Pathological changes in bones like osteoarthritis and osteoporosis are among the most frequent outcomes of age and aging. Presently, little is known about the genetic basis of peak bone mass or rate of bone loss, or on the genetics of bone formation and resorption. This paper reviews modern studies, dealing with the genetic aspects of bone formation and bone aging. The currently most popular measures of bone aging are: osteometric measurements (OSM) including measures of cortical thickness, bone mineral density (BMD), and osteographic scores (OSS) basing on descriptive criteria of bone age. These three are important clinical tools for predicting chronic degenerative disease and estimating biological age of individuals. Despite abundant data on ethnic and racial differences in these bone aging measures, modern knowledge regarding the genetics of the processes came primarily from family studies of BMD which point to strong familial and probably also genetic effects on bone mass. Regardless of the measurement technique or skeletal site selected, heritability estimates of BMD in most studies account for about 60% of the total variation in bone mass. Similarity of heritability estimates in most studies suggests that the same genetic factors operate on both weight-bearing and nonweight-bearing bones. However, genetic heritability may be overestimated in some family studies due to underestimation of common environmental effects. Segregation analysis, performed to date, reveals strong effect of potential major locus on BMD of both compact and trabecular bone, but much remains to be clarified. Genetic factors affecting BMD may be mediated through biochemical turnover of bone. Hence, segregation, linkage, and molecular biology are the staples of any genetic analysis of BMD, while the study of biochemical factors regulating bone turnover should elucidate the full picture of bone formation and aging. Am. J. Hum. Biol. 10:421–438, 1998.© 1998 Wiley-Liss, Inc.
eterious, and significantly affect normal functioning. The pathological changes are deemed to be a chronic degenerative disease (Crews and Gerber, 1994; Plato et al., 1994), and are referred to as osteoarthritis and osteoporosis.

Little is known presently about the genetic basis of peak bone mass or the rate of bone loss. Neither is the genetic basis of bone formation and resorption fully documented. Yet, and this should be stressed, osteoporosis, osteoarthritis and other osteopathies are among the most frequent diseases which are closely associated with age and the aging process. Understandably, therefore, elucidating the genetic mechanism of bone formation and bone aging as well as establishing the chromosomal location of potential major genes could pave the way for genetic engineering and should also contribute importantly to the understanding of degenerative bone diseases and the development of new therapeutic approaches.

**Roentgenographic measures of bone aging**

One way to assess bone health or age status is roentgenography. In practice, only select parts of the skeletal system can be evaluated for bone aging; most investigators use the femur, lumbar spine, metacarpals, and forearm in attempts to assess age-related changes in bone (e.g., Lovejoy et al., 1985; Kaur and Jit, 1990; Plato et al., 1994; Loth and Iscan, 1994). This also includes radiograms of hand bones, which are convenient and useful for studying bone aging (Kobyliansky et al., 1985). Hand roentgenography provides three different types of bone information: osteometric measurements (OSM) including measures of length and breadth of bones and their cortical thickness; bone mineral content or bone mineral density (BMD); and osteographic scores (OSS) that entail descriptive criteria of bone health status or age, as for example, the appearance of osteophytes, exostoses, osteoporotic foci, resorption lacunae, nuclei of sclerosis, and joint deformations (Pavlovsky, 1987; Kobyliansky et al., 1995). The enumerated measurements as well as those shown below, are all correlated with age and are considered bone age characteristics. They are all relatively easy to perform and relatively inexpensive with large samples. They are also important clinical prognosticators of osteoporosis, osteosclerosis, and other pathological conditions related to bone aging.

OSS measurements have recently been described in detail (Pavlovsky, 1987; Kobyliansky et al., 1995). To evaluate individual OSS, the occurrence of the following is recorded: (1) osteophytes or nodes of Heberden in the periarticular regions of the bone and at sites of muscle-tendon attachment; (2) manifestations of osteoporosis; (3) osteosclerosis, and (4) nontraumatic articular deformities. Curves of bone aging, as measured by OSS, are nearly identical in males and females and have a typical sigmoid shape, showing an early “slow” phase followed by a phase of rapid increase in OSS (after 30–35 years of age in both sexes). As shown by Kobyliansky et al. (1995, 1997) for numerous samples from various populations, there is a strong and significant correlation between OSS and age in both sexes in the age range of 30–95 years ($r = 0.84–0.94$, $P < 0.01$). An example of the correlation between OSS and age in the Turkmenian population is shown in Figure 1a.

The OSM (quantitative radiogrammetry) entails caliper measurement of cortical bone thickness from a simple radiograph (Garn, 1970; Plato and Norris, 1980; Thompson, 1980; Kaur and Jit, 1990). Dequeker (1976) argued that basic measurements of cortical area thickness could serve as indirect evaluators of bone mass, and indeed the most frequent terms used in association with OSM include cortical thickness, cortical index (which is the ratio of the combined cortical thickness on both sides of the bone image to the total diameter of the bone), and the percent of cortical area, which takes into account the size of the bone.

Radiogrammetry reflects changes in endosteal bone resorption, but fails to measure cancellous bone, whose features are better indices of bone turnover (Dequeker, 1976). Photodensitometry, which is a radiographic densitometry method to evaluate BMD, employs radiographic film and a wedge of standard variability, proportional to the mineral mass (Ostlere and Gold, 1991). In recent years, several more sophisticated, precise, and noninvasive methods of BMD measurement have been developed (Genant et al., 1991; Nickoloff, 1992; Adams, 1992). None of these, however, is readily adaptable to field conditions. Even so, radiographic densitometry of the hand bones is highly accurate and precise (error of estimation <2%,
according to Virtama, 1957; Mack et al., 1959; Trouerbach et al., 1988), on par with single-photon absorptiometry and quite comparable also with dual-photon absorptiometry (Nickoloff, 1992), and is being less expensive. Moreover, radiographic film allows one to take measurements of OSM and OSS, procedures which are not possible with other techniques of BMD assessment. This explains why photodensitometry is still widely used (Kimura, 1990, 1992; Oyster, 1992; Trouerbach et al., 1993).

Both measures, BMD and OSM, show bone mass decrease with age, in particular after the fourth decade. Kimura (1992) used factor analysis to compare radiogrammetric and microdensitometric parameters in assessing BMD of the second metacarpal. The parameters related to the factors indicating cortical mass and BMD are evidently age-associated. Kimura (1992) therefore concluded that a combination of metacarpal cortical thickness, cortical index and BMD are the most useful means for predicting age. The data further suggested that in both sexes cortical thickness and cortical BMD

Fig. 1a. Relationship between three radiological measures of bone age and chronological age in a Turkmenian sample (data from Kobyliansky et al., 1996). Means for target age (±5 year interval) for OSS (1a), BMD (1b), and OSM (1c) are shown.
are linearly correlated with age from age 30 onwards ($r = -0.41$ and $r = -0.39$, respectively; $P < 0.01$). Figures 1b and 1c show BMD and OSM age-related changes in the Turkmenian sample. Figure 1b shows that the BMD in females generally decreases with age more sharply than in males. There is a moderate negative correlation with age throughout, due to a possible lack of age 'trend' between the third and fifth decades of life (Kobyliansky et al., 1997). In various samples, correlations of mean cortical BMD in 5-year cohorts with age are within the range $-0.54$ to $-0.65$ ($P < 0.01$).

The curves of OSM indices are very similar to that of BMD and have a typical shape, showing an early phase of increase, followed by a phase of gradual decrease. Figure 1c, using the data on the Turkmenian population as an example, shows obvious nonlinear correlations. Indeed, nonlinear functions (cubed, squared, Gompertz’ and logarithmic regressions) detected a significant correlation between age and the mean OSM index in several age groups. For instance, the OSM of the metacarpals in Turkmenian females were well described by a cubed model (multiple $R^2 = 0.55$) and a squared function (multiple $R^2 = 0.55$) (Kobyliansky et al., 1997). Note that correlation between
age and mean age cohort OSM and BMD after age 40–45 became practically linear, strong and significant (Fig. 1b and c).

The prediction of biological age using the aforementioned bone measures has been recently studied by Kobyliansky et al. (1997). The study entailed two different populations: Israelis: 365 cadavers, aged 17 to 96 years, 206 males and 159 females; and Turkmenians: 643 living subjects, aged 17 to 98 years, 254 males and 389 females. Multiple regression analysis was used to assess the possibility of predicting chronological age by the simultaneous use of OSS, OSM and BMD (Table 1). The equations elaborated using different combinations of age-related traits led to polynomial functions. In all combinations the major age predictor is OSS, which alone predicts 46.2% to 77.8% of age variation.

Ethnic variation in bone age measurements

Ethnic and racial differences in bone age measures have been subjected to careful examination for many years and are presently well documented. Thus, for example, Garn et al. (1964) showed that Chinese and Japanese had significantly less cortical bone than did American Whites, even when they were born in the U.S. or any other country.
Guggenheim et al. (1971) have established that osteoporosis occurred more often among Israeli Jews of North African and Asian origin in Israel than among those of European or North American origin, and no significant association existed between the extent of osteoporosis and either the calcium or protein intake. Dequeker (1976) discovered population differences in periosteal diameter and cortical area of the metacarpals in seven ethnically different populations. Another study showed that Eskimos start losing bone at an early age and at a greater rate compared to other populations (Harper et al., 1984). Garn (1970) found considerably less medullary expansion in the second metacarpals of American Black than White females after the sixth decade. Bone density also proved significantly higher in Blacks than in Whites: by 7% in men and 13% in females (Pollitzer and Anderson, 1989). Furthermore, American Black men and women also had higher age-adjusted BMD than did their White counterparts. For example, in Black men the lumbar vertebrae, radial and femoral neck BMD were higher by 5–20% than in White men of the same age (Nelson et al., 1995). The latter authors suggest that differences in BMD are largely due to differences in body fat, and support the endocrine explanation of this phenomenon, i.e., that adipose tissue serves as a depot of estrogen.

Bell (1988) hypothesized that differences in bone mass between Blacks and Whites are due to cellular mechanisms of bone formation. An earlier study on Blacks and Whites raised on the same diet, showed the Blacks to have significantly higher serum immunoreactive parathyroid hormone and 1,25-dihydroxyvitamin D concentrations, while their serum 25-hydroxyvitamin D and bone GLA-protein (osteocalcin) concentrations were lower (Bell et al., 1985). These biochemical differences in Blacks of either sex are more likely to be genetic than environmental according to Nelson et al. (1995). Based on an extensive review, Pollitzer and Anderson (1989) concluded that along with environmental factors, such as diet and physical exercise, ethnic factors are strong determinants of BMD and OSM variation. Plato et al.’s (1994) later review also provides data illustrating considerable ethnic variation in both BMD and OSM measurements, the ethnic variation being particularly marked with regard to percent of cortical area in the second metacarpal.

Ethnic and sex variation of OSS in an array of samples containing information on more than 30 Eurasian populations was recently reported (Kobyliansky et al., 1995). The age dependence of OSS can be fairly accurately described by a stochastic model, operating by parameters $T$, a specific age after which OSS starts to increase, and $b$, the rate of bone change per time (age) unit, i.e., a regression coefficient. In addition, the term $TM$, the mean age in the population at which there is transition into the visible

### TABLE 1. Multiple regression analysis of the relationship between measures of bone aging and age

<table>
<thead>
<tr>
<th>Variables</th>
<th>Age range (yr)</th>
<th>N</th>
<th>$\beta \pm$ S.E.</th>
<th>$R^2$</th>
<th>S.E.E.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Israeli males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OSS</td>
<td>20–95</td>
<td>182</td>
<td>0.871 ± 0.126</td>
<td>0.773</td>
<td>10.44</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>OSM</td>
<td></td>
<td></td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMD</td>
<td></td>
<td></td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turkmenian males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OSS</td>
<td>17–99</td>
<td>246</td>
<td>0.882 ± 0.176</td>
<td>0.778</td>
<td>16.30</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>OSM</td>
<td></td>
<td></td>
<td>−0.032 ± 0.014</td>
<td>0.057</td>
<td>5.98</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>BMD</td>
<td></td>
<td></td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Israeli females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OSS</td>
<td>20–95</td>
<td>55</td>
<td>0.753 ± 0.152</td>
<td>0.462</td>
<td>12.10</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>OSM</td>
<td></td>
<td></td>
<td>−0.171 ± 0.017</td>
<td>0.259</td>
<td>10.98</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>BMD</td>
<td></td>
<td></td>
<td>0.206 ± 0.013</td>
<td>0.069</td>
<td>10.51</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Turkmenian females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OSS</td>
<td>17–95</td>
<td>268</td>
<td>0.857 ± 0.162</td>
<td>0.735</td>
<td>11.81</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>OSM</td>
<td></td>
<td></td>
<td>−0.044 ± 0.017</td>
<td>0.078</td>
<td>6.02</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>BMD</td>
<td></td>
<td></td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$\beta$ = standardized regression coefficients and their associated standard errors.

$R^2$ = proportion of variance, attributable to the predictor bone aging measure.

S.E.E. = standard error of estimate (predicted years).

NS = nonsignificant value.
Livshits et al. (1996a) undertook a study which examined to what extent observed ethnic variation in OSS can be attributable to ethnic, linguistic and environmental (e.g., climatic) factors. The total sample included 3,611 adult men and 3,949 adult women, belonging to two major human groups, Europeans and Asians, and to seven language families, and from 32 geographic locations in the former USSR, Egypt, India and Israel. Analysis of the data showed that a substantial portion of the differences in \( T \) and \( TM \) was due to climate, much more so than the ethnic or genetic differences per se (Fig. 2). Multiple regression analysis, for example, revealed that 37.5% of \( TM \) variation could be explained by climatic factors, such as annual amount of precipitation, geographic longitude and its interaction with mean temperature and the mean monthly atmospheric pressure in January. Longitude per se cannot be a factor exerting a direct effect on \( TM \), but it is quite possible that there are some other factors linked with longitude, like exposure to sun or mineral composition of the water or soil which could directly influence the bone aging process.

There is a growing body of published evidence suggesting that sun exposure (mostly UV-radiation) affects the rate of vitamin D synthesis, and this in turn influences the rate of bone loss (e.g., Stumpf and Privette, 1991; Holick, 1996). Climatic data were recently converted into a Bioclimatic Index of Severity of the Climatic Regime (BISCR) and correlated with OSS parameters. A statistically significant correlation (\( r = -0.35, P < 0.01 \)) was detected between the BISCR and the parameter \( TM \) (Belkin et al., in preparation). The BISCR index includes a temperature coefficient, barometric pressure, speed of wind, relative humidity coefficient, and a coefficient of direct solar radiation. It ranks climatic zones according to their comfortability for human organism (Belkin, 1992). Interestingly, Livshits et al. (1996a) found a very small environmental effect on the rate of bone change \( b \) (\( R^2 = 0.1 \)), but substantial correspondence in \( b \) and genetic differences between populations (Fig. 2).

**Genetic studies: Additive heritability estimates**

Bone mass at a given point in time is influenced by the peak mass achieved and the bone loss that has occurred. Peak BMD (maximum amount of bone) is reached by individuals of both sexes at a young age [27 years according to Kaur and Jit, (1980), or between 25 and 34 years according to Dequeker, (1976)]. It seems reasonable to believe that peak bone mass results from a complex interaction between genetic and environmental factors, especially diet and exercise, during the entire period of growth and maturation (Seeman et al., 1989; Nelson et al., 1995; Kelly et al., 1995). Thus, Lutetz et al. (1990) suggested that genetic factors have a greater effect on the acquisition of peak BMD than on its preservation. Christian et al. (1989) observed no genetic effect in forearm BMD change with age in a longitudinal study of male twins. However, Kelly et al. (1995) criticized their choice of placement for the BMD gauge, and performed a study of similar design in predominantly female twins. The study showed a genetic influence on age-related changes in the lumbar spine and Ward's triangle. In general, it may be that a female sample is more informative on this issue because of higher rates of bone loss with age in women.

What is presently known regarding the genetics of bone aging stems primarily from family studies of BMD, all of which point to strong familial, probably genetic, effects on bone density and bone mass. Such studies were based mostly on twin or parent-child correlations of BMD aimed at determining whether observed correlations are consistent with a simple additive genetic model. On the basis of the roentgenographic examination of the second metacarpal, Garn (1963) suggested a strong hereditary influence on bone mineralization. There were good correlations between cortical thickness in sibs aged 16 years (\( r = 0.33, P < 0.05 \)), the midparent with offspring, and monozygotic twins (\( r = 0.67 \)), which suggested that more than 50% of the total variation in bone mass was attributable to hereditary factors. Later roentgenographic investigations of total and cortical width of three metacarpal bones (Moller et al., 1978) revealed four to five times greater intrapair variance among elderly dizygotic (DZ) than monozygotic (MZ) twin pairs, suggesting a heritability of...
Since then, many studies using various skeletal sites, as well as different methods of BMD assessment, confirmed these earlier observations. The major findings of the earlier studies are reviewed by Pollitzer and Anderson (1989). Family correlations detected in more recent studies are summarized in Table 2. Figure 3 shows the distribution of $h^2$ values which resulted from MZ, DZ, midparent/child and sibling correlations obtained in numerous studies listed in Pollitzer and Anderson (1989) and in Table 2. In Figure 3, the Y axis refers to the number of heritability estimates on the various sites of the skeleton. The multiple sites were considered as independent, each as a single measurement. Regardless of the method of heritability esti-
offspring, suggests the possible existence of a
ample, heritability $h^2 > 1$, computed by sev-
equity in $h^2$ estimates may be noted. For ex-
may simply reflect measurement variability
values among the different skeletal sites
bones. Conceivably, the range of heritability
weight-bearing and nonweight-bearing
tion of the same genetic factors on both
estimates in most studies suggest the opera-
properties in family correlations and heritability
arm, contradict this view. Rather, similari-
al. (1994) on the distal and proximal fore-
relations obtained recently by Hustmyer et
influenced by life style. However, family cor-
ners and therefore, more
mechanical loading and are, therefore, more
influenced by life style. However, family cor-
ations obtained recently by Hustmyer et
on the distal and proximal fore-
arm, contradict this view. Rather, similari-
ties in family correlations and heritability
estimates in most studies suggest the opera-
tion of the same genetic factors on both
weight-bearing and nonweight-bearing
bones. Conceivably, the range of heritability
values among the different skeletal sites may simply reflect measurement variability
(Krall and Dawson-Hughes, 1993).

Nevertheless, some causes for heteroge-
ity in $h^2$ estimates may be noted. For ex-
ample, heritability $h^2 > 1$, computed by sev-
eral authors (Slemenda et al., 1991; Hust-
myer et al., 1994), as well as $r_{sibs} > r_{parents} \text{ offspring}$, suggests the possible existence of a
common family and common sibling envi-
ronment. The additive heritability may be
overestimated when environmental covari-
ance is greater within MZ pairs compared to
DZ pairs (Slemenda et al., 1991), and in
family studies as a result of common envi-
ronmental effects and nonadditive genetic
variance (Krall and Dawson-Hughes, 1993).

There are substantial differences in twin
correlations between age cohorts, which
possibly stem from environmental influ-
ences and tend to alter (generally, to dimin-
ish) heritability estimates. Thus, calcula-
tion of heritability of bone mass yielded 0.75
for juvenile twins but only 0.49 for adult
twins (Smith et al., 1973). A similar conclu-
sion was reaching also by Gueguen et al.
(1995), who found in nuclear families that
heritability increases from childhood until
the age of 26.5 (i.e., age of BMD acquisition).
After this age there occurs an increase in
individual specific variability. Previously,
Pocock et al. (1987) also pointed out that
environmental factors are of increasing im-
portance after menopause and/or with ad-
vancing age. Christian et al. (1988) sug-
gested that environmental factors contrib-
ute greatly to the bone loss in both MZ and
DZ twins. Kelly et al. (1993) showed that
bone loss processes occur at different rates
with age at several sites in the same twin
pairs, as noted in a 3-year follow-up. Sowers
et al. (1992) found that age and the body
mass index explained 13%–15% of the vari-
ability of peak femoral BMD in women of
child-bearing age.

A comparison of potential peak bone mass
in daughters and their premenopausal
mothers (Tylavsky and Bortz, 1989) has
provided evidence for strong genetic influ-
ence, and also that a good diet and reason-
able physical exercise are needed for the
optimization of genetic expression. Both com-
mon family environment as well as
differences between generations, were con-
sidered in a recent study by Kahn et al.
(1994), where 27 triads including mother,
daughter and grandmother were sampled
for possible genetic influence on nondomi-
inant forearm bone density. There was a sig-
nificant correlation in BMD between moth-
ers and grandmothers ($r = 0.50, P < 0.01$),
but less between mothers and daughters
($r = 0.33, P < 0.1$). These findings suggested
that although there were similarities in
bone mass between the three generations,
genetic factors could not be conclusively
proven to be the major determinant of bone
density in preference to lifestyle and envi-
ronmental factors. McKay et al. (1994) com-
pared the maternal adult BMD with that of
the growing children at various stages of
maturity (the children were categorized by
use of maturational indices). The authors
suggested that strong familial resemblance
within the family groups is based on both
environment and genetics.

Although these and other studies usefully
foreshadow a potential genetic contribution
to the rate of bone loss, they suffer from sev-
eral shortcomings. For one, they do not un-
**TABLE 2. Summary of literature on family correlations of BMD**

<table>
<thead>
<tr>
<th>Site</th>
<th>Method</th>
<th>Sample description</th>
<th>Genetic results</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radius</td>
<td>SPA</td>
<td>Twins, females</td>
<td>( r_{ma} = .72 ) ( r_{fa} = .31 )</td>
<td>Slemenda et al. (1991)</td>
</tr>
<tr>
<td>Femoral neck</td>
<td>DPA</td>
<td>MZ: 124 pairs (30-74 yrs)</td>
<td>( r_{ma} = .73 ) ( r_{fa} = .17 )</td>
<td></td>
</tr>
<tr>
<td>Lumbar spine (L₂-L₅)</td>
<td>Same</td>
<td>DZ: 47 pairs (25-80 yrs)</td>
<td>( r_{ma} = .80 ) ( r_{fa} = .19 )</td>
<td></td>
</tr>
<tr>
<td>Ward's triangle</td>
<td>Same</td>
<td></td>
<td>( r_{ma} = .68 ) ( r_{fa} = .17 )</td>
<td></td>
</tr>
<tr>
<td>Femoral trochanter</td>
<td>Same</td>
<td></td>
<td>( r_{ma} = .64 ) ( r_{fa} = .15 )</td>
<td></td>
</tr>
<tr>
<td>Lumbar spine</td>
<td>DPA</td>
<td>Twins, females</td>
<td>( r_{ma} = .96 ) ( r_{fa} = .55 )</td>
<td>Kally et al. (1993)</td>
</tr>
<tr>
<td>Femoral neck</td>
<td>MZ: 21 pairs (24-75 yrs)</td>
<td>( r_{ma} - r_{fa} = 0 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ward's triangle</td>
<td>DZ: 19 pairs (25-65 yrs)</td>
<td>( r_{ma} = .91 ) ( r_{fa} = .64 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal femur (trochanter)</td>
<td>SPA</td>
<td>Nuclear families</td>
<td>( r_{mbdch} = .51 ) ( r_{mbdh} = .57 )</td>
<td>Krall and Dawson-Hughes (1993)*</td>
</tr>
<tr>
<td></td>
<td>Same</td>
<td>40 families</td>
<td>( r_{mbdch} = .64 ) ( r_{mbdh} = .86 )</td>
<td></td>
</tr>
<tr>
<td>Os calcis</td>
<td>Same</td>
<td>(m.: 60 ± 6 yrs)</td>
<td>( r_{mbdch} = .70 ) ( r_{mbdh} = .63 )</td>
<td></td>
</tr>
<tr>
<td>(f.: 63 ± 6 yrs)</td>
<td>(d.: 31 ± 6 yrs)</td>
<td>( r_{mbdch} = .50 ) ( r_{mbdh} = .35 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar spine</td>
<td>Same</td>
<td>(s.: 32 ± 5 yrs)</td>
<td>( r_{mbdch} = .69 ) ( r_{mbdh} = .80 )</td>
<td></td>
</tr>
<tr>
<td>Total body</td>
<td>Same</td>
<td></td>
<td>( r_{mbdch} = .80 ) ( r_{mbdh} = .43 )</td>
<td>Hustmyer et al. (1994)**</td>
</tr>
<tr>
<td>Distal forearm</td>
<td>SPA</td>
<td>Twins, females</td>
<td>( r_{ma} = .80 ) ( r_{fa} = .43 )</td>
<td></td>
</tr>
<tr>
<td>Proximal forearm</td>
<td>Same</td>
<td>MZ: 86 pairs (46.9 ± 11.8 yrs)</td>
<td>( r_{ma} = .84 ) ( r_{fa} = .64 )</td>
<td></td>
</tr>
<tr>
<td>Femoral neck</td>
<td>DPA</td>
<td></td>
<td>( r_{ma} = .77 ) ( r_{fa} = .43 )</td>
<td></td>
</tr>
<tr>
<td>Ward's triangle</td>
<td>Same</td>
<td>DZ: 39 pairs (43.0 ± 11.7 yrs)</td>
<td>( r_{ma} = .80 ) ( r_{fa} = .38 )</td>
<td></td>
</tr>
<tr>
<td>Femoral trochanter</td>
<td>Same</td>
<td></td>
<td>( r_{ma} = .77 ) ( r_{fa} = .26 )</td>
<td></td>
</tr>
<tr>
<td>Lumbar spine</td>
<td>Same</td>
<td></td>
<td>( r_{ma} = .85 ) ( r_{fa} = .41 )</td>
<td></td>
</tr>
<tr>
<td>Proximal femur (L₂-L₅)</td>
<td>SPA</td>
<td>Pairs, 27 m. - d., 27 m. - g., (g.: 76.9 ± 6.7 yrs) (m.: 49.7 ± 3.6 yrs) (d.: 23.9 ± 4.1 yrs)</td>
<td>( r_{mb} = .50 ) ( r_{mb} = .33 )</td>
<td>Kahn et al. (1994)</td>
</tr>
<tr>
<td></td>
<td>SPA</td>
<td></td>
<td>( r_{mb} = .50 ) ( r_{mb} = .33 )</td>
<td></td>
</tr>
<tr>
<td>Femoral neck</td>
<td>DEA</td>
<td>Pairs, 41 m. - d., 42 m. - s., 24 m. - g., (g.: 57.1 - 78.9 yrs) (m.: 26.5 - 49.1 yrs) (d.: 8.5 - 15.6 yrs)</td>
<td>( r_{mb} = .31 ) ( r_{mb} = .22 )</td>
<td>McKay et al. (1994)***</td>
</tr>
<tr>
<td></td>
<td>Same</td>
<td></td>
<td>( r_{mb} = .31 ) ( r_{mb} = .22 )</td>
<td></td>
</tr>
<tr>
<td>Femoral trochanter</td>
<td>Same</td>
<td>(g.: 57.1 - 78.9 yrs) (m.: 26.5 - 49.1 yrs) (d.: 8.5 - 15.6 yrs) (s.: 8.9 -16.1 yrs)</td>
<td>( r_{mb} = .32 ) ( r_{mb} = .09 )</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Same</td>
<td></td>
<td>( r_{mb} = .32 ) ( r_{mb} = .09 )</td>
<td></td>
</tr>
<tr>
<td>Proximal femur</td>
<td>Same</td>
<td></td>
<td>( r_{mb} = .36 ) ( r_{mb} = .26 )</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Same</td>
<td></td>
<td>( r_{mb} = .36 ) ( r_{mb} = .26 )</td>
<td></td>
</tr>
</tbody>
</table>
equivocally resolve the issue as to whether familial resemblance in bone density is due primarily to genetic effects or rather to a common environment. The standard genetic analysis employed in the studies assumes the existence of a large number of minor loci with additive interaction, allowing no other types of interaction among or within loci.

<table>
<thead>
<tr>
<th>Site</th>
<th>Method</th>
<th>Sample description</th>
<th>Genetic results</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spine (L2)</td>
<td>Same</td>
<td></td>
<td>$r_{ma} = .26$</td>
<td>Jouanny et al. (1995)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$r_{ma} = .22$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$r_{fa} = .30$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$r_{fa} = -.01$</td>
<td></td>
</tr>
<tr>
<td>Spine (L1-4)</td>
<td>Same</td>
<td></td>
<td>$r_{ma} = .44$</td>
<td>Gueguan et al. (1995)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$r_{ma} = .25$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$r_{fa} = .21$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$r_{fa} = -.23$</td>
<td></td>
</tr>
<tr>
<td>Total body</td>
<td>DEA</td>
<td>Pedigrees, 129 parents, 85 s., 98 d. (m: 41.9 ± 3.6 ys) (f: 44.0 ± 3.8 ys) (d: 18.1 ± 2.0 ys)</td>
<td>$r_{ma} = .24$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$r_{fa} = .18$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$r_{fa} = .29$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$r_{fa/nobh} = .27$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$r_{fa} = .30$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$r_{fa} = .23$</td>
<td></td>
</tr>
</tbody>
</table>
| Distal phalanx        | X-ray  | Nuclear families, Turkmenia, 213 pedigrees (males: 18 - 89 yrs) (fem.: 16 - 96 yrs) | $r_{ma} = .380 ± .004$ | Livshits et al. (1996b)**
|                       |        |                    | $r_{ma} = .298 ± .077$ |                             |
|                       |        |                    | $r_{fa} = .295 ± .115$ |                             |
| Cancellous bone       |        |                    | $r_{ma} = .291 ± .098$ |                             |
|                       |        |                    | $r_{ma} = .261 ± .078$ |                             |
|                       |        |                    | $r_{fa} = .411 ± .129$ |                             |
| Cortical bone         |        |                    | $r_{ma} = .249 ± .043$ |                             |
|                       |        |                    | $r_{ma} = .323 ± .061$ |                             |
| Distal phalanx        | Same   | Nuclear families, Chuvashia, 119 pedigrees (males: 19 - 91 yrs) (fem.: 16 - 96 yrs) | $r_{ma} = .249 ± .043$ | Livshits et al. (in review) |
| Cortical bone         |        |                    | $r_{ma} = .323 ± .061$ |                             |

mz = monozygotic twins.
dz = dizygotic twins.
p = parent.
f = father.
m = mother.
g = grandmother.
d = daughter.
s = son.
mch = midchildren.
mmp = midparent.
ch = offspring.
fem = females.
yrs = years.
Mean age ± S.D. or age range are shown in parenthesis.
SPA = single-photon absorptiometry.
DPA = double-photon absorptiometry.
DEA = dual energy absorptiometry.
BMD values were: *adjusted on age, height and environmental covariates; **adjusted on age, height and weight;
***adjusted on age only; ****adjusted on age and sex.
Such an assumption is mostly untestable against the genetic hypothesis, especially the Mendelian mode of transmissibility.

**Genetic studies: Major gene effect**

Segregation analysis tests the hypothesis of Mendelian transmissibility, and provides evidence for the existence of a locus (or loci) governing variation in a trait. Indeed, results of complex segregation analyses by Livshits et al. (1996b) on an array of Turkmenian pedigrees, strongly support the existence of a single Mendelian locus with a large effect on BMD. The Mendelian hypothesis with two codominant alleles was chosen as the best fitting and most parsimonious. Under this hypothesis, about 61% of the variance in cortical BMD and 51% of that in cancellous BMD of the phalanges of the third finger are attributable to a major gene effect. No residual intragenotype multifactorial transmissibility was detected in this study. Gueguen et al. (1995), in the independent study of total body BMD, strongly rejected a mixed environmental model ($\chi^2 = 9.8; \text{d.f.} = 2, P < 0.01$), but was unable to reject the mixed Mendelian hypothesis ($\chi^2 = 7.3; \text{d.f.} = 3, P > 0.05$). Yet,
the polygenic inheritance (multifactorial) model fit their data better. Interestingly, this model explained about 64% of the age-adjusted variation in BMD, and no common environment for family members was identified in further analysis.

In a new sample of pedigrees in the Chuvashia district (Russia), 564 individuals, belonging to 135 pedigrees, were assessed for cortical and cancellous BMD of the phalanges of the third finger (Livshits et al., in preparation). The main results of the complex segregation analysis of cortical bone are shown in Table 3, and are consistent with those obtained on the Turkmenian sample (Livshits et al., 1996b). The hypothesis of non-Mendelian transmissibility (environmental model) as well as a model denying transmissibility altogether (sporadic model) are both strongly rejected at $P < 0.01$. On the other hand, the Mendelian inheritance hypothesis showed a very small and insignificant difference from the general model ($x^2 = 0.02; \text{d.f.} = 3, P >> 0.05$).

As in the study of Livshits et al. (1996b), exploration of the likelihood surface using the codominant hypothesis with no residual transmission, revealed that this model is the best-fitting and most parsimonious for BMD of the phalanges (Table 3).

Despite the above studies, it is still not known if the three indicators of bone aging (OSM, BMD, OSS) are controlled by a single (or a few) major gene(s) and where it (they) may be located. It is also not known whether potential loci involved in determining genetic variation of OSM, BMD or OSS possess a significant pleiotropic effect, or to what extent they are possibly linked to each other. Morrison et al. (1994) showed that pathophysiological processes deemed amenable to complex multifactorial regulation may also be modulated by a single gene with pleiotropic transcriptional capability. Genetic factors affecting BMD may be mediated through biochemical turnover in a bone, i.e., through effects on collagen synthesis or degradation. Therefore, these aspects of bone aging require further attention.

### Biochemical markers of bone aging and first genetic findings

Several biochemical indices may adequately describe the rate of bone remodeling. Biochemical markers reflect the rate of bone turnover rather than absolute BMD. If genetic effects on adult bone density and its rate of change are mediated through bone formation and/or resorption, then the genetic effect on the biochemical indices may

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Genetic model</th>
<th>General (1)</th>
<th>Arbitrary (2)</th>
<th>Mendelian (3)</th>
<th>Environmental (4)</th>
<th>Sporadic (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_1$ frequency</td>
<td>.382</td>
<td>.428</td>
<td>.574</td>
<td>.540</td>
<td>.000</td>
<td></td>
</tr>
<tr>
<td>$\gamma(A_1A_1)$</td>
<td>-.840</td>
<td>-.835</td>
<td>-.836</td>
<td>-.985</td>
<td>.114</td>
<td></td>
</tr>
<tr>
<td>$\gamma(A_1A_2)$</td>
<td>.315</td>
<td>.143</td>
<td>.142</td>
<td>-.952</td>
<td>.114</td>
<td></td>
</tr>
<tr>
<td>$\gamma(A_2A_2)$</td>
<td>1.183</td>
<td>1.120</td>
<td>1.120</td>
<td>.794</td>
<td>.114</td>
<td></td>
</tr>
<tr>
<td>Variance</td>
<td>.484</td>
<td>.523</td>
<td>.523</td>
<td>.604</td>
<td>.997</td>
<td></td>
</tr>
<tr>
<td>$g_{12}$</td>
<td>1.000</td>
<td>1.000</td>
<td>[1.000]</td>
<td>.434*</td>
<td>[1.000]</td>
<td></td>
</tr>
<tr>
<td>$g_{13}$</td>
<td>.512</td>
<td>[.500]</td>
<td>.434*</td>
<td>[.500]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$g_{23}$</td>
<td>.001</td>
<td>.001</td>
<td>.434*</td>
<td>[.000]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual family correlations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$r_{ps}$</td>
<td>-.073</td>
<td>[.000]</td>
<td>[.000]</td>
<td>[.000]</td>
<td>[.000]</td>
<td></td>
</tr>
<tr>
<td>$r_{ps}$</td>
<td>-.073</td>
<td>[.000]</td>
<td>[.000]</td>
<td>[.000]</td>
<td>[.000]</td>
<td></td>
</tr>
<tr>
<td>$r_{sp}$</td>
<td>.070</td>
<td>[.000]</td>
<td>[.000]</td>
<td>[.000]</td>
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<td></td>
</tr>
<tr>
<td>$-\ln$ likelihood</td>
<td>721.97</td>
<td>723.46</td>
<td>723.47</td>
<td>743.87</td>
<td>744.40</td>
<td></td>
</tr>
<tr>
<td>$x^2$</td>
<td>2.98NS</td>
<td>0.02NS</td>
<td>40.82SS</td>
<td>41.88SS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D.F.</td>
<td>3</td>
<td>310</td>
<td>210</td>
<td>610</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^{1}(A_1A_1)$ denotes genotypic mean.

$^{2}g_{12}$ is the transmission probability of allele $A_1$ by three respective genotypes $A_1A_1$, $A_1A_2$ and $A_2A_2$.

$^{3}g_{12}$, $g_{13}$, $g_{23}$, $g_{12}$, $g_{13}$, $g_{23}$, $g_{12}$, $g_{13}$, $g_{23}$.

$^{4}r_{sp}$, spouse.

$^{5}r_{sp}$, parent/offspring.

$^{6}r_{sp}$, siblings.

$^{7}$Values in brackets indicate parameters set to values in the models.

$^{8}$Indicate parameters constrained to values equal to one another.

$^{9}$NS corresponds to $P > 0.05$.

$^{10}$SS corresponds to $P < 0.01$.

$^{11}$Compared with Model 1.

$^{12}$Compared with Model 2.
be demonstrable and should prove of clinical
importance.
BMD depends on a delicate balance be-
tween bone formation and resorption. The
sequence of events in bone turnover is in
general as follows: activation (and recruit-
ment) of bone cell populations in response to
a stimulus; resorption of old bone by osteo-
clasts; and new bone formation by osteo-
blasts. Regulation of remodeling involves lo-
cal regulatory factors, a combination of
physical factors such as weightbearing
stress, and the effects of calcium-regulating
hormones (Martin and Burr, 1989; Kiebzak,
Two types of cells are directly involved in
bone formation and resorption: osteoblasts
and osteoclasts. The scheme of these pro-
cesses is diagrammatically shown in Figure
4. The osteoblasts are associated with the
formation of bone and are particularly re-
sponsible for synthesis, deposition, and min-
eralization of bone matrix (Rodan and Ro-
dan, 1984). Their surfaces are rich in alka-
line phosphatase, and this enzyme, located
at the plasma membrane, can be of use in
detection of rapid bone formation or turn-
over. Osteoblasts synthesize, secrete, and
play a role in mineralization of the major
part of organic bone matrix, i.e., type I col-
lagen (T1C).
Serum carboxyterminal propeptide of
T1C was suggested as a marker of bone col-
lagen synthesis and osteoblastic activity
(Tokita et al., 1994; Garnero et al., 1996),
while hydroxyproline, amino- or carboxy
terminal crosslinked telopeptide and deoxy-
pyridinoline were considered as markers of
T1C degradation (Kelly et al., 1993; Tokita
et al., 1994; Garnero et al., 1996). Because
both skin (dermis) and bone are developmentally derived from connective tissue, whose extracellular matrix mainly contains T1C, there is a significant correlation between skinfold thickness and BMD in various skeletal sites (Chappard et al., 1991; Kahn et al., 1995). The association between thin skin and osteoporosis has thus been shown as a useful test for osteoporosis screening (Orme and Belchetz, 1994).

Osteocalcin (bone GLA-protein) is another glycoprotein synthesized by osteoblasts, which is bound to the mineral. Circulating osteocalcin levels are assumed to reflect the intensity of bone formation (Garcia-Carrasco et al., 1988; Kelly et al., 1991). A regulator of the rate of osteocalcin synthesis in vitro and in vivo is 1,25-dihydroxyvitamin D3 (1,25(OH)2D3). Once formed in the skin, vitamin D3 is metabolized first in the liver and then in the kidneys to 1,25-dihydroxyvitamin D3 or calcitriol, which influences intestinal transport of calcium and phosphate (Holick, 1996). Calcitriol stimulates the synthesis of osteocalcin through interaction with a vitamin D response element in the osteocalcin gene (Howard et al., 1995). A rise in intracellular calcium leads to inactivation of the osteoclasts. Osteoclast activity is strongly related to bone resorption, and increases urinary excretion of calcium and hydroxyproline. The urinary concentration of these chemicals is considered an index of the rate of bone resorption (Nordin, 1978; Hodgkinson and Thompson, 1982). The opposite process, wherein there is chronic low serum calcium, leads to increased levels of parathyroid hormone, decreased renal excretion of calcium, an increase in the active form of calcitriol, and activation of the bone resorption by osteoclasts, all of which are adaptations geared to retain calcium in serum. Excess parathyroid hormone (primary hyperparathyroidism) stimulates osteoclastic erosion of bone (Gray, 1995). According to Anderson and Kissane (1977), secondary hyperparathyroidism and a resultant skeletal demineralization usually accompany the vitamin D-deficient state.

Osteoblasts, which during bone deposition may inhibit osteoclast activity, in presence of parathyroid hormone release various intermediaries, such as interleukin-6, the cytokine which activates osteoclasts. On the other hand, production of these intermediaries is inhibited by estrogens (Manolagas and Jilka, 1995). Thus, decrease in estrogen level during menopause is one of the etiologic mechanisms of postmenopausal osteoporosis.

The genetic effect on bone turnover remains unclear. Thus far, little and contradictory data have been obtained regarding indices of bone turnover, albeit attempts to study the involvement of genetic factors in the determination of levels or rates of some of the above-mentioned biochemical indices of bone turnover have been made. Observations of Kelly et al. (1989) yielded cross-sectional data on a large group of women and demonstrated that serum osteocalcin increases at menopause, but subsequently declines with advancing age without a corresponding decline in either the index of bone resorption, or the fasting urinary calcium/creatinine and calcium/hydroxyproline. More recently, Kelly et al. (1991) used a modest sample of MZ and DZ twins to test possible genetic effects on these biochemical indices of bone turnover. The results suggest that genetic effects on variation in urinary levels of both hydroxyproline and calcium are not detectable. Yet, potential genetic effects on serum osteocalcin level are strong and highly significant, and the data show that about 80% of the variance in serum osteocalcin could be explained by genetic factors. Garnero et al. (1996) reported intraclass correlations in MZ twins higher than in DZ twins for both serum osteocalcin and bone specific alkaline phosphatase. It is further possible that genetic factors may be mediated also through effects on collagen synthesis or degradation (Kelly et al., 1993). Garnero et al. (1996) implemented several additional markers of T1C turnover: serum propeptide of T1C, a marker of its synthesis, and urinary markers of bone degradation, deoxypiridinoline and crosslinked T1C peptides. The authors found a significant genetic influence only for markers which do not change at menopause, suggesting a small contribution of genetic factors to the postmenopausal bone turnover.

Morrison et al. (1992) discovered a highly significant correlation between the serum level of osteocalcin and vitamin D receptor genotypes in Caucasian women. Kelly et al. (1993) have also identified restriction fragment-length polymorphisms in the vitamin D receptor gene that predicts osteocalcin levels in normal subjects. Indeed, Morrison et al. (1994) estimated that common alleles
in the gene encoding the vitamin D receptor can explain up to 75% of the genetic variation in BMD in healthy individuals.

It has been shown also (Morrison et al., 1992; Kelly et al., 1993), that the vitamin D receptor allele B determines low BMD. This correlation remained significant both when unrelated women as well as twins were compared. Specifically, analysis of twin pairs showed that individuals homozygous for the absence of the restriction endonuclease BsmI site (BB homozygotes) had the lowest spinal BMD. These results were confirmed recently by Fleet et al. (1995), who studied young unrelated premenopausal White and Black women, and found that despite the significant ethnic differences in BMD values, BB genotypes in both groups were strongly associated with decreased BMD of the femoral neck and the lumbar spine.

Howard et al. (1995) hypothesized that the net result of an altered vitamin D receptor functional capacity in the BB genotype drives calcitriol to higher levels. This in turn may also force an elevated bone turnover resulting in a lowered BMD. However, Looney et al. (1995), in their case/control study comparing vitamin D receptor genotype distributions, found no evidence to support their hypothesis regarding prediction of lower BMD by vitamin D receptor alleles; the authors concluded “that the polymorphism in vitamin D receptor gene that defines the BB genotype is not a good predictor of severe osteoporosis. . .”

To evaluate the relative effects of other hormones on premenopausal bone mass, Armamento-Villareal et al. (1992) studied the impact of lifelong estrogen exposure, assessed by an estrogen score on vertebral bone density, in 63 premenopausal women. The findings clearly showed that estrogen hormonal status is strongly related to bone mass. Thus, women with low bone density, when compared to women with normal bone density, yielded a significantly lower estrogen score (15.1 vs. 18.7, $P = 0.001$), as well as lower serum estradiol (46.9 vs. 86.6 pg/ml, $P < 0.05$) and estrogen (107.4 vs. 178.8 pg/ml, $P = 0.05$) levels. Positive family history of osteoporosis was also significantly more frequent in women with low bone density (86% vs. 61%, $P < 0.05$), suggesting the involvement of genetic factors. Note, however, that hormonal levels can by themselves be genetically determined, so that the indirect involvement of hereditary factors may even be considerably higher. Nevertheless, segregation analysis of biochemical indices, in particular, products of collagen degradation and osteocalcin, has yet to be carried out. Such results should contribute importantly to the understanding of the genetic basis of the physiological mechanisms of bone formation.

In summary, attention should focus on the identification of molecular biologic factors that may regulate bone turnover. Identifying genes that determine BMD through the use of molecular genetic techniques could have wideranging implications and applications for the prevention and treatment of osteoporosis. Moreover, it could contribute to the development of methods for increasing skeletal mineralization or identifying individuals at risk, so as to act prophylactically to prevent the clinical disease. Much is known about the heredity of BMD; yet segregation analysis has only been performed twice. There is need to target the genetic analysis of BMD and the biochemical regulating factors of bone turnover via segregation, linkage and molecular biology techniques.

LITERATURE CITED


