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Determination of Bone Mineral Density of the Hip and Spine in Human Pedigrees by Genetic and Life-Style Factors

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In 40 human pedigrees with 563 subjects, we evaluated the contribution of genetic and life-style factors (exercise, smoking, and alcohol consumption) and the interactions between non-genetic factors in determining bone mineral density (BMD) of the hip and spine. In our analysis, we adjusted for age, weight, height, menopausal status in females, life-style factors, and the significant interactions among these factors. For the spine and hip BMD, heritabilities (h^2) (± SE) were, respectively, 0.68 (0.21) and 0.86 (0.28) in males and 0.64 (0.13) and 0.67 (0.14) in females. Exercise had significant beneficial effects for male spine BMD and female hip BMD. Alcohol consumption experienced in our sample had significant beneficial effects on hip BMD in both sexes. Although the main effect of smoking was not significant, there were significant interaction effects between smoking and other important factors (e.g., exercise, weight, alcohol consumption). For example, for female spine BMD, exercise had significant beneficial effects in smokers; however, its effect in non-smokers was non-significant. This result indicates that exercise may reduce deleterious effects of smoking (if any) on BMD, but may have minor effects in increasing BMD in non-smokers. The various interaction effects among risk factors explicitly revealed here for the first time indicate that the detailed effects and direction of individual risk factors

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may depend on the presence and magnitude of other factors. Weight invariably affected BMD of the hip and spine in both sexes. Age effects were significant for hip BMD, but not for male spine BMD. Genet. Epidemiol. 19:160–177, 2000.

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INTRODUCTION

Bone mineral density (BMD) is a quantitative trait, which is measured on a continuous scale by methods such as dual X-ray absorptiometry (DXA). 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]. Osteoporosis results in more than 1.3 million osteoporotic fractures a year, with an estimated direct cost of $13.8 billion [Ray et al., 1997] in the United States alone.

Decades of research have identified an array of factors underlying BMD variation. The importance of gender, race, age, weight, height, and menopausal status in females has been well established [Liel et al., 1988; Krall and Dawson-Hughes, 1993; Pouilles et al., 1995; Deng et al., 1998a]. The relevance of life-style factors (such as smoking, exercise, and alcohol consumption) has also been demonstrated. However, despite a moderately large body of studies, a consensus has yet to be developed. For example, while most studies report detrimental effects of smoking in reducing BMD and increasing fracture risks [e.g., Bauer et al., 1993; Hopper and Seeman, 1994; Slemenda, 1994; Kiel et al., 1996], several studies have failed to demonstrate the detrimental bone effects of smoking [e.g., Hemenway et al., 1988; McDermott and Witte, 1988; Glynn et al., 1995]. For exercise, the majority of studies indicate that moderate exercise can increase and/or preserve BMD and reduce fracture risks [Kohrt et al., 1997; Uusi et al., 1998]; however, several studies have failed to demonstrate any significant effect [Bauer et al., 1993; Young et al., 1995]. Even in the same study [Khan et al., 1998], the same physical activity increased BMD at some skeletal sites (spine and/or hip) but not the forearm. This differential responsiveness to exercise at different skeletal sites was also revealed by a meta-analysis [Berard et al., 1997]. Similarly, for alcohol consumption, studies showing beneficial [Felson et al., 1995; Kroger et al., 1994], detrimental [Laitinen and Valimaki, 1993], and no or uncertain effects [Bauer et al., 1993; Hu et al. 1994] have all been reported.

Despite extensive data accumulated over years, few studies addressed the combined effects among the potentially important factors. Particularly, the interaction effects among important factors have seldom been studied. The interactive effects of smoking with other environmental factors have been suggested [Law and Hackshaw, 1997; Forsen et al., 1994; Kiel et al., 1992; Kusec et al., 1998]. For example, smoking was found to have a larger effect in increasing osteoporotic fracture risk in thinner women [Forsen et al., 1994]. Smoking may eliminate the protective effects of oral estrogen for hip fracture and bone mass loss among women [Kiel et al., 1992] or hormone replacement therapy may conceal the possible adverse effect of smoking [Kusec et al., 1998]. Without studies on the interaction, the detailed effects (direction and magnitude) of a particular risk factor cannot be accurately ascertained. This

is because in the presence of interactions, the detailed effects of a risk factor would critically depend on the specific background of other factors.

Recently, rapidly accumulating data have unequivocally established that BMD variation is also under genetic control with heritability (h^2) estimated 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., 1999b]. Almost all of these results are from twin studies [Dequeker et al., 1987; Slemeda et al., 1991], correlation studies of relatives [Deng et al., 1999b], or small nuclear families [Gueguen et al., 1995]; few are from large human pedigrees. Results from different approaches using different types of samples may provide a mechanism to confirm and generalize the conclusions of each other. Importantly, in previous h^2 studies, few adjusted for the effect of important factors (such as age) on BMD. Failure to adjust for the age effect may bias the interpretation of the h^2 estimates obtained [Deng et al., 1999b]. Adjusting for significant covariates in genetic analyses can generally increase the genetic signal to noise ratio (i.e., h^2 estimates) by decreasing the proportion of the residue phenotypic variation attributable to random environmental factors [Deng et al., 1999a] and this can increase statistical power in our subsequent linkage analyses. In addition, since genes clearly account for a substantial proportion of phenotypic variation, simultaneous characterization of h^2 and selected environmental factors (as covariates) can result in more accurate characterization of the selected environmental factors.

Our purposes here were twofold. First we estimated the h^2's by adjusting for some significant non-genetic factors. The estimation was performed for males and females, respectively, to account for the gender difference in BMD [McCormick et al., 1991], the potential gender-specific effects of non-genetic factors, and the potential difference of genetic variance in two genders that have been revealed in other organisms [Lynch and Walsh, 1998]. Second, we evaluated the significance of the non-genetic factors, such as weight, age, menopause in females, and, particularly, the life-style factors (smoking, exercise, and alcohol consumption) on BMD simultaneously when estimating h^2. The study was performed for the hip and spine BMD, respectively, since there is substantial heterogeneity of BMD at different skeletal sites [Deng et al., 1998a] and the determination of BMD at different skeletal sites may not be the same [Deng et al., 1999b].

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.

The study subjects in this analysis came from an expanding database being created for a whole genome linkage study aimed at searching for genes underlying BMD variation that is underway in the Osteoporosis Research Center of Creighton University. Only healthy people [defined in detail in Deng et al., 1999b] were included in the analysis. All the study subjects were Caucasians of European origin. Forty pedigrees with 212 male and 351 female subjects from two to four generations were included in analyses. Each pedigree was identified through a proband having BMD Z-scores ≤ -1.28 at the hip or spine. BMD values expressed as Z-scores adjust for age, gender, and ethnic difference in general referent healthy populations.

Measurement

BMDs of spine and hip 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. We chose the hip and spine because they are the most common osteoporotic fracture sites [Cummings et al., 1985]. Short-term precision in humans is 0.7% for spine BMD and 1.0% for hip BMD. We maintain constant quality assurance procedures that track potential confounding events such as X-ray tube replacement, arm re-alignments, collimator changes, and software version updates. Technicians maintain scan-by-scan surveillance for quality control. We chose BMD rather than bone mineral content as our bone mass phenotype, because BMD is the measure most closely correlated with fracture risk [Black et al., 1992]. For the spine, our quantitative phenotype was combined BMD of L_{1-4}. For the hip, it was combined BMD of the femoral neck, trochanter, and intertrochanteric region. All DXA machines report hip and spine BMD in g/cm^2. Height and weight were measured at the same visit when the BMD measurements were taken.

All subjects completed a nurse-administered risk factor questionnaire to assess the information concerning smoking, alcohol consumption, physical activity, and menopausal status, etc. For smoking, the ages of starting and stopping smoking and the average packs of cigarettes smoked per day were recorded. For exercise and alcohol consumption, the number of episodes of exercise and the number of drinks per week were recorded. The practice of asking respondents to recall past levels of smoking and alcohol consumption has been shown to be valid [Krall et al., 1989; Longnecker et al., 1992].

Statistical Analyses

The variance component analysis [Lange et al., 1976] for quantitative traits with a polygenic component of variation was performed. The analysis assumed multivariate normal distribution of phenotypic values, additive genetic effects, and no interaction between genes and the residual. The common familial environmental effects were assumed to be negligible, which is reasonable and supported by previous studies [Sowers et al., 1992; Krall and Dawson-Hughes, 1993; Gueguen et al., 1995; Deng et al., 1999b]. The program employed is SOLAR (Sequential Oligogenic Linkage Analysis Routines) available on the internet (http://www.sfbr.org/sfbr/public/software/solar/solar.html). The theory of pure polygenic analysis by SOLAR is based on that of Lange et al. [1976]. The ascertainment bias of pedigrees based on the low BMD values of probands was corrected in the SOLAR program by identifying to the program the probands for each pedigree.

The general analysis model assumed that BMD was determined by the mean of the trait, a polygenic background representing genetic effects, a number of non-genetic covariates and the interactions among them (modeled as multiplication of values of potentially interacting covariates), and a residual representing the effects of environmental factors that were not analyzed in the model. The non-genetic covariates include weight, age, age$^{\wedge 2}$, height, menopausal status (for females), smoking, exercise, and alcohol consumption. The residues after adjusting for covariates were tested by graphic methods [Sokal and Rohlf, 1995] and found not to deviate from a normal

distribution. Hypotheses testing for genetic effects and non-genetic covariates was conducted by the maximum likelihood method, which compares the maximum likelihood obtained in full and nested models using the likelihood ratio statistic. Under the null hypothesis that the reduced model (null hypothesis, in which some effects are assumed to be non-significant) is correct, the likelihood ratio statistic approximately follows a χ^2 distribution with the degrees of freedom equal to the number of constrained parameters. Weight, menopausal status (for females), smoking, exercise, and alcohol consumption were always included in the model. Various interactions among these non-genetic factors were tested. Only the significant interactions were included in the final analyses. After extensive testing for different models, final results on the mean, standard errors, and the significance levels of the coefficient of various effects are reported.

For testing the smoking effects, study subjects were first classified as smokers and non-smokers, with smokers numerically coded as 1 and non-smokers as 0. Then analyses were performed for smokers only, in which the detailed effects of the number of packs of cigarettes smoked per day, the starting age of smoking (representing the differential effects of smoking on BMD at various developmental stages), and the time duration of smoking were tested. To demonstrate intuitively the detailed effects of smoking and/or other risk factors, when there were significant interactions between them, separate analyses were performed under the same model to reveal the differential effects of the other factors in smokers versus non-smokers. We also tested the quadratic effects of the numbers of times of exercise and alcohol drinking on BMD.

Tables I and II and Figure 1 are employed to show intuitively the interaction effects between smoking and weight for female hip BMD, and between drinking and age for male spine BMD, respectively. Table III demonstrates the differential effects of age on BMD in pre- and post-menopausal females in the presence of a significant interaction between age and menopausal status for spine BMD.

RESULTS

The basic characteristics of the study subjects stratified by age and sex are summarized in the Appendix.

Heritability (h^2) of Spine and Hip BMD

After adjusting for a number of non-genetic covariates (Table I), the h^2's (±SE) estimated for spine and hip BMD were 0.68 (0.21) and 0.86 (0.28), respectively, for males, and were 0.64 (0.13) and 0.67 (0.14), respectively, for females. SE denotes one standard error. Although the h^2 tends to be higher in males than in females, it also tends to be higher for hip BMD than for spine BMD; none of these differences was significant in light of the standard errors associated with the point estimates.

Effects of Non-genetic Factors and Their Interactions on Hip and Spine BMD

Generally (Table I), it can be easily noticed that the effects of the same non-genetic factors may be different for BMD at spine and hip and for males and females. For example, the main effects of exercise were significant for male spine BMD and for female hip BMD; however, they were not significant for male hip

TABLE I. Determination of BMD by Genetic and Non-genetic Factors[†]

	Spine BMD		Hip BMD	
	Female	Male	Female	Male
Mean	1.02 (0.02)	1.03 (0.019)	0.883 (0.0138)	0.980 (0.0147)
Weight	2.49E-3*** (5.23E-4)	3.30E-3*** (8.31E-4)	4.50E-3*** (4.31E-4)	4.44E-3*** (7.24E-4)
Menopause	–0.0725*** (0.0238)	— —	–0.0204 (0.0185)	— —
Age	1.63E-3 (1.88E-3)	2.51E-4 (8.24E-4)	–4.28E-3*** (6.87E-4)	–1.28E-3** (6.09E-4)
Age^2	1.02E-4* (5.02E-5)	4.13E-5 (4.05E-5)	–4.14E-5 (0.07) (2.29E-5)	8.88E-5*** (3.12E-5)
Smoking	5.98E-3 (1.32E-2)	–2.21E-3 (1.79E-2)	0.0159 (0.0109)	–0.0116
Drinking	6.39E-3) (6.30E-3)	0.0108 (0.00721)	0.0121* (0.00506)	4.90E-3*** (5.17E-3)
Exercising	4.31E-4 (3.41E-3)	0.0137*** (0.00463)	7.04E-3* (2.81E-3)	3.02E-3
Height	—	—	–0.105 (0.0836)	6.17E-3*** (0.134)
Menopause & age	–9.10E-3*** (2.96E-3)	—	—	—
Exercising & smoking	0.0143* (0.00570)	—	—	—
Exercising & age	–4.98E-4* (2.12E-4)	—	—	—
Drinking & weight	–9.20E-4* (4.17E-4)	—	—	—
Drinking & age	—	1.11E-3* (4.46E-4)	—	—
Smoking & drinking	—	—	–0.0161* (0.00702)	—
Smoking & weight	—	—	–1.32E-3* (6.30E-4)	—
Drinking & height	—	—	0.131* (0.0578)	—
Smoking & height	—	—	–0.245 (0.08) (0.139)	—
Weight & height	—	—	–0.0129* (0.00614)	—
h^2	0.639 (0.126)	0.684 (0.212)	0.68 (0.14)	0.858 (0.276)

[†]The numbers given as the coefficients and associated one standard error for each effect indicated. Effects significant at *P* values of 0.05 (*), 0.01 (**), and 0.005 (***) are indicated. & indicates an interaction between the two factors involved. — indicates that the effects were not significant and thus not in the final model presented or that the effects are not appropriate (such as menopause for males). As standard, E-*n* where *n* is a number from 1 to 9 indicates a multiplication by 10^{-n}.

BMD and for female spine BMD. The only exception was weight, which invariably had highly significant effects on BMD of the hip and spine in both genders. It needs to be pointed out that the different effects of various factors at the spine and hip were detected with the same sample size and with the same analyses. Thus, it is unlikely

TABLE II. Analysis of Smoking Effects on Female Hip BMD[†]

	Smokers			Non-smokers
	Model I	Model II	Model III	Model III
Mean	0.870 (0.0136)	0.871 (0.0133)	0.871 (0.0138)	0.874 (0.0132)
Weight	4.02E-3*** (6.17E-4)	4.15E-3*** (5.94E-4)	4.15E-3*** (5.79E-4)	4.82E-3*** (6.27E-4)
Age	–7.24E-3*** (1.07E-3)	–7.31E-3*** (1.06E-4)	–6.29E-3*** (7.82E-4)	–4.51E-3** (5.86E-4)
Age^2	–5.27E-5 (4.45E-5)	–5.49E-5 (4.02E-5)	–5.63E-5 (4.54E-5)	–3.70E-5 (2.76E-5)
Drinking	6.10E-3 (7.19E-3)	5.18E-3 (7.19E-3)	6.20E-3 (7.15E-3)	0.0163804* (0.00723)
Exercising	0.0143*** (0.00437)	0.0141*** (0.0043)	0.0131*** (0.0044)	3.66E-3 (3.79E-3)
Height	–0.305* (0.145)	–0.284* (0.141)	–0.275(0.059) (0.145)	–0.0135 (0.0865)
Packs per day	0.0265(0.09) (0.0157)	0.0268(0.09) (0.0158)	—	—
Starting year	4.35E-3 (3.03E-3)	4.67E-3 (3.03E-3)	—	—
Years smoking	4.97E-4 (9.63E-4)	4.91E-4 (9.68-04)	—	—
Drinking & height	0.104 (0.0868)	—	—	—
Weight & height	–5.99E-3 (1.03E-2)	—	—	—
h^2	0.322 (0.259)	0.27 (0.245)	0.368 (0.235)	0.692 (0.214)
Ln likelihood	215.96	215.00	212.82	333.75
Alternative model		Model I	Model II	
χ^2		1.92	4.36	
DF		2	3	
P value		0.383	0.225	
Conclusion		Model II is acceptable	Model III is acceptable	

[†]See footnote to Table I. Models I, II, and III differ in the detailed smoking effects and the interactions analyzed. Models I, II, and III do not differ from each other significantly. Thus, model III is chosen for comparison between smokers and non-smokers.

that the different effects detected are entirely owing to the different statistical powers and sampling effects involved and are more likely owing to the true differential effects of various factors on different skeletal sites.

Specifically (Table I), for spine BMD in females, weight, menopausal status, age^2, the interactions (between menopausal status and age, between exercise and smoking, between exercise and age, and between drinking and weight) all had significant effects. The other factors were non-significant. From here on, non-significant factors are generally not mentioned and should be obvious in Tables I–III. Only significant effects are highlighted. For the spine BMD in males, the effects of weight and exercise were highly significant, and the interaction between drinking and age was significant. For the hip BMD in females, weight, age, exercise, drinking, the interaction

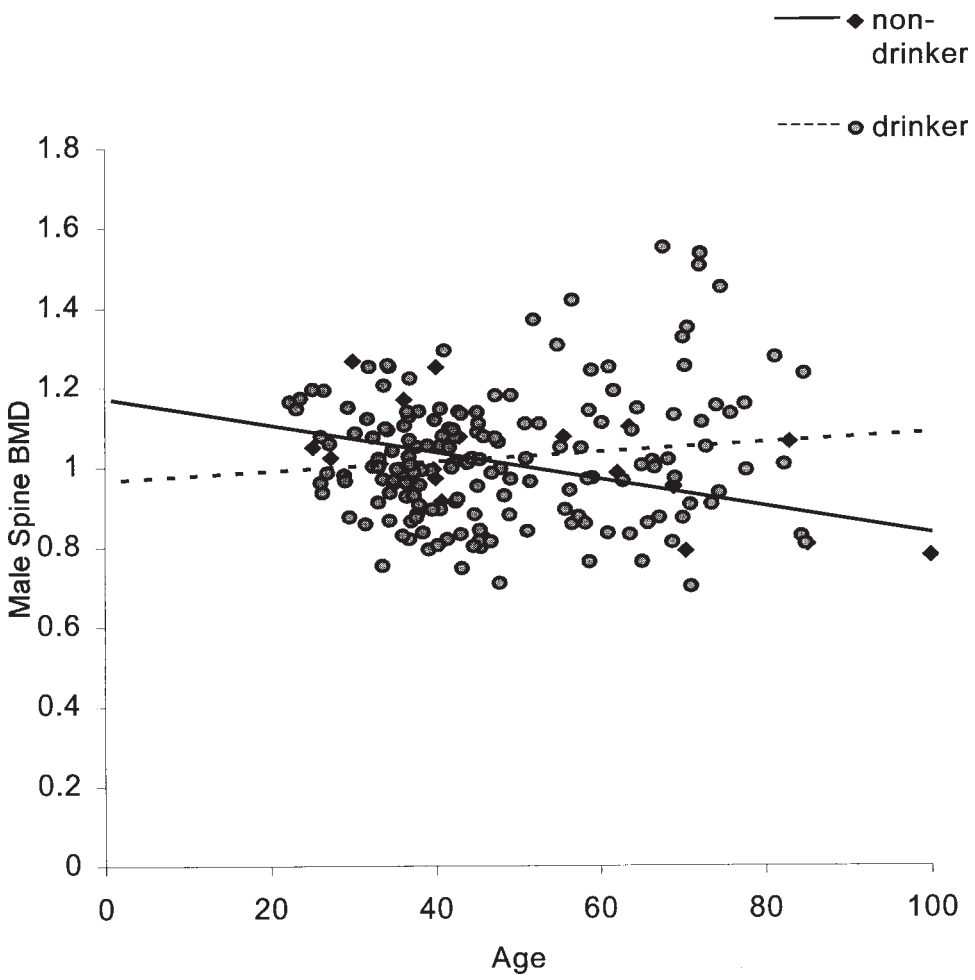

Fig. 1. The interaction effects between alcohol drinking and age.

between smoking and drinking, the interaction between smoking and weight, the interaction between drinking and height, and the interaction between height and weight all had significant effects. In addition, age$^{\wedge 2}$ (P = 0.07) and the interaction between smoking and height both had nearly significant effects (P = 0.08). For the hip BMD in males, weight, age, age$^{\wedge 2}$, drinking, and height had significant effects.

For the detailed smoking effects analyzed for smokers, the packs of cigarettes smoked per day, the starting age of smoking, and the duration of smoking did not have significant effects. These non-significant effects are demonstrated in Table II for female hip BMD. In Table II, for smokers, models I, II, and III differ in the factors that were constrained to be zero, which involved the null hypotheses concerning these factors. These factors are the packs of cigarettes smoked per day, the starting age of smoking, the duration of smoking, and the interactions. The three models tested did not differ from each other and the coefficients for these factors were not significantly

TABLE III. Analysis of Menopausal Effects on Female Spine BMD[†]

	Pre-menopausal data		Post-menopausal data	
	Model I	Model II	Model II	Model I
Mean	1.02 (0.0125)	1.02 (0.0126)	0.857 (0.0181)	0.855 (0.0186)
Weight	1.71E-3*** (5.75E-4)	1.73E-3*** (5.79E-4)	4.56E-3*** (9.59E-4)	4.15E-3*** (1.00E-4)
Age	–1.52E-3 (1.26E-3)	–1.63E-3 (1.26E-3)	–4.68E-3*** (1.12E-3)	–5.07E-3*** (1.13E-3)
Age^2	–1.52E-4 (1.26E-4)	–1.63E-4 (1.26E-4)	1.68E-4** (6.38E-5)	1.60E-4* (6.43E-5)
Smoking	3.44E-3 (1.61E-2)	3.74E-3 (1.60E-2)	–1.51E-4 (2.06E-2)	3.64E-4 (2.04E-2)
Drinking	6.12E-3 (7.31E-3)	7.04E-3 (7.31E-3)	0.0211* (0.0107)	0.0178 (0.0111)
Exercising	3.78E-3 (3.96E-2)	3.29E-3 (3.95E-3)	–3.10E-4 (5.93E-3)	–1.24E-3 (5.90E-3)
Exercising & smoking	0.0132 (0.078) (0.00747)	0.0119 (0.00742)	0.0193* (0.000922)	0.0161 (0.086) (0.00933)
Exercising & age	–4.97E-4 (5.40E-4)	—	—	–5.53E-4 (5.52E-4)
Drinking & weight	–3.84E-4 (4.64E-4)	—	—	–1.06E-3 (8.22E-4)
h^2	0.615 (0.197)	0.629 (0.204)	0.47 (0.194)	0.533 (0.197)
Ln likelihood	311.88	311.13	198.13	199.66
Alternative model		Model I	Model I	
χ^2		1.5	3.06	
DF		2	2	
P value		0.472	0.217	
Conclusion		Model II is acceptable	Model II is acceptable	

[†]See footnote to Table II.

different from zero. Thus, the packs of cigarettes smoked per day, the starting age of smoking, and the duration of smoking did not have significant effects for female hip BMD. This result justified the analysis of smoking effects by classifying subjects as smokers and non-smokers. The interactions between drinking and height and between weight and height were not significant in smokers, although they were significant in the combined data of smokers and non-smokers. This discrepancy may be due to the larger sample size in the combined data than the data of smokers alone.

The direction of the main effects (such as weight, age, exercise, and drinking) can be easily inferred from the coefficients associated with these factors. For example, from the hip BMD in males (Table I), from the signs of the associated coefficients, it can be inferred that larger weight, higher height, and more drinking had significantly beneficial effects to increase and/or to preserve BMD. While BMD decreased significantly with increasing age for the male hip BMD, the decrease was less than linear as reflected by the significant positive age$^{\wedge 2}$ effects.

The interaction effects were less obvious. When there is a significant interaction between two factors, the magnitude and/or the direction of the effects of one

factor depend on those of the other factor involved in the interaction. Some examples of this kind of effect dependency in the presence of interactions are given in Table II and Figure 1. The significant interaction effects between smoking and drinking and between smoking and weight for female hip BMD (Table I) were manifested as differential effects of drinking and weight in smokers and non-smokers (Table II). While drinking did not have significant effects in smokers, it had significant beneficial effects in non-smokers to preserve BMD (Table II). The non-smokers tended to have higher baseline (mean) values and the effects of weight to increase BMD in non-smokers were higher (as reflected by the higher positive coefficient for weight in non-smokers [Table II]). For males, the significant interaction between drinking and age (Table I) was manifested in that spine BMD was relatively stable in drinkers with aging, and it decreased with aging in non-drinkers (Fig. 1).

Quadratic terms of the amount of exercise and drinking, indicative of inflection of change in these covariate factors, were not significant for BMD. Therefore, the effects (if any) of exercise and drinking on BMD were roughly linear. There were no optimum intermediate levels of exercise and drinking within the range and the extent of exercise and drinking that were experienced in our study subjects (quantified in the Appendix).

The non-significant effects of age on female spine BMD were a bit surprising (Table I). However, when the female study subjects were stratified according to their menopausal status (Table III), the analyses revealed significant age effects (age and age$^{\wedge 2}$) for post-menopausal females and no age effects for pre-menopausal females).

DISCUSSION

In 40 human pedigrees with 212 male and 351 female subjects from two to four generations, we evaluated the contribution of genetic and life-style factors (exercise, smoking, and alcohol consumption) and the interactions between non-genetic factors in determining BMD of the hip and spine. h^2 estimates were high after adjusting for age, weight, height, menopausal status in females, life-style factors, and the significant interactions. Exercise had significant beneficial effects for male spine BMD and for female hip BMD. Normal alcohol consumption seen in our random sample (with respect to alcohol consumption) had significant beneficial effects on hip BMD in both sexes. The normal alcohol consumption experienced in our sample is quantified in the Appendix for various age groups. Although the main effect of smoking was not significant, there were significant interaction effects between smoking with other important factors (e.g., exercise, weight, alcohol consumption). To the best of our knowledge, the various interaction effects among risk factors of BMD were explicitly studied here for the first time. We have shown that, in the presence of significant interactions, the detailed effects and direction of individual risk factors may depend on the presence and magnitude of other factors. Weight invariably affected BMD at the hip and spine in both sexes significantly. Age effects were significant for hip BMD, but not for spine BMD in males.

The high h^2s revealed here unambiguously demonstrates that the major determining factors of BMD variation in healthy people are genetic in nature. The high h^2s of BMD indicate the importance of family history of osteoporosis (characterized by low BMD and/or osteoporotic fractures) in predicting the osteoporotic status for

other family members, particularly for first-degree relatives such as children. Thus, practically speaking, the high h^2s estimated here reinforce the importance of prevention and early intervention among offspring who have osteoporotic parents or grandparents [Danielson et al., 1999].

Owing to the difference in genetic composition and environmental background of different populations, there may be variation in the importance of genetic determination of BMD in populations as reflected by different h^2 s. However, the slight differences of the point estimates of the h^2 in this study and in previous studies were not statistically significant after accounting for sampling errors. This is true even though our estimates adjust for a number of nongenetic factors, which probably reflects the fact that the non-genetic factors studied are just a small subset of a large number of non-genetic factors influencing BMD variation and they only explain a relatively small proportion of non-genetic variation of BMD. h^2 tends to be higher in males than in females, and it also tended to be higher for hip BMD than for spine BMD. However, there were no significant differences for the h^2s estimated for spine and hip and for males and females in light of the standard errors associated with the point estimates of h^2 as stated in Results. Therefore, interaction between polygenes and sex may be absent although our main interests here are not on the polygenes by sex interaction and just to account for it in heritability estimation if it does indeed exists. Separate analyses of two genders are necessary to see the detailed differential effects of various non-genetic factors and their interactions on BMD in males and females, respectively, and it is important that some differential effects were detected for males and females (see Results). Since the work of Morrison et al. [1994], tremendous effort has been spent on unraveling the molecular genetic basis of BMD variation by several approaches. The subjects for this study were from an ongoing large-scale whole genome linkage study designed to search for genomic regions underlying BMD variation. The results from molecular studies of BMD should not only provide bases for genetic counseling and molecular genetic diagnoses for people prone to low BMD, but also pave the way for discovering effective interventions based on the genotypes [Deng et al., 1998b] and properties of mutation products.

As with many earlier studies [Hui et al., 1982; Pouilles et al., 1995; Deng et al., 1998a], age, weight, and menopausal status of women generally have significant effects on BMD. Thus, we do not elaborate all these effects and only briefly discuss the age effects. Age had significant effects for hip BMD in both sexes. For female spine BMD, even though the main effect of age was not significant, the effects of age$^{\wedge 2}$ and the interaction between menopausal status and age were significant (Table I). When separate analyses were performed for females before and after menopause (Table III), the age effect was significant for post- but not for pre-menopausal women. This result reflects our knowledge about the dynamics of BMD with aging. Bone mass increases during growth and development until peak bone mass is reached about age 20 [Recker et al., 1992; Cooper et al., 1995]. It remains more or less stable until about age 50 (or the onset of menopause) [Pouilles et al., 1995] and then declines gradually during the rest of life [Slemenda et al., 1996]. Loss of bone mass accelerates for 5–8 years in women who become estrogen deplete [Recker et al., 1992; Slemenda et al., 1996]. The subjects in this study were all over 20 years of age. Thus, BMD in the pre-menopausal women represents peak bone mass and remains relatively inert to the aging effects. It should be noted from our results that, for the

same sample analyzed, the same factor may have differential effects at different skeletal sites. This substantiates the belief [Krall and Dawson-Hughes, 1993; Matkovic et al., 1990] that different skeletal sites may differ to a certain extent in their responsiveness to specific environmental influences.

There have been extensive studies on the effects of life-style factors on BMD (see Introduction), though a consensus is yet to emerge. The results here are consistent with earlier studies [Kohrt et al., 1997; Uusi et al., 1998; Felson et al., 1995; Kroger et al., 1994] that revealed beneficial effects of exercise and alcohol drinking on preserving or increasing BMD, and are inconsistent with those [Bauer et al., 1993; Young et al., 1995; Laitinen and Valimaki, 1993] that revealed different results. The discrepancies among different studies might be partially owing to the type and the extent to which exercise and alcohol drinking were experienced by the study subjects. This partial explanation was suggested by the evidence that 1) even in the same study [Khan et al., 1998; Berard et al., 1997], the same physical activity was shown to increase BMD at some skeletal sites but not the others. This result might be owing to differential responsiveness to various types of exercise at different skeletal sites. 2) The type and the extent of alcohol consumption were different for some studies that revealed different effects on BMD [Felson et al., 1995; Kroger et al., 1994; Laitinen and Valimaki, 1993]. In our study sample, there may not be optimum intermediate levels of exercise and drinking and the beneficial effects of exercise and drinking increase (or decreases) with the amount that are experienced in our study sample. This conclusion is suggested by the fact that in the presence of significant linear effects of exercise and drinking, we failed to reveal any significant quadratic effects of the times of exercise and drinking on BMD.

Although the smoking effect was often not detected as a main effect in the sample, a significant smoking effect was often manifested through its interaction with other risk factors, such as weight for female spine BMD, and drinking and weight for female hip BMD. For example, owing to the significant interaction between smoking and exercise and between smoking and weight, for female hip BMD in smokers, the exercise will preserve BMD; however, weight has a smaller effect per unit in preserving BMD compared to non-smokers. In non-smokers, exercise has no significant effect. It should be pointed out that the beneficial effect of exercise in smokers may not be to increase BMD; rather it may be to preserve BMD to counteract the detrimental effects of smoking. This is suggested by the lower baseline BMD value in smokers than non-smokers (Table II). The interaction between smoking and weight coincides with the earlier finding that smoking was found to have a greater effect in thinner women [Forsen et al., 1994].

Despite extensive data accumulated over years, few studies address the joint effects of potentially important factors. Particularly, the interactions among important factors have seldom been explicitly studied. The interactive effects of smoking with other environmental factors revealed here, and suggested in earlier studies [Law et al., 1997; Forsen et al., 1994; Kiel et al., 1992; Kusec et al., 1998], pinpoint the necessity to study joint and interactive effects of factors underlying BMD variation. Without these studies, the detailed effects (direction and magnitude) of a particular risk factor cannot be accurately ascertained. Technically, from a statistical point of view, ignoring an existing interaction in analyses may, erroneously, cause the main effect of the factors involved to appear non-significant [Deng et al., 1998b, 1999a;

Ottman, 1990], and thus the importance of the risk factors can be easily overlooked. The discrepancies concerning the effects of individual risk factors in earlier studies may also be partially owng to the facts that 1) the level of other factors was not properly controlled so that the conclusion may be easily different if these factors are important and 2) the interaction effects were not incorporated in the analyses so that the importance of some factors may be overlooked in some studies and the conclusions may be different.

Despite some inconsistent results concerning the importance of life-style factors on BMD variation, some detailed effects of these factors have begun to emerge, although more studies are needed. For example, the mechanism for increased BMD by exercise may be owing to both decreased bone resorption and increased bone formation in cancellous bone and to increased bone formation in cortical bone [Iwamoto et al., 1998]. For smoking, nicotine [Ramp et al., 1991] inhibited collagen synthesis in chick calvarial osteoblast-like cells. An inhibition of collagen synthesis was also observed [Galvin et al., 1988] in embryonic chick tibial cultures exposed to smokeless tobacco extract. Smokers exhibit lower luteal phase circulating estrogen levels [MacMahon et al., 1982] and experience an earlier menopause than non-smokers [Jick and Purter, 1977]. Post-menopausal smoking women on estrogen replacement therapy have lower estrogen levels than comparable non-smokers [Jensen et al., 1985]. The protective effect of estrogen on bone resorption may thus be reduced in smokers, owing either to decreased endogenous estrogen formation [Barbieri et al., 1986] or to increased endogenous/exogenous degradation [Jensen et al., 1985; Michnovicz et al., 1986], leading to increased bone loss. Another smoking effect leading to high bone turnover and subsequent loss may be decreased calcium absorption [Krall and Dawson-Hughes, 1991]. These emerging mechanisms being revealed for the life-style factors coincide well with the studies that demonstrated the importance of the life-style factors.

In summary, this study revealed significant genetic and non-genetic effects on BMD. Our results reinforce the importance of prevention and early intervention among offspring who have osteoporotic parents or grandparents. The life-style factors revealed as important here for BMD are also important for other aspects of human health. For example, exercise can improve cardiorespiratory fitness [Dunn et al., 1999]. Given that some detrimental life-styles are being increasingly adopted [Wechsler et al., 1998], our results indicate the importance of preserving or increasing BMD by changing to life styles of moderate drinking, frequent exercise, and no smoking.

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APPENDIX. Basic Characteristics of the Study Subjects Stratified by Age of Each Decade*

Age Group (years)	Ages (years)	Spine BMD (g/cm^2)	Hip BMD (g/cm^2)	Height (m)	Weight (kg)	M#[a]	Exercise (#/week)	Smoke (packs/ day)	Alcohol drinking (#/week)
20–29	25.6(3.0)	1.02(0.09)	0.97(0.10)	1.66(0.06)	68.3(15.2)	(0)	3.29(1.45)	0.63(0.36)	1.80(0.96)
F	[30]	[28]	[30]	[30]	[30]	[30]	[21]	[9]	[25]
	26.5(2.5)	1.08(0.10)	1.08(0.10)	1.83(0.07)	84.7(11.7)	—	3.38(1.26)	0.88(0.25)	3.00(1.57)
M	[20]	[20]	[20]	[20]	[20]		[13]	[4]	[14]
30–39	35.6(2.7)	1.03(0.12)	0.92(0.13)	1.67(0.08)	69.0(11.5)	(8)	3.65(1.23)	0.93(0.60)	1.62(0.84)
F	[82]	[82]	[82]	[82]	[82]	[73]	[54]	[25]	[65]
	35.9(2.5)	1.01(0.12)	0.99(0.11)	1.79(0.09)	86.7(14.3)	—	3.32(1.44)	0.65(0.41)	2.52(1.31)
M	[64]	[64]	[64]	[64]	[64]		[28]	[15]	[54]
40–49	44.1(2.8)	0.99(0.11)	0.90(0.13)	1.64(0.07)	72.3(17.6)	(19)	3.98(1.49)	1.01(0.57)	2.04(1.23)
F	[94]	[94]	[92]	[94]	[94]	[69]	[41]	[39]	[70]
	44.0(2.6)	0.99(0.14)	0.98(0.14)	1.78(0.07)	87.7(13.6)	—	4.05(1.51)	1.14(0.62)	3.05(1.34)
M	[49]	[49]	[49]	[49]	[49]		[19]	[25]	[43]
50–59	54.9(2.7)	0.93(0.16)	0.83(0.15)	1.64(0.05)	70.9(14.6)	(35)	4.15(1.22)	0.95(0.58)	1.79(1.23)
F	[42]	[42]	[40]	[42]	[42]	[4]	[26]	[20]	[28]
	55.1(3.0)	1.05(0.17)	1.01(0.11)	1.78(0.07)	87.8(10.7)	—	3.55(1.57)	1.33(0.68)	2.70(1.49)
M	[26]	[26]	[26]	[26]	[26]		[11]	[13]	[20]
60–69	64.6(2.8)	0.85(0.20)	0.76(0.13)	1.62(0.06)	69.8(15.9)	(57)	3.97(1.38)	1.00(0.59)	1.97(1.07)
F	[62]	[60]	[55]	[62]	[62]	[0]	[35]	[21]	[35]
	65.6(3.0)	1.02(0.17)	0.97(0.11)	1.75(0.08)	88.2(12.8)	—	4.44(1.67)	0.92(0.39)	2.14(1.11)
M	[27]	[27]	[26]	[27]	[27]		[16]	[13]	[21]

70–79	73.8(2.3)	0.75(0.17)	0.67(0.10)	1.54(0.17)	63.1(10.0)	(30)	4.53(1.62)	0.97(0.87)	1.68(1.06)
F	[33]	[33]	[28]	[33]	[33]	[0]	[17]	[10]	[19]
	73.1(2.4)	1.12(0.25)	1.05(0.16)	1.74(0.05)	84.6(12.0)	—	4.64(1.82)	1.15(0.41)	2.33(1.40)
M	[16]	[16]	[16]	[16]	[16]		[14]	[10]	[15]
80–89	83.0(2.6)	0.87(0.25)	0.67(0.17)	1.59(0.04)	67.1(22.9)	(5)	5.00(0.00)	2.00(0.00)	1.67(1.15)
F	[6]	[6]	[6]	[6]	[6]	[0]	[1]	[1]	[3]
	84.1(2.1)	1.01(0.19)	0.93(0.13)	1.70(0.08)	76.3(6.1)	—	5.20(1.79)	1.25(0.35)	1.40(0.55)
M	[8]	[8]	[8]	[8]	[8]		[5]	[2]	[5]
90–99	92.7(3.4)	0.85(0.20)	0.46(0.31)	1.46(0.03)	52.9(10.1)	(2)	0	0	0
F	[2]	[2]	[2]	[2]	[2]	[0]	[0]	[0]	[0]
	99.9(0.0)	0.78(0.0)	0	1.57(0.00)	70.5(0.0)	—	0	2.00(0.00)	0
M	[1]	[1]	[0]	[1]	[1]	—	[0]	[1]	[0]

*Within each cell with three numbers, data are the mean, the standard deviation (the number within parentheses), and the same size (the number within brackets). For each age strata, F and M indicate data from females and males, respectively. The numbers of people who exercise, or smoke, or drink are indicated in brackets in the columns for exercise, smoke, and drink. The rest (if any) did not exercise, smoke, or drink, or did not provide the information on these life-style characters.
[a]In the M# column are the numbers of females before (the number within brackets) and after (the number within parentheses menopause in the age group. The menopausal status for the rest (if any) of the female is unknown.