZ (Yes–No) *n* = 35 pairs
Hip	.79 (.73–.85)	.50 (.37–.64)	.88 (.79–.97)	.03 (.0–.44)	.64 (.42–.85)	.33 (.03–.63)
NOF	.81 (.75–.87)	.49 (.35–.62)	.93 (.87–.98)	.0 (.0–.41)	.75 (.58–.91)	.20 (.0–.52)
Arm	.72 (.64–.80)	.29 (.12–.45)	.82 (.69–.95)	.25 (.0–.64)	.71 (.53–.89)	.40 (.12–.68)
Lumbar	.78 (.72–.85)	.46 (.32–.60)	.78 (.62–.94)	.30 (.0–.68)	.70 (.52–.88)	.23 (.0–.55)

Note: Yes = > 60 months of exposure to HRT, No = ≤ 60 months of exposure to HRT

that could influence BMD variance such as socioeconomic status, physical activity and other lifestyle factors. In addition, a previous analysis on this population of twins showed that for models of the lumbar spine, data in twins aged over 50 were of poorer fit because of the effects of osteoarthritis on lumbar spine BMD measurements (Naganathan et al., 2002).

The genetic components of total hip and NOF BMD variance did not appear to be significantly influenced by HRT use. Any HRT influence on BMD variance did not involve a HRT-specific genetic variance component as was seen in the forearm. In HRT-concordant users the common use of HRT did not result in this common environment factor becoming significant in the path analyses models (compare this with the lumbar spine). One explanation is that HRT use has less of an influence on hip BMD than forearm and lumbar BMD, which have a higher trabecular bone component. Clinical trials, however, have shown that HRT use does have significant effects on hip BMD (Wells et al., 2002). It could be that the biological mechanisms of HRT effects on bone are not the same at all sites.

The biological mechanisms for how estrogen effects on bone and bone loss could be influenced by genetic polymorphisms are not clear (Riggs et al., 2002). The authors are unaware of any direct studies that show how estrogen biological effects on bone are influenced by genetic factors. Allelic variations in the estrogen-receptor-α gene has been shown in some but not all studies to be a determinant of BMD (Albagha et al., 2001; Becherini et al., 2000). The next step would be for the hypothesis tested in this study to be investigated in studies specifically designed to look directly for gene–environment interaction with estrogen. One way would be to look for specific interaction between genotype and estrogen use in longitudinal epidemiological studies that have BMD change as the main outcome. The other would be to design clinical trials of estrogen use so that the influence of genotype on BMD change could be determined.

With regards to possible gene × smoking interaction, it has been shown that cigarette-smoking condensates can activate estrogen receptors resulting in induction of estrogen-regulated genes (Meek & Finch, 1999). There have, however, not been any studies that have looked specifically at whether smoking modulates the association between specific genotypes and BMD. Our study, admittedly with limited power to do so, did not show any significant interaction between genetic influences on BMD variance and smoking.

The limitations of this study relate to the small size of the study. The main limitation of the genetic modeling analysis is that only a small number of twin pairs concordant for a positive exposure (e.g., for HRT, 22 MZ and 11 DZ) and discordant for exposure (e.g., for HRT, 28 MZ and 49 DZ) were seen. As a result the confidence intervals for correlation coefficients are wide. Much larger numbers in these groups may have made a difference to the choice of best fitting models. Again due to the limitations of sample size it was necessary to categorize 'no HRT exposure' as being less than 60 months of exposure rather than as never having HRT. The majority of people in the 'no HRT' group had never taken HRT but the inclusion of people with some exposure would have 'diluted' differences between groups based on HRT use and zygosity and biased the results towards the null hypothesis. It is also important to note that the methodology used in this study has determined the genetic and environmental components of BMD variance in a population. This is not the same as determining the genetic and environmental factors that determine mean BMD.

To conclude, this study is unique in that none of the previous twin studies on bone mass looked directly at whether gene–environment interaction had a role to play in determining BMD and bone quality. The main evidence for G × E was the finding that HRT influences forearm BMD variance via a unique genetic path.

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