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ORIGINAL RESEARCH

Heritability of Bone Mineral Density in a Multivariate Family-Based Study

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Abstract There is evidence for a genetic contribution to bone mineral density (BMD×). Different loci affecting BMD have been identified by diverse linkage and genome-wide association studies. We studied the heritability of and the correlations among six densitometric phenotypes and four bone mass/fracture phenotypes. For this purpose, we used a family-based study of the genetics of osteoporosis, the Genetic Analysis of Osteoporosis Project. The primary aim of our study was to examine the roles of genetic and environmental factors in determining osteoporosis-related phenotypes. The project consisted of 11 extended families from Spain. All of them were selected through a proband with osteoporosis. BMD was measured using dual-energy X-ray absorptiometry. The proportion of variance of BMD attributable to significant covariates ranged from 25 % (for femoral neck BMD) to 48 % (for whole-body total BMD). The vast majority of the densitometric phenotypes had highly significant heritability, ranging from 0.252 (whole-body total BMD) to 0.537 (trochanteric BMD) after correcting for covariate effects. All of the densitometric phenotypes showed high and significant genetic correlations (from −0.772 to −1.000) with a low bone mass/osteopenia condition (Affected 3). Our findings provide additional evidence on the heritability of BMD and a strong genetic correlation between BMD and bone mass/fracture phenotypes in a Spanish population. Our results emphasize the importance of detecting genetic risk factors and the benefit of early diagnosis and especially therapeutic and preventive strategies.

Keywords Heritability · Bone mineral density · Family-based genetic study · Osteoporosis · Genetic variation

The authors declare that they have no competing interests.

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Introduction

Osteoporosis is one of the world's most significant health issues and inflicts substantial social, economic, and clinical burdens [1]. It is a common systemic skeletal disorder characterized by low bone mass and microarchitectural deterioration of bone tissue, resulting in increased bone fragility and susceptibility to fracture, usually hip, vertebral, and wrist [2]. It has been estimated that osteoporosis affects 200 million people worldwide [3] and that over 9 million osteoporotic fractures occur every year [4].

The diagnosis of osteoporosis and the assessment of fracture risk are based on the measurement of bone mineral density (BMD) [5]. There are various causes of osteoporotic fractures, but the most important is low BMD. Since low BMD is a risk factor for fracture, therapeutic decisions to prevent fracture are based often on BMD measurements. Thus, for effective prevention of osteoporosis, it is necessary to identify factors involved in determining BMD.

There is strong evidence that genetic factors contribute to osteoporosis risk, such as BMD, bone turnover markers, as well as structural and strength properties of the hip [4,

6–11]. Also, there is significant evidence from studies of peak bone mass that genes contribute to the variation of BMD. At older ages, a large genetic contribution to BMD has been also demonstrated [12]. Environmental factors such as physical activity, lifestyle, and calcium intake may affect BMD also [13–15].

Thus, it is clear that risk of osteoporosis depends upon genetic and environmental factors acting jointly. With recent advances in genetics and epidemiology it is possible to quantify the genetic determinants of osteoporosis. The data indicate that 60–90 % of BMD variation can be explained by genetic factors [16, 17]. In addition, there is evidence for a genetic contribution to BMD, and different loci affecting BMD have been identified by diverse linkage [18–20] and genome-wide association studies [21]. Furthermore, variance component analysis increases the statistical power of genetic studies on families with extensive genealogy. For this reason, we designed a family-based study of the genetics of osteoporosis, the Genetic Analysis of Osteoporosis (GAO) Project. We studied a sample of extended families ascertained through individuals with osteoporosis. The primary aim of our study was to examine the relative roles of genetic and environmental factors in determining osteoporosis-related phenotypes.

Methods

The GAO Project included 11 extended families from Spain. We selected these families primarily on the basis of pedigree size, to maximize the statistical power of detecting genetic effects. In particular, to be included in the project, a family had to have at least 10 living individuals distributed in three or more generations. The structure of the families was verified by use of microsatellite genotyping and control for Mendelian inconsistencies [22]. Large pedigrees have comparably more power per sampled individual than small families, partially compensating for small sample sizes [23].

The enrollment period for our study was between March 2009 and March 2012. All of the families were selected through a proband with osteoporosis, which was defined as (1) hip neck, total hip, or spine BMD yielding a T score < -2.5 or (2) the occurrence of at least one osteoporotic fracture in subjects over 21 years of age.

A medical history was obtained from all of the participants. It included menstrual period, history of all clinical fractures (traumatic and nontraumatic), and current medications with a negative (e.g., corticoids, heparin, proton pump inhibitors, insulin, or thiazolidinediones) or a positive (e.g., bisphosphonates, calcium, strontium, parathyroid hormone, thiazide diuretics, vitamin D) effect on bone remodeling. Coffee, alcohol, and smoking habits; dietary calcium intake; sun exposure; and physical activity were recorded also. The questionnaire and definitions are available in the Appendix.

Table 1 Description of the phenotypes studied in the GAO Project

Trait abbreviation	Description
HipTotBMD	Total BMD of hip (g/cm^2)
InterBMD	BMD of intertrochanteric area (g/cm^2)
NeckBMD	BMD of femoral neck (g/cm^2)
SpineBMD	Total BMD of spine (g/cm^2)
TrochBMD	BMD of trochanteric area (g/cm^2)
WBTotBMD	Total BMD of the whole body (g/cm^2)

BMD bone mineral density

The ethics committee of our institution approved all recruitment protocols (08/015/281). Adult subjects gave informed consent for themselves and for their minor family members.

Spine, femur, and whole-body dual-energy X-ray absorptiometry (DXA) scans were performed on all participants using a Discovery DXA system with APEX v2.3 software (Hologic, Bedford, MA, USA), following the manufacturer's recommendations. The measurement of total-hip BMD is a conglomerate of femoral neck, trochanteric, Ward's triangle, and other components. To analyze strength and geometrical properties of the hip, we used the hip structural analysis software included in APEX. Scans were performed and reviewed by the same technician and physician, both of them certified by the International Society for Clinical Densitometry. Our study focused on six densitometric phenotypes that we considered clinically relevant. Table 1 contains a description of the phenotypes as well as a guide for the abbreviations.

Apart from the six osteoporosis-related quantitative traits mentioned previously, we studied also four categorical phenotypes of particular clinical interest:

1. Phenotype "Affected 1," corresponding to low bone mass according to the most common definition of osteoporosis; it included individuals $\geq$21 years old who presented one or more of the following characteristics: (1) T score ≤ -2.5 (column, hip neck, or total hip), (2) at least one osteoporotic (nontraumatic) fracture, (3) antiresorptive or forming agent treatment.
2. Phenotype "Affected 2," corresponding to patients who suffered at least one osteoporotic fracture.
3. Phenotype "Affected 3," corresponding to a broad spectrum of skeletal conditions, encompassing (1) "Affected 1" individuals and (2) patients with a T score ≤ -1 (column, hip neck, or total hip).
4. Phenotype "Affected 4," corresponding to an extension of "Affected 1" as it includes also individuals $<$21 years old who presented Z scores ≤ -2.5 (column, hip neck, or total hip).

We checked the 11 pedigrees for Mendelian inconsistencies with FBAT v2.0.3 [24], and we corrected most of

them by genotyping. However, in two family branches involving nine individuals, we discovered systematic inconsistencies for more than one microsatellite marker, so those participants were excluded from our study [22].

For usual statistical analysis, SPSS 21 software was used (SPSS, Chicago, IL, USA).

We used a variance component analysis to determine the contribution of genetic and individual-specific environmental factors to the variation of intermediate (i.e., quantitative traits) and final (i.e., status) osteoporotic phenotypes in the GAO pedigrees.

We modeled the level of a trait y for individual i (y_i) as a linear function as follows:

$$y_i = \mu + \Sigma\beta_j x_{ij} + g_i + e_i$$

where μ is the trait mean, x_{ij} is the jth covariate and β_j is its regression coefficient.

Covariates included age, age^2, gender, body mass index (BMI), age at menopause for postmenopausal women, alcohol intake, smoking, and use of osteoporosis-related medication, as well as interactions of age and age^2 with gender. Age-related covariates were scaled so that their regression coefficients represented the effect produced by a 10-year deviation from the mean age. Discrete covariates (gender, alcohol intake, and smoking) were scaled so that regression coefficients represented the effect of the covariate presence versus absence. A special case is the use of osteoporosis-related medication, which was scaled so that its regression coefficient represented a positive, negative, or no effect of the covariate.

The remaining variables, g_i and e_i, represent the random deviations from μ for individual i that are attributable to additive genetic and residual error effects, respectively. The residual error component included true random error, measurement error, and any nonadditive genetic components. The effects of g_i and e_i were assumed to be not correlated with one another and normally distributed with mean $= 0$ and variances σ^2_g and σ^2_e. The likelihood of the phenotypes of the family members is assumed to follow a multivariate normal distribution with a phenotypic covariance matrix that is a function of the kinship between individuals and the additive genetic and environmental variances.

We used the maximum-likelihood methods implemented in SOLAR v4.3.1 [23] to estimate simultaneously the mean and variances, as well as the covariate and genetic effects, for each trait. We assessed the significance of such effects with a likelihood-ratio test [25, 26]. Finally, we estimated the heritability for each trait as the proportion of the total phenotypic variability attributable to additive genetic effects. For this particular estimation, we considered only environmental covariates (i.e., we performed the analysis without BMI and age at menopause for postmenopausal women).

To study the genetic relationships between status phenotypes and quantitative variation in osteoporosis-related intermediate phenotypes, we used a modified variance component method for mixed discrete/continuous traits [27] incorporated in SOLAR. This method allowed for the phenotypic correlations between pairs of traits to be separated into common genetic influences and common environmental influences. The separation of phenotypic correlations (ρ_p) into genetic (ρ_g) and environmental (ρ_e) components is a valuable tool because it reveals hidden relationships among traits [28].

Results

We enrolled 681 individuals from the 11 extended families. Once we excluded the deaths, children of early age (<5 years), and individuals with incomplete data or who we were unable to recruit, there remained 376 individuals available for the study. Finally, another 9 of the 376 subjects were excluded due to Mendelian inconsistencies, yielding a final sample size of 367 individuals.

The general characteristics of the 11 pedigrees as well as those of the probands used for recruitment are described in Table 2. Sample size per family ranged from 15 to 91, and the male to female gender ratio was 1.07. The ages ranged from 5 to 93 years (median 41). Moreover, the age of the 11 probands ranged from 40 to 89, and only one of the probands was male, while three probands presented multiple osteoporotic fractures. The total number of individuals with osteoporotic fractures in the cohort was 24 (6.5 % of the total sample size). Their distribution in the 11 pedigrees was nonrandom: 14 fractures occurred in families 1, 3, and 11, whereas no fractures were reported in families 4 and 8.

The covariates that had significant effects on BMD ($p < 0.05$) appear in Table 3. From the covariates that were initially included in the model, smoking and use of osteoporosis-related medication did not have any significant effect on the final phenotypes and, therefore, are not shown. Alcohol consumption was dichotomized between two groups: no consumption plus low consumption (group 0) and consumption above 30 g/day (group 1), even though there was no significant correlation with BMD. The rest of the environmental covariates collected and described in the Appendix did not show significant correlation. Table 3 shows the proportion of variance of BMD that is attributed to significant covariates, ranging from 25 % (for Neck-BMD) to 48 % (for WBtotBMD).

The heritability of each of the densitometric phenotypes is shown in Table 4 and is based on the most parsimonious model of variance component analysis for each phenotype, including only significant sources of variation. The remaining variance not accounted for in Table 4 is

Table 2 Characteristics of the 11 probands and distribution of individuals by pedigree

Pedigree	*n*	M:F ratio	Median age	Age range	Proband age	Proband sex	Proband HipNeckT T	Proband HipTotT	Proband SpineT	Proband Fx
1	57	0.73	40	7–75	75	F	−2.2	−1.3	−3.1	2
2	91	1.76	38	10–89	89	F	−4.2	−3.3	−1.9	0
3	23	0.77	41	12–91	67	F	−2.4	−1.6	−2.8	0
4	34	1.00	53.5	14–93	84	F	−3.6	−3.4	−1.3	5
5	19	0.90	40	6–76	76	F	−3.1	−1.9	−4.2	0
6	31	1.58	44	8–80	80	F	−1.4	−1.1	−4.7	0
7	22	1.00	47	10–86	86	F	−3.2	−2.0	−2.0	1
8	15	1.14	46	7–80	78	F	−3.4	−2.7	−3.3	0
9	30	0.58	37.5	5–78	78	F	−3.0	−2.2	−3.2	0
10	30	1.31	50.5	8–82	73	F	−0.9	−0.4	−2.7	0
11	15	0.67	39	5–69	40	M	−2.8	−2.8	−4.4	2
Total	367	1.07	41	5–93						

n family size, *M* male, *F* female, *T T* score, *Fx* fractures

Table 3 Regression coefficients for statistically significant covariate effects

Trait	Age	Female sex	BMI	Menopause age	Var. expl.
Mineral density/content					
HipTotBMD		−0.075	0.012	−0.002	0.3804
InterBMD		−0.089	0.014	−0.003	0.4138
NeckBMD	−0.002	−0.043	0.008	−0.002	0.2538
SpineBMD	0.003		0.008	−0.003	0.3459
TrochBMD	−0.001	−0.047	0.008	−0.001	0.2653
WBTotBMD	0.002	−0.043	0.008	−0.002	0.4835
Disease status					
Affected 1	−0.048		0.064		–
Affected 2					–
Affected 3	−0.053		0.084		–
Affected 4	−0.028		0.057		–

Only significant *p* values are shown ($p < 0.05$). Empty spaces mean that the effect was not significant ($p \geq 0.05$)

var. expl. variance explained by adjusted covariates, – not analyzable

attributable to random individual-specific environmental influences and random error. All of the results were statistically significant ($p < 0.05$). The spine and hip densitometric scans of two participants were excluded due to previous bilateral total hip replacement surgery and vertebral fractures.

The vast majority of the densitometric phenotypes showed highly significant heritability, ranging from 0.252 (WBTotBMD) to 0.537 (TrochBMD) after correcting for covariate effects.

Heritability of the phenotype (Affected 1–4) was generally higher when compared to the six quantitative traits. We observed a heritability of 0.501 for osteoporosis (Affected 1), whereas the highest heritability (0.827) was observed for both osteoporotic fractures (Affected 2) and a compound status condition including osteoporosis and low bone mass (Affected 3).

Table 5 shows the correlation of each of the six densitometric traits with the four different status phenotypes on the phenotypic (ρ_P), genetic (ρ_G), and environmental (ρ_E) levels. In general terms, the phenotypic correlation can be considered the result of the mathematical combination of genetic and environmental correlation. Any correlation $\geq$0.70 was considered strong (Table 5). The majority of the correlations were negative.

The most significant correlations were observed on the genetic level (Table 5). We observed the highest correlations for TrochBMD (from −0.704 to −0.891), with three different status phenotypes (Affected 1, 3, and 4). Moreover, all densitometric phenotypes showed high and significant genetic correlations (from −0.772 to −1.000) with low bone mass/osteoporotic condition (Affected 3). Few high and significant correlations were observed on the environmental level and none on the total phenotypic level. The highest correlations on the environmental level were identified between Affected1 and NeckBMD (−0.692) and between Affected4 and NeckBMD (−0.726).

Discussion

The aim of the GAO Project, based on extended pedigrees, is to examine the role of genetic and environmental factors in determining osteoporosis-related phenotypes. One of the most important advantages of our study design is the extent and variety of phenotypic traits that we included. In contrast, most studies have examined a relatively low number

of phenotypes. Our results include six quantitative phenotypes and four status phenotypes, all of them highly relevant clinically.

From the covariates that were initially included in our model, a statistically significant correlation with mineral density traits was observed for females, age, BMI, and age at menopause. The variance explained by these covariates ranged from 0.25 to 0.48. These findings are in accordance with previous data [18, 29–36]. The correlation of age and BMD was surprisingly positive. It has to be taken into account that in our sample one-third of the population was younger than 30 years (before peak bone mass is reached), and it is known that the projected area of the spine in children's DXA can be lower than reality. Moreover, about 10 % of the population was older than 70 years, and the spinal degenerative changes in these patients could interfere with an accurate estimation of BMD, leading to overestimation.

The status phenotypes were significantly influenced by age and BMI, with age exerting a negative and BMI exerting a positive influence. High BMD was related to young age and high BMI. These results agree with those from other studies [35]. This relation was not present in Affected 2 (osteoporotic fracture), possibly due to the small sample and homogeneity of the age of patients with fractures.

In our study, the estimates of heritability of mineral density traits and phenotype status were clearly significant. Our estimates of heritability were generally lower than those reported elsewhere [6, 37], which could be explained by the fact that our study used family data. It is well known that family-based designs provide more conservative estimates of heritability compared to linkage [18, 20, 30, 31, 37–39] and genome-wide association [21]. Another possible explanation for the low h^2 values could be that children were included in our sample. When we analyzed the heritability of different phenotypic statuses, we obtained estimates that were higher (50–82 %) than those observed in other studies, although another study found that osteoporotic fracture had a lower heritability for wrist (54 %) and hip (68 %) in perimenopausal women [40]. These data cannot be compared directly to our data because of the heterogeneity of classification of fractures. The reviews carried out in genome-wide association studies for fractures concluded that many limitations exist in these results because the genetics of fracture risk is poorly understood, and much progress is likely to be made through the dissection of fracture risk, independently of BMD [21].

To our knowledge, our study is the largest Spanish family study to examine genetic correlations between various pairs of BMD traits (measured at different body sites) and phenotype status. We observed strong and significant genetic correlations between diverse BMD traits and statuses, especially Affected 3. Other studies have examined the relation between BMD traits at two different sites (demonstrating high heritability [>0.5], much higher than the environmental contribution [9, 34, 37, 41, 42]).

We found that the lowest genetic correlation of densitometric BMD traits was with the Affected 2 phenotype (presence of fractures), probably reflecting the fact that fractures depend less on genetic than on environmental factors. This information has been reflected in diverse studies [21, 41]. Our study confirms that genetic variants contributing to the low bone mass do not appear to

Table 4 Heritability of the phenotypes in the GAO Project

Trait	h^2 (h^2 s)	p
Mineral density/content indices		
HipTotBMD	0.427 (0.101)	1.6×10^{-06}
InterBMD	0.364 (0.101)	1.79×10^{-05}
NeckBMD	0.492 (0.101)	1×10^{-07}
SpineBMD	0.414 (0.100)	2.1×10^{-06}
TrochBMD	0.537 (0.108)	1×10^{-07}
WBTotBMD	0.252 (0.085)	2.59×10^{-04}
Disease status		
Affected 1	0.501 (0.268)	2.77×10^{-02}
Affected 2	0.827 (0.384)	1.41×10^{-02}
Affected 3	0.827 (0.217)	1.4×10^{-05}
Affected 4	0.582 (0.233)	5.78×10^{-03}

(h^2 s) h^2 standard error

Table 5 Phenotypic, genetic, and environmental correlations of intermediate phenotypes with four different disease phenotypes

Trait	Affected 1			Affected 2			Affected 3			Affected 4		
	ρ_p	ρ_g	ρ_e	ρ_p	ρ_g	ρ_e	ρ_p	ρ_g	ρ_e	ρ_p	ρ_g	ρ_e
HipTotBMD	−0.512	−0.659	−0.452	−0.250	−0.414	−0.177	−0.365	−1.000*	−0.046	−0.513	−0.599	−0.491
InterBMD	−0.420	−0.532	−0.394	−0.220	−0.501	−0.117	−0.257	−0.984*	0.063	−0.429	−0.486	−0.436
NeckBMD	−0.640	−0.572	−0.692	−0.281	−0.208	−0.326	−0.524	−0.772*	−0.372	−0.623	−0.500	−0.726*
SpineBMD	−0.421	−0.503	−0.406	−0.036	0.237	−0.166	−0.171	−0.796*	0.164	−0.424	−0.457	−0.442
TrochBMD	−0.571	−0.779*	−0.408	−0.269	−0.272	−0.267	−0.446	−0.891*	−0.127	−0.554	−0.704*	−0.432
WBTotBMD	−0.344	−0.516	−0.333	−0.078	0.140	−0.147	−0.051	−0.911*	0.246	−0.359	−0.469	−0.381

* Results with high and significant genetic correlations

influence the risk of fracture. As a consequence, there is a need to investigate other genetic loci that could influence osteoporotic fractures independently from BMD [41, 43].

One of the strengths of our study is its design, based on the analysis of extended pedigrees, which may better estimate the genetic influences than other studies. For example, twin studies tend to overestimate the genetic contribution to the phenotype because environmental factors are more likely to be shared between twins than between nontwin siblings [38, 39]. At the same time, the inclusion of many members of the same family makes the separation of genetic from common environmental effects more challenging [30].

The strong genetic influences on BMD and status observed in our population provide a strong motivation for pursuing gene-mapping strategies, such as genome-wide linkage and association analyses. Recently, genome-wide association studies have had considerable success in identifying replicated loci that are associated with low bone mass and osteoporotic fractures [21].

In conclusion, our findings provide additional evidence of the statistically significant heritability of and strong genetic correlation between BMD and phenotypes status in a Spanish population. Our results emphasize the importance of detecting risk factors as well as developing early diagnosis for therapeutic and preventive strategies.

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Disclosure The authors have nothing to disclose.

Appendix

Definitions

- Level of sun exposure was defined as the weekly number of hours of exposure between 11:00 am and 2:00 pm.
- Dietary calcium intake was defined as the number of glasses of milk or portions of yogurt or cheese that were consumed weekly.
- Physical activity was quantified through the International Physical Activity Questionnaire [44]. Activity was classified as high, moderate, or low on the categorical score.
- Smoking habit was evaluated as either ongoing or finished. Consumption was calculated as packs per year, and when finished, nonsmoking time was measured in years.
- Alcohol habit was defined as nonconsumption, low consumption (less than 30 g/day or 3 units), moderate consumption (30–40 g/day or 3–4 units), and high consumption (more than 40 g/day or 4 units).
- Coffee intake was estimated as 0, 1–2, 2–4, or >4 cups of coffee per day. Weight was measured in kilograms (within 0.1 kg of accuracy), height in centimeters (within 0.5 cm of accuracy), and BMI (kg/m^2).

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