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Evidence on Major Gene Control of Cortical Index in Pedigree Data From Middle Dalmatia, Croatia

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ABSTRACT It was recently reported that the inheritance of the metacarpal cortical index (CI) in the Chuvashian population can be described in terms of a major gene (MG) model. By applying transmission probability tests, the hypothesis was accepted that not only baseline level of CI but also its sex-specific dependence on age were under control of the same putative large-effect gene. Using a pedigree sample from the population of the islands of Middle Dalmatia, Croatia (847 observed individuals in 278 pedigrees), data are presented to support the above findings. The following hypotheses were accepted: (i) inheritance of baseline CI in the Croatian population can be attributed to the effect of a MG responsible for about 42% of the variation; (ii) the same MG takes part in the control of the dependence of CI on age, particularly the age at onset of involutive bone changes (inflection point), and of the rate of decrease in CI with age (slope coefficient). Issues related to the assortative mating effect on CI and the determination of the most parsimonious model are discussed. Am. J. Hum. Biol. 13:398–408, 2001.

Most genetic studies of osteoporosis and bone fracture focus on genetic contributions to bone density, a major determinant of bone strength and fracture risk. However, bone density is not the only determinant of skeletal fragility, as the architectural properties, overall size, and geometry of bone also influence skeletal strength (Arden et al., 1996).

The osteometric dimensions of the metacarpals are an efficient and practical method for investigating and monitoring bone mass (Barnett and Nordin, 1960; Garn et al., 1976; Exton-Smith et al., 1969). Many efforts have been directed toward identification of genetic and environmental factors that influence bone-remodeling processes (Matković et al., 1979; Plato and Noris, 1980; Kobyliansky et al., 1995; Kušec et al., 1990; Behluli et al., 1991; Stini et al., 1994; Livshits et al., 1999; Pavlovsky and Kobyliansky, 1999; Škarić-Jurić et al., 1998). There is special interest in the genetics of osteometric dimensions, which are a reflection of changes related to osteoporotic processes. Even though many family and twin studies have been undertaken and increased prevalence of osteoporosis has been established in relatives of affected persons (Krall and Dawson-Hughes, 1993; Jouanny et al., 1995; Škarić-Jurić and Rudan, 1997; Livshits et al., 1999), the question of the relative importance and the mode of transmission of genetic and environmental family factors needs further study.

Previous population structure analyses carried out in population groups of Middle Dalmatia, Croatia, based on the distances analysis of various measures of biological [polygenic (anthropometrical, physiological, and dermatoglyphic) and monogenic traits], socio-cultural (linguistic), and biocultural (kinship coefficients) traits, showed that morphometric dimensions of the metacarpals may be good indicators of population structure (Rudan et al., 1987a, 1990a,b, 1992; Waddle et al., 1998; Martinović et al., 1999). The results of factor analysis provide indirect evidence that genetics has an important role in the variability of osteometric traits and suggest that it is likely that several different loci with major effects (or different sets of linked polygenes) are involved (Šimić et al., 1992). Heritability estimates based on family data of the populations of the Middle Dalmatian islands (Škarić-Jurić and Rudan,

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1997) provide further evidence for a strong influence of genetic factors on medullary canal width formation (H^2 = 54%–71%).

Recently, Karasik et al. (2000a) reported genetic control of the metacarpal cortical index (CI), which provides the information on the percentage of the cortical bone in one-dimensional space. Using complex segregation analysis of pedigree data from Chuvashia population in Russia, the results suggest major gene (MG) pleiotropic control of the baseline values of CI and a sex-genotype specific dependence of CI on age.

To test whether the results of Karasik et al. (2000a) can be taken as a general phenomenon, this study used segregation analysis of the CI based on pedigree data from the population of the Middle Dalmatia islands in Croatia.

MATERIALS AND METHODS

Original sample

Osteometric dimensions of metacarpal bones and information on family relations are derived from material collected between 1978 and 1987 from the population of the islands of Brač and Hvar and the Pelješac peninsula in Croatia. The data were gathered from randomly sampled individuals, encompassing 7.7%–10.7% of the total population of the islands and peninsula. This research was approved by the Ministry of Science and Technology of the Republic of Croatia, and all subjects gave their informed consent.

The Middle Dalmatian islands of Brač and Hvar and the Pelješac peninsula occupy a rather small area (7.460 km^2), and the inhabitants share the same overall environmental conditions (climate, professions, economy, culture, health service, and life style). The modern population of the islands and peninsula is composed partially from the ancient Croatian population, which dates to the 7th century and partially from the new Croatian population which intensively migrated to this area from the east during the Turkish wars from the end of 15th to the end of 17th centuries. Various ethno-historical and biological properties of the population have been intensively investigated (Rudan, 1980; Rudan et al., 1987a,b, 1990a,b, 1992; Smolej-Narančić, 1988; Smolej-Narančić et al., 1990; Sujoldžić, 1991, 1997; Janićijević et al., 1994; Waddle et al., 1998; Barać et al., 1999).

Pedigree data

The number of subjects included in the present study, 847 examinees (391 males and 456 females), 18–85 years, was determined by the coincidence that two (or more) participants of the original random sample were the members of the same family. The sample available for analysis consisted of 278 two- and three-generation pedigrees. Their size was distributed as follows: 150 pedigrees having 2 individuals, 66 having 3 individuals, 50 having from 4 to 6 individuals, and 12 families having from 7 to 17 individuals each.

The morphometry of the metacarpals was performed on hand–wrist radiographs of both hands. Total diaphysis width (D) and medullary canal width (d) of the second, third, and fourth metacarpals were measured on the left and right hands, after Barnett and Nordin (1960). All measurements were performed by one investigator within a short time, using a millimeter ruler and a magnifying glass (×10) with a scale permitting 0.05-mm accuracy. Measurements were rounded to 0.1 mm. For each individual and for each bone, the cortical index was computed as CI = $(D - d)/D$ (Barnett and Nordin, 1960). Table 1 shows the age distribution of the CI of metacarpals II–IV for males and females. To remove possible scale differences between the sub-populations, CI values were standardized separately for each metacarpal bone, within each sex and each of the four sub-populations (Hvar, Pelješac, Brač-east, and Brač-west). The standardized indices from all measured metacarpals (II–IV) of both hands were then used to calculate an average CI value for each individual. The pooled CI data were used in the segregation analysis.

Statistical analysis

Following the assumption that the predomination of bone resorption over bone formation as a natural part of the aging process begins after a certain sex-specific age, a two-interval function for adjustment of CI for age was used:

$$\begin{aligned} x_s(t) &= x_s \text{ if } t \le T_s \text{ and } x_s(t) \\ &= x_s + a_s(t - T_s), \quad (1) \end{aligned}$$

where $x_s(t)$ is the CI value for an individual of sex s ($s = m$ or f for males and females, respectively) and age t (in years); x_s is the CI value expected in individuals whose age

TABLE 1. Descriptive statistics of II–IV metacarpal CI (mean values of both hands) in males and females by age groups (years)

	II[nd] metacarpal		III[rd] metacarpal		IV[th] metacarpal		
Age group	Mean	SD	Mean	SD	Mean	SD	Number
			Males				
18–29	0.60	0.08	0.58	0.09	0.59	0.09	71
30–39	0.62	0.08	0.59	0.10	0.60	0.11	63
40–49	0.59	0.07	0.56	0.08	0.57	0.07	64
50–59	0.59	0.07	0.56	0.08	0.58	0.09	124
60–69	0.58	0.08	0.53	0.08	0.55	0.07	46
70+	0.54	0.10	0.48	0.10	0.54	0.09	23
						Total	391
			Females				
18–29	0.66	0.10	0.61	0.11	0.60	0.11	62
30–39	0.68	0.08	0.63	0.10	0.66	0.11	48
40–49	0.64	0.08	0.60	0.08	0.65	0.08	107
50–59	0.59	0.09	0.55	0.09	0.59	0.09	132
60–69	0.54	0.08	0.49	0.08	0.53	0.08	79
70+	0.50	0.07	0.46	0.06	0.50	0.06	28
						Total	456

does not exceed a certain sex-specific threshold T_s (Fig. 1) and a_s is a slope coefficient measuring the annual rate of the bone loss. Estimates of parameters x_s, T_s, and a_s were obtained by minimizing the sum of residual squares (least-square estimates) separately for males and females. Due to the absence of an additional sample of trait–age pairs not structured by pedigrees, and assuming no family-specific effect on the trait dependence on age, the parameters of the adjusting function (1) were estimated using the whole set of pedigree data.

Genetic analysis

Segregation analysis was performed by implementation of the program package MAN, developed by Ginsburg (1997). Two types of MG models were tested:

(A) Model 1 describes the segregation of CI values adjusted for age and sex effects by equation (1) prior to the segregation analysis. This model is determined by the following genetic parameters:

p is the population frequency of the first of two MG alleles (A_1 and A_2);

μ_g is the average trait value (genotypic value) in all individuals having genotype g; $g = 1$, 2, and 3, corresponding to genotypes A_1A_1, A_1A_2, and A_2A_2, respectively; $\mu_1 \leq \mu_3$;

σ_g^2 is the trait variance in individuals having the same MG genotype g, which estimates trait variation due to the effect of all possible environmental factors and potential minor genes;

ρ,β,ε are partial correlations between trait residual values (i.e., values after adjustment for MG effects) in spouses, in parents and offspring, and in sibs, respectively. The assumption was made that the partial residual correlation between any two individuals who do not belong to the same nuclear pedigree equals zero. The pairwise correlations between residual values in any pair of pedigree members [as in program packages SAGE (Elston, 1995) and PAP (Hasstedt, 1994)] are expressed through those three parameters and depend additionally on the structure of the particular pedigree and the position of this pair.

(B) Model 2 describes segregation of raw data, i.e., CI values without preliminary adjustment for age and sex effects. The corresponding parameters determining the genotype–sex–age interaction are explicitly incorporated into this model and they are simultaneously estimated with all other parameters. The genotypic value in individuals having MG genotype g, sex s, and age t was determined as

$$\mu_{gs}(t) = \mu_{gs} + a_{gs}(T_{gs} - \bar{t}_{gs}),$$
$$\text{if} t \geq T_{gs} \text{ and} = \mu_{gs} + a_{gs}(t - \bar{t}_{gs}), \qquad (2)$$

where μ_{gs} is the genotypic value (the expected trait value averaged over the age range) in individuals having the same geno-

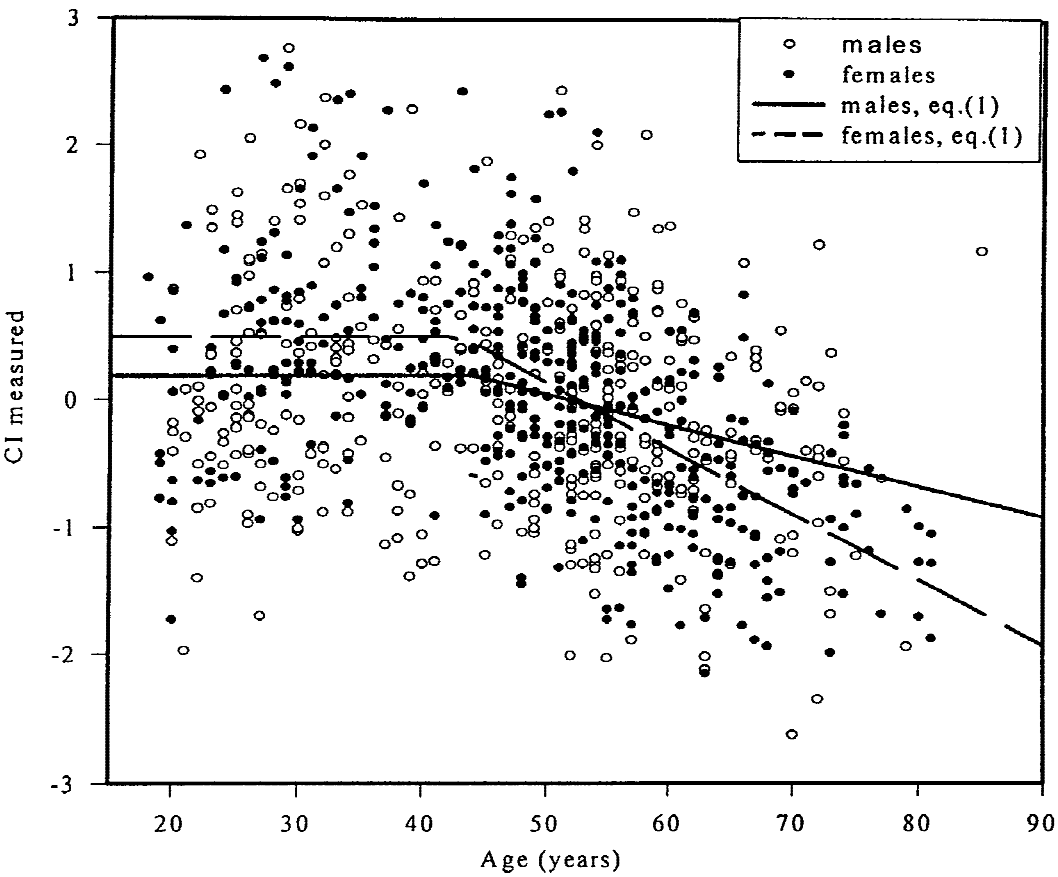

Fig. 1. Two-interval dependence of cortical index on age for both sexes, as given by equation (1).

type g and sex s; $\bar{t}_{gs}$ is the mean age for the given genotype g and sex s; T_{gs} is the genotype–sex specific age threshold. Equation (2) describes age dependence when the trait value expected in individuals of sex s and genotype g linearly decreases (or increases if $a_{gs} > 0$) with age, only after the latter exceeds a certain threshold age, T_{gs}. If age is lower than T_{gs}, the expected trait value is constant and equals: $\mu^0_{gs} = \mu_{gs} + a_{gs}(T_{gs} - \bar{t}_{gs})$. The genotype and sex specific dependence of the trait values on age in Model 2 corresponds to the trait dependence on age as defined by (1).

Instead of three parameters μ_g, defined in Model 1, 18 new parameters are introduced in Model 2: 6 of them are genotypic values μ_{gs}, 6 are threshold ages T_{gs}, and 6 of them are the slope coefficients a_{gs}. Other parameters of Model 2 are the same as in Model 1. The basic assumption of Model 2 is that the hypothetical MG controls not only the baseline levels of the trait variation (μ_{gs}) but also the age at the onset of the age-dependent changes in trait expression (T_{gs}) and the rate of changes (a_{gs}) since the moment they begin. All three MG effects are defined separately for males and females.

There are additional characteristics of the two described models that evaluate their fit to the analyzed pedigree data: the proportions of the trait variance attributable to MG and non-MG effects. For Model 1 these are the trait heritability, $H^2 = \sigma^2_\mu/\sigma^2_x$, i.e., the proportion of phenotypic variance attributable to the putative MG effect, and $D^2 = H^2 + d^2$, the proportion of the trait variation attributable to both, the MG and the multifactorial (non-MG) effects, quantified by partial correlations ρ, β, and ε (described earlier). Here, $\sigma^2_\mu = \sum p_g \mu^2_g - (\sum p_g \mu_g)^2$ is the variance of genotypic values. The d^2 is de-

fined as the average (over all nuclear pedigrees in the sample) value of the squared multiple correlation between the residual value in an individual and those in his/her parents and in siblings. Obviously, $0 \le d^2 \le 1 - H^2$ and $H^2 \le D^2 \le 1$, because $d^2 = 0$ only when all three parameters, ρ, β, and ε, equal zero.

For Model 2, a more detailed decomposition of the trait variance is possible in the standard manner of a three-way classification (genotype, sex, and age). The main variance proportions can be presented as follows. H^2 is the proportion of the trait variance attributable to the within-sex difference between the MG–genotypic values; D^2_{G} is the proportion of the variance attributable to the all genotypic effects, including the MG control of the CI dependence on age; D^2_{GSA} is the proportion of the variance due to the combined effect of the three sources of the trait variation, namely, MG, sex, and age; and $D^2 = D^2_{\mathrm{GSA}} + d^2$ is the proportion of the trait variance ascribed to all the effects included in Model 2. By definition, $H^2 \le D^2_{\mathrm{G}} \le D^2_{\mathrm{GSA}} \le D^2$ (see Ginsburg, 1997).

The MG hypotheses in both types of models were tested using two transmission probability tests (Elston and Stewart, 1971), $\chi^2_A = 2[LH(\hat{\tau}) - LH(\tau_0)]$ and $\chi^2_E = 2[LH(\hat{\tau}) - LH(\bar{\tau})]$, where $LH(\tau)$ is the maximal log-likelihood value obtained with transmission probabilities $\tau_g = Pr(\mathrm{A}_1 \,|\, g)$; τ_0 denotes a triplet of Mendelian transmission probabilities 1.0, 0.5, and 0.0 for the parent's genotypes $g = 1$, 2, and 3, respectively; $\hat{\tau}$ denotes the triplet of the maximal likelihood estimates of these probabilities, and $\bar{\tau}$ is the maximal likelihood estimates for transmission probabilities constrained to be equal. The MG model of the trait inheritance is accepted if (1) χ^2_E exceeds the critical value corresponding to df = 2 and the a priori established type I error $\alpha = 0.01$ (the hypothetical independence of offspring's MG genotype from the genotypes of his/her parents is rejected) and (2) concurrently, χ^2_A does not exceed the critical value corresponding to df = 3 and $\alpha = 0.05$ (the hypothesis of Mendelian transmission probabilities is accepted).

Once the general MG model was accepted, then the most parsimonious model containing only statistically significant genetic and non-genetic effects was constructed. As usual, this was achieved by constraining the model parameters to the expected values. However, as illustrated below, different "most parsimonious" models (i.e., those models for which no further statistically insignificant parameter constraint is possible) can be obtained by choosing different sequences of tested and accepted parameter constraints. To avoid this ambiguousness in the most parsimonious model construction, a certain optimizing procedure was accepted here as follows. The whole set of parameters (13 parameters in Model 1 and 28 in Model 2) was divided into the following groups (some of them are absent in Model 1): genotypic values within each sex, residual genotype-specific variances, genotype-specific slope coefficients within each sex, genotype-specific inflection points (thresholds) within each sex, and three groups represented by only one parameter, ρ, β, and ε. For each of the latter three parameters, only a single constraint was tested, namely, its equality to zero (meaning absence of the corresponding effect). For each of the previous groups, the following constraints were tested: no MG effect $E(\mathrm{A}_1\mathrm{A}_1) = E(\mathrm{A}_1\mathrm{A}_2) = E(\mathrm{A}_1\mathrm{A}_2)$; additive $E(\mathrm{A}_1\mathrm{A}_2) = 0.5[E(\mathrm{A}_1\mathrm{A}_1) + E(\mathrm{A}_2\mathrm{A}_2)]$, recessive $E(\mathrm{A}_1\mathrm{A}_2) = E(\mathrm{A}_1\mathrm{A}_1)$, and dominant MG effect $E(\mathrm{A}_1\mathrm{A}_2) = E(\mathrm{A}_2\mathrm{A}_2)$, and a specific heterozygotic effect (heterosis) $E(\mathrm{A}_1\mathrm{A}_1) = E(\mathrm{A}_2\mathrm{A}_2) \ne E(\mathrm{A}_1\mathrm{A}_2)$, where $E(\mathrm{A}_i\mathrm{A}_j)$ denotes one of the above-defined characteristics of the MG genotype $\mathrm{A}_i\mathrm{A}_j$, namely, genotypic value, residual variance, inflection point, or slope coefficient. The most parsimonious model was then constructed successively: on each stage, all possible (not accepted on previous stages) constraints were tested. If a constraint was found, which had a minimal likelihood ratio test value not exceeding the corresponding χ^2 critical value, this constraint was included in the model and the next stage began. Otherwise, the procedure ended.

Finally, χ^2_A and χ^2_E tests were used to justify the obtained most parsimonious MG model. No ascertainment correction of likelihood was made because our method of pedigree collection was in no way connected with an individual's bone properties.

RESULTS

Age dependence

Figure 1 shows age-related CI values in the sample by sex. The linear correlation of the CI with age was significant, −0.213 ($P < 0.025$) for males and −0.482 ($P < 0.01$) for females. The age dependence can be pre-

TABLE 2. Model 1: best fitting and most parsimonious major gene model for metacarpal cortical index in pedigree sample from Croatia[a]

Parameter		General models			Most parsimonious models		
		General (1)[b]	Mendel. (2)	Equal τ's (3)	Arbitrary (4)	Mendel. ± SD (5)	Equal τ's (6)
1	p	0.546	0.671	0.285	0.592	0.672 ± 0.057	0.416
2	μ_1	−0.770	−0.642	−1.006	−0.706	−0.652 ± 0.097	−0.034
3	μ_2	0.161	0.295	−0.080	0.216	0.303 ± 0.144	−0.063
4	μ_3	1.253	1.546	0.458	1.439	1.566 ± 0.219	0.320
5	σ_1^2	0.526	0.557	0.555	0.551	0.546 ± 0.063	1.085
6	σ_2^2	0.390	0.391	0.519	0.373	0.377 ± 0.055	0.341
7	σ_3^2	0.681	0.572	0.994	0.551[c]	0.546[c]	1.085[c]
8	ρ	−0.243	−0.263	−0.188	−0.329	−0.292 ± 0.195	−0.093
9	β	0.061	0.057	0.235	[0.000]	[0.000]	[0.000]
10	ε	0.175	0.182	0.242	0.181	0.180 ± 0.057	0.222
11	τ_1	1.000[d]	[1.000]	0.400	1.000[d]	[1.000]	0.944
12	τ_2	0.628	[0.500]	0.400[c]	0.614	[0.500]	0.944[c]
13	τ_3	0.000[d]	[0.000]	0.400[c]	0.000[d]	[0.000]	0.944[c]
Log LH		−1,126.43	−1,128.34	−1,134.50	−1,126.81	−1,128.53	−1,143.54
χ^2		–	3.82[NS] (1)	16.14* (1)	0.76[NS] (1)	3.44[NS] (4)	33.46* (4)

*$P < 0.01$.
[a]NS, corresponds to $P > 0.05$.
[b](N), number indicating the comparative column; [ ], parameter is fixed to shown value.
[c]Parameter is constrained to be equal to the parameter above in the Table.
[d]Parameter estimate achieved its limit. For parameter definitions see Materials and Methods.

sented as follows. A certain age threshold (point of inflection) can be indicated for each sex after which CI decreases gradually with age, while practically no age dependence of the CI is expected for ages lower than this threshold. Using function (1), CI values were adjusted for age with the following least-square estimates: $x_s = 0.182$ and 0.488; $T_s = 44.00$ and 43.00, and $a_s = -0.024$ and −0.052 for males and females, respectively. The age-adjusted CI values showed a negligible correlation with age (0.007 in males and 0.037 in females). The variance of the adjusted trait values in the pooled sample was 0.812 of the initial trait variance. To evaluate the efficacy of this two-interval adjustment, note that the linear regression produced only 15% of CI variation accounted for by sex and age. The two-interval adjustment was then compared with another performed by polynomial regression including age values up to the fourth power. This polynomial regression produced a slightly larger proportion, 21.2% of CI variation accounted for by age and sex, but was obviously less parsimonious: 5 estimated parameters instead of 3.

Familial correlations between the age- and sex-adjusted CI values were as follows: $R_{SPO} = -0.058$ ($n = 86$; $P > 0.05$) between spouses, $R_{PO} = 0.281$ ($n = 353$; $P < 0.01$) between parents and offspring, and $R_{SIB} = 0.391$ ($P < 0.01$) between sibs. The last value was an intra-class correlation. The number of nuclear pedigrees having at least 2 offspring was 220, and the total number of offspring was 490. The correlations indicate strong involvement of familial factors in variation in the CI.

Model 1. Table 2 shows the results of segregation analysis performed using Model 1. The first three columns provide maximal likelihood estimates of the model parameters and corresponding maximum likelihood values for the general model, the general Mendelian model, and the model with equal τ's, respectively. By the transmission probability tests, $\chi^2_A = 3.82$, df = 3, $P > 0.05$ and $\chi^2_E = 16.14$, df = 2, $P < 0.01$, the MG model can be accepted.

Further constraining of the model parameters led to the most parsimonious Mendelian model presented in column 5. Only two parameter constraints have been accepted, namely, no residual correlation between parents and offspring and the heterosis effect on residual variance: $\sigma_2^2 < \sigma_1^2 = \sigma_3^2$. Standard deviations for each non-constrained parameter are shown in Table 2. They were derived through the inverse matrix of the second derivatives of the pedigree log-likelihood. Columns 4 and 6 present two additional variants of this model, one with arbitrary estimates of τ's and another with equal transmission probabilities. Comparison of the general model (column 1) with the

TABLE 3. Model 2: best fitting and most parsimonious major gene model for metacarpal cortical index in pedigree sample from Croatia[a]

Parameter		General models				Most parsimonious models			
		General (1)[b]	Mendel. (2)	Equal τ's (3)	Linear (4)	Arbitrary (5)	Mendel. (6)	Equal τ's (7)	2nd MP (8)
1	P	0.591	0.638	0.587	0.537	0.658	0.698 ± 0.038	0.49	0.680
2	μ_{1m}	−0.612	−0.611	−0.345	−0.663	−0.669	−0.651 ± 0.075	−0.525	−0.626
3	μ_{2m}	−0.057	−0.080	−0.112	−0.082	0.320[d]	0.345[d]	0.246[d]	0.278[d]
4	μ_{3m}	1.126	1.177	0.951	0.978	1.310	1.341 ± 0.111	1.018	1.182
5	μ_{1f}	−0.473	−0.453	−0.229	−0.436	−0.474	−0.452 ± 0.068	−0.306	−0.456
6	μ_{2f}	0.182	0.209	−0.004	0.057	0.369[d]	0.392[d]	0.217[d]	0.354[d]
7	μ_{3f}	1.115	1.147	1.011	1.011	1.211	1.236 ± 0.101	0.739	1.165
8	σ_1^2	0.342	0.360	0.524	0.477	0.289	0.296 ± 0.032	0.379	0.310
9	σ_2^2	0.242	0.246	0.305	0.250	0.224	0.228 ± 0.030	0.415	0.254
10	σ_3^2	0.264	0.255	0.314	0.302	0.224[c]	0.228[c]	0.415[c]	0.254[c]
11	ρ	−0.213	−0.213	−0.213	−0.120	−0.339	−0.312 ± 0.165	−0.163	[0.000]
12	β	0.137	0.137	0.137	0.133	[0.000]	[0.000]	[0.000]	[0.000]
13	ε	0.221	0.221	0.221	0.211	0.244	0.246 ± 0.003	0.240	0.246
14	A_{1m}	−0.036	−0.035	−0.046	−0.016	−0.029	−0.029 ± 0.007	−0.026	−0.018
15	A_{2m}	−0.021	−0.026	0.205	−0.004	−0.023	−0.022 ± 0.006	−0.024	−0.018[c]
16	A_{3m}	−0.026	−0.024	−0.036	−0.023	−0.023[c]	−0.022[c]	−0.024[c]	−0.052
17	A_{1f}	−0.053	−0.052	−0.059	−0.014	−0.050	−0.052 ± 0.005	−0.047	−0.049
18	A_{2f}	−0.048	−0.048	−0.047	−0.036	−0.050[c]	−0.052[c]	−0.047[c]	−0.049[c]
19	A_{3f}	−0.090	−0.089	−0.078	−0.065	−0.102	−0.105 ± 0.013	−0.092	−0.104
20	T_{1m}	46.09	47.00	47.00	0.000	44.21	45.38 ± 1.320	43.48	44.55
21	T_{2m}	52.64	57.00	79.15	0.000	44.21[c]	45.38[c]	43.48[c]	44.55[c]
22	T_{3m}	29.00	29.00	32.00	0.000	44.21[c]	45.38[c]	43.48[c]	44.55[c]
23	T_{1f}	44.22	45.24	44.00	0.000	44.21[c]	45.38[c]	43.48[c]	44.55[c]
24	T_{2f}	46.71	46.87	46.61	0.000	44.21[c]	45.38[c]	43.48[c]	44.55[c]
25	T_{3f}	42.50	42.54	39.63	0.000	44.21[c]	45.38[c]	43.48[c]	44.55[c]
26	τ_1	0.967	[1.000]	0.640	1.000[e]	0.989	[1.000]	0.691	[1.000]
27	τ_2	0.600	[0.500]	0.640!	0.855	0.588	[0.500]	0.691[c]	[0.500]
29	τ_3	0.000	[0.000]	0.640!	0.000[e]	0.000[e]	[0.000]	0.691[c]	[0.000]
Log LH		−908.17	−908.97	−922.17	−935.17	−916.44	−917.50	−943.64	−918.62
χ^2		–	1.60[NS] (1)	28.00* (1)	54.00* (1)	16.54[NS] (1)	2.12[NS] (5)	54.40* (5)	19.30[NS] (2)

*$P < 0.01$.
[a]NS, corresponds to $P > 0.05$.
[b](N), number indicating the comparative column; [ ], parameter is fixed to shown value.
[c]Parameter is constrained to be equal to the parameter above in the table.
[d]Model is additive.
[e]Parameter estimate achieved its limit. For parameter definitions see Materials and Methods.

arbitrary model (column 4) showed a non-significant difference; the likelihood ratio test was 0.76 with df = 2 ($P > 0.05$). The same was true in the comparison of the general and most parsimonious Mendelian models (columns 1 and 5, respectively: $\chi^2 = 4.20$, df = 5, $P > 0.05$). The transmission probability tests of the most parsimonious Model 1 (column 5) against the arbitrary model (column 4) showed again that the Mendelian hypothesis can be accepted ($\chi^2 = 3.44$, df = 3, $P > 0.05$), while the hypothesis of independence between parental and offspring genotypes (column 6) was strongly rejected ($\chi^2 = 33.46$, df = 2, $P < 0.01$).

About 51.25% of the adjusted CI variance was attributable to the MG effect in this model (H^2), and 52.30% was attributable to all (genetic and non-genetic) model effects (D^2). This explained, respectively, 0.812 × 0.5125 = 0.416 or 41.6% and 0.812 × 0.5230 = 0.425 or 42.5% of the original CI variance, i.e., not adjusted for age and sex.

Model 2. Results of segregation analysis of the CI not adjusted for sex and age, performed on the same sample of pedigrees were as follows. The equal τ's model was rejected: $\chi^2_E = 12.59$, df = 2; $P < 0.01$. The Mendelian transmission was also rejected: $\chi^2_A = 9.83$, df = 3; $P < 0.05$. However, excluding only one family changed the results (Table 3). The first three columns present the maximal likelihood estimates of parameters and maximal likelihood values for the general model, its Mendelian version and the sub-model with equal τ's, respectively. As seen from the transmission probability tests, the MG model was accepted, while the equal τ's model was rejected at $P < 0.01$. To evaluate the choice of the sex- and genotype-

specific age dependence of CI presented by equation (2), the hypothesis about a linear sex-genotype specific dependence of CI on age (without any inflection point) was tested. The results are presented in column 4 of Table 3. The age dependence function was strongly rejected: comparing the log-likelihood from this column and from the first one, $\chi^2 = 54.00$, df = 6, $P < 0.01$.

Further sequential constraining of the parameters performed according to the above-described algorithm permitted construction of the most parsimonious Mendelian model. It can be described as follows (Table 3, column 6; the standard deviation is shown for each unconstrained parameter):

(1) An additive MG–allele interaction was found for genotypic values, both in males and in females.
(2) The dominance effect was found ($\sigma_2^2 = \sigma_3^2$) for the genotype-specific residual variances (note that the heterosis effect was found in Model 1).
(3) The partial correlation between residuals in parents and offspring (β) could be constrained to zero; the same result was obtained also in Model 1.
(4) Slope coefficients showed dominance MG control in males ($A_{2m} = A_{3m}$) and recessive in females ($A_{1m} = A_{2m}$).
(5) No sex or age effect on the age threshold was significant: $T_{gs} \equiv T = 45.38$ years. This value slightly exceeded the inflection points found by equation (1): 44 for males and 43 years for females. As seen in the standard deviation of parameter 20, this difference was within the confidence interval.

The most parsimonious model (Table 3, column 6) did not differ significantly either from the most parsimonious model with arbitrary τ's (column 5; $\chi^2 = 2.12$, df = 3, $P > 0.05$) or from the general Mendelian model (column 2; $\chi^2 = 17.06$, df = 11, $P > 0.05$). The most parsimonious model with arbitrary τ's (column 5) also did not differ significantly from the general model (column 1; $\chi^2 = 16.54$, df = 11, $P > 0.05$). On the other hand, the equal τ's model was much poorer ($P < 0.001$) than either the general or arbitrary models.

The proportions of the CI variance attributable to the effects included in Model 2 were: $H^2 = 0.455$, $D^2_G = 0.464$, $D^2_{GSA} = 0.663$, and $D^2 = 0.684$. The comparison of H^2 and D^2 with corresponding proportions of Model 1 showed that Model 2 yielded a slightly larger proportion of trait variance attributable to the MG effect, and that the total amount of the CI variation as accounted by Model 2 was much higher than that by Model 1. Thus, Model 2 provides a more complete and more accurate explanation of the variation in the CI.

Most parsimonious models

The algorithm of the most parsimonious model construction, through successive acceptance of only one constraint per stage producing the minimal change in the sample log-likelihood, makes the term "most parsimonious model" a little bit less ambiguous. In Model 2, this algorithm resulted in the acceptance of as many as 11 parameter constraints. As expected, the sequence of the accepted parameter constraints was as follows. The group of threshold parameters appeared to be the most liable for constraints—it produced smaller log-likelihood changes than other parameter groups. The next two groups included the slope coefficients and partial residual correlations, respectively, followed by the residual variances. The last group to accept the constraints consisted of MG genotypic values. Thus, when describing CI inheritance in terms of Model 2, the statistical estimation of the parameters determining the trait dependence on age was less accurate than that for MG genotypic values.

Using other sequences of the tested and accepted constraints, it is possible to construct several models, which can be called "most parsimonious" simply because they do not permit any further statistically insignificant parameter constraints. One such model is presented in Table 3, column 8. Its genetic interpretation differs from that of the model in column 6. In particular, it was acceptable to constrain the residual correlation between spouses to zero (in addition to the zero correlation between parents and offspring). The slope coefficient in males then came under recessive MG control instead of the dominant one as in the most parsimonious model in column 6. This model had slightly larger log-likelihood than that presented in column 6 because it was described by one less parameter (13 instead of 14). And, using the standard transmission probability tests, this model was also accepted.

DISCUSSION

After comparing the results of the two types of segregation analysis performed in

the present study (Model 1 and Model 2) with those obtained by Karasik et al. (2000a), the results obtained on two ethnically different pedigree samples were consistent.

By using standard transmission probability tests, there was strong evidence in support of the hypothesis that baseline levels of CI are controlled by a single large-effect gene responsible for about 45% of the trait variation (42%–46% in the Croatian and 45%–48% in the Chuvashian samples).

Model 2 was based on the assumption that a single MG controls pleiotropically the three facets of CI inheritance described by baseline CI levels, by inflection points, and by slope coefficients. This assumption seems reasonably parsimonious. The alternative model, implying separate MGs in control of above three characteristics of bone aging, would appear more complicated and it would be difficult to interpret the results obtained by such a complex model. A significant sex-genotype specific effect on the change in CI with age has been accepted in both populations. Moreover, the chosen two-interval sex-genotype specific function of age dependence was statistically grounded; it fit better than the linear one. Although the threshold in the Croatian sample did not appear to be dependent on sex and genotype (in contrast to the finding in Chuvashian sample), the fact that in both populations it was statistically accepted and showed a threshold at the ages of 44–46 years appears to be a convincing observation. Moreover, the sex and genotype effects on the slope coefficients were statistically accepted in both populations. Even taking into account the results of the most parsimonious model construction, in particular the relative liability of the age function coefficients to constrain, the statement about the two-interval age dependence on sex and genotype specific parameters seems quite probable. Model 2 fits the pedigree data much better and explains a substantially larger proportion of the trait variation (68.4%) compared with Model 1 (42.5%).

These results are in good agreement with those reported earlier. Peak bone mass is reached by individuals of both sexes at a relatively young age [between 25 and 40 years of age (Dequeker, 1976; Oyster, 1992)]. In both sexes, medullar cavity expansion thins the cortical bone to a sufficient extent to be conducive to fracture. The rate of bone loss and decrease in the CI is much higher in women compared with men of the same ethnic origin (Rudan et al., 1987a, 1990a,b; Behluli et al., 1991; Stini et al., 1992; Plato et al., 1994). Taking into account ethnic variation in age of inflection points in bone development and diminution (Kobyliansky et al., 1995; Pavlovsky and Kobyliansky, 1999; Karasik et al., 2000b), it does not seem unexpected that the monogenic control of a crucial moment in bone aging, i.e., the age from which the processes decreasing bone integrity begin, was statistically accepted in independent analyses of two ethnically different pedigree samples.

The most parsimonious model is usually constructed to provide an economic interpretation of the process studied. However, the most parsimonious model is a result of some compromise between model specificity, including the number of parameters, and the specific sample. Evidently, there is not a unique way to compromise, and the algorithm proposed here is the only one possible. The ambiguous nature of the most parsimonious model is a statistical effect of insufficient sample information, as can be the difference between the general Mendelian model and the most parsimonious one. Given a very large and genetically informative pedigree sample, only one most parsimonious model could be constructed. However, for any pedigree sample it is not necessarily so. Here, a more detailed description of the trait inheritance in Model 2 was provided by an additional number of genetic parameters: 18 in Model 2 instead of only 3 in Model 1. Because these parameters are estimated on the same limited pedigree sample, it seems reasonable to expect lesser accuracy of the estimates compared with those in Model 1. Note that strong genetic constraint was placed upon these additional parameters, namely, it was assumed that the same two-allele MG controls all groups of these parameters, the genotypic values, the genotype-specific residual variances, the slope coefficients, and the inflection points in males and in females. However, this constraint does not disavow the statistical effect of the sharp increase in the number of model parameters, the expected loss in accuracy of their estimates.

The basic assumption in Model 2 was that the same MG pleiotropically controls three aspects of bone aging. This model was ac-

cepted, though the putative MG affects the three facets of CI variability differently. In the most parsimonious model, there was a recessive MG effect on slope coefficients in females and a dominant one in males, and additive genotypic values in males and females, but no genotype (or sex) effect on the inflection points. In the construction of the most parsimonious model, the hypotheses of the same type of MG control (additive, recessive, or dominant) of the three CI traits were tested statistically and rejected. Thus, the sample appeared sufficiently informative to reject these hypotheses about no genetic effect. It was possible that the hypothesis about no genotype and sex effect on the thresholds could be accepted not because it was true but because the sample was not sufficiently informative regarding this very effect. Moreover, different types of MG allele interaction found for other parameters can also be only a statistical effect and can change by enlarging the sample. In this context and given the specificity of the statistical instrument used in segregation analysis (likelihood ratio test), only the qualitative results, namely, the acceptance or rejection of the MG model, permit a certain genetic interpretation. The interpretation of more subtle, secondary, accessory results, such as the particular values of model parameters, should be made under certain stipulations. Thus, it seems premature to offer a genetic interpretation of particular results in these terms.

The process of constructing more detailed models of trait inheritance can be extended by including additional effects into the model, such as different triplets of the residual variances for males and females, or a genotype-sex-specific dependence of the residual variance on age similar to that for the genotypic values. These more detailed and, accordingly, more complicated trait inheritance models provide possibilities for testing statistical hypotheses about more intimate details of trait inheritance. However, the above consideration shows that the model specification is limited statistically, i.e., the number of model parameters should always be brought into proper correlation with the sample information. This is the reason that only one particular model specification was introduced and statistically tested in the present study—the genotype-sex control of the trait dependence on age. The dependence was expressed by the two-interval function. It appeared that the sample was sufficiently informative to reject hypotheses about no sex and genotype effects.

The robustness of the primary result should be noted. The MG control of CI variation was accepted using different MG models of trait inheritance. In addition to Model 1 (with a preliminary adjustment of the trait for age and sex) and Model 2 (assuming pleiotropic control of the CI baseline levels and the CI dependence on age), other versions of Model 2 were tested. In particular, segregation analysis using Model 2 with non-Hardy–Weinberg distribution of MG genotypes and an assortative mating effect (Ginsburg, 1997; Ginsburg et al., 1998) resulted in the same acceptance of the MG mode of trait inheritance (results not shown).

The basic conclusion of the segregation analysis can be summarized as follows. The hypothesis about a pleiotropic MG effect on baseline level of CI and on the dependence of the CI on age was accepted in pedigree samples from two ethnically and geographically different populations. The fact that the same results are obtained in two remote populations may play a pivotal role in subsequent linkage analysis.

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