Complex Segregation Analysis of the Radiographic Phalanges Bone Mineral Density and Their Age-Related Changes

GREGORY LIVSHITS, DAVID KARASIK, and EUGENE KOBYLIANSKY

ABSTRACT

The complex segregation analyses performed in our previous studies revealed a significant major gene (MG) effect on the age-adjusted cortical and cancellous bone mineral density (BMD) in two ethnically different populations, Chuvasha and Turkmenians. The aim of the present study was to test the hypothesis of pleiotropic MG control of three components of bone aging, that is, the baseline level of BMD ($\mu_{gs}$), the age at onset of the bone mass loss ($T_{gs}$), and the rate of this loss over the years ($a_{gs}$). Nuclear and more complex pedigrees from the same two ethnic samples were assessed for hand phalangeal BMD (Chuvasha, 1208 individuals, and Turkmenians, 643 individuals), and complex segregational analysis incorporating age and sex effects directly into MG penetrance function was carried out. The results of the present analysis clearly confirmed the existence of the putative MG and showed that the proportion of BMD variation attributable to this MG effect within the sex was remarkably similar in both populations and ranged between 34.7% and 35.2%. The most parsimonious model for BMD transmission in Chuvasha pedigrees additionally indicated significant residual correlation between siblings and clear sex differences in the annual rates of bone loss $a_{gs}$. The latter was more than twice as high in females than that in males (0.086 SD vs. 0.033 SD per year). In Turkmenian pedigrees the most parsimonious model presented obvious evidence of the MG control of BMD baseline levels in both sexes with significantly lower baseline levels and younger age at onset ($T_{gs}$) in females. No clear MG effects were inferred on $T_{gs}$ and/or $a_{gs}$ in either sample, either in males or in females. That is, the present study does not suggest MG $\times$ SEX $\times$ AGE interaction. We suppose that if the rate of age-related changes in phalangeal BMD is genetically determined, then these are not the same genes as those affecting the BMD baseline levels. (J Bone Miner Res 2002;17:152–161)

Key words: bone mineral density, osteoporosis, segregation analysis, hand bones, age dependence

INTRODUCTION

Involvement of genetic factors in determination of bone mineral density (BMD) is beyond doubt, although not fully elucidated. (1–7) The review of family studies showed that some 30–80% of the total variation in BMD could be explained by genetic factors. (5,7) Our meta-analysis of the published data indicated that despite the ethnic differences in BMD measures, and regardless of the measurement technique or skeletal site selected, heritability estimates of BMD in most studies account for about 60% of the total variation, adjusted for age and sex effects. (7) However, the genes and allelic variants conferring osteoporotic risk are largely unidentified, as well as their chromosomal location and possible interaction between genes and with other risk factors. (7–10) Moreover, the question of monogenic or poly-
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genic mode of transmission of BMD levels is not yet unequivocally answered and is still disputable.\(^{(3,4,7,9–11)}\)

Studies of our team produced strong evidence in support of the single-gene mode of inheritance of the hand bones BMD in two ethnically different populations, that is, Chuvasha and Turkmenians.\(^{(12,13)}\) These results were recently confirmed on a sample of pedigrees of young probands suffering from early idiopathic osteoporosis.\(^{(10)}\) The involvement of large-effect gene(s) in determination of metacarpal cortical index had also been tested recently in two ethnic samples, Chuvasha and Croatians.\(^{(14,15)}\) These studies showed possible major gene effect not only on baseline cortical index but also on sex-specific age at the beginning of cortical bone loss and on annual rate of bone loss once it is started. The rationale for such modeling is a well-recognized fact that bone mass decrease advances with age and is characterized by a sex-specific pattern of such change: bone loss starts at different ages and continues gradually with varying intensity in males and females.\(^{(16–19)}\) Potential sex- and age-genotype-specific interactions in genetic control of BMD were assumed, in a number of publications,\(^{(3,18,20,21)}\) as apparent incomplete expression of genes at certain ages or differences in gene action at different ages. However, these effects were not addressed in direct study until the present time.

Here, we present results of complex segregation analysis of hand fingers compact BMD, obtained in two arrays of pedigrees. Cross-sectional familial data were tested to infer possible major gene effect on the baseline level of the trait, the age at onset of the BMD diminution, and the rate of this loss in males and females, separately. Thus, in the present study we attempted to examine the hypotheses pertaining to possible major gene (MG), sex (S), and age (A) interaction.

MATERIALS AND METHODS

Pedigree data

Pedigree data analyzed here were described in detail elsewhere.\(^{(12–14)}\) Three different samples were included in the present study:

1. Chuvasha I, 547 individuals (293 males and 254 females; mean age of 45.9 years [range 18–91] and 50.2 years [range 18–91], respectively, who live in several small villages in the Chuvasha autonomy, Russian Federation. The data included 135 two- and three-generation pedigrees. The pedigree size was distributed as follows: 9 pedigrees, having 2 observed individuals each; 52, having 3 individuals; 67, from 4 to 6 individuals; and 7 families, from 7 to 12 individuals each. This material was gathered during August/December of 1994.

2. Chuvasha II, 661 individuals (343 males and 318 females; mean age of 45.5 years [range 18–89] and 45.4 years [range 18–81], respectively), who live in eight small villages in the Bashkortostan autonomy, Russian Federation. The data included 161 two- and three-generation pedigrees. The pedigree size was distributed as follows: 7 pedigrees, having 2 observed individuals each; 41, having 3 individuals; 103, from 4 to 6 individuals; and 10 families, from 7 to 15 individuals each. This material was gathered during the summer of 1999. Two Chuvasha samples are ethnically identical; however, the geographic distance between the two locations (~1000 km) and consequent difference in climatic zones may cause some dissimilarity in their data.

3. Turkmenians from rural regions of the Central Asian republic, Turkmenia. The sample was collected during the summer of 1993, consisting of 643 individuals, 257 males and 386 females, with the corresponding mean age and range as follows: 49.0 years (range 18–82) and 48.4 years (range 17–83). These individuals belong to 231 pedigrees of the following sizes: 156, 2 observed individuals; 55, 3 individuals; and 20 with 4 to 6 individuals each.

The studied populations are whites, living in geographically distant regions with strictly different climatic environments: forested or hilly portions of the Volga riverside for both Chuvasha I and Chuvasha II versus arid zone of Central Asia for Turkmenians. Originally, the Chuvasha language group is considered as Finno-Ugor, whereas the Turkmenian language family is Altaic. All study populations are characterized by demographically stable family structure with traditional relations between family members. They have lived at least for a number of generations under the same environmental conditions and have not been exposed to outside influences, such as genetic flow. As usual in rural communities, members of each sample share quite similar living, economic, and professional conditions in the majority of families. No individuals with known bone diseases and risk factors for increased BMD loss (such as steroid hormone therapy, diabetes, and hyperparathyroidism) were included in the study.

The study families in all three samples were collected randomly, through direct contact of the research group staff with practically all households in the small villages where the present project was carried out. All nuclear families who agreed to participate in the study were recruited for further assessment. All subjects who agreed to participate in the study signed informed consent and the project was approved by the Tel Aviv University ethics committee.

BMD measurement

Plain radiographs of the hand bones were taken in all the studied individuals and the standard BMD assessment of the finger bones was performed as fully described in our previous studies.\(^{(12,22)}\) Briefly, in this study, radiographs of both hands were taken in the posteroanterior position with X-ray source 60 cm above, at exposition time of 5–10 s, at 100–150 mA and 50 kV, without intensifying screens. We used the average standardized values of BMD from the compact compartment of middle and distal phalanges of the third finger on the left and right hands, obtained via optic densitometer. The helium neon laser densitometer LKB 2202 (UltrScan XL, Sweden) was used. Radiographic densitometry of hand bones is a precise and accurate method, which is actually on a par with dual-photon absorptiometry
and dual-energy X-ray absorptiometry (DXA), having a CV ranging from 0.6% (23) to 2% (24), a possible accuracy error around 4% (25) and an effective radiation dose of <1 mSv (24). It shows a strong correlation with BMD of other parts of the skeleton (26, 27). Moreover, roentgenographic densitometry is highly suitable for field conditions.

Statistical and genetic analysis

Before genetic analysis the data were studied for age correlations and dependence on stature, body mass, and body mass index (BMI). BMD measures in both Chuvasha samples and in the Turkmenians showed age dependence similar to the well-known trend observed previously in many other samples, that is, no significant change until some sex-specific age threshold \( T_s \) and then gradually decreased in both sexes with a sex-specific slope coefficient \( \alpha_s \). (8, 15–17) A two-interval function was therefore applied to fit BMD loss:

\[
x_i(t) = x_i \quad \text{if} \quad t \leq T_s \quad \text{and} \quad x_i(t) = x_i + \alpha_i(t - T_s), \quad \text{otherwise}, \quad (1)
\]

where \( x_i(t) \) is the BMD value for an individual of sex \( s \) (\( s = m \) or \( f \) for males and females, respectively) and age \( t \) (in years); \( x_i \) is the BMD value expected in individuals with age lower than a sex-specific threshold \( T_s \); and \( \alpha_i \) is a sex-specific slope coefficient measuring the rate of the bone loss per year.

Multiple regression analysis was also performed in both Chuvasha samples to examine the effect of body weight, stature, and BMI on BMD variation in each sex, separately. To avoid the bias in regression coefficients estimates resulting from nonindependence of the related individuals in the samples, the computation of regression coefficients was performed using a maximum-likelihood estimate for the parameters. These computations take into account the pedigrees’ size and structure. (13) In Turkmenians, the correlations between compact BMD and these potential predictor variables were negligible. However, in Chuvasha males, body weight and stature significantly and independently correlated with BMD, whereas multiple regression analysis did not retain BMI at the significant level \( p > 0.05 \). Also in Chuvasha females, the independent correlation between BMD and BMI was virtually zero in both samples; however, stature and body weight again showed significant correlation with BMD. Because of these results, bone density data in each sample were adjusted for corresponding significant confounding variables for further genetic modeling. This was accomplished in two ways: first, the standardized residuals in each ethnic sample were created by adjustment of BMD for the potential covariates, that is, age, weight, and stature, in each sex, separately. Second, partial regression coefficients for weight and stature from the multiple regression analysis were used for BMD adjustment, whereas age and sex effects were preserved.

Complex segregation analysis of the data was carried out using the statistical-genetic package MAN. (28) The detailed description of the method was given in numerous publications including our recent studies. (12, 13, 30) Segregation analysis was undertaken in this study with age and sex effects incorporated directly into the genetic model.

Our general model assumed the existence of two alleles \( (A_1 \) and \( A_2) \) at single autosomal locus affecting BMD levels. The Hardy–Weinberg equilibrium of the corresponding hypothetical genotypes, \( A_iA_i \), \( A_iA_j \), and \( A_jA_j \) (denoted by \( g = 1, 2 \), and 3, respectively), was assumed. The following genetic parameters were estimated in the general model: (1) \( p \) is the population frequency of the first of two major alleles, \( A_1 \). This allele is supposed to cause a low trait value; (2) \( \mu_{gs}(t) \) is the mean genotypic value in individuals having major gene genotype \( g \), sex \( s \), and age \( t \). It was determined as

\[ \mu_{gs}(t) = \mu_{gs} + \alpha_{gs}[1 - \delta(T_{gs})] + \tau_{gs} \delta(T_{gs}) - \bar{t}_{gs}], \quad (2) \]

where \( \mu_{gs} \) is the genotype–sex-specific baseline level of BMD, that is, the average BMD value in individuals of the given genotype and sex before the beginning of age-related BMD diminution; \( \bar{t}_{gs} \) and \( T_{gs} \) are the genotype–sex-specific mean age in the sample and age at onset of the beginning of bone loss, respectively. \( T_{gs} \) reflects the assumed average age of the individuals at inflection point (threshold) after which age-related BMD changes occurred. In equation 2, \( \delta(T_{gs}) = 0 \) if \( t \geq T_{gs} \) and has a value of 1, otherwise; \( \alpha_{gs} \) measures the genotype-specific slope coefficient of the BMD changes within each sex, separately. Thus, equation 2 shows that the BMD changes with age only after the latter exceeds a certain age threshold, \( T_{gs} \). If the individual’s age \( t \) is less than \( T_{gs} \), the expected BMD value is constant. As observed, the model assumes that a hypothetical major gene may control not only the baseline of the trait \( (\mu_{gs}) \) but also the age of the onset of age-dependent changes in trait expression \( (T_{gs}) \) and the rate of changes per year \( (\alpha_{gs}) \) since they began. (3) \( \sigma^2_g \) is the trait variance in individuals having the same major gene genotype \( g \); it estimates the trait variation attributable to all possible environmental factors and potential minor genes. (4) \( \rho, \beta \), and \( \epsilon \) are the partial correlations between the trait residuals adjusted for major gene effect, in spouses, in parents and offspring, and in siblings, respectively. (5) \( \tau_{gs} \) is the probability of transmission of the allele \( A_1 \) to the offspring generation, by each of three possible genotypes.

Thus, the general model encompasses major gene, multifactorial (polygenic), and environmental effects. To evaluate proportions of the trait variance attributable to age, sex, major gene, and residual multifactorial effects, the total explained variance was decomposed into the following components: \( H^2 \) is the proportion of variance attributable to the within-sex differences between the genotypic values; \( D^2_G \) is the proportion of variance attributable to all genotypic effects, including the change of the genotypic value with the age; \( D^2_{GSA} \) is the proportion of variance attributable to the combined effect of the three explicitly formulated sources of the trait variation, that is, major gene, sex, and age; \( d^2 \) is the proportion of variance attributable to residual familial correlations. \( D^2 = D^2_{GSA} + d^2 \) is the proportion of variance ascribed to all the effects included in the genetic model. By
Table 1: Statistical Characteristics of Three Studied Samples

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Chuvasha I</th>
<th>Chuvasha II</th>
<th>Turkmenians</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td></td>
<td>Parameter</td>
<td>p_s</td>
<td>Parameter</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>63.49 ± 0.67</td>
<td>63.93 ± 0.57</td>
<td>64.82 ± 0.98</td>
</tr>
<tr>
<td>Stature (m)</td>
<td>1.658 ± 0.069</td>
<td>1.635 ± 0.054</td>
<td>1.666 ± 0.070</td>
</tr>
<tr>
<td>BMI</td>
<td>23.06 ± 0.13</td>
<td>23.29 ± 0.10</td>
<td>23.39 ± 0.19</td>
</tr>
<tr>
<td>Mean BMD (mg/mm³)</td>
<td>0.968 ± 0.032</td>
<td>0.925 ± 0.040</td>
<td>0.839 ± 0.018</td>
</tr>
<tr>
<td>Initial correlation with age</td>
<td>0.424 ± 0.042</td>
<td>0.579 ± 0.057</td>
<td>0.937 ± 0.021</td>
</tr>
<tr>
<td>1.541 ± 0.034</td>
<td>1.541 ± 0.032</td>
<td>25.39 ± 0.29</td>
<td>25.39 ± 0.29</td>
</tr>
<tr>
<td>n, (0.008)</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Residual correlation with age</td>
<td>0.098 ± 0.020</td>
<td>0.098 ± 0.020</td>
<td>0.098 ± 0.020</td>
</tr>
</tbody>
</table>

*Note: p (p < 0.05); S, significant (p < 0.001); SS, significant (p < 0.0001) value.*

To avoid the ambiguity in the most parsimonious model construction, a certain order of the reduced models testing was undertaken. The array of the general model parameters was divided into (I) sex-genotype-specific means (μg), (II) genotype-specific variances (σ²), (III) sex-genotype-specific slope coefficients (αg), (IV) sex-genotype-specific inflection points (T_g), and (V) residual familial correlations ρ, β, and γ. For each of the first four groups of parameters, the following constrains were tested: No major gene effect, E(A1A1) = E(A1A2) = E(A2A2); additive, dominant, and recessive major gene effect: E(A1A2) = 0.5[E(A1A1) + E(A2A2)]; E(A1A1) = E(A1A2) = E(A2A2), respectively. Here, E(Aij) denotes one of the four above-defined groups of the parameters. Additionally, the significance level of differences between sexes was tested by constraining parameters of females equal to those in males. Finally, the most parsimonious model was constructed by inclusion of the assumed constrains. The obtained most parsimonious major gene model was compared with the corresponding model with arbitrary τ's with equal τ's model, by likelihood ratio tests.

No ascertainment correction of likelihood was made because our method of the pedigree collection was not connected with the individual’s bone properties.

RESULTS

Age dependence

Table 1 provides basic descriptive statistics and shows significant sex differences for anthropometric traits in all three samples. In addition, body weight and BMI were significantly higher in Chuvasha II females than in Chuvasha I females. The linear correlation of the BMD with age was negative and significant (p < 0.001) in both Chuvasha samples and in Turkmenians (Table 1). Detailed exploration of BMD change with age allows one to indicate a certain age threshold (a point of inflection) for each sex, after which BMD decreases gradually with age, whereas practically no age dependence of the BMD values is notable for ages before this threshold. Table 1 also provides the maximum-likelihood estimates of the age-fitting parameters obtained using equation 1, for males and females in three studied samples, separately. As observed, there were notable sex differences in parameter estimates within each sample, and also significant differences in these parameters between the two Chuvasha samples. Linear and polynomial regressions of BMD with age were also examined in each of the study.
samples. All regressions, excluding the polynomial equation with age values up to the 4th power, were characterized by a worse fit to the data than those obtained by the two-interval function. However, the polynomial model was obviously less parsimonious (five estimated parameters instead of three) and more difficult to interpret.

**Familial correlations**

The pairwise correlations between family members were computed after adjustment for age and sex differences according to equation 1. The obtained correlations clearly indicated a strong involvement of familial factors in the compact BMD variation in all three samples. The corresponding family correlations were generally of the same magnitude in all samples. Thus, parent/offspring correlations varied between 0.24 and 0.27 ($p < 0.01$ in all instances), whereas the sibling correlations ranged between 0.26 and 0.41 ($p < 0.01$). As observed, sibling correlations were consistently highest. Marital correlations were lowest (0.06–0.11) and not significant statistically ($p > 0.05$).

**Genetic homogeneity test**

Because two Chuvasha samples showed a significant difference in BMD measurements, we first examined whether the major gene effect could be established in the second population. We also wondered, given the positive results of this analysis, whether the parameter estimates would be comparable in two samples. To solve this task, the data were first adjusted for sex, age, body weight, and height. Sex and age interactions with putative major gene effect, therefore, were not tested at this stage of analysis. Segregation analysis was performed as described under Materials and Methods, with initial testing of Mendelian mode of inheritance and consequent constructing of the most parsimonious model and its confirmation. Table 2 shows the best-fitting and most parsimonious models in both samples. Despite the apparent differences in average BMD values between the two Chuvasha samples, the major gene effect was definitely inferred in both samples. The equal $t_2$ model was strongly rejected ($\chi^2 = 35.2$ in Chuvasha I and $\chi^2 = 58.6$ in Chuvasha II, with df = 2 and $p < 0.001$), whereas the Mendelian model was accepted ($p > 0.05$). As observed in the table, the parameter estimates in both most parsimonious models were remarkably similar. Because of this fact and the same ethnic background, we deemed it possible to combine two samples together for further statistical genetic analysis.

**Major gene effect, age, and sex interactions**

Complex segregation analysis of phalangeal BMD, non-adjusted for sex and age, produced unambiguous results regarding the major gene mode of inheritance in both samples of pedigrees, combined Chuvasha and Turkmenians. In Chuvasha, maximum log-likelihood (log-LH) values were $-1372.30$, $-1373.16$, and $-1383.49$, for the general, Mendelian, and “environmental” models, respectively (Table 3). This resulted in acceptance of the Mendelian hypothesis and
in rejection of the nongenetic (equal $\tau$’s) model. The transmission probability estimates ($\tau$’s) in the general model differed nonsubstantially from the expected Mendelian probabilities: 1.000, 0.418, and 0.000 for $g = 1$, 2, and 3, respectively. A similar pattern was also observed in Turkmenians (Table 4).

The additional models denying the major gene transmission of BMD were also examined and consistently rejected: (1) the model with $\tau_g = p$ (column 4, Tables 3 and 4) was clearly rejected in both samples when compared with the corresponding general model: $\chi^2 = 24.22$, df = 3, $p < 0.001$ in Chuvasha and $\chi^2 = 13.42$, df = 3, $p < 0.001$ in
The maximum-likelihood test indicated that the sex differences in the model in both samples: $2\log\text{LH}$.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>General</th>
<th>Mendelian</th>
<th>Equal $\tau$'s</th>
<th>$\tau$'s = p</th>
<th>Multifactorial</th>
<th>M.P. Mendelian</th>
<th>No gender effect on $\alpha_{gs}$ and $T_{gs}$</th>
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</thead>
<tbody>
<tr>
<td>$p$</td>
<td>0.565</td>
<td>0.574</td>
<td>0.588</td>
<td>0.560*</td>
<td>[0.000]</td>
<td>0.628 ± 0.051</td>
<td>0.649</td>
</tr>
<tr>
<td>$\mu_{1m}$</td>
<td>-0.447</td>
<td>-0.480</td>
<td>-0.467</td>
<td>-0.482</td>
<td>0.326</td>
<td>-0.474 ± 0.134</td>
<td>-0.463</td>
</tr>
<tr>
<td>$\mu_{2m}$</td>
<td>0.356</td>
<td>0.342</td>
<td>0.369</td>
<td>0.285</td>
<td>0.326</td>
<td>0.557</td>
<td>0.600</td>
</tr>
<tr>
<td>$\mu_{3m}$</td>
<td>1.550</td>
<td>1.529</td>
<td>1.270</td>
<td>1.257</td>
<td>0.326</td>
<td>1.587 ± 0.018</td>
<td>1.675</td>
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<tr>
<td>$\mu_{1f}$</td>
<td>-0.859</td>
<td>-0.883</td>
<td>-0.864</td>
<td>-0.906</td>
<td>-0.203</td>
<td>-0.887 ± 0.121</td>
<td>-0.879</td>
</tr>
<tr>
<td>$\mu_{2f}$</td>
<td>-0.120</td>
<td>-0.135</td>
<td>0.002</td>
<td>-0.041</td>
<td>-0.203</td>
<td>0.088</td>
<td>0.115</td>
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<tr>
<td>$\mu_{3f}$</td>
<td>0.908</td>
<td>0.921</td>
<td>0.807</td>
<td>0.765</td>
<td>-0.203</td>
<td>1.063 ± 0.123</td>
<td>1.109</td>
</tr>
<tr>
<td>$\sigma^2$</td>
<td>0.314</td>
<td>0.305</td>
<td>0.348</td>
<td>0.342</td>
<td>0.754</td>
<td>0.277 ± 0.054</td>
<td>0.303</td>
</tr>
<tr>
<td>$p$</td>
<td>0.146</td>
<td>0.240</td>
<td>0.188</td>
<td>-0.064</td>
<td>-0.154</td>
<td>[0.000]</td>
<td>[0.000]</td>
</tr>
<tr>
<td>$\beta$</td>
<td>0.065</td>
<td>0.066</td>
<td>0.216</td>
<td>0.312</td>
<td>0.236</td>
<td>[0.000]</td>
<td>[0.000]</td>
</tr>
<tr>
<td>$\epsilon$</td>
<td>0.273</td>
<td>0.267</td>
<td>0.325</td>
<td>0.224</td>
<td>0.219</td>
<td>[0.000]</td>
<td>[0.000]</td>
</tr>
<tr>
<td>$\sigma_{1m}$</td>
<td>-0.031</td>
<td>-0.029</td>
<td>-0.026</td>
<td>-0.026</td>
<td>-0.045</td>
<td>-0.045 ± 0.005</td>
<td>-0.043</td>
</tr>
<tr>
<td>$\sigma_{2m}$</td>
<td>-0.066</td>
<td>-0.065</td>
<td>-0.066</td>
<td>-0.042</td>
<td>-0.045</td>
<td>-0.045 ± 0.005</td>
<td>-0.043</td>
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<td>$\sigma_{3m}$</td>
<td>-0.034</td>
<td>-0.031</td>
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<td>-0.045</td>
<td>-0.045 ± 0.005</td>
<td>-0.043</td>
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<tr>
<td>$\sigma_{1f}$</td>
<td>-0.026</td>
<td>-0.025</td>
<td>-0.031</td>
<td>-0.034</td>
<td>-0.045</td>
<td>-0.045 ± 0.005</td>
<td>-0.043</td>
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<td>$\sigma_{2f}$</td>
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<td>-0.067</td>
<td>-0.056</td>
<td>-0.051</td>
<td>-0.045</td>
<td>-0.045 ± 0.005</td>
<td>-0.043</td>
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<tr>
<td>$\sigma_{3f}$</td>
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<td>-0.070</td>
<td>-0.063</td>
<td>-0.065</td>
<td>-0.045</td>
<td>-0.045 ± 0.005</td>
<td>-0.043</td>
</tr>
<tr>
<td>$T_{1m}$</td>
<td>48.10</td>
<td>48.49</td>
<td>50.44</td>
<td>52.00</td>
<td>56.85</td>
<td>56.57 ± 2.97</td>
<td>46.41</td>
</tr>
<tr>
<td>$T_{2m}$</td>
<td>67.00</td>
<td>67.00</td>
<td>67.00</td>
<td>57.00</td>
<td>56.85</td>
<td>56.57 ± 2.97</td>
<td>46.41</td>
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<td>57.00</td>
<td>55.00</td>
<td>55.00</td>
<td>56.85</td>
<td>56.57 ± 2.97</td>
<td>46.41</td>
</tr>
<tr>
<td>$T_{1f}$</td>
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<td>21.00</td>
<td>22.87</td>
<td>32.26</td>
<td>38.00</td>
<td>38.00 ± 2.84</td>
<td>46.41</td>
</tr>
<tr>
<td>$T_{2f}$</td>
<td>41.28</td>
<td>42.00</td>
<td>42.00</td>
<td>40.00</td>
<td>38.00</td>
<td>38.00 ± 2.84</td>
<td>46.41</td>
</tr>
<tr>
<td>$T_{3f}$</td>
<td>46.99</td>
<td>47.49</td>
<td>47.28</td>
<td>47.31</td>
<td>38.00</td>
<td>38.00 ± 2.84</td>
<td>46.41</td>
</tr>
<tr>
<td>$\tau_1$</td>
<td>1.000</td>
<td>[1.000]</td>
<td>0.597</td>
<td>0.560*</td>
<td>—</td>
<td>[1.000]</td>
<td>[1.000]</td>
</tr>
<tr>
<td>$\tau_2$</td>
<td>0.579</td>
<td>[0.500]</td>
<td>0.597*</td>
<td>0.560*</td>
<td>—</td>
<td>[0.500]</td>
<td>[0.500]</td>
</tr>
<tr>
<td>$\tau_3$</td>
<td>0.000</td>
<td>[0.000]</td>
<td>0.597*</td>
<td>0.560*</td>
<td>—</td>
<td>[0.000]</td>
<td>[0.000]</td>
</tr>
<tr>
<td>log-LH</td>
<td>-546.58</td>
<td>-546.91</td>
<td>-552.92</td>
<td>-553.29</td>
<td>-577.14</td>
<td>-553.32</td>
<td>-567.48</td>
</tr>
<tr>
<td>$\chi^2$ (column)</td>
<td>—</td>
<td>0.66NS (1)</td>
<td>12.68** (1)</td>
<td>13.42** (1)</td>
<td>61.12** (1)</td>
<td>13.48NS (1)</td>
<td>41.80** (1)</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 3.

Turkmens; (2) multifactorial transmission model (column 5, Tables 3 and 4) was also much worse than general model in both samples: $\chi^2 = 76.00$, df = 16, $p < 0.001$ in Chuvasha and $\chi^2 = 61.12$, df = 16, $p < 0.001$ in Turkmenians.

Further sequential constraining of the parameters in both samples yielded the “most parsimonious” Mendelian models. Thus, in Chuvasha, by the standard likelihood ratio tests, the basic BMD levels ($\mu_{gs}$) for each putative genotype were not significantly different between sexes (column 6, Table 3). The differences between sexes, however, were significant with respect to the rate of bone loss. Coefficients $a_{gs}$ in females were almost three times higher than those in males. The age at onset of bone loss, that is, threshold parameter $T_{gs}$, was also significantly different between sexes. Indeed, the model denying the sex differences in the pattern of BMD loss was strongly rejected in both ethnic samples (column 7, Tables 3 and 4). Moreover, the threshold parameters differed significantly between genotypes in Chuvasha males. The maximum-likelihood test indicated the possibility of the additive major gene control of the age threshold in males, whereas no genetic influence at $T_{gs}$ was shown in females. Finally, in Chuvasha pedigrees, the residual partial correlations between spouses and between siblings ($p$ and $\beta$) were statistically significant, whereas the residual parent/offspring correlation ($\beta$) did not differ significantly from zero (column 6, Table 3).

The parameter estimates in the most parsimonious model in Turkmenians are shown in Table 4 (column 6). As mentioned before, Table 4 shows that all models, assuming no major gene effect (columns 3–5), were rejected at $p < 0.01$, whereas the Mendelian transmission model (column 2) was accepted. The most parsimonious model suggests significant sex differences and an additive major gene control of the baseline levels of BMD ($\mu_{gs}$) in both men and women. However, no major gene effect was found on the inflection points and slopes of the age-related BMD change in either males or females. The parameters of BMD loss, $T_{gs}$ and $\alpha_{gs}$, were obviously sex specific. The corresponding model, assuming no sex effects on $T_{gs}$ and $\alpha_{gs}$ (column 7,
COMPLEX SEGREGATION ANALYSIS OF BMD

Table 4), was statistically rejected ($\chi^2 = 41.80$, df = 18, $p < 0.001$).

The proportions of BMD variance attributable to the effects of the various factors included in the most parsimonious model (see Materials and Methods) in the Chuvasha pedigrees were as follows: $H^2 = 0.347$, $D_G^2 = 0.378$, $D_{GSA}^2 = 0.632$, and $D^2 = 0.652$. In Turkmenians, these proportions were: $H^2 = D_G^2 = 0.352$ and $D_{GSA}^2 = D^2 = 0.608$.

DISCUSSION

Although today it is unambiguously recognized that genetic factors strongly affect BMD variation, the question about polygenic or monogenic mode of inheritance of this trait is still disputable. (7,10,11,18) The majority of publications consider BMD as a complex trait, which presumably is influenced by multiple genes. The dominating view was formulated by Cooper(11) as follows: “A single gene... is unlikely to play a large role in complex, chronic disease such as osteoporosis. A polygenic model is more likely...” (p. 1647).

In accordance with this point of view, the apparently unimodal distribution of BMD in the population is a composite of the separate overlapping distributions consistent with the polygenic model (e.g., Refs. 5, 32). Our statistical-genetic studies, on the contrary, provided clear-cut evidence supporting the hypothesis that a major genetic locus influences the levels of radiographic phalanges BMD in the same two human populations that were studied here. (12,13) The most recent segregation analysis of spinal BMD, as assessed by DXA in pedigrees with idiopathic osteoporosis, (6) provided strong support to our findings obtained in the general population. Cardon and colleagues (60) found that the codominant major gene model with a relatively high allele frequency for low BMD ($p_{A1} = 0.52$) fit the data significantly better than concurrent models and explained as much as 78% of the total variance in BMD, adjusted for age and sex differences. In addition, in accord with these results of segregation analysis were the data of Spotila et al. (35) on Z scores of BMD in osteopenic families. The bimodal distribution of Z scores in the selected pedigrees was best compatible with a monogenic dominant pattern of BMD inheritance.

Moreover, it was also shown that several osteoporosis-related phenotypes may be linked to one particular chromosomal segment. Thus, loci for both high (35) and low (30) bone mass, as well as an autosomal recessive osteoporosis, (35) were mapped to chromosome 11q. Similarly, the normal BMD variation of the femoral neck was found linked to chromosome 11q. (36) Even in pedigreed baboons, evidence had also been obtained for a locus linked to BMD on chromosome homologous to human chromosome 11q. (37) and yet some other chromosomal regions might also be involved in determination of BMD levels. (9,10,38)

Nevertheless, the genetic effects on pattern of bone aging, and particularly on rate of bone loss, were not inferred in these studies. The potential sex– and age– genotype-specific pattern of BMD heritability was suggested several times. (3,20,21) To comprehend the nature of age– genotype interactions in genetic control of compact BMD, one could recognize certain crucial moments in the process of bone maturation and aging. On completion of growth, there is a period of skeletal consolidation. Peak bone mass is reached by individuals of both sexes at the age of 25 to 40 years. (16,17) After the peak, the BMD values show a clear decrease with age in cross-sectional studies. This decrease, meaning the cortical bone loss, is more pronounced in women, appearing at perimenopausal age and being followed by a postmenopausal acceleration. (18,38 – 40)

In this study, we presented results of complex segregation analysis of compact BMD, which simultaneously examined the possible major gene control on three facets of hand phalangeal BMD variation, that is, the baseline level, the age when bone loss starts, and the rate of this loss over years. Using the standard transmission probability tests, consistent evidence was obtained in support of the hypothesis that baseline levels of the phalangeal BMD are controlled by a single large-effect gene responsible for a substantial part of the trait variation. The allele frequency for low BMD is between 0.411 in Chuvasha and 0.628 in Turkmenians. Between 34.7% and 35.2% of the baseline BMD variance may be explained by the effect of this putative locus in studied ethnic samples. This result supports our previous conclusions about the monogenic inheritance of age-adjusted BMD in the same populations. (12,13) In addition, the present investigation found in both populations substantial differences between the slope coefficients in males and females (Table 1), which is also in good agreement with many previous publications. (18,19,39,40) However, age– sex– genotype interactions were not so unambiguous and as clear as major gene effect on the BMD levels before age-related decrease. In Chuvasha pedigrees, the maximum-likelihood test indicated the additive major gene control of the age threshold in males but no significant age– genotype interaction in females. The model in which genotype-specific regression slopes within the sex were constrained to be equal was accepted by the maximum-likelihood ratio test (Table 4). This test suggested that the annual rate of bone loss is independent of inferred putative major gene.

In the Turkmenian sample, both major gene and sex were important factors of baseline BMD variation. However, we were unable to detect any genotype by age interaction (thresholds and slope coefficients were equal in three putative genotypes) within the sex. Moreover, slope coefficients did not differ between the sexes. These results may be attributable to the relatively small sample size of Turkmenians and simple structure of pedigrees. However, this was not the case in Chuvasha. Still, even in the latter, the majority of genotype– age interactions were not significant. It could be concluded therefore that the major gene obviously affects baseline BMD levels, but likely does not influence rate of BMD loss or even the age of the beginning of such involutive bone process. However, involvement of the additional genetic sources in the pattern of bone loss is possible. In this respect, substantial population differences observed in the present study definitely do not contradict this assumption. It should also be stressed here that all inferences concerning the MG × S × A interactions were
inferred on cross-sectional data and are not the results of longitudinal follow-up.

The results obtained in our study, nevertheless, make an important contribution to understanding of the epidemiology of osteoporosis. First, we now have a well-grounded expectation for the penetrance model-based genome search to discover a single genetic locus involved into compact BMD variation. Second, the risk for low BMD, predisposing to development of osteoporosis can now be attributed, not only to age and sex of the individual, but also to his (her) putative genotype. The findings of the present analysis are in good agreement with the hypothesis that a major gene influences substantially (≥35% in this study) interindividual baseline variation of BMD. Such a high estimate of the single-gene effect suggests that its chromosomal location should be an imperative aim for further studies.

ACKNOWLEDGMENTS

This study was partly supported by grant 4240 from the Ministry of Health, Israel and grant 960470 from Sacker Faculty of Medicine, Tel Aviv University, Israel.

REFERENCES


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Received in original form September 27, 2000; in revised form May 7, 2001; accepted June 15, 2001.