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Genetic and environmental determinants of bone mineral density in Mexican Americans: results from the San Antonio Family Osteoporosis Study

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Abstract

Osteoporosis is a major cause of disability in the United States. Numerous factors contribute to the decline in bone mineral density (BMD) that characterizes this disease, and the importance of heredity is now widely appreciated. We evaluated the joint contributions of genes and environmental factors on variation in BMD in 895 participants of the San Antonio Family Osteoporosis Study (SAFOS). Participants of the SAFOS ranged in age from 18 to 96 years and were members of 34 large families of Mexican American ancestry. BMD was measured at the spine, hip, and forearm by dual-energy X-ray absorptiometry. Information about medical history, lifestyle habits, dietary intake, and physical activity patterns was obtained by questionnaire. Age and body mass index were strongly associated with BMD at nearly every site; these and other measured risk factors accounted in aggregate for up to 46% of the total variation in BMD. In general, the environmental risk factors accounted for proportionately more of the total variation in BMD in men than in women. Genes accounted for 65–80% of the residual variation in spine and hip BMD, and 25–55% of the residual variability in forearm BMD. Although residual heritabilities were generally comparable between men and women across all ages combined, heritabilities at all sites tended to be higher in premenopausal women than in men younger than 50 years of age. Identifying the individual genes involved will shed insights into the processes that govern bone remodeling and may suggest strategies for the prevention of osteoporosis.
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Introduction

Osteoporosis is a major cause of disability throughout the world. It is estimated that 10 million Americans are currently affected with this disorder and another 18 million have low bone mass, placing them at future risk for becoming affected [1]. Osteoporosis is responsible for 1.5 million fractures per year in the United States, including an estimated 700,000 vertebral and 300,000 hip fractures [2,3]. Nearly one-quarter of those experiencing an osteoporotic hip fracture will die within 1 year [4,5]. The economic burden associated with health care costs due to osteoporotic fractures in the United States alone ranges from 10 to 15 billion dollars [6].

Osteoporosis is defined as bone mineral density (BMD) that is reduced by >2.5 standard deviations below peak bone mass [7,8]. Because BMD is such an important predictor of future fracture, concerted efforts have been undertaken to understand the factors that influence BMD. How-

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ever, these efforts have been complicated by the need to consider the dynamic properties of bone growth because bone density at any given point in time reflects the cumulative balance of processes contributing to bone formation and bone resorption. Nevertheless, epidemiologic studies have revealed a number of environmental and lifestyle factors to be associated with reduced BMD, such as lean body size, cigarette smoking, steroid use, nutritional deficiency, and early menopause [9,10]. There also appears to be ethnic variation in BMD, with Americans of African and Mexican ancestry having on average increased BMD relative to individuals of non-Hispanic Caucasian background [11,12]. However, at least some of this ethnic variability can likely be accounted for by ethnic differences in other known risk factors for BMD (e.g., body size).

Over the past 2 decades, family and genetic studies have clearly established an important genetic influence on BMD [13]. The heritability of BMD has been estimated from twin [14–16] and family [10,17] studies to be in the range of 40 to 80%. However, the nature of the genetic contribution to BMD is at present unclear. Although there appears to be a strong genetic influence affecting acquisition of peak bone mass [16,18,19], genes may also influence the maintenance of bone mass and/or the rate at which bone loss occurs in later ages. Moreover, the extent to which genetic influences may be sex-dependent is not known.

In order to elucidate further the genetic epidemiology of the determinants of BMD, we initiated the San Antonio Family Osteoporosis Study (SAFOS), a population-based family study of BMD and its determinants in large extended families of Mexican American ancestry. This report describes the study design and initial findings from this study, including the relative contributions of lifestyle variables and genetic factors on BMD. Because our study included men and women across a wide range of ages, we were further able to assess the genetic and environmental influences on BMD in men and women separately.

Materials and methods

Subjects

Families enrolled into the SAFOS were selected because of their concurrent participation in a follow-up examination of the San Antonio Family Heart Study (SAFHS), a population-based prospective family study of atherosclerosis and its risk factors. The baseline phase of the SAFHS was carried out between 1991 and 1996, during which time 1431 individuals from 41 large families were recruited. Probands for these families were identified from a low-income neighborhood using a house-to-house recruitment procedure. Eligibility criteria for study probands were that they be 40–60 years of age and have large families in the San Antonio area. All first, second, and third degree relatives of the proband and the probands' spouse were invited to participate; the invitation was extended regardless of the probands' (or relatives') medical history. Details of the sampling and recruitment procedures for the SAFHS have been previously described [20].

Recruitment into the SAFOS was held in conjunction with a 4- to 5-year follow-up examination of the SAFHS families. In 1997, all individuals from the 34 largest SAFHS families were invited back to participate in a 5-year follow-up examination. Participating subjects received a medical examination in our clinic in the morning following a 12-h fast. Fasting blood samples were collected for biochemical analysis and a 2-h glucose tolerance test was then performed following ingestion of a 75-g glucose equivalent load. Diabetes was diagnosed using the plasma glucose criteria of the World Health Organization [21] and self-report of current use of antidiabetic medications. Relevant to this report, the basic medical examination also included measurement of height and weight (after the participant removed his or her shoes), from which body mass index was calculated as weight (in kilograms) divided by height (in meters) squared. Pregnant women were not eligible to participate; women reporting that they were pregnant were rescheduled for examination following their pregnancy. All procedures were approved by the Institutional Review Board at the University of Texas Health Science Center at San Antonio.

Phenotypes

Bone mineral content was measured using a dual energy X-ray absorptiometer (DXA) at the spine (L1–L4) and at multiple sites within the hip (trochanter, intertrochanter, neck, and Ward's triangle) and forearm (the 1/3, mid-, and ultradistal sites of the radius and ulna). All subjects were measured on the same machine (Hologic 1500W, Hologic, Inc., Bedford, MA). The areal bone mineral density (BMD; grams per square centimeter) was determined by dividing the bone mineral content (BMC; grams) by the projected area of the region scanned (square centimeters). Total hip BMD was defined as the sum of the BMC at the neck, trochanter, and intertrochanter sites divided by the total area of these three sites. All measurements were obtained and analyzed using standard protocols provided by the manufacturer. The short-term in vivo precision of the BMD was determined for our technician on 27 subjects who were examined twice on the same day. The precision of the lumbar spine was 0.009 gm/cm^2 (CV% = 1.0%) and precision of the total hip was 0.007 gm/cm^2 (CV% = 0.87%). The precision of the manufacturer's spine phantom was 0.0017 gm/cm^2 (CV% = 0.17%).

Covariates

A questionnaire was administered to obtain information about subjects' medical history, medication use, dietary habits, physical activity patterns, and smoking and alcohol

consumption behaviors. An extensive reproductive history questionnaire was administered to women that included questions about menstrual cycles and current use of oral contraceptives and estrogens. Women were considered to be menopausal if more than 1 year had elapsed since their last menstrual period or if they had undergone surgical menopause, defined as having both ovaries removed. Total months of breastfeeding was coded as the total number of months the woman had spent breastfeeding, summed across all of her children.

Dietary calcium intake was assessed by a 104-item food frequency questionnaire designed for this population [20]. Participants were further questioned about all dietary supplements they were taking, including vitamin use. Supplemental calcium intake was defined as the number of milligrams of calcium the subject consumed per day as a result of multivitamin or supplemental calcium pills.

Physical activity was assessed using a modified version of the Stanford 7-day Physical Activity Recall Instrument [22,23]. Subjects reported the weekly number of hours they slept and engaged in moderately strenuous, heavy, and very heavy physical activities. Examples of activities corresponding to each category were provided to assist the subject's responses. Light physical activity is defined as the difference between the total possible hours of weekly activity (i.e., 7 days × 24 h/day = 168 h) and the number of hours accounted for by sleep and moderate, heavy, and very heavy activity. Each category of physical activity was scored in metabolic equivalents, or METS (one MET equals the energy expenditure of 1 kg body wt/h), and expressed on a per-week basis.

Statistical analyses

The overall aim of these analyses was to determine the extent to which genes and measured environmental factors contribute to variation in BMD. As previously described in detail [20], we used quantitative genetic methods to model the total variation in the trait as a function of the mean trait value, effects attributable to the measured covariates, and the proportions of the remaining variation that could be attributed to the residual genetic and unmeasured environmental effects, respectively. For each BMD trait, we estimated the effects of the following environmental covariates: education (years), smoking status (current vs not), alcohol consumption (current drinker vs not), physical activity level (in METS), dietary calcium intake (in milligrams per deciliter), supplemental calcium intake (yes/no), and BMI (kilograms per square meter). In women, we also estimated effects associated with the following variables: menopausal status (postmenopausal vs not), oral contraceptive use (current user vs not), hormone replacement therapy (current user vs not), number of live births, and total months of breast feeding. The effects of age and age squared were included in each model, allowing for the age effects to vary by sex.

Genetic effects were estimated simultaneously along with the environmental effects using a pedigree-based likelihood approach [24,25]. Only additive polygenic effects were estimated so that we defined heritability as the proportion of the total trait variance (σ^2_T) attributable to the additive effects of genes (σ^2_G) (i.e., "narrow sense" heritability; $h^2 = \sigma^2_G/\sigma^2_T$). Estimation of the additive genetic heritability follows basic quantitative genetic theory, which models the phenotypic covariances (conditional upon covariate effects) between two individuals in a pedigree as a function of their degree of biologic relatedness. Specifically, under the assumption of multivariate normality, we modeled the observed phenotypic covariances between any two individuals within the pedigree as the sum of their expected coefficient of relationship (which is twice the kinship coefficient) times the additive genetic variance plus the environmental variance. (In this model, the additive genetic variance plus the environmental variance equals the total phenotypic variance.) Maximum likelihood methods were used to estimate the values of the parameters (including the heritability) that resulted in the highest likelihood obtained across all of the pedigrees. These analyses were conducted using the SOLAR software program [26].

We performed likelihood ratio tests to determine whether the set of environmental covariates included in each analysis accounted for a significant component of the phenotypic variation in that trait. This test compares the likelihood of a full model (all covariates and additive genetic effects) with that of a nested model in which the covariate being tested is removed from the model. The likelihood ratio statistic is distributed asymptotically as a χ^2 statistic with one *df*. Because a major aim of our analysis was to estimate the proportion of unexplained variance that could be explained by the genetic factors, we used a liberal threshold of 0.10 to establish significance levels for covariate effects.

The final models included all environmental covariates, including age, age squared, and sex, which were significantly associated with BMD in univariate analysis, as well as an additive genetic effect modeled as a random effect. We computed the relative proportions of the variance explained by the measured environmental covariates and genes as the variance attributable to that particular component divided by the total phenotypic variance. The residual variance that was not accounted for by the two components corresponds to the residual environmental variance or the proportion of the variance attributable to unmeasured environmental factors [20].

The analysis of each phenotype was restricted to those individuals for whom all covariate data were complete. Of the original 895 subjects who received DXA scans, 2 were excluded because they reported that they were currently taking corticosteroids, and 8 were excluded due to poor quality of the BMD measurements. Of the remaining 885 individuals, 89 individuals were missing information on one or more of the covariates and were excluded from analyses involving that variable.

Table 1
Clinical characteristics of the study sample according to gender[a]

Variable	Males (n = 344)	Females (n = 551)
Age (years)	42.3 ± 16.6	43.1 ± 15.5
% with diabetes	19.0%	19.8%
Education (years)	10.9 ± 3.4	10.4 ± 3.3
% Smoke	31.4%	17.0%
% Alcohol	60.1%	29.2%
METS (per week)	276 ± 58	251 ± 37
Diet Calcium (mg/dl)	1013 ± 553	882 ± 407
% Supplemental Calcium	12.8%	25.2%
BMI (kg/m^2)	29.8 ± 6.2	31.5 ± 7.6
% Menopause	—	29.8%
% Oral Contraceptives	—	13.4%
% HRT	—	13.6%
No. of Live Births	—	3.1 ± 2.5
Duration of Breastfeeding (months)	—	8.7 ± 22.8

Abbreviations: METS, metabolic equivalent units; BMI, body mass index; HRT, hormone replacement therapy.

[a] Means ± standard deviations presented for continuous variables and percentages with trait presented for categorical variables

Results

A total of 895 individuals (344 men and 551 women) from 34 families were enrolled in the San Antonio Family Osteoporosis Study. The median number of individuals examined per pedigree was 25. The sample included a total of 358 distinct sibships (i.e., same mother and same father), with sizes ranging from 1 to 10.

Because we recruited extended families, the sample of examined individuals included a very large number of relative pair types. The sample included information on 1561 pairs of first-degree relatives (733 parent–offspring pairs and 828 sib pairs), 1983 pairs of second-degree relatives (1646 avuncular pairs [aunt/uncle–niece/nephew], 210 grandparent–grandchild pairs, and 127 half-sibling pairs), and 2571 pairs of third-degree relatives (1921 cousin pairs, 396 great-avuncular pairs, 250 half-avuncular pairs, and 4 great-grandparent–great-grandchild pairs).

Characteristics of the study sample are summarized in Table 1 according to gender. The prevalence of diabetes was 19.0% in men and 19.8% in women, and the mean body mass index ranged from 29.8 kg/m^2 in men to 31.5 kg/m^2 in women. The average level of formal education was 10.9 years in men and 10.4 years in women. Men were more likely than women to drink alcohol and smoke cigarettes. Men reported higher levels of dietary calcium intake than women, although women reported higher rates of calcium supplementation. Nearly 30% of the female study subjects were postmenopausal. Thirteen percent of women reported current use of contraceptives, and 13.6% reported that they were currently taking estrogens.

We contrasted mean BMD at the hip (total) in Mexican Americans from the SAFOS with corresponding published estimates from a representative national sample of the non-Hispanic white population [27] (see Fig. 1). In general, BMD decreased with increasing age in both sexes and ethnic groups, with the exception of Mexican American men, in whom BMD was highest in the oldest age category, although this category included only six individuals. In each age group, mean BMD was higher in Mexican Americans than in the corresponding sample of non-Hispanic whites.

The effects of environmental and lifestyle variables on BMD were analyzed separately in men and women. Results of these analyses are shown in Table 2 for the spine and hip. In men, BMI was the only variable significantly associated with spine BMD ($P < 0.01$), and after accounting for the effects of BMI, the residual heritability of spine BMD was estimated to be 53% (i.e., 53% of the residual variation in spine BMD could be attributed to the additive effects of genes). In women, decreased spine BMD was associated with increasing age ($P < 0.01$), presence of diabetes ($P < 0.10$), higher education ($P < 0.05$), higher BMI ($P = 0.01$), menopause ($P = 0.01$), and less time spent breastfeeding ($P = 0.10$). After accounting for these variables, the residual heritability in spine BMD was estimated to be 78%.

The relationship between environmental covariates and hip BMD is also shown in Table 2. In men increased BMD was associated with younger age, higher physical activity, and higher BMI at each site. After accounting for these variables, the residual heritability for BMD in men ranged from 66% for total hip BMD to 76% for BMD at Ward's triangle. In women, higher BMD at all hip sites was significantly associated with younger age, higher BMI, and less time spent breastfeeding. In addition, increased BMD at the

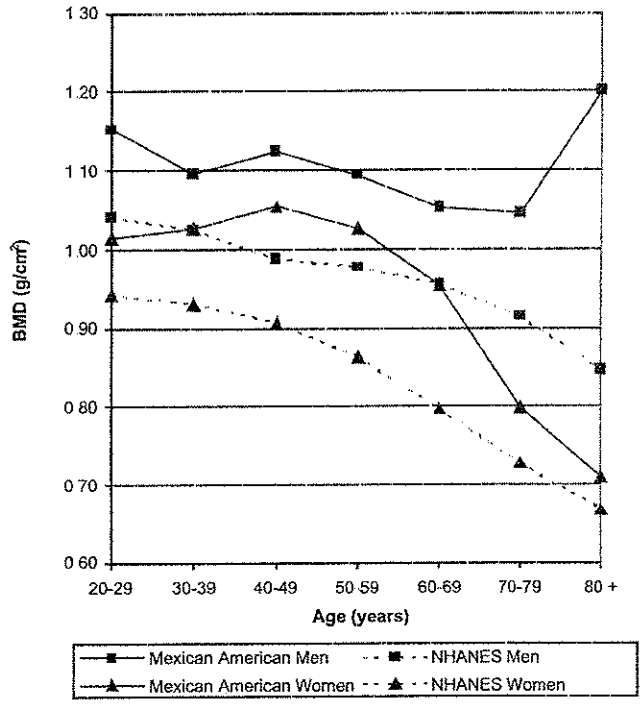

Fig. 1. Mean BMD at the hip (total) in Mexican Americans and in non-Hispanic whites from the NHANES.

Table 2
Environmental correlates of BMD in spine and hip

Variable	Spine (L1–L4)		Hip (Neck)		Hip (Troch)		Hip (Inter)		Hip (Wards)	
	Men	Women	Men	Women	Men	Women	Men	Women	Men	Women
Age		−0 0222†	−0 0740‡	−0 0517‡	−0 0287‡		−0 0310†		−0 1393‡	−0 0615‡
Age²		−0 0263‡	0 0279‡		−0 0230‡	−0 0194‡	0 0171†	−0.0376‡	0 0401‡	
Diabetic (years)		0 0210*								−0 0398†
Education (years)		0.0138†		0 0108*						0 0159†
Smoking (years)										
Alcohol (years)										
Total METS			0 0126†		0 0093*		0.0154†		0 0088*	
Dietary Ca										
Supp Ca (years)										
BMI	0 0313‡	0 0439‡	0 0536‡	0 0722‡	0 0341‡	0 0432‡	0.0896‡	0 0866‡	0 0371‡	0 0500‡
Menopause (years)		−0 0464‡				−0 0318†	—			−0 0503‡
OC (years)							—			
HBT (years)							—			
No. of Live Births							—			
Total Months BF		−0.0091*		−0 0130†		−0 0121†	—	−0.0220†		−0.0161†
h2r	0 53 ± 0 12	0 78 ± 0 11	0 66 ± 0 14	0 67 ± 0 10	0 70 ± 0 14	0 76 ± 0 10	0 67 ± 0.14	0 71 ± 0 10	0 76 ± 0 14	0 70 ± 0 10

Abbreviations: METS metabolic equivalent units; BMI body mass index; OC, oral contraceptive use; HRT, hormone replacement therapy
* $P < .10$
† $P < .05$
‡ $P < .01$

total hip and Ward's triangle was associated with higher level of education, and BMD at the trochanter and Ward's triangle was inversely associated with menopause After accounting for all covariates, the residual heritability for BMD in women ranged from 67% for total hip BMD to 76% for BMD at the trochanter

Table 3 summarizes the relationships between environmental covariates and forearm BMD In men, increased BMD at all forearm sites was associated with younger age and increased BMI ($P < 0.01$). Alcohol intake was associated with higher BMD at the radius 1/3 ($P < 0.05$), but not at any other sites. In addition, higher physical activity was also associated with higher BMD at the radius UD and at all three ulna sites ($P < 0.01$) In women, increased BMD was associated with younger age ($P < 0.01$) and higher BMI ($P < 0.01$) at all six sites, with less time spent breastfeeding at all sites except radius and ulna 1/3, and inversely associated with menopause at all sites except ulna UD. Higher BMD at the radius UD and ulna UD was also associated with a larger number of live births, and BMD at the ulna UD was also

Table 3
Environmental correlates of BMD in forearm

Variable	Radius 1/3		Radius Mid		Radius UD		Ulna 1/3		Ulna Mid		Ulna UD	
	Men	Women	Men	Women	Men	Women	Men	Women	Men	Women	Men	Women
Age		−0.0204‡		−0 0153‡	−0 0220‡	−0.0150‡	—	−0 0150‡		−0.0163‡	−0 0105†	−0.0171‡
Age²	−0.0128‡	−0 0174‡	−0 0145‡	−0 0179‡		−0 0150‡	−0 0088‡	−0.0211‡	−0.0114‡	−0 0179‡		−0.0141‡
Diabetic (y)			−0.0311†				−0 0227*				−0.0114†	
Education (y)												
Smoking (y)												
Alcohol (y)	0 0147†											
Total METS					0.0069*		0.0079*		0.0064*		0 0071*	
Dietary Ca												−0 0031*
Supp. Ca (y)				0.0039*		0.0053†						
BMI		0 0063‡	0.0121‡	0.0120‡	0 0167‡	0.0220‡	0 0128‡	0.0071‡	0.0108‡	0 0072‡	0 0108‡	0 0116†
Menopause (y)		−0 0234†		−0.0107†		−0.0118†	—	−0.0082†		−0 0082†		
OC (y)							—					−0 0084*
HRT (y)							—					0 0084*
No. of Live Births						0 0039*	—					0 0053†
Total Months BF				−0.0057†		−0.0077†	—			−0 0039*		−0 0062*
h2r	0 56 ± 0 14	0 38 ± 0 09	0 36 ± 0 17	0.42 ± 0.09	0 30 ± 0 14	0 48 ± 0 10	0 56 ± 0 15	0 34 ± 0 09	0 41 ± 0 15	0 36 ± 0 09	0 25 ± 0 13	0.56 ± 0 10

Abbreviations: METS metabolic equivalent units; BMI body mass index; OC oral contraceptive use; HRT hormone replacement therapy
* $P < 10$
† $P < 05$
‡ $P < 01$

Table 4
Components of variance for BMD in the spine, hip, and forearm

Variable	Males					Females				
	n	Measured Covariates		Genetic	Residual Environment	*n*	Measured Covariates		Genetic	Residual Environment
		All*	(Age only)				All*	(Age only)		
Spine	328	0 05	(0)	0 50	0 45	492	0 31	(0 22)	0 53	0 15
Neck	307	0 29	(0 17)	0 47	0 24	494	0 38	(0 18)	0 41	0 21
Trochanter	307	0 11	(0 05)	0 62	0 26	530	0 26	(0 12)	0 56	0 18
Intertrochanter	307	0 22	(0 01)	0 52	0 26	532	0 35	(0 13)	0 46	0 19
Wards	307	0 47	(0 43)	0 40	0 13	489	0 40	(0 32)	0 42	0 18
Radius 1/3	336	0 07	(0 06)	0 52	0 41	539	0 46	(0 40)	0 21	0 33
Radius Mid	310	0 14	(0 08)	0 31	0 55	539	0 44	(0 36)	0 24	0 30
Radius UD	315	0 15	(0 09)	0 26	0 59	540	0 41	(0 26)	0 28	0 31
Ulna 1/3	306	0 09	(0 04)	0 51	0 40	541	0 40	(0 34)	0 20	0 40
Ulna Mid	314	0 10	(0 05)	0 37	0 53	540	0 44	(0 38)	0 20	0 36
Ulna UD	307	0 12	(0 04)	0 22	0 66	510	0 37	(0 30)	0 35	0 28

* Measured covariates eligible for inclusion in each model included for men: age, age^2, diabetes (present/absent), alcohol consumption (yes/no) smoking (yes/no) education (yrs), BMI, METS calcium supplementation (g/day) calcium intake (g/day); and for women all above variables and menopause status, total number of months breastfeeding, oral contraceptive use (current user vs not), and hormone replacement therapy (currently taking estrogens vs not)

associated with lower dietary calcium intake (P = 0 10), lack of oral contraceptive use (P = 0 10), and current use of estrogen (P = 0 10) After accounting for covariates, the residual heritability of forearm BMD ranged from 25 to 56% in men and from 34 to 56% in women

Table 4 shows the components of variance for BMD at each site, analyzed separately in men and women Each row in the table describes the proportion of the total phenotypic variance in that trait that can be attributed to the combined effects of the measured covariates (including age), additive genetic effects, and unmeasured (residual) environmental factors The residual environmental component is computed as the remainder of the phenotypic variance that cannot be explained by the measured covariates and genetic effects The proportion of the variance attributable to additive genetic effects estimated from this analysis differs from the residual heritability estimated in the previous analyses (summarized in Tables 2 and 3) because the residual heritability (shown in the prior analyses) corresponds to the proportion of the *unexplained* variation accounted for by genes (that is, after accounting for all covariate effects), whereas the proportion of the variance attributable to genetic effects (shown in this analysis) reflects the proportion of the *total* phenotypic variation accounted for by genes With only a single exception (i e , BMD at Ward's triangle), measured covariates accounted for a larger proportion of the total phenotypic variation in women than in men This is due largely to the fact that the two variables that account for most of the variation in BMD, namely age and body mass index, account for a larger proportion of the phenotypic variance in women than in men (see Table 4 for proportion of variation explained by age alone) For BMD at both the spine and hip, genetic factors accounted for approximately similar proportions of the phenotypic variation in men and women (e g , 50–53% of the total variation in BMD at the spine and 40–60% of the variation in BMD at the hip) In contrast, genes accounted for a larger proportion of the variation in forearm BMD in men (22–52%) than in women (20–35%)

Figure 2 shows the residual heritability of BMD at the spine, hip (neck) and forearm (radius mid) for premenopausal women and men younger than age 50 separately.

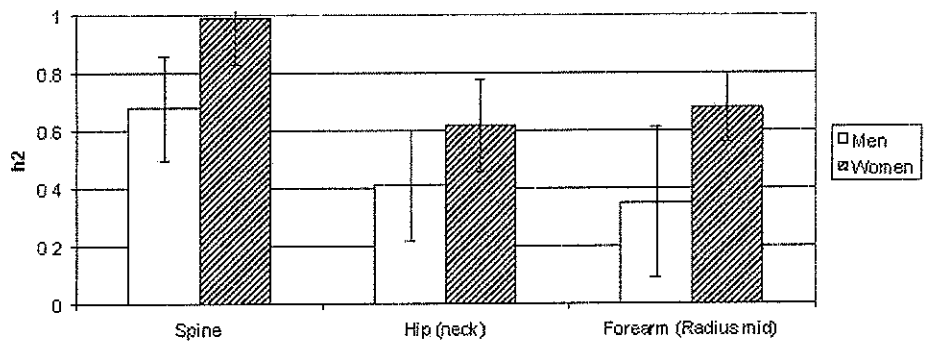

Fig 2 Residual heritability (h^2) of BMD in premenopausal women (n = 339) and men younger than 50 years of age (n = 230)

Premenopausal women ranged in age from 18 to 53 years. Residual heritability of BMD was considerably higher in women than in men at these ages across all three sites. Further comparison of the genetic variances between men and women (estimated by multiplying the residual heritability by the residual trait variance, since $h^2 = \sigma^2_G/\sigma^2_P$) revealed the genetic variances to be higher in younger women than in men at each site. This result suggests that after accounting for known, measured covariates, such as age, genes have a larger effect on phenotypic variation in younger women than in younger men (data not shown). Residual heritability estimates are not presented for postmenopausal women and men older than age 50 because precise estimates could not be obtained most likely due to the relatively small numbers of relative pairs in these categories.

Discussion

The SAFOS is one of the largest family studies of BMD carried out to date. The design of this study is unique from several perspectives, including its focus on large extended families, its inclusion of both men and women, and its restriction to families of Mexican American ancestry. One interesting feature of the Mexican American population, at least in terms of bone health, is that the risk of hip and vertebral fracture appears to be lower in this population than in the non-Hispanic white population [28–30], although at least some of this lower risk may be explained by the greater level of obesity in this population. The relative protection against fracture experienced by Mexican Americans is small; it could be related to a relatively higher BMD observed in Mexican Americans [11], greater protection against trauma among those experiencing falls, or to some other feature related to bone structure.

We have simultaneously estimated the contributions of genes and a variety of epidemiologic covariates to phenotypic variation in BMD. In men, the covariates and genetic effects (heritability) together accounted for 55% of the total variation in spine BMD, 75% of the total variation in hip BMD, and 34–60% of the total variation in forearm BMD. The corresponding proportions in women were 85% (spine), 79–82% (hip), and 60–72% (forearm). In men, genes accounted for a far larger proportion of the total variation in BMD than did measured environmental risk factors, with the sole exception of Ward's triangle, where measured covariates accounted for 47% of BMD variation compared to 40% for genes. In women, genes accounted for slightly more of the variation than measured environmental risk factors in BMD at the spine and hip but not at the forearm, where there was a marked decrease in BMD following menopause. Measured covariates accounted for considerably larger proportions of the total variation in BMD in women than they did in men, especially at the spine and hip. At most sites, most of the measured covariate effects could be accounted for by age.

The strong genetic influences on BMD observed in this Mexican American population are consistent with results obtained from many other populations. In general, high trait heritabilities provide a strong motivation for pursuing gene-mapping strategies, such as genomewide linkage analysis, although some caveats are in order. First, genetic influences on BMD may be age-specific, whereas heritabilities reflect only the aggregate effects of genes that influence BMD throughout life. Some of these genes may exert their effects primarily later in life (e.g., they may influence bone loss), whereas others may exert their effects younger in life (e.g., they may influence acquisition or maintenance of peak bone mass). Second, there may be genes whose effects on BMD are sex-specific, such as genes influencing estrogen or androgen production and/or balance. Gene-mapping strategies should therefore consider the possibility that genetic influences on BMD may include some genes whose effects may be detected throughout the population structure, as well as others whose effects may be detectable primarily in specific subsets.

A unique feature of our sample is its composition of large extended pedigrees with large numbers of different types of relative pairs, which enabled us estimate heritabilities of BMD for subsets of our data. In women, genes accounted for 78% of the unexplained variation in spine BMD compared to only 53% in men. There was little difference in residual heritability between the sexes for both hip and forearm BMD, where genes accounted for 66–76% and 25–56% of the unexplained variation in men and women, respectively. The apparently higher heritability in BMD at the hip and spine than at the forearm may represent a stronger genetic effect at these sites or a greater precision in measurement at these sites. The higher heritability in spine observed in women than in men, both across all ages and for younger age groups separately, appears to reflect a greater genetic effect in women in acquisition of peak bone mass.

A modifying role of gender on the genetic determinants of whole body BMD has recently been reported in mice. Overall, the heritability, of weight-corrected BMD was greater in male than female mice (45% vs 22%), and a genome scan revealed gender-specific linkages with only a few overlapping regions of linkage between the two sexes [31]. In one of the few human studies to explore this issue directly, Naganathan and colleagues reported lower correlations among opposite-sex twins than among same-sex dizygotic twins in forearm BMD, although not hip BMD, suggesting the presence of unique gender-specific genetic effects, at least at the forearm site [32]. Our analyses based on extended families are consistent with this interpretation, although the gender-specific genetic effects in our data were most evident at younger ages (i.e., premenopausal women and men younger than 50), where they were observed across all sites.

It is likely that we have underestimated the effects of some of the environmental covariates. Nutritional background, physical activity, and reproductive history (in women) are widely regarded as important contributors to bone health. Our assessments of these exposures, although reasonable for epidemiologic settings, were not designed to capture lifetime exposures to these variables, nor have we considered possible interactions among the measured environmental factors, such as between dietary intake and sex. Consequently, we almost have certainly underestimated the "true" effects of these variables on BMD. The likely effect of these shortcomings is to underestimate variance to the measured environmental factors and overestimate variance to the unmeasured (residual) environmental factors.

The assessment of environmental determinants, although imperfect, offers several opportunities for future gene-mapping efforts. For example, identification of individual-specific covariates may increase the power of gene-mapping efforts by decreasing the amount of unexplained phenotypic variance, and thereby increasing the strength of the genetic signal. Additionally, covariate information may ultimately prove useful for detection of specific genetic mutations whose effects are manifested primarily in the context of a particularly beneficial (or detrimental) environment.

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