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Relevance of the Genes for Bone Mass Variation to Susceptibility to Osteoporotic Fractures and Its Implications to Gene Search for Complex Human Diseases

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We investigate the relevance of the genetic determination of bone mineral density (BMD) variation to that of differential risk to osteoporotic fractures (OF). The high heritability (h^2) of BMD and the significant phenotypic correlations between high BMD and low risk to OF are well known. Little is reported on h^2 for OF. Extensive molecular genetic studies aimed at uncovering genes for differential risks to OF have focussed on BMD as a surrogate phenotype. However, the relevance of the genetic determination of BMD to that of OF is unknown. This relevance can be characterized by genetic correlation between BMD and OF. For 50 Caucasian pedigrees, we estimated that h^2 at the hip is 0.65 ($P < 0.0001$) for BMD and 0.53 ($P < 0.05$) for OF; however, the genetic correlation between BMD and OF is nonsignificant ($P > 0.45$) and less than 1% of additive genetic variance is shared between them. Hence, most genes found important for BMD may not be relevant to OF at the hip. The phenotypic correlation between high BMD and low risk to OF at the hip (approximately –0.30) is largely due to

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an environmental correlation (ρ_E = –0.73, $P < 0.0001$). The search for genes for OF should start with a significant h^2 for OF and should include risk factors (besides BMD) that are *genetically* correlated with OF. All genes found important for various risk factors must be tested for their relevance to OF. Ideally, employing OF per se as a direct phenotype for gene hunting and testing can ensure the importance and direct relevance of the genes found for the risk of OF. This study may have significant implications for the common practice of gene search for complex diseases through underlying risk factors (usually quantitative traits). Genet. Epidemiol. 22:12–25, 2002.

Key words: bone mineral density; genetic correlation; heritability; human pedigrees; osteoporotic fractures

INTRODUCTION

It is a common practice in human genetics that underlying risk factors (usually quantitative traits) are studied as surrogate phenotypes in the search for genes of complex diseases. This practice is usually based on (1) the observation that the underlying risk factors are phenotypically correlated with the diseases, so that low or high values of the risk factors may confer higher risk to the diseases; and (2) the argument that dissecting a complex disease into its various underlying risk factors may facilitate the search for genes of complex diseases, since underlying risk factors may be less complex and thus may be simplified phenotypes for study. We will show, with data from studies of osteoporotic fractures (OF) and bone mineral density (BMD), an important risk factor of OF, that problems can occur when underlying risk factors are used as surrogate phenotypes without solid evidence for a genetic correlation between a disease and a risk factor.

Low BMD is an important risk factor for fracture, and osteoporosis is mainly characterized by low BMD [Cummings et al., 1985; Melton et al., 1989; Deng et al., 2000a]. Osteoporosis results in more than 1.3 million OF a year, with an estimated direct cost of 13.8 billion dollars [Ray et al., 1997] in 1995 in the United States alone. Extensive data have established that BMD variation is under strong genetic control with heritability (h^2) estimates ranging from 0.5–0.9 [Dequeker et al., 1987; Slemeda et al., 1991; Sowers et al., 1992; Gueguen et al., 1995; Deng et al., 1999a, 2000b]. Recently, extensive molecular genetic studies [Morrison et al., 1994; Johnson et al.,1997; Gong et al., 1999; Koller et al., 1998; Deng et al., 1998a, 1999b] have been launched to search for genes underlying BMD variation. The results so far have been largely inconsistent [Gong et al., 1999; Deng et al., 2001]. Molecular genetic studies of other major risk factors (such as bone loss and bone size) have been scarce, even if the importance of the genetic determination has been revealed for them [Kelly and Negyun, 1994; Krall et al., 1995; Heaney et al., 1996; Zmuda et al., 1997; Harris et al., 1998]. Compared with hundreds of genetic studies of BMD, direct molecular genetic studies of OF *per se* are relatively rare [but see Langdahl et al., 1998; Feskanich et al., 1998; Uitterlinden et al., 1998; Ensrud et al., 1999; Gennari et al., 1999; Cauley et al., 1999; Weichetova et al., 2000]. Direct evidence for genetic determination of OF is extremely scarce and the few available data have yielded inconsistent conclusions [Fox et al., 1998; Kannus et al., 1999; Keen et al., 1999; Deng et al., 2000a].

One major goal of our effort in bone molecular genetic studies is to find genes underlying the differential susceptibility to OF whether OF per se is the phenotype under study or a major risk factor (such as BMD) is studied as a surrogate phenotype. Therefore, it is crucial to demonstrate unequivocally the genetic determination of OF. If OF is heritable and a heritable risk factor (such as BMD) is studied as a surrogate phenotype, it is critical to demonstrate that the genes found important for this risk factor are relevant to OF. Although BMD and OF are *phenotypically* correlated so that lower BMD values are associated with higher risk to OF [Cummings et al., 1985; Melton et al., 1989], whether they are *genetically* correlated is unknown. It is well known [Lynch and Walsh, 1998] that for two complex traits (such as OF and BMD), phenotypic correlation (ρ_p) may be caused by both genetic and environmental factors as reflected respectively by genetic (ρ_G) and environmental (ρ_E) correlations. Therefore, significant ρ_E between BMD and OF does not necessarily imply significant ρ_G (see Discussion).

The main purpose of this study is to evaluate the relevance of genetic determination of BMD variation to that of OF by measuring the degree of their shared genetic determination as indexed by their genetic correlation, ρ_G. In addition, we characterize the genetic determination of OF as indexed by heritability h^2 of the hip, spine, and wrist. There is substantial heterogeneity of BMD and OF at different bone sites [Cummings et al., 1985; Deng et al., 1998a] and the determination of BMD at different bone sites may not be the same [Deng et al., 1999a].

MATERIALS AND METHODS

Subjects

The study was approved by the Creighton University Institutional Review Board. All the study subjects signed informed-consent documents before entering the project. All the study subjects were Caucasians of European origin; 50 pedigrees with 703 subjects (263 males and 440 females) from 2–4 generations were analyzed. Each pedigree was identified through a single proband having BMD Z-scores ≤ -1.28 at the hip or spine. BMD values that were expressed as Z-scores adjust for age, gender, and ethnic difference in a general referent healthy population. The exclusion criteria for the study subjects were: (1) serious residuals from cerebral vascular disease; (2) diabetes mellitus, except for easily controlled, non-insulin dependent diabetes mellitus; (3) chronic renal disease manifest by serum creatinine >1.9 mg/dl; (4) chronic liver disease or alcoholism; (5) significant chronic lung disease; (6) corticosteroid therapy at pharmacologic levels for more than 6 months duration; (7) treatment with anticonvulsant therapy for more than 6 months duration; (8) evidence of other metabolic or inherited bone disease such as hyper- or hypoparathyroidism, Paget's disease, osteomalacia, osteogenesis imperfecta or others; (9) rheumatoid arthritis or collagen disease; (10) recent major gastrointestinal disease (within the past year) such as peptic ulcer, malabsorption, chronic ulcerative colitis, regional enteritis or any significant chronic diarrhea state; (11) significant disease of any endocrine organ that would affect bone mass; (12) hyperthyroidism; (13) any neurologic or musculoskeletal condition that would be a non-genetic cause of low bone mass; (14) any disease, treatment or condition that would be a non-genetic cause for low bone mass. The exclusion criteria were assessed by nurse-administered questionnaires and/or medical records.

Measurement

BMDs of spine, hip, and wrist were measured by a Hologic 1000, 2000+, or 4500 scanner (Hologic corporation, Waltham, MA). All machines are calibrated daily, and long-term precision is monitored with external spine and hip phantoms. Hip, spine, and wrist are chosen because they are the most common osteoporotic fracture sites [Cummings et al., 1985]. Short-term precision in humans is 0.7% for spine BMD, 1.0% for hip BMD, and 1.2% for wrist BMD. We maintain constant quality assurance procedures that track potential confounding events such as X-ray tube replacement, arm realignments, collimator changes, and software version updates. Technicians maintain scan-by-scan surveillance for quality control. We have chosen BMD rather than bone mineral content as our bone mass phenotype, because BMD is the measure most closely correlated phenotypically with fracture risk [Black et al., 1992]. For the spine, our quantitative phenotype was the combined BMD of $L_{1\text{-}4}$ (denoting the first to the fourth lumbar vertebrae). For the hip, it was the combined BMD of the femoral neck, trochanter and intertrochanteric region. For the wrist, it was the ultra distal BMD. All DXA (dual energy X-ray absorptiometry) machines report BMD in g/cm^2. Data obtained from different machines were transformed to a compatible measurement by an algorithm developed by us (Recker et al., unpublished data) based on the measurements of BMD with different scanners on the same referent population. Members of the same pedigree were usually measured on the same type of machine. Weight was measured on the visit when the BMD measurements were taken.

All subjects completed a nurse-administered questionnaire to assess the information concerning OF of the spine, hip, and wrist. It has been shown [Bush et al., 1989; Nevitt et al., 1992; Pagnini and Chao, 1993; Ismail et al., 2000] that self-reported symptomatic fractures are reliable, especially those involving extensive pain and requiring medical treatment, such as OF at wrist and hip. For example, Ismail et al. [2000] found that the false negative report rate for those who did not recall sustaining a hip or wrist fracture is only about 3% or less and the false positive rate is 0 and 6%, respectively, for those who did not have a hip and wrist fracture. Circumstances leading to fractures were ascertained by a research nurse and those cases of self-reported OF that were not due to low trauma (i.e., fall) were excluded. Inadvertent inclusion of fracture cases due to high trauma and/or inaccurate report on OF status will render our estimation of genetic determination of OF conservative since random accidents and inaccuracy may reduce familial co-occurrence of OF.

Statistical Analyses

The variance component analysis [Lange et al., 1976] for quantitative traits with additive genetic and random, individual-specific components of variation was performed. For a qualitative trait (such as OF), a continuously distributed underlying quantitative trait liability [Falconer, 1989; Lynch and Walsh, 1998] is assumed. The analysis assumed joint multivariate normality of phenotypic values and no interaction between genes and the environment. The common familial environmental effects were assumed to be negligible, which is supported by previous studies for BMD [Sowers et al., 1992; Krall and Dawson-Hughes, 1993; Gueguen et al., 1995; Deng et al., 1999a; but see Hopper et al., 1998]. The relevant results for OF are currently

lacking. The program employed is SOLAR (Sequential Oligogenic Linkage Analysis Routines: http://www.sfbr.org/sfbr/public/software/solar/solar.html. We performed both univariate and bivariate analyses, respectively, for BMD and OF at the hip, spine, and wrist.

The theory and method for the bivariate mixed quantitative-qualitative analyses of BMD and OF is described in detail in Williams et al. [1999a] and an example of its application is found in Williams et al. [1999b]. The ascertainment scheme of pedigrees based on the low BMD values of probands was accounted for in the SOLAR program by identifying the proband for each pedigree. SOLAR accounted for the ascertainment scheme based on the cutoff BMD value and proband status by use of a conditional likelihood approach. The general bivariate analysis assumed that BMD and liability of OF were each determined by a mean of the trait, an additive genetic component**,** covariates**,** and random environmental components. The additive genetic components were allowed to be correlated, as were the environmental components. For OF, a positive fracture history is coded as 1 and a negative fracture status is coded as 0. The covariates included sex, weight, age, and age^2 for males and females. For sex, male is coded as 0 and females coded as 1. Simultaneous estimation of covariate effects with the variance components can generally increase the genetic signal to noise ratio (i.e., h^2 estimates) by decreasing the proportion of the residual phenotypic variation attributable to random environmental factors [Deng et al., 1999a, 2000b]. The BMD data were evaluated by graphic methods [Sokal and Rohlf, 1995] and found not to deviate from normal distributions. Hypothesis testing was conducted using the likelihood ratio statistic. The likelihood ratio statistic approximately follows a χ^2-distribution with the degrees of freedom equal to the number of constrained parameters. In analyses, for computational ease by the SOLAR program, BMD values were multiplied by 100. This does not affect the estimation of ρ_G, ρ_E, and h^2.

RESULTS

The basic characteristics of the study participants stratified by age and sex demonstrate that BMD and OF are both age dependent (data available from us upon request). The results not presented in detail here show that univariate analyses for OF demonstrate significant h^2 for the hip but not for the spine and wrist when the parameters such as the population prevalence are estimated from the sample. The h^2 for OF from univariate analyses are respectively 0.15 (±SE 0.06) at the hip and bounds to zero at the spine and wrist when all the parameters are estimated from the sample. Since the results of our univariate and bivariate analyses are qualitatively the same and the bivariate analysis results contain those information (such as environmental correlation) that are not revealed in the univariate analyses, we will only elaborate and discuss the results of bivariate analyses in order to avoid redundancy in presentation.

At the hip (Table I), there are significant heritabilities ($h^2 \pm$ SE) for both BMD (0.65 ± 0.07) and OF (0.53 ± 0.40) after adjusting for the covariates. Although the SE for h^2 of hip OF is large relative to its maximum likelihood estimate, the h^2 of hip OF is significantly ($P = 0.048$) different from zero when comparing Models III and IV, respectively, with Model I (Table I). In spite of the high and significant h^2 for

TABLE I. Bivariate Analyses of BMD and OF at Hip*

	Model I (unrestricted)	Model II ($\rho_G = h^2_{BMD} = h^2_{OF} = 0$)	Model III ($\rho_G = h^2_{OF} = 0$)	Model IV ($\rho_G = 0$)	Model V ($\rho_E = 0$)	Model VI ($\beta_{weight} = 0$)
BMD						
Mean	66.48	70.00	66.55	66.43	66.97	102.69
	(3.44)	(0.00)	(2.85)	(2.91)	(2.85)	(1.99)
Sex	–2.20	–2.80	–2.22	–2.18	–2.36	–9.49
	(1.90)	(2.98)	(2.17)	(1.19)	(2.04)	(2.42)
Age (male)	–0.40	–0.52	–0.41	–0.40	–0.41	–0.17
	(0.14)	(0.20)	(0.15)	(0.12)	(0.14)	(0.16)
Age (female)	–0.25	–0.38	–0.25	–0.25	–0.26	–0.0084
	(0.10)	(0.14)	(0.10)	(0.09)	(0.11)	(0.13)
Age^2 (male)	0.0053	0.0071	0.0053	0.0053	0.0054	0.090
	(0.0025)	(0.0037)	(0.0026)	(0.0023)	(0.0025)	(0.003)
Age^2 (female)	–0.0043	–0.0028	–0.0043	0.0043	–0.0042	–0.0092
	(0.0018)	(0.0024)	(0.0019)	(0.0018)	(0.0019)	(0.0023)
Weight	0.44	0.42	0.44	0.44	0.44	—
	(0.03)	(0.03)	(0.03)	(0.03)	(0.03)	—
H^2	0.65	—	0.64	0.65	0.65	0.66
	(0.08)	—	(0.07)	(0.07)	(0.07)	(0.07)
OF						
Mean	1.00	1.00	1.00	1.00	1.00	1.46
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.36)
Sex	0.78	0.85)	0.83	0.78	0.83	0.72
	(0.48)	(0.55)	(0.50)	(0.48)	(0.49)	(0.54)
Age (male)	0.033	0.035	0.034	0.033	0.041	0.043
	(0.032)	(0.032)	(0.033)	(0.031)	(0.031)	(0.033)
Age (female)	0.016	0.016	0.016	0.015	0.021	0.023
	(0.030)	(0.032)	(0.030)	(0.029)	(0.030)	(0.030)
Age^2 (male)	–0.047	–0.052	–0.049	0.033	–0.064	–0.065
	(0.057)	(0.056)	(0.057)	(0.031)	(0.054)	(0.056)
Age^2 (female)	–0.063	–0.064	–0.065	0.015	–0.070	–0.075
	(0.047)	(0.048)	(0.046)	(0.029)	(0.046)	(0.046)
Weight	0.061	0.065	0.0066	0.0062	0.0059	—
	(0.0042)	(0.0041)	(0.0042)	(0.0039)	(0.0040)	—
H^2	0.52	—	—	0.53	0.35	0.38
	(0.42)	—	—	(0.40)	(0.54)	(0.37)
ρ_G	–0.05	—	—	—	–0.61	–0.24
	(0.30)	—	—	—	(0.44)	(0.31)
ρ_E	–0.73	–0.34	–0.51	–0.78	—	–0.48
	(0.48)	(0.10)	(0.14)	(0.39)	—	(0.30)
Ln likelihood	–2577.39	–2630.93	–2580.43	–2577.40	–2580.12	–2665.42
Alternative model		Model I	Model I	Model I	Model I	Model I
χ^2		107.1	6.1	0.0	5.5	176.1
d.f.		3	2	1	1	2
P value		<0.0001	0.048	0.941	0.020	<0.0001

*The numbers given are maximum likelihood estimates, with standard errors under each estimate. β_{weight} is the partial regression coefficient for weight effect. In the above analyses, the best fit and most parsimonious model is Model IV.

both BMD and OF of the hip, the genetic correlation ρ_G between them is very small (0.05) and nonsignificant (P = 0.94). If the ρ_G were significant, this would suggest that OF risk and BMD variation may be influenced by a common set of genes and less than 1% of the genetic variation would be shared by BMD and OF at the hip. By comparison of Models I and VI (Table I), weight affects BMD variation and OF susceptibility significantly ($P < 0.0001$).

Although the genetic correlation (ρ_G) between BMD and OF at hip is low and nonsignificant, there is a significant environmental correlation (ρ_E) between them; ρ_E = –0.73 (P = 0.020 by comparing Models I and V, Table I). With the h^2, ρ_G and ρ_E computed from pedigree data for BMD and OF, we can compute the phenotypic correlation (ρ_p) between them by the following formula [Falconer, 1989; Lynch and Walsh, 1998]:

$$\rho_P = \sqrt{h_1^2}\sqrt{h_2^2}\rho_G + \sqrt{(1-h_1^2)}\sqrt{(1-h_2^2}\rho_E, \quad (1)$$

where h_1^2 and h_2^2 are the heritabilities for the BMD and OF, respectively. By the data from Model I and Equation 1, we estimate that ρ_p = –0.30. A negative phenotypic correlation indicates that increasing BMD values are associated with decreasing susceptibilities to OF at the hip, which is consistent with previous results [Cummings et al., 1985; Melton et al., 1989; Deng et al., 2000a].

Examination of the accepted model from the bivariate analyses of BMD and OF at the spine (Model III in Table II) and wrist (Model IV in Table III), reveals that h^2 estimates are 0.58 (±0.06) for spine BMD and 0.71 (±0.04) for wrist BMD. Comparison of Models III and V (Tables II and III) for both of these sites shows that weight significantly affects BMD variation and OF susceptibility ($P < 0.0001$). The ρ_E between BMD and OF is significant at the spine (ρ_E = –0.39, P = 0.0007) and nonsignificant at the wrist (ρ_E = –0.09, P = 0.38). However, in contrast with the results at the hip, h^2 of OF at the spine and the wrist are both not significantly different from zero by comparing Models I and III (Tables II and III). Similarly, ρ_G between BMD and OF is not significant for both the spine and the wrist. The phenotypic correlation (ρ_p) between BMD and OF is –0.25 at the spine and –0.05 at the wrist.

DISCUSSION

In summary, for 703 subjects from 50 Caucasian pedigrees, we have attempted to characterize the shared genetic components of BMD and OF at the hip, spine, and wrist. At the hip, we estimated that 64% of the residual phenotypic variance in BMD and 52% of the residual phenotypic variance in susceptibility to OF is attributable to additive effects of genes. However, less than 1% of additive genetic variance is shared between hip BMD and hip OF. Hence, in this population, genes found important for BMD may not be relevant to OF at the hip. The phenotypic negative correlation between hip BMD and hip OF is largely due to a highly significant negative environmental correlation. Analyses of the spine and the wrist did not detect significant h^2 for OF, thus no estimate of ρ_G is available. As with many previous studies, weight significantly affects BMD and OF risk [e.g., Hui et al., 1982; Pouilles et al., 1995;

TABLE II. Bivariate Analyses of BMD and OF at Spine*

	Model I (unrestricted)	Model II ($\rho_G = h^2_{BMD} = h^2_{OF} = 0$)	Model III ($\rho_G = h^2_{OF} = 0$)	Model IV ($\rho_G = h^2_{OF} = \rho_E = 0$)	Model V ($\beta_{weight} = 0$)
BMD					
Mean	82.06	78.79	80.95	80.17	105.21
	(3.56)	(4.50)	(3.77)	(11.82)	(2.50)
Sex	4.23	4.72	4.53	4.82	–0.45
	(2.47)	(3.73)	(2.81)	(8.81)	(2.57)
Age (male)	–0.12	–0.27	–0.12	–0.13	0.04
	(0.17)	(0.21)	(0.17)	(0.44)	(0.17)
Age (female)	–0.22	–0.31	–0.24	–0.26	–0.06
	(0.13)	(0.16)	(0.14)	(0.25)	(0.14)
Age^2 (male)	0.0017	0.0050	0.0018	0.0019	–0.0013
	(0.0030)	(0.0034)	(0.0029)	(0.0063)	(0.0030)
Age^2 (female)	–0.0041	–0.0024	–0.0037	–0.0038	–0.0073
	(0.0024)	(0.0028)	(0.0025)	(0.0039)	(0.0028)
Weight	0.28	0.31	0.29	0.30	—
	(0.04)	(0.04)	(0.04)	(0.08)	—
h^2	0.60	—	0.58	0.60	0.63
	(0.07)	—	(0.06)	(0.06)	(0.07)
OF					
Mean	2.77	2.65	2.69	2.48	1.93
	(0.76)	(0.75)	(0.75)	(1.06)	(0.43)
Sex	0.05	0.09	0.09	0.31	0.20
	(0.58)	(0.46)	(0.45)	(0.71)	(0.59)
Age (male)	0.0093	0.0051	0.0046	0.011	0.0067
	(0.031)	(0.030)	(0.029)	(0.040)	(0.031)
Age (female)	0.030	0.025	0.025	0.0099	0.030
	(0.029)	(0.027)	(0.026)	(0.051)	(0.031)
Age^2 (male)	–0.03	–0.021	–0.020	–0.035	–0.024
	(0.047)	(0.046)	(0.044)	(0.061)	(0.046)
Age^2 (female)	–0.085	–0.077	–0.076	–0.055	–0.080
	(0.046)	(0.044)	(0.043)	(0.08)	(0.049)
Weight	–0.0095	–0.0084	–0.0089	–0.0065	—
	(0.0072)	(0.0074)	(0.0074)	(0.014)	—
h^2	0.10	—	—	—	—
	(0.00)	—	—	—	—
ρ_G	–0.98	—	—	—	—
	(0.51)	—	—	—	—
ρ_E	–0.16	–0.30	–0.39	—	–0.17
	(0.16)	(0.08)	(0.11)	—	(0.13)
Ln likelihood	–2728.53	–2781.55	–2730.32	–2736.16	–2758.46
Alternative model		Model I	Models I/II	Model III	Model III
χ^2		106.0	3.6/102.5	11.7	56.3
d.f.		3	2/1	1	2
P value		<0.0001	0.169/<0.0001	0.0007	<0.0001

*In Tables II and III, the alternative model given is either the general model against which the current model is tested as a restricted model or is the restricted model for which the current model serves as a general model for testing. For example, for Model III in this table, in the alternative Models I and II, model I is the general model against which Model III is tested and Model II is the restricted model for which Model III serves as a general model for testing. The restricted models are nested within the general models. In the above analyses, the best fit and most parsimonious model is Model III.

TABLE III. Bivariate Analyses of BMD and OF at Wrist*

	Model I (unrestricted)	Model II ($\rho_G = h^2_{BMD} = h^2_{OF} = 0$)	Model III ($\rho_G = h^2_{OF} = 0$)	Model IV ($\rho_G = h^2_{OF} = \rho_E = 0$)	Model V ($\rho_G = h^2_{OF} = \beta_{weight} = 0$)
BMD					
Mean	60.61	70.00	60.57	60.57	68.11
	(0.28)	(0.00)	(1.76)	(0.02)	(1.04)
Sex	–6.76	–11.26	–6.76	–6.75	–8.26
	(0.25)	(1.17)	(1.26)	(0.02)	(1.21)
Age (male)	0.28	0.09	0.28	0.28	0.33
	(0.06)	(0.09)	(0.08)	(0.05)	(0.08)
Age (female)	0.16	0.20	0.16	0.16	0.21
	(0.05)	(0.08)	(0.06)	(0.04)	(0.07)
Age^2 (male)	–0.0079	–0.0050	–0.0079	–0.0079	–0.0089
	(0.0012)	(0.0016)	(0.0013)	(0.10)	(0.0015)
Age^2 (female)	–0.0078	–0.0087	–0.0079	–0.0079	–0.0089
	(0.097)	(0.0014)	(0.0011)	(0.089)	(0.0013)
Weight	0.091	0.019	0.091	0.091	—
	(0.0090)	(0.012)	(0.018)	(0.0078)	—
H^2	0.71	—	0.71	0.71	0.73
	(0.08)	—	(0.06)	(0.04)	(0.07)
OF					
Mean	1.03	1.00	0.96	0.97	1.26
	(0.68)	(0.00)	0.47)	(0.07)	(0.48)
Sex	–0.19	–0.16	–0.14	–0.14	–0.20
	(0.49)	(0.28)	(0.31)	(0.16)	(0.65)
Age (male)	–0.0065	–0.0059	–0.0047	–0.0053	–0.0035
	(0.027)	(0.024)	(0.023)	(0.019)	(0.039)
Age (female)	0.019	0.019	0.019	0.018	0.020
	(0.019)	(0.017)	(0.017)	(0.016)	(0.022)
Age^2 (male)	0.020	0.019	0.017	0.018	0.015
	(0.048)	(0.046)	(0.043)	(0.038)	(0.060)
Age^2 (female)	–0.045	–0.045	–0.045	–0.044	–0.048
	(0.032)	(0.030)	(0.030)	(0.029)	(0.037)
Weight	0.0027	0.0028	0.0030	0.0031	—
	(0.0060)	(0.0029)	(0.0051)	(0.0026)	—
h^2	0.05	—	—	—	—
	(0.00)	—	—	—	—
ρ_G	0.44	—	—	—	—
	(0.34)	—	—	—	—
ρ_E	–0.21	–0.02	–0.09	—	–0.11
	(0.16)	(0.07)	(0.11)	—	(0.12)
Ln likelihood	–1817.40	–1890.48	–1817.62	–1818.01	–1830.99
Alternative model		Model I	Models I/II	Model III	Model III
χ^2		146.1	0.4/145.7	0.80	26.7
d.f.		3	2/1	1	2
P value		<0.0001	0.80/<0.0001	0.38	<0.0001

*In Tables II and III, the alternative model given is either the general model against which the current model is tested as a restricted model or is the restricted model for which the current model serves as a general model for testing. For example, for Model III in this table, in the alternative Models I and II, model I is the general model against which Model III is tested and Model II is the restricted model for which Model III serves as a general model for testing. The restricted models are nested within the general models. In the above analyses, the best fit and most parsimonious model is Model IV.

Deng et al., 1998a, 2000b]. Age effects on BMD and OF have been examined extensively by many previous studies [e.g., Deng et al., 1998a, 2000a,b], thus we wish not to pursue to elaborate them here. The SE associated with age effects may convey to some extent the significance of the age effects. For the dichotomous variable OF, the sample size employed here is small compared with the extensive previous studies on examination of the age effect on OF [Deng et al., 2000a]. This may have led to the large standard errors for the age effects on OF in Tables I–III.

Given these results, a genome search for OF ideally should start with unequivocal evidence of significant heritability for OF. However, direct evidence for genetic determination of OF is extremely rare [but see Deng et al., 2000a]. Even the evidence for familial aggregation of OF [Fox et al., 1998; Kannus et al., 1999; Keen et al., 1999] is scarce with the few results being largely inconsistent with each other. Due to the dichotomous outcome for OF, it usually needs much larger sample sizes to detect a significant h^2 than for continuously distributed BMD. With a moderate sample size of about 700 subjects from 50 human pedigrees, we detected significant and relatively high h^2 for OF at the hip, but failed to do so for the spine and wrist. In a previous study [Deng et al., 2000a], with a much larger sample size of 8,745 individuals from 2,471 nuclear families (mothers and daughters) from the same study population in the Midwest of the United States, we detected a significant and moderate h^2 for wrist OF (0.254 ± 0.118). Therefore, the inability to detect a significant h^2 for OF at wrist in the current study may be due to the relatively small sample size employed. It should be pointed out that ascertainment of OF here is based on self-reports in questionnaires. It has been shown [Bush et al., 1989; Nevitt et al., 1992; Pagnini and Chao, 1993; Ismail et al., 2000] that self-reported symptomatic fractures (such as OF at the wrist and hip) are quite reliable. However, there are still some degrees of discrepancies between self-reported OF status and those that are confirmed by radiography or medical record [Ismail et al., 2000]. Some incidents of OF with minor effects may have been ignored or forgotten by subjects. This, together with the relatively small sample size (703 people from 50 pedigrees) and inadvertent inclusion of fractures due to accidental high trauma, may underlie the nonsignificant h^2 revealed for the spine and wrist.

Without solid evidence of a significant genetic component to variation in susceptibility to OF**,** extensive molecular genetics studies [Morrison et al., 1994; Johnson et al.,1997; Koller et al., 1998; Langdahl et al., 1998; Feskanich et al., 1998; Uitterlinden et al., 1998; Gong et al., 1999; Gennari et al., 1999; Cauley et al., 1999; Deng et al., 1998b, 1999b; Ensrud et al., 1999; Weichetova et al., 2000] have been launched to search for genes underlying OF susceptibility. BMD has most often been used as a surrogate phenotype [Morrison et al., 1994; Johnson et al.,1997; Koller et al., 1998; Gong et al., 1999; Deng et al., 1998b, 1999b]. This is largely because the genetic determination of BMD is strong [Dequeker et al., 1987; Slemeda et al., 1991; Sowers et al., 1992; Gueguen et al., 1995; Deng et al., 1999a, 2000b], and there are significant *phenotypic* correlations between high BMD and low risk to OF [Cummings et al., 1985; Melton et al., 1989; Deng et al., 2000a]. However, these two facts DO NOT imply either of the following links that are *necessary* in order to study BMD as a surrogate phenotype in search for genes underlying OF risk. First, OF is significantly heritable. Second, the genes found for BMD variation is important and relevant for differential susceptibility to OF. Establishment of the above two links is

important, in light of the facts [Allolio, 1999] that OF cannot be exclusively attributable to BMD and other skeletal factors (such as bone size and bone loss rate) and non-skeletal factors such as propensity to fall (susceptibility to low trauma) play a critical role in OF risk and incidents.

Genetic correlation is an important index in genetic studies of complex traits [Falconer, 1989; Deng and Kibota, 1995; Lynch and Walsh, 1998; Deng et al., 1999c]. As is well known in genetics for complex traits [Falconer, 1989; Lynch and Walsh, 1998], phenotypic correlation is caused by genetic and environmental factors**,** so that a significant phenotypic correlation does not necessarily imply significant shared genetic effects between the two traits. Significant genetic correlations are usually interpreted as evidence of shared genetic effects on the variance of two traits, i.e., pleiotropy; however, they also can indicate linkage disequilibrium between very closely linked genes that influence the two traits [Lynch and Walsh, 1998; Williams et al., 1999a]. While the latter seems an unlikely confounder for complex traits like those analyzed here, discrimination between pleiotropy and co-incident linkage can best be accomplished at the linkage screen stage [Williams et al., 1999a]. Estimation of genetic correlation usually has large sampling errors [Robertson, 1959]. While significant and high genetic correlation indicates that some of the genes influencing two concerned traits are the same or are in strong linkage disequilibrium [Lynch and Walsh, 1998; Falconer, 1989], low genetic correlations may not unequivocally exclude some shared gene(s) influencing variation in the two traits [Carey, 1988].

It is clear from Equation 1 that a significant phenotypic correlation and a significant heritability estimate for the one trait (such as BMD) do not imply a significant heritability estimate for the second trait (such as OF). This point is demonstrated by our result for the spine for which a statistically significant phenotypic correlation of –0.25 is estimated between BMD and OF when the estimated heritability of 0.58 for BMD is significantly different from zero, but the heritability estimate for OF is not. Even when the heritability estimates for BMD and OF both are significant and there is a high phenotypic correlation between them, it is not necessary that the genetic correlation is significantly different from zero. This is demonstrated by our result for the hip. At the hip, heritability estimates for both BMD and OF are significant and the phenotypic correlation between BMD and OF is also high; however, there appears to be little shared genetic determination between BMD and OF at the hip as revealed by the low (0.05) and nonsignificant ρ_G when weight is adjusted in analyses. Therefore, our results indicate that genes found for BMD variation may be of little relevance to differential susceptibility to OF. On the other hand, our results of significant and negative environmental correlations are consistent with the observations that low BMD is associated with high risk to OF [Cummings et al., 1985; Melton et al., 1989] and with current therapeutic practice [Deng et al., 1998b] to increase BMD with the aim to reduce OF risk.

In conclusion, gene search for complex diseases via underlying risk factors should ideally first establish genetic correlation between a disease and its underlying risk factors and only those underlying risk factors genetically correlated with the disease should be employed as study phenotypes to search genes for the disease. The relevance of the genes found for underlying risk factors should ultimately be tested through studying diseases per se as phenotypes.

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