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Genetics of Osteoporosis

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Osteoporosis is a common multifactorial disorder of reduced bone mass. The disorder in its most common form is generalized, affecting the elderly, both sexes, and all racial groups. Multiple environmental factors are involved in the pathogenesis. Genes also play a major role as reflected by heritability of many components of bone strength. Quantitative phenotypes in bone strength in the normal population do not conform to a monogenetic mode of inheritance. The common form of osteoporosis is generally considered to be a polygenic disorder arising from the interaction of common polymorphic alleles at quantitative trait loci, with multiple environmental factors. Finding the susceptibility genes underlying osteoporosis requires identifying specific alleles that coinherit with key heritable phenotypes in bone strength. Because of the close correspondence among mammalian genomes, identification of the genes underlying bone strength in mammals such as the mouse is likely to be of major assistance in human studies. Identification of susceptibility genes for osteoporosis is one of several important approaches toward the long-term goal of understanding the molecular biology of the normal variation in bone strength and how it may be modified to prevent osteoporosis. As with all genetic studies in humans, these scientific advances will need to be made in an environment of legal and ethical safeguards that are acceptable to the general public. (*Endocrine Reviews* 23: 303–326, 2002)

Abbreviations: BMC, Bone mineral content; BMD, bone mineral density; Chr, chomosome; COL1A1, collagen type 1 α 1; COL1A2, collagen type 2 α 1; DXA, dual x-ray absorpiometry; Dz, dizygotic; ER, estrogen receptor; IBD, identity by descent; LRP5, lipoprotein acceptor-related protein; Mz, monozygotic; QTL, quantitative trait loci; RI, recombinant inbred; SAM, senescence-accelerated mice; SNP, single-nucleotide polymorphism; TDT, transmission disequilibrium test; VDR, vitamin D receptor.

I. Introduction

OSTEOPOROSIS IS A common multifactorial disorder of reduced bone mass manifesting clinically as fragility fracture. Fracture arises as a stochastic event from minor trauma acting on a skeleton that has reduced bone strength (1) (Fig. 1). The pathogenesis of fragility fracture almost always involves trauma and is not necessarily associated with reduced bone mass. Thus, fragility fracture should neither be used synonymously nor interchangeably as a phenotype for osteoporosis.

Osteoporosis in its most common form is generalized, affecting the elderly, both sexes, and all racial groups. Multiple environmental risk factors are involved in the pathogenesis. Genetic risk factors, however, also play a major role as reflected by the high heritability of many components of bone strength. Although there are a small number of cytogenetic (2, 3) and monogenetic diseases causing osteoporosis (4–9), quantitative traits in bone strength in the normal population do not conform to a monogenetic mode of inheritance. Thus, the common form of osteoporosis is generally considered to be polygenic, arising from the interaction of common polymorphic alleles at quantitative trait loci (QTL) with multiple environmental factors. Finding the genes underlying osteoporosis typically requires identification of its key heritable phenotypes and demonstrating in family and population studies that these phenotypes are coinherited with specific alleles. With progress in developing statistical methods to detect QTL and biochemical techniques to identify and map abundant polymorphisms throughout the genome (10, 11), studies to identify the susceptibility genes for osteoporosis are timely. The recent publication of the initial sequencing and analysis of the human genome (12, 13) has added a strong impetus to such studies. The sequence provides a very large number of new polymorphisms, particu-

larly in the form of single-nucleotide polymorphisms (SNPs) (14) that are central for identification of QTL. Because of the close correspondence among mammalian genomes, it is hoped that identification of the genes underlying bone strength in mammals such as the mouse (15) will be of major assistance in human studies. The identification of susceptibility genes for osteoporosis is expected to be a major contributing factor toward the long-term goal of understanding the molecular biology of the normal variation in bone strength and how it may be modified to prevent osteoporotic fractures. As with all genetic studies in humans, these scientific advances will need to be made in an environment of legal and ethical safeguards that are acceptable to the general public (16).

II. Osteoporosis

A. Normal variation in bone mass and structure

Bone mass and skeletal proportions exhibit a wide range in the normal population (Fig. 2). This variation is further magnified by differences due to age, sex, and race. Skeletal size and mass increase into adolescence. With closure of the epiphyses, the skeleton achieves adult size, although further accumulation of bone mineral continues for several years thereafter. The range in peak bone mineral density and content at the femoral neck in white women measured by dual x-ray densitometry is 50% and 60% of the mean, respectively (Normal White Population Database, DPX-IQ Reference Manual, Documentation Version 5/96, Lunar Corp., Madison, WI). After the third decade, bone mass is steadily lost until the end of life. About 22% of the bone mineral density is lost at the femoral neck in white women from age 30 to age 80 yr (Normal White Population Database, DPX-IQ Reference Manual, Documentation Version 5/96, Lunar Corp., Madison, WI) (17). The main determinants of bone mass in the elderly, who are at greatest risk of osteoporosis, are peak bone mass and the rate of age-related bone loss (18). At all ages, the variance remains relatively stable (19). Furthermore, bone mass among different skeletal sites is highly correlated (20, 21). Age-related bone loss is accompanied by deterioration in bone architecture (22, 23) and an overall expansion of the skeleton (24). Men on average have larger skeletons and have more bone mass at all ages than women (Fig. 2). American blacks have more bone mass than American and European whites (25, 26), who in turn have more bone mass than Asians (27, 28). Common polymorphisms probably underlie much of the normal variation in bone mass and structure. Thus, bone mass and structure phenotypes are key quantitative traits that are used for searching for the susceptibility genes for osteoporosis.

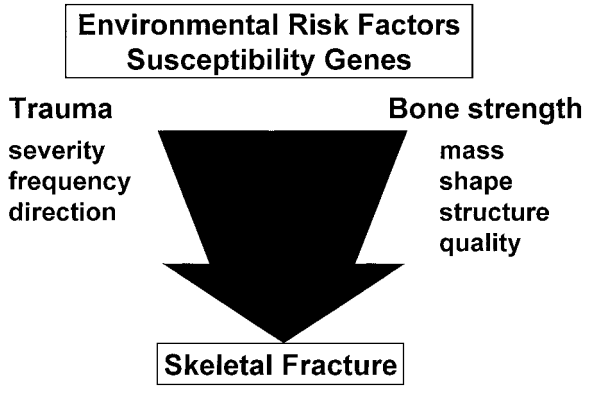


FIG. 1. Schema of the effect of environmental and genetic risk factors on the interaction between bone strength and trauma that leads to osteoporotic fracture.

B. Definition of osteoporosis

The term osteoporosis encompasses a number of disorders of the skeleton, the essential feature of which is a reduced amount of bone tissue in bone as an organ (29–31). The bone mass deficit reduces bone strength, which in turn increases fracture risk. When the disorder is severe, fractures result from mild trauma and are frequently referred to as fragility fractures. Osteoporosis is a complex disorder with a large number of environmental risk factors including diet, life style, and disease, often interacting in combinations (Table 1). In common forms of the disorder, the reduced bone mass is generalized. Both cortical and cancellous bone are affected, although not always equally. The bone deficit results from an

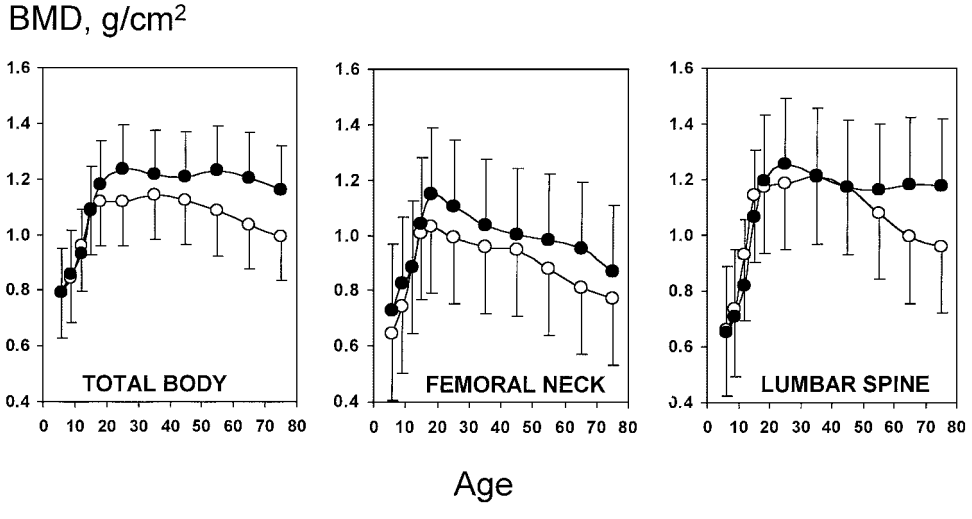


FIG. 2. Normal variation (mean and 2 SD) and change in BMD with age in healthy men (*black circle*) and women (*open circle*) (Normal Population Database, DPX-IQ Reference Manual, Documentation Version 5/96, Lunar Corp., Madison, WI). Peak bone mass at hip and spine for measurement on Lunar machines is taken as the mean BMD between age 20 and 40 yr, but this age range varies with DXA machine manufacturer.

TABLE 1. Environmental risk factors for osteoporosis

Risk Factor	Reference
Nutrition	
Calcium	238–240
Vitamin D	239, 240
Vitamin C	241, 242
Protein	243, 244
Lifestyle	
Physical activity	245, 246
Smoking	247–249
Alcohol	250
Pregnancy	251
Anorexia nervosa	252
Endocrine disorder	
Estrogen deficiency	253, 254
Testosterone deficiency	255
Cushing's syndrome	256, 257
Primary hyperparathyroidism	258, 259
Thyrotoxicosis	260
GH deficiency	261, 262
Malabsorption disorder	
Gastrectomy	263, 264
Small bowel resection	265
Celiac disease	266, 267
Crohn's disease	268, 269
Cystic fibrosis	270
Bone marrow disorder	
Myeloma	271, 272
Mastocytosis	273
Inflammatory disease	
Rheumatoid arthritis	274
Lupus erythematosis	275
AIDS	276
Depression	277
Drug	
Corticosteroids	41, 278
Anticonvulsants	279
Immunosuppressants	280
Chemotherapy	281
T_4	282
Heparin	283

imbalance in the normal relationship between bone formation and bone resorption, causing too little bone to be formed, too much removed, or both. The effect on cortical bone includes thinning of the cortex (20, 32) and increased intracortical porosity (32, 33). In cancellous bone, the effect includes trabecular thinning (22, 23) and loss of trabecular connectivity (34, 35). Although bone mass is the major component of bone strength, other characteristics contribute to strength and to fracture risk. These include structural elements that form the architecture and overall geometric shape of the bone (36–39). In addition, bone quality, a characteristic that cannot currently be measured *in vivo*, contributes to strength. Indeed, in some conditions under which fracturing is prominent, such as organ transplant (40), oral glucocorticosteroid treatment (41), and diabetes in elderly subjects (42), deterioration in bone quality appears to be a major cause of fractures because they may occur largely unrelated to changes in bone mineral density. Furthermore, fragility fractures occur in conditions of increased bone mass such as fluoride treatment (43) and osteopetrosis (44). Therefore, although fragility fracture is the clinical outcome of osteoporosis, fragility fracture can neither be used synonymously nor interchangeably as a phenotype for osteoporosis. Thus, the genes underlying fragility fracture and those underlying osteoporosis will not necessarily be the same.

C. Diagnosis of osteoporosis

An inevitable outcome of the reduced amount of mineralized bone is that osteoporosis is characterized by a decrease both in bone mass and in bone mineral density. However, these two parameters need to be distinguished. Noninvasive diagnosis of osteoporosis currently relies heavily on measurement of bone mineral content (BMC) and bone mineral density (BMD) by imaging techniques (45). Dual x-ray absorptiometry (DXA), the most commonly available technique, assesses bone mass as BMC in grams of calcium phosphate within the area of bone that is scanned. Because bone size varies among individuals, BMC is a function of skeletal size. In an attempt to reduce the variance among individuals due to the area of bone scanned, BMC is converted to an areal density in grams per cm^2 (BMD) by dividing BMC by the projected scanned area. Quantitative computed tomography (QCT), currently a less accessible technique, measures BMD as a volume density, grams per cm^3. In addition, QCT provides BMD of cortical and trabecular compartments separately and, if resolution is sufficient, of the material density of bone tissue (46). Because of the marked effect of age, sex, and race on BMD, it is expressed for clinical purposes most usefully as a Z score in SD units in relation to a healthy population matched for sex and race (Fig. 2). However, because peak bone mass represents the skeleton at its maximum strength, BMD is also expressed in relation to peak bone mass as a T score to assess fracture risk. The age of achieving peak bone mass is taken as sometime between 20 and 40 yr but varies according to DXA machine manufacturer and skeletal site. Epidemiologically, osteoporosis in white women is currently diagnosed as a T score on DXA of less than −2.5 at any skeletal site, with a T score between −1.0 and −2.5 being referred to as osteopenia (47). It should be stressed, however, that −2.5 is not only an arbitrary level but is also sensitive to the skeletal site measured and the technique of measurement (21, 48). Furthermore, this threshold does not necessarily apply to men (49) or to all races. Thus, it should not be used in genetic studies as an absolute level for the diagnosis of osteoporosis. Other techniques, including bone biopsy, are unsuitable for measuring phenotypes for genetic studies because of the invasive nature of the procedure.

D. Phenotypes predicting fracture risk

Measurement of BMD by DXA predicts fracture risk (50), particularly when it is made at the skeletal site of future fracture (51). Although there are inherent inaccuracies in the technique (52), it is widely used as a key phenotype in searching for susceptibility genes for osteoporosis. The hip and spine are commonly measured sites because of their high incidence of osteoporotic fracture. For each SD decrease in T score, the lifetime risk of fragility fracture about doubles (50). However, skeletal structure also contributes independently to fracture risk and can be obtained from radiographs (20), QCT (53), and DXA images (54). Although phenotypes based

on direct measurements of biomechanical strength cannot be made in humans, a variety of parameters related to bone strength can be derived from structural variables (55, 56). Deterioration in bone quality also leads to fracture. By definition, this is not measurable except by destructive biomechnical tests. New techniques using ultrasound (57, 58) and magnetic resonance (59) may capture some quantitative components of bone quality. Although not all studies agree, fracture risk in elderly women may also be predicted from bone turnover as assessed by biochemical markers (60). Thus, key bone phenotypes involved in fracture risk relate not only to bone mass but also to bone structure, bone loss, and possibly to bone turnover. Because of the wide variety of key phenotypes and because it is not known how the susceptibility genes for osteoporosis affect the skeleton, measurement of multiple skeletal phenotypes is essential. However, it should also be appreciated that in addition to these skeletal risk factors, the frequency of falls (61–63), the direction of falling (63, 64), and the occurrence of previous fracture (61) (65) are also risk factors for osteoporotic fractures.

E. Bone strength and physical activity

Bone strength cannot be directly measured *in vivo* in humans. However, it may be assessed indirectly from measuring components of mass and the distribution of structure. Such measures can be used as quantitative traits in searching for the susceptibility genes for osteoporosis (66) and are of particular interest at skeletal sites such as the hip and spine where fragility fractures are common. The strength of bone is normally maintained in balance with the amount of physical activity the skeleton is subject to through mechanisms collectively known as the mechanostat (67). However, the effectiveness of the mechanostat to achieve this balance may also be under genetic influences. Muscle mass, an important covariate of bone strength and an integral component of the mechanostat, is a key phenotype and can be measured simultaneously with BMD by DXA and QCT.

F. Fragility fractures

Fragility fractures may affect any bone. However, they are common at the vertebra (65, 68) and the upper end of the femur (61, 69) (Fig. 3). The incidence of fracture rises steeply with age after the age of 50, and hip fracture is higher in women than men and lower in black than white Americans (70, 71). Thus, fragility fracture incidence inversely tracks bone mass. However, although bone mass predicts fracture risk within discrete populations, it does not identify individuals who will fracture (50). This is explained in part by the fact that other factors such as bone structure predict fracture independent of bone mass (37). However, it is mainly due to the fact that fracture itself is a complex disorder with multiple underlying risk factors (72), many of which are unrelated to bone strength. Fragility fracture, therefore, is a highly complex phenotype in its own right, which should not be used by itself to diagnose or to evaluate risk factors for osteoporosis. As such, it is unlikely to be a useful phenotype in searching for the genes underlying osteoporosis.

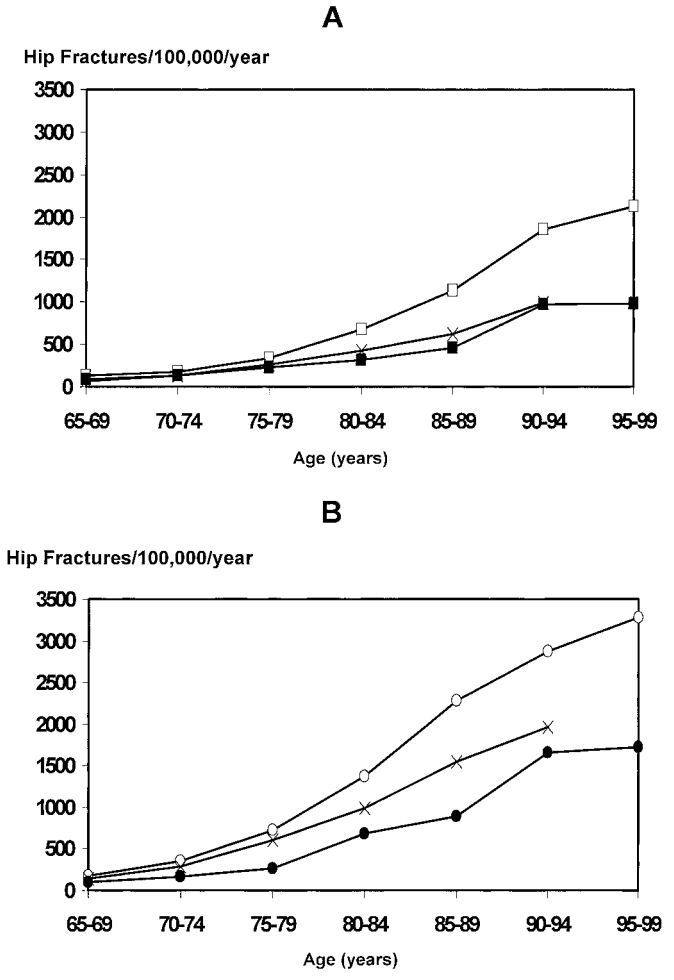


FIG. 3. Incidence of hip fracture in white (*open square*), Asian (×), and black (*black square*) men (panel A) and white (*open circle*), Asian (×), and black (*black circle*) women (panel B) (71, 339).

III. Heritability

A. Estimation of heritability

In multifactorial diseases, population variance in a quantitative phenotype is determined by the interaction of genotype with environment. An estimate of heritability takes into account the population variance due to genetic and environmental factors (73). Perhaps the easiest heritability to appreciate is that calculated from studies in twins. Monozygotic (Mz) twins have 100% of alleles in common, whereas dizygotic (Dz) twins have on average only 50% of alleles in common. Thus, any decreased variance in a phenotype in Mz twins as compared with Dz twins reflects the underlying genetic contribution. An assumption in this model is that the degree to which Mz twins share a common environment is the same as that for Dz twins. This is rarely the case and often leads to overestimates of heritability. In some twins studies (74, 75) heritabilities above unity have been achieved. These may reflect violations of the assumptions that Mz and Dz twins have similar contributions from their shared environments. The extent of similarity for the phenotype is measured by the correlation between pairs of twins. An estimate of the variance between twin pairs in relation to the variance

among all twin pairs provides the intraclass correlation. It is calculated as: (mean squares among pairs − mean squares between pairs)/(mean squares among pairs + mean squares between pairs). The heritability statistic is $H^2 = 2 \times (rMz - rDz)$ where rMz and rDz are the intraclass correlations for Mz and Dz twins, respectively. H^2 is an estimate of the proportion of the variation in the phenotype that is genetically determined. This model can be extended to estimate heritability among first-degree relatives. In this case, $H^2 = 2 \times r$ is for first-degree relatives such as sib pairs or parent offspring pairs. In our studies in twins (76) and sib pairs (77), the heritability estimates for bone mass phenotypes are very similar.

B. Heritability of bone mass

Studies over the last 30 yr in healthy twins (74, 75, 78–80) consistently demonstrate a large genetic contribution to bone mass even into old age (Fig. 4). A number of family studies using healthy parent-children pairs (81–86), healthy sister pairs (87), and parent-children pairs in whom the parent had osteoporosis (88–90) have corroborated the major role of genes in determining bone mass. Furthermore, heritability of bone mass can be detected during childhood even though the skeleton is undergoing major changes in both skeletal size and mass (78, 80, 91, 92).

Most studies have sampled white women, and not enough studies have been done in men to establish whether or not there are significant gender differences in the heritability of certain bone phenotypes. It could be that some of the sex differences in bone mass are accounted for by gender-specific genes. In this regard, it is perhaps significant that in inbred mice, of five QTL for bone mass, only three were linked in both females and males, strongly suggesting sex-specific loci (93).

C. Heritability of bone size and structure

Height and other anthropometric variables related to skeletal size have been known for many years to be highly heritable (94–96; see Table 2). In the early reports on the heritability of bone mass, variables related to bone size and structure such as forearm width (78) and phalangeal cortical and medullary width (79) were shown to be as highly heritable as bone mass. More recent reports examining structural variables that are important in maintaining bone strength at skeletal sites where osteoporotic fracture is common, highlight the strong heritability of bone structure at both the hip (97, 98) and at the spine (99). Hip axis length measured from DXA images (97) and femur axis length measured from radiographs (98) are both highly heritable and predict risk for fracture at the hip.

D. Heritability of bone loss

In contrast to the extensive studies on heritability of bone mass, few studies on heritability of bone loss have been reported (100, 101). No evidence of a genetic component to loss of BMD at the midshaft of the radius was found in 25 Mz and 21 Dz elderly twin men followed over a 16-yr period (100). Although the length of the study period was satisfactory to detect rates of loss, the sample size was small, and the skeletal site measured is not a site of osteoporotic fracture in men. A genetic component to the change, but not loss, in BMD at the spine and hip was found in 21 Mz and 19 Dz twins measured over a 3-yr mean period (101). However, the sample size was small, the period of study was relatively short, ranging from only 1–5 yr, the subjects were a mixture of men and premenopausal and postmenopausal women, and the age range was wide, extending from 25–65 yr. Thus, there are no current studies that powerfully address the important question of whether the rate of bone loss is heritable at skeletal sites where osteoporotic fracture is common.

E. Heritability of bone turnover

Bone formation and resorption can be assessed from a number of biochemical markers in blood and urine (102). These vary with age (103), race (104), and disease (102) and its treatment (105). Formation and resorption markers show a strong correlation among themselves (103). These markers do not measure bone mass or structure but in some circumstances may reflect bone gain (103) or bone loss (106, 107). In elderly white women, they may also predict hip fracture (60). Their relationship to bone strength is tenuous. Nevertheless, a number of studies have reported that there is a heritable component to bone turnover markers although with little consistency in the turnover markers across studies and little corroboration among markers within studies (108, 109).

F. Heritability of fracture

The heritability of fracture, as expected with such a complex phenotype, is not strong. In a large prospective study of white American women 65 yr of age or older, a history of hip fracture in their mother doubled the risk of hip fracture (110). The increased risk remained after adjusting for BMD, indicating that factors other than bone mass are involved. In a study of 2308 Mz and 5241 Dz male and female twins followed prospectively in Finland, although the concordance in the rate of fracture (111) was greater in Mz twins than Dz twins, the magnitude of difference was small and osteoporotic fracture was not strongly influenced by genes. When these data were reanalyzed using a variance components approach (112), genetic factors were estimated to account for, at most, only about one third of the variance in the liability to fracture. In a questionnaire study of white American women older than 40 yr identified because they had participated in any type of bone study, there was a significant history, recalled by the proband, of a forearm fracture in their mother and their sister. The heritability of forearm fracture was calculated to be less than one third (113). Although there appears to be a genetic component to fragility fracture, it seems equally likely from these studies that the underlying genes may not relate to bone strength but to the risk factors for falls, which are also highly heritable (114, 115).

G. Heritability of falls

Trauma is an essential component in most fractures (Fig. 1). The most common source of trauma in the elderly is falls. Most age-related osteoporotic fractures, particularly at the

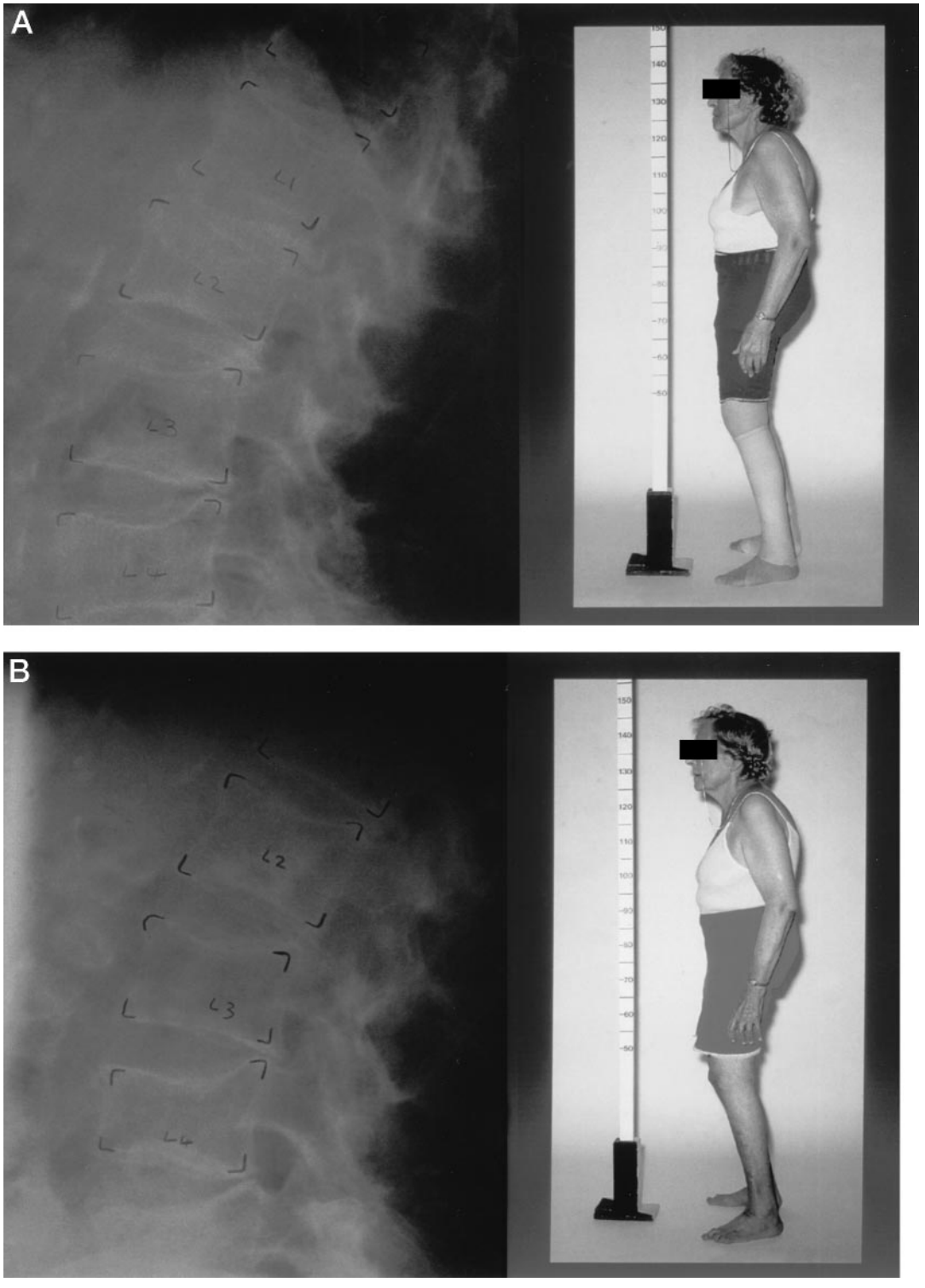


FIG. 4. Pair of elderly Mz twins showing striking similarity both in height, weight, and body dimensions and in vertebral shape, structure, and mineral density. Sister A presented with backache, whereas sister B was asymptomatic.

hip, result from simple falls from a standing height (61–63, 116, 117). Falls increase with age (118) and are more common in women than men (116). Although the minority of falls result in hip fracture, the majority of hip fractures result from a fall. The incidence of hip fracture is greatly reduced if the energy of the fall is attenuated by padding around the hip (119, 120). The number of falls along with decreased bone strength, age, and previous fracture are major predictors of hip fracture (61, 63). Falls are a highly complex phenotype with multiple environmental risk factors (62, 72, 116, 118, 121–123). Although falls also have a heritable component (114, 115), the susceptibility genes for fracture resulting from

TABLE 2. Heritability (H^2) estimates

Phenotype	Sibling pairs (n)	H^2
Lumbar spine BMD	425	0.89
Femoral neck BMD	425	0.77
Pelvic axis length	309	0.83
Femoral neck axis length	309	0.81
Femoral head width	309	0.75
Femoral calcar width	309	0.68
Femoral medulla width	302	0.63
Femoral neck width	309	0.61
Femoral shaft width	302	0.58
Lumbar vertebral middle height	206	0.83
Lumbar vertebral posterior height	206	0.66
Lumbar vertebral anterior height	206	0.68
Lumbar vertebral upper width	206	0.72
Lumbar vertebral lower width	206	0.61

See text for BMD (77, 284) and structural phenotypes in white sister pairs (98, 99).

falls are unlikely to relate to the genes underlying bone strength.

IV. Locating the Susceptibility Genes

A. Candidate gene approach

For phenotypes with a substantial genetic contribution to phenotypic variability, studies to identify the genetic loci influencing the phenotypes are more likely to be eventually successful. However, high heritability does not ensure large individual gene effects. Thus, the power to detect a QTL contributing to a multifactorial phenotype is directly proportional to the magnitude of the specific effects of the QTL. For example, regardless of the average heritability of the phenotype, there is greater power to detect a QTL accounting for 50% of the phenotype variance as compared with a QTL explaining only 5% of the overall variation of the phenotype. There are several experimental approaches that can be employed to identify genetic loci contributing to the risk for osteoporosis. One of the most commonly employed experimental designs is that of candidate gene analysis, which seeks to test the association between a particular genetic variant (*i.e.*, allele) and a specific phenotype. Many of these candidate gene studies use population-based association methods. As applied to the study of osteoporosis, two samples are collected: a group of osteoporotic patients and a control group of nonosteoporotic subjects. The allele frequencies at a polymorphism within or near the candidate gene are then compared in the two groups. Ideally, the two groups should be matched so that they differ only in their disease status. In theory, evidence of differences in allele frequencies within the two populations (association) may be the result of linkage disequilibrium with the candidate gene or possibly with another gene in close proximity; however, in practice, the candidate gene is thought likely to be causative for disease.

Because of its simplicity, the population-based association study of candidate genes has been widely used and perhaps abused. There are a number of major problems with this approach. The first is the choice of candidate gene. A large number and variety of proteins are involved in skeletal biology, and their genes are all potential candidates. Thus, the number of candidates is large (124) and multiplies daily with the application of new technologies to bone such as gene expression microarray analysis. Second, analysis of each candidate in isolation of the others is difficult to interpret statistically (10, 125, 126). Third, association studies often use simple polymorphisms in introns with doubtful biological effect. Fourth, studies in multiple populations are required. Fifth, there is no chance of finding genes outside those hypothesized. Sixth, spurious associations are common due to racial admixture and to the failure to use polymorphisms in genes known not to be involved in bone biology as a negative control (127, 128). Finally, disequilibrium appears to exist in blocks separated by regions of excess recombination (129–131), suggesting that complete characterization of the boundaries of linkage disequilibrium is essential for the accurate interpretation of results that could lead to erroneous interpretation of apparent linkage disequilibrium with candidate genes. About 20 candidate genes have been shown to be associated with BMD (Table 3). None, however, have been replicated over all populations. Unfortunately, meta-analysis (132) does not overcome this lack of consistency because it fails to take into account the many unpublished negative studies and also results in the problem of racial admixture. Another common disturbing feature of the candidate gene approach is that in studies that fail to detect the original association such as with BMD, other associations, some quite unrelated to BMD, emerge and when the polymorphism is examined in other disorders, new associations emerge. The vitamin D receptor (VDR), a candidate gene that has been extensively studied in relation to BMD, has been reported to be associated with phenotypes as diverse as body size (133), height (134), infant growth (135), age at menarche (136), muscle strength (137), calcium absorption (138), calcium intake (139), urinary calcium excretion (140), blood lead levels (141), blood pressure (142), primary hyperparathyroidism (143), type 1 diabetes mellitus (144), severity of hyperparathyroidism in renal failure (145), calcium rickets (146), calcified aortic valve stenosis (147), multiple sclerosis (148), thyrotoxicosis (149), intervertebral disc degeneration (150), osteoarthritis (151), biliary cirrhosis (152), breast cancer (153), rheumatoid arthritis (154), benign prostatic hyperplasia and prostatic cancer (155), and tuberculosis (156).

B. Candidate gene association studies with BMD

Over the last decade a large number of association studies have been performed with candidate genes (see Table 3). The first candidate gene was examined by its product, α_2HS-glycoprotein, a major protein in bone matrix. Since then, candidate gene products have ranged from structural proteins to regulatory proteins to factors apparently unrelated to bone (Table 3).

α_2HS-glycoprotein is present in bone matrix and was the first candidate gene shown to be associated with BMD (157, 158). It is preferentially concentrated in bone matrix (159) and functions as an immunoregulator (160).

The VDR is largely responsible for the broad range of actions of 1,25-$(OH)_2$ vitamin D including its effect on cal-

TABLE 3. Association studies with candidate genes and BMD

Candidate gene	Protein	Chromosome	References
AHSG	α_2HS-glycoprotein	3q27	157, 158
VDR	VDR	12q12–q14	76[a], 163, 164[a], 165
ESR1	ER 1 (α)	6q25.1	171, 172, 173[a], 174[a]
ESR2	ER 2 (β)	14q23	175
COL1A1	Collagen, type 1, α 1	17q21.3–q22.1	176, 177, 178[a], 179[a]
COL1A2	Collagen, type 1, α 2	7q22.1	179[a]
IL6	IL-6	7p21	185, 285, 286[a]
TGFB1	TGFβ	19q13.2	287, 288, 289
CALCR	Calcitonin receptor	7q21.3	290, 291
IGFI	IGF-1	12q22–q23	292, 293[a], 294[a]
BGLAP	Bone γ-carboxyglutamide protein (osteocalcin)	1q25–q31	295, 296
MTHFR	Methylenetetrahydrofolate reductase	1p36.3	297
IL1RN	IL-1β receptor antagonist	2q14.2	298, 299, 300[a]
TNFRGF5	TNF receptor superfamily/1β	1p36.3–p36.2	301
CASR	Calcium-sensing receptor	3q21–q24	302
CYP19	Aromatase (cytochrome P450)	15q21.1	303
P57, KIP2	Cyclin-dependent kinase inhibitor 1c	11p15.5	304
HLA DRB1	Major histocompatibility complex, class 11, DR β1	6p21.3	180
APOE	Apolipoprotein E	19q13.2	181, 182[a], 183[a]

[a] No association.

TABLE 4. Results of sibling pair linkage analysis of markers close or within candidate genes with BMD (184)

Candidate gene	Protein	Chromosome	Marker	LOD score	
				Hip BMD	Spine BMD
PTHR1	Parathyroid hormone receptor 1	3p22–p21.1	D3S3559	1.5	1.3
			D3S1289	2.7	0.3
EGF	Epidermal growth factor	4q25	D4S430	1.3	0.4
			D4S429	1.8	0.2
IL4	IL-4	5q31.3	D5S2057	1.1	0.0
			D5S2017	1.2	0.3
IL6	IL-6	7p21	D7S503	0.6	1.2
COL2A1/	Collagen, type II, α 1/VDR	12q13.11–q13.2	D12S1586	1.0	0.7
VDR		12q12–q14	D12S83	0.0	1.7
COL1A1	Collagen, type 1, α 1	17q21.3–q22.1	D17S807	1.7	0.5

cium transport and homeostasis and bone resorption (161). Mutations in the gene result in vitamin D-resistant rickets (162). Polymorphisms in the introns of VDR were initially said to account for about 80% of the variability in bone mass in twins (163). Subsequent studies, however, were unable to confirm linkage in twin samples (76, 164), and the original observation of linkage was retracted because of genotyping errors (165). Despite these negative linkage studies, VDR polymorphisms have been extensively studied in association studies. More than 50 association studies have been published, none of which show a consistent association with BMD and many showing associations with various nonskeletal phenotypes. In two large sib pair studies, no evidence of linkage with the VDR locus at 12q12–14 was found (87, 166).

The estrogen receptors (ERs) are responsible, in large part, for the broad range of actions of estrogenic steroids on target tissues including those in skeletal tissues. There are two ER genes, ER1 and ER2. ERα (167) and ERβ (168) have a wide tissue distribution. Estrogen is essential for closure of the bone epiphyses in adolescent girls (169) and for maintaining bone mass in women (170). In men, mutations in the ER result in prolonged skeletal growth and osteoporosis (7). Polymorphisms in the ER1 (171–174) have been most extensively studied, but more recently ER2 (175) has also been examined. There is no consistent association between ER1 or ER2 polymorphisms and phenotypes of bone mass across studies. In a large sib pair study, no evidence of linkage with the ER1 locus and ER2 locus was evident (87).

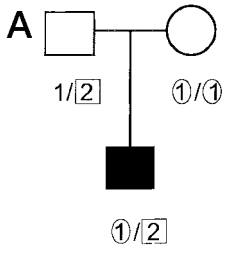
Collagen type 1 α I (COL1A1) along with collagen type 1 α 2 (COL1A2) make up bone collagen, which is the main structural protein in the skeleton. Mutations in COL1A1 and COL1A2 cause a dominant monogenetic osteoporotic disease, osteogenesis imperfecta (4). Polymorphisms outside the coding region but in an SP1 binding site in COL1A1 were reported to account for part of the variance in BMD in the normal population and to be associated with fragility fracture (176, 177). Subsequent studies have not replicated an association between COL1A1 polymorphisms with phenotypes of bone mass (178, 179). No association was found with polymorphisms in COL1A2 (179). In a large sib pair study, no evidence of linkage of BMD with the COL1A1 locus was evident (87).

At least another 16 candidate genes have been examined for association with BMD (Tables 3 and 4). They have been selected because their products are known either to be involved in some aspect of the metabolism or structure of bone. In addition, association studies have also been performed with bone phenotypes whose heritability has not been clearly established such as bone loss and vertebral fracture. "Noncandidate" genes have also been studied including HLA DRB1 (180) and apolipoprotein E (181–183) because of their known associations with other diseases.

Candidate genes may also be used to test whether they behave as QTL (Table 4). Multiple microsatellite polymorphisms located within or in close proximity to 23 candidate genes were examined for linkage in families identified with a member having a BMD T score less than 2.5 (184). Suggestive linkage was found for BMD at hip and spine with PTH receptor 1, and moderate evidence was found for linkage with seven of the other candidate genes (Table 4). Using a similar approach, BMD at the radius was shown to be linked with a microsatellite close to the IL-6 locus but not to microsatellites close either to the IL-6 receptor, calcium-sensing receptor, or the matrix gla protein loci (185). In the same population, a later study found that a microsatellite close to the TNFα gene also showed significant linkage with radial BMD (186). In neither of these latter two publications, however, was the designation of the markers provided. The use of microsatellite markers overcomes the problems of low numbers of alleles at the locus, and the simultaneous use of multiple candidate genes in one study increases the breadth of the study. However, the problem of selecting candidate genes remains. Candidate genes cannot be distinguished from genes in linkage disequilibrium, the expense of the linkage studies is high compared with that of association studies, and the need for multiple comparisons decreases power for detecting linkage.

C. Family-based association approach: the transmission disequilibrium test

To avoid the pitfalls of population-based association studies, a family-based association test, the transmission disequilibrium test or TDT (126, 187, 188) was developed. The primary advantage of the TDT is that it avoids the necessity of collecting a matched control sample. As originally proposed, the TDT analyzes a nuclear trio consisting of an affected individual and his/her parents (Fig. 5). These three individuals are genotyped at the polymorphism in or near the candidate gene. The alleles transmitted by the genotyped parents to the affected offspring are the "affected" sample, and the alleles not transmitted from these two parents are then used as "control" alleles. Through the use of such a within-family design, the control sample of alleles is perfectly matched to the affected sample of alleles, because they are transmitted from the same two parents. Thus, spurious association results due to population stratification are avoided. When the TDT is performed with one affected offspring from each family, it is a valid test of linkage and association (linkage disequilibrium), because affected individuals are unrelated and provide independent meiotic information toward the test of association. Application of the TDT with multiple affected siblings remains a test of linkage, but due to the lack of independent meiotic data from siblings, it is no longer a valid test of association (126).



B

	Not Transmitted	
Transmitted	Allele 1	Allele 2
Allele 1	-	10
Allele 2	90	-

FIG. 5. Schema of the TDT. A, TDT trio: The individual (II: 1) is affected with osteoporosis. His father (I: 1) is heterozygous at the marker and transmits allele 2 but not allele 1 to his affected son. This result provides the data for the TDT and would be tabulated in a table such as that shown in panel B. The mother (I: 2) is homozygous and can only transmit to her affected son allele 1 and, therefore, does not provide information toward the TDT. B, Hypothetic data from 100 trios. When considering informative transmissions from a parent heterozygous at a marker (genotype 1/2), allele 2 was preferentially transmitted to the affected offspring in 90 of 100 meioses. If no association exists, the expectation is that the two alleles will be equally transmitted. If association exists, there would be an excess of one of the two alleles transmitted to affected offspring.

Recently, a series of novel methodological extensions of the TDT have been proposed that allow data from affected and unaffected siblings to be used in family-based association tests (189–191). Results from the sibling-based tests can be combined with those from the traditional TDT to extract greater power to detect linkage disequilibrium. In general, for families of equal sibship size, the sib-TDT is less powerful than the conventional TDT, in part because unaffected siblings may inherit the disease susceptibility allele, but due to reduced penetrance or multilocus effects, may not have the disorder. Other recent modifications of the TDT have allowed the inclusion of data from extended pedigrees while still testing for linkage disequilibrium, even in the presence of population substructure (192).

Further extensions of the TDT methodology have been developed to enable the investigator to utilize family-based disequilibrium methods to analyze quantitative phenotypes (193). A series of quantitative TDT methods have been proposed depending on the type of ascertainment employed in the collection of the proband. Subsequently, additional modifications of the quantitative TDT have been developed providing greater flexibility if parental DNA is not available (194, 195) or if data are available from multiple generations (196).

The testing of candidate genes using the TDT or other association methodologies has not proven, to date, to provide consistent results across populations. However, as demonstrated in Crohn's disease (129), a multifactorial disorder, the application of the TDT approach in chromosomal regions previously identified to be in linkage to the disease phenotype can be a powerful means to narrow, and potentially identify, disease susceptibility loci.

D. Linkage approach

Few genes influencing complex traits have been identified by the study of candidate genes alone. Therefore, researchers in the field of osteoporosis have used other experimental designs to identify genes contributing to the risk of osteoporosis. To improve the likelihood that a gene influencing osteoporosis might be identified, investigators search the genome, testing polymorphic markers evenly spaced on all chromosomes. A strength of the genome-wide approach is that it may allow susceptibility genes to be identified that are not candidates based on the current understanding of the pathophysiology of osteoporosis.

Identification of the genes contributing to polygenic traits

can be extremely complex, even for a phenotype such as BMD with substantial heritability. Therefore, several types of genetic studies have been employed to dissect the genetic contribution to BMD. One technique has been the identification of families with extreme BMD phenotypes. The rationale for such studies is that genes with a substantial contribution to BMD are more likely to be segregating in families with extreme BMD measurements. This strategy has been employed to identify families with either abnormally high or low BMD. An advantage of this approach is that statistical tests of linkage can be employed that model the genetic contribution to BMD as a single gene effect. Such studies typically employ parametric linkage analyses [*i.e.*, computer package: FASTLINK (197); VITESEE (198)], the most powerful study design for identification of genetic loci contributing to extreme BMD phenotypes. Unfortunately, there are several limitations to this particular experimental design. First, and perhaps most importantly, the genes found to contribute to the extreme BMD phenotypes observed in these unusual pedigrees may not contribute substantially to the normal variation in BMD phenotype observed in the general population. A second limitation of the identification of extreme pedigrees is their rarity in the population, which makes the identification of such families very expensive. A third limitation is the likely faulty assumption that families with low bone mass, but within the normal range, are segregating as a single-gene disorder, whereas the phenotype is due to more than one gene as would occur in a multifactorial disorder.

An alternative to the identification of pedigrees with extreme phenotype is the ascertainment of families with members having BMD within the normal range. In such pedigrees, BMD is inherited in a complex, non-Mendelian fashion, with multiple genes and environmental factors contributing to the phenotype. As a result, a particular model for BMD inheritance may be difficult to specify. In addition, the time and effort required to correctly specify more complex penetrance-based linkage models may outweigh the slight advantage those approaches may have over some penetrance-free linkage approaches (199). Such model-free nonparametric linkage analyses typically involve studying a large number of related subjects thought to be segregating for genes that influence BMD.

All statistical tests of linkage using nonparametric methods are based on "identity by descent" (IBD) marker allele sharing (Fig. 6). Alleles are IBD if siblings inherit the same marker allele from the same parent. If the marker being tested is in close physical proximity to a gene influencing the phenotype, then siblings with similar phenotypic values would be expected to share marker alleles IBD. Conversely, siblings with dissimilar phenotypes would be expected to share fewer marker alleles IBD near the gene influencing the phenotype. An advantage of quantitative linkage methods as employed here is that no arbitrary cutoff for "high" or "low" phenotypic values is necessary; therefore, all sibling pairs are included in the analysis [*i.e.*, computer package: Mapmaker/Sibs (200)].

More recently, nonparametric linkage methods, which allow the inclusion of more extended pedigrees beyond simply sibling pairs in the genetic analysis, have been developed.

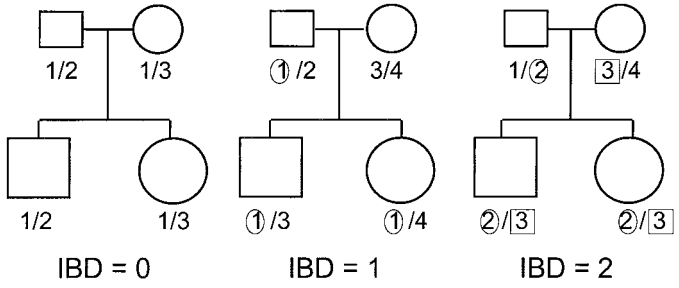


FIG. 6. Schema of IBD. In each nuclear family, the genotype for a marker with four alleles is shown. In the *left panel*, the two siblings have both inherited allele 1. However, the brother inherited this allele 1 from his mother while his sister inherited this allele 1 from her father. Therefore, they have no alleles IBD (IBD = 0). In the *middle panel*, both siblings inherited the 1 allele from the father (IBD = 1). In the *right panel*, both siblings inherited the 2 allele from their father and the 3 allele from their mother (IBD = 2).

These methods typically rely on variance component-based approaches [*i.e.*, computer package: SOLAR (201)]. An important advantage of these techniques is the ability to include data from large numbers of informative individuals within a pedigree and estimate the genetic contribution from a particular chromosomal region as well as the residual genetic variance.

Linkage analyses for complex diseases are commonly performed using affected sibling pairs or other types of affected relative pairs. In the case of osteoporosis, these might be relatives diagnosed with osteoporosis. Rather than employing a quantitative phenotype such as BMD, these studies classify individuals as "affected" or "unaffected." Tests of linkage are still based on the sharing of marker alleles identical by descent; however, in this type of analysis, because both relatives are affected (*i.e.*, not discordant), linkage tests only for increased sharing of marker alleles. The use of a dichotomous rather than quantitative phenotype is a less powerful approach for gene mapping.

E. Family studies

A family with high bone mass has been reported in which high BMD segregates as an autosomal dominant phenotype (202). The proband was identified on radiographs taken after a car accident. Affected individuals have spine BMD greater than 3 SD above the mean and are perfectly healthy (203) with no evidence of the sequelae of a sclerosing bone dysplasia (204). Using a genome screen approach, linkage to chromosome (Chr) 11q12-l3 was identified, and further fine mapping and sequencing identified the responsible gene as low-density lipoprotein receptor-related protein 5 (LRP5) (205) This is the same gene responsible for the Mendelian disorder autosomal recessive osteoporosis-pseudoglioma syndrome (9). The high-bone mass syndrome results from a mutation causing gain of function whereas the osteoporosis-pseudoglioma syndrome results from a mutation causing loss of function. There is a dosage effect for Lrp5 function because heterozygous carriers of osteoporosis-pseudoglioma syndrome have reduced bone mass (9). Lrp5 is involved in Wnt signal transduction (206, 207) and, as such, Lrp5 represents

a new regulatory pathway in osteoblast function and bone mass regulation.

Low-bone mass families ascertained through a proband with low BMD (Z score < -2.0 or radiographic evidence of osteoporotic fracture) consisted of seven families comprising 149 members who had parametric linkage analysis performed to evaluate three candidate genes: COL1A1, COL1A2, and the VDR. Linkage to all three candidate genes was excluded with LOD scores below -2.0 (208). Subsequently, parametric and nonparametric linkage analysis was performed in these seven families using data from a genome screen (209). The maximum parametric LOD score was obtained on Chr 11q. Nonparametric linkage analysis using 74 independent sibling pairs derived from these same families supported linkage to Chr lp36 (LOD = 2.29), Chr 2p23–24 (LOD = 2.25), and Chr 4q (LOD = 2.28).

An independent sample of eight families has been ascertained through a proband under the age of 35 yr with a history of two or more crush fractures and a spinal BMD at least 2.5 SD below the mean for age and sex, *i.e.*, severe early-onset osteoporosis (210). Segregation analyses performed in this sample suggest a major gene of codominant inheritance for spinal BMD. Linkage studies have not been reported in these families. Whether the gene in these rare families will be relevant to the common form of osteoporosis or BMD in the general population is uncertain. In contrast, segregation analyses performed in healthy nuclear families rejected the hypothesis of a single major gene and, instead, supported a polygenic model underlying BMD (86).

F. Genome scans in sibling pairs

BMD. A study in 374 healthy premenopausal white and black sister pairs reported linkage of femoral neck BMD to Chr 11q12–13 (211). This region harbors the LRP5 gene responsible for autosomal high bone mass trait (205) and the autosomal recessive osteoporosis-pseudoganglioma syndrome (9) and the TCIRG1 subunit of the vacuolar proton pump responsible for a subset of autosomal recessive osteopetrosis (212), suggesting that the same locus may also regulate BMD in the normal population. However, a subsequent analysis of this region in 464 white and 131 black sister pairs weakened the evidence of linkage (87), and analysis in a sample ascertained through a low bone mass proband did not support linkage to 11q12–13 (213).

A 10-cM autosomal genome screen identified six possible QTL (LOD >1.85) in 429 white premenopausal sister pairs (Table 5 and Ref. 87). The linkages on Chr 1, 5, 6, and 22 were at or near a marker locus and were reexamined in an expanded sample of 595 sister pairs. The initial genome screen in 429 sibling pairs had a LOD score of 3.11 at Chr 1q, which increased to 3.86 when the 595 sister pairs were included. This is not the same region of linkage reported on Chr 1 in a genome screen employing pedigrees ascertained on the basis of an osteoporotic proband (209). With the addition of sibling pairs, linkage to Chr 5p also increased from 1.9 to 2.2, linkage to Chr 6p was not substantially increased, and linkage to Chr 22 decreased. These results provide substantial evidence that genetic loci influencing BMD can be detected.

TABLE 5. Linkage of BMD using a genome screen in pairs of sisters (211)

Chromosome	Phenotype	Marker	LOD score
1q21–23	Lumbar spine	D1S484	3.11
5q33–35	Femoral neck	D5S422	1.87
6p11–12	Lumbar spine	D6S257	1.94
11q-12–13	Lumbar spine	D11S987	1.97
14q31–34	Femoral trochanter	D14S78	1.99
22q12–13	Lumbar spine	D22S423	2.13

The chromosome locations identified on the genome screen do not harbor any of the candidate genes itemized in Tables 3 and 4.

A genome wide screen for linkage to BMD in 153 Asian sib pairs who were originally identified as sibling pairs for extreme blood pressure values showed that proximal forearm BMD had a LOD score of 2.15 over a more than 50-cM large region on Chr 2 (214). This region includes a region previously identified in families ascertained through an individual with low BMD (209).

Structure. In the sister pair sample used to detect linkage of BMD to Chr 1, 5, 6, and 11 reported for linkage to BMD, seven QTL were found for various measures of structure at the proximal femur (Table 6 and Ref. 98). Two chromosomal regions were identified with significant LOD scores (>3.6) for at least one femoral structure phenotype. The maximum LOD score of 4.3 was obtained for femoral neck axis length on Chr 5q. Evidence of linkage to Chr 4q was found with both femoral neck axis length (LOD = 3.9) and midshaft width (LOD = 3.5). Significant linkage was found to Chr 17q with femoral head width (LOD = 3.6). Chromosome 3q showed linkage with pelvic axis length (LOD = 3.1), midshaft femoral width (LOD = 2.8), and femoral head width (LOD = 2.3). Chromosome 19p showed linkage with femoral neck axis length (LOD = 2.8) and femoral head width (LOD = 2.8).

V. Identification of the Susceptibility Genes

Genome wide linkage scans at about a 10-cM marker density have already provided evidence that there are several regions that harbor genes affecting both peak bone mass and femoral structure. As these studies expand and progress, they will confirm or refute the initial results, and they may also identify new regions for study. Once these data are firm, the next step is to "fine map" these regions. However, the regions are very large, encompassing 30–50 cM of genomic DNA and containing between 20–70 megabases of DNA, with several hundred genes. Furthermore, because the follow-up studies require substantial resources, the regions

TABLE 6. Linkage of bone structure using a genome screen in pairs of sisters (98)

Chromosome	Phenotype	Marker	LOD score
5q11–12	Femoral neck axis length	D5S647	4.3
4q11–12	Femoral neck axis length	D4S428	3.9
4q12–13	Femoral shaft width	D4S392	3.5
17q21–23	Femoral head width	D17S791	3.6
3q22–24	Pelvic axis length	D3S1569	3.1
3q11	Femoral shaft width	D3S1271	2.8
19p13	Femoral neck axis length	D19S226	2.8
19p13	Femoral head width	D19S226	2.8
9q22–23	Femoral neck width	D9S157	2.4
7q31–32	Femoral head width	D7S2502	2.3

must be prioritized for fine mapping. Criteria for prioritization include the strength of the initial linkage data, the consistency of linkage across populations, and studies in animal models that support linkage of the phenotypes in syntenic regions (Tables 7 and 8).

The goal of fine mapping is to limit the region containing the gene of interest to as small a region as possible. Unlike fine mapping for Mendelian disorders, fine mapping for complex traits is not recombination based. Thus, it is not possible to limit the region of interest to less than 1 or 2 megabases of genomic DNA before examining the region for candidate genes. Currently, data to guide the investigator as to how many polymorphic genetic markers should be used to fine map a complex trait locus are limited. The efficiency of a multistage approach was explored recently in a data set obtained from patients with multiple sclerosis (215). The results suggested that increasing the marker density to a 2.5–5 cM efficiently extracted additional IBD information. However, increasing the marker density to less than 2 cM between markers did not substantially improve the resolution of fine mapping, because of confounding effects of marker order and genotyping errors. Thus, in the absence of more comprehensive data, a multistage approach is reasonable. After the initial genome scan, generally at a 10-cM marker density, follow-up genotyping is performed at about 5-cM intervals using highly polymorphic microsatellite repeat markers. After analysis of the resulting data with some narrowing of the interval, further genotyping at 2-cM intervals over a somewhat smaller distance is performed. This approach requires that the markers are highly polymorphic. Genotyping with SNPs requires a higher density map because they are less polymorphic than microsatellite markers. Our simulation studies suggest that follow-up genotyping is more accurate if performed on a sample size that is larger than the sample used in the original genome screen. Once the candidate region is limited to the smallest amount of DNA possible, subsequent efforts are directed toward identifying candidate genes within the linkage interval. The first step in the process is to examine databases, such as OMIM (http://www3.ncbi.nlm.nih.gov:80/Omim) and Unigene (http://www.ncbi.nlm.nih.gov/UniGene/index.html), for known genes that may be excellent candidate genes. Although the number of known genes is rapidly expanding, investigators still have to identify unknown genes from raw genomic sequence to identify the susceptibility genes for osteoporosis. A rough draft sequence of the human genome is now available (12, 13), and a finished sequence will be available in the near future. However, having the complete sequence does not mean that all of the coding sequences have been identified. In fact, it will take much longer to identify all the genes, and much of this work will need to be done by individual investigators. Currently, there are several methods to identify novel genes in a candidate interval. These include using the expressed sequence tag databases; exon prediction programs such as GRAIL (http://compbio.ornl.gov/Grail-1.3/) and GENSCAN (http://CCR-081.mit.edu/GENSCAN.html); and sequence comparison programs such as PIPmaker (http://bio.cse.psu.edu), which identify exons by comparing sequence between two or more species. However, all of these informatic approaches require laboratory follow-up studies to fully assess

TABLE 7. QTL for BMD in mice from four different laboratories (223–226)

Chromosome	Marker	Map position	Method and skeletal site	Human syntenic region
1	Mit14	81.6	pQCT; femur, L5	1q21–q31 (228)
1	Mit15	87.9	pQCT; femur	1q21–q31 (224)
2	Mit456	86.3	pQCT; femur	20q11 (228)
2	Ncvs42	87.0	DXA; total body	20q11–q12 (223)
2	Mit464	9.5	DXA; spine	10p13–p11; 2q14; 9q34 (226)
3	Mit23	4.6	pQCT; femur	1q24–q32; 8q12–q22 (224)
4	Mit51	82.7	pQCT; femur, L5	1p36 (228)
4	Mit124	57.4	pQCT; femur, L5	13q14–q21 (228)
5	Mit112	42.0	pQCT; femur	4p14–p12; 4q11–q13 (224)
6	Mit150	51.0	pQCT; femur	3p26–p25; 3q21–q24; 19q13; 10q11 (228)
7	Mit332	65.6	pQCT; L5	10q25–q26 (228)
7	Mit234	44.0	DXA; total body	15q24–q26; 11q13–q21 (223)
7	Mit210	11.0	DXA; spine	19q12–q13 (226)
9	Mit196	48.0	pQCT; L5	6q12–q16; 15q24 (228)
11	Mit242	31.0	pQCT; femur	5q31–q32; 17p12–p11 (228)
11	Mit90	42.0	CTI; femur	17p-pter; 17q-qter (225)
11	Mit284	52.0	DXA; spine	17q21–q22 (226)
12	Mit215	2.0	pQCT; femur	2p25–p22 (228)
13	Mit266	16.0	pQCT; femur	6p25–p21 (228)
13	Mit135	10.0	CTI; femur	7p15–p13; 6p22; 9q22 (225)
13	Mit16	10.0	pQCT; femur	7p15–p13; 6p22; 9q22 (224)
13	Mit13	35.0	pQCT; femur, L5	5pq22–q35 (228)
13	Mit20	22.0	DXA; spine	6p24–22 (226)
14	Mit160	40.0	pQCT; femur, L5	13q14–q21 (228)
14	Ptprg	2.0	DXA; total body	3p14; 10q21–q24; 8p23 (223) 15
Mit29	pQCT femur	42.8		8q24; 22q12–q13 (224)
16	Mit12	27.6	pQCT; femur	3q13–q29 (228)
16	Mit39	29.1	DXA; spine	3q13–q29 (226)
18	Mit36	24.0	pQCT; femur, L5	5q21–q33 (228)

Map position given in centimorgans; human syntenic regions ± 3 cM of published best marker. CTI, Cortical thickness index. [Courtesy of Dr. Wesley Beamer.]

TABLE 8. Skeletal phenotypes in knockout and transgenic mice

Candidate gene	Protein	Human chromosome	Manipulation	Phenotype
Tnfrgf1 1b	Osteoprotegerin	8q24*[a]	Knockout	Osteoporosis (305)
Tgfb2	TGFβ2	1q41	Targeted overexpression	Osteoporosis (306)
K1	Klotho	13q12	Knockout	Osteoporosis (307)
Abl1	v-able Abelson murine leukemia viral oncogene homolog 1	9q34.1*	Knockout	Osteoporosis (308)
Col1a1	Collagen type 1, α1	17q21.3–q22.1*	Mutation	Osteopenia, fractures (309)
Col1a1	Collagen type 1, α1	17q21.3–q22.1*	Knockout (+/−)	Bone fragility (310)
Col1a1	Collagen type 1, α1	17q21.3–q22.1*	Knock-in mutation	Osteopenia, fractures (311)
Col1a2	Collagen type 1, α2	7q22.1	Mutation	Osteopenia, fractures (312)
Lrp5	Low density lipoprotein receptor-related protein 5	11q13.4	Knockout	Osteoporosis (313)
Tgfb1	TGFβ1	19q13.2*	Knockout	Osteopenia (314)
Nos3	Nitric oxide synthase 3	7q36	Knockout	Osteopenia (315)
Sparc	Osteonectin	5q31.3–q32*	Knockout	Osteopenia (316)
Bgn	Biglycan	Xq28	Knockout	Osteopenia (317)
Mmp 14	Matrix metalloproteinase	1414q11–q12	Knockout	Osteopenia (318)
Igf1	IGF	112q22–q23	Targeted overexpression	Increased bone mass (319)
Tgfbr2	TGFβ receptor II	3p22	Targeted truncation	Increased bone mass (320)
Vdr	Vitamin D receptor	12q12–q14	Targeted overexpression	Increased bone mass (321)
Lep/Lepr	Leptin/leptin receptor	7q31.3/1p31*	Knockout	Increased bone mass (322)
Gsn	Gelsolin	9q33	Knockout	Increased bone mass (323)
Fosl 1	FOS-like antigen 1	11q13*	Overexpression	Osteosclerosis (324)
Fosb	δFosB	19p13.2–p12	Overexpression	Osteosclerosis (325)
Traf6	TNF receptor-associated factor 6	11pter-p15.5	Knockout	Osteopetrosis (326, 327)
Ctsk	Cathepsin K	1q21*	Knockout	Osteopetrosis (328)
Itgb3	Integrin, β3	17q21.32*	Knockout	Osteopetrosis (329)
Fos	v-*fos* FBJ murine osteosarcoma viral oncogene homolog	14q24.3	Knockout	Osteopetrosis (330)
Tnfrgfl 1a	RANK	18q22.1	Knockout	Osteopetrosis (331)
TcirgI	T cell immune regulator 1	11q13.4–q13.5*	Knockout	Osteopetrosis (212, 332)
Nfkb1/2	Nuclear factor of κ light polypeptide gene enhancer in B cells 1/2	4q24/10q24*	Double knockout	Osteopetrosis (333)
Src	v-*src* avian sarcoma viral oncogene	20q12–q13*	Knockout	Osteopetrosis (334)
Csf1	Colony stimulating factor 1	1p21–p13	Mutation	Osteopetrosis (335)
Sfpi 1	Spleen focus forming viral (SFVN) proviral integration oncogene SPI-1	11p11.2	Knockout	Osteopetrosis (336)
Tnfrsfl 1b	Osteoprotegerin	8q24*	Overexpression	Osteopetrosis (337)

Mutation in CA2 (Carbonic anhydrase II) 8q22* in humans produces osteopetrosis (338), but no knockout has been produced in mice.
[a] *Asterisks* indicate genes in a region of linkage to BMD in mice.

the transcriptional content of the candidate region. Importantly, none of the computer programs are entirely sensitive for exon detection, and they can also falsely predict exons. Therefore, it is critical to combine informatic approaches with laboratory approaches to ensure that all exons for a new gene are identified and to ensure that predicted exons are true exons. Despite the continued need for follow-up laboratory experiments, these programs are already adequate to allow successful identification genes from the candidate regions and are extremely useful in positional cloning studies. It is anticipated that these programs will be substantially improved over time.

Normal genetic variation in complex traits, such as peak BMD, is generally not due to deleterious mutations but to common polymorphisms resulting in more subtle changes in gene function or expression. The large number of genes and the intensity with which each gene must be examined for sequence variation mandates that a logical strategy for ranking candidates is pursued rather than examining in sequence every gene that lies within the region of interest. However, there are pitfalls in ranking candidate genes. First, ranking genes is based largely on current models of the pathophysiology of osteoporosis, which are incomplete. Second, rankings are based on knowledge of the function of the genes, which is also incomplete. An example of the former is the PHEX gene, which is a member of the neutral endopeptidase family and is responsible for X-linked hypophosphatemic rickets (216). Before demonstrating that PHEX mutations were responsible for XLH, investigators had never considered that an enzyme defect could be responsible for the disease. Therefore, the goal of ranking genes should be to analyze genes in a systematic fashion from the most likely to least likely, rather than exclude genes based on current notions of pathophysiology. Indeed, one of the strengths of positional cloning studies is the potential to dramatically alter the field by identifying genes that were not previously known to be involved in the pathophysiology of osteoporosis. Thus, it is reasonable to initially study genes that are expressed in bone and genes that by virtue of homology have a high likelihood of being involved in the pathophysiology of osteoporosis. However, subsequent studies may need to examine candidate genes that are not obviously related to the pathophysiology of osteoporosis.

Once the candidate genes in a fine mapped region are identified and ranked, the next task is to identify polymorphisms in these genes. This can be done by searching the SNP databases, such as the NCBI SNP database (http://www.ncbi.nlm.nih.gov/SNP) and the HGBASE (Human

Genic Bi-Allelic Sequences, http://hgbase.interactiva.de/) for known polymorphisms, focusing on polymorphisms that are likely to have functional significance such as those that result in amino acid changes. Although these databases currently have limitations, they are expanding rapidly and are already very useful. Finally, once the polymorphisms are identified, DNA from the subjects can be genotyped using a variety of different methods and the results analyzed (217, 218).

VI. Animal Studies

In searching for the susceptibility genes for osteoporosis, complementary studies in animals are essential. Not only do they allow breeding strategies that cannot be performed in humans, but they also provide important bone strength phenotypes that cannot be measured *in vivo* in humans. Two animal models, the mouse (219) and the baboon (220), have been used for identifying genes underlying bone strength. More recently, the rat has been used (221).

The most intensively studied animal model is the mouse. It is ideally suited for genetic analysis because of its short generation time and its ability to produce large litters in the laboratory (222). Its contribution to the genetics of osteoporosis and skeletal biology is already substantial. A variety of inbred mouse strains have been used in genetic studies. A mouse strain is considered inbred when virtually every genetic locus in its genome is homozygous. Typically, this has been produced from 20 or more consecutive generations of brother-sister mating. As a result, all animals within the inbred mouse strain are genetically identical. This situation is analogous to twin studies in humans. Also, founder effects in genetically isolated populations can be amenable to similar approaches to those employed in mouse studies, again emphasizing the similarity between human and mouse genetics studies.

Many of the genetic mapping studies in mice designed to identify chromosomal regions contributing to osteoporosis or BMD were initially performed in recombinant inbred (RI) strains. RIs are created from an F_2 (second-generation offspring) sample by completing multiple generations of brother-sister mating. As a result, each RI strain is not only inbred but also unique in its genetic composition from each of the inbred founders. The power of the RI methodology to identify genes underlying phenotypic variability lies in the vast amount of genotyping already completed in the various RI lines. However, the limited number of available RI lines compromises the power of these lines to localize and identify genetic loci. As a result, whereas RI studies can detect regions of possible linkage, most researchers have pursued additional confirmation studies in backcross or F_2 progeny derived from inbred animal lines.

The most powerful strategy for mapping QTL involves the intercross of two strains discordant for the relevant phenotype of interest. Presumably, these mouse strains are discordant because they have fixed differing alleles at loci relevant to the phenotype. The discordant inbred progenitor strains are mated to produce F_1 hybrid mice. These mice are likely to be obligate heterozygotes at loci contributing to the phenotype. The F_1 mice are then intercrossed (brother-sister mated) to produce an F_2 population. In the F_2 population, the alleles at the loci contributing to the phenotype (the QTL) are segregating, meaning that each F_2 has different combinations of the alleles at the loci contributing to the phenotype. This can be observed in the wide variation in the phenotype which is observed in the F_2 sample with the extreme of the phenotype distribution often exceeding that observed in the progenitor lines. Therefore, the F_2 sample is considered to be segregating for the relevant QTL and is an ideal sample in which to perform QTL mapping. The intercross strategy, as well as the less powerful recombinant inbred strategy, has been used to create extensive data regarding the likely position of QTL contributing to BMD phenotypes (Table 7). Linkage analysis and subsequent fine mapping can provide corroborating information on important QTL syntenic with the human. The effects of individual gene products on skeletal biology can be evaluated using knockout and transgenic technology (Table 8). These techniques can also be used to identify candidate genes for human studies. A number of transgenic or knockout mice have clear skeletal phenotypes. This is an active area of research, and the list will undoubtedly grow in the future.

The first QTL for skeletal BMD in mice were reported (223) using the recombinant inbred approach for the BXD lines (C57BL/6J and DBA/2J cross). Subsequently, femoral BMD QTL were reported using the intercross approach (C57BL/6J and CAST/EiJ cross) (224), as were QTL for femoral cortical thickness index (225) and QTL for spinal BMD in senescence accelerated mice (SAM) using an intercross strategy (SAMP6 and SAMP2 cross) (226). Femoral BMD QTL for both the C57BL/6J and CAST/EiJ cross (225) and the C57BL/6J and C3H/HeJ cross have been reported (227). One QTL was found to be at the same location for both crosses (Chr 1, see Table 7). Interestingly, the two crosses produced several different QTL even though the BMD phenotype is identical. The major QTL for femoral density from the C57BL/6J and CAST/EiJ cross are on Chr 1, 3, 5, 13, and 15 and from the C57BL/6J and C3H/HeJ cross are on Chr 1, 2, 4, 6, 11, 12, 13, 14, 16, and 18. These differences may be due to each strain having fixed differing alleles at the relevant loci or the two progenitor strains in one cross fixing the same QTL allele, resulting in the F_2 sample not segregating for this QTL. This behavior emphasizes the importance of collecting data from several mouse crosses to assure that all QTL contributing to a phenotype are uncovered. As illustrated in Table 7, there are BMD QTL on mouse Chr 1, 2, 13, and 16 that have been uncovered in at least two different linkage studies.

Spinal BMD QTL for the cross of C57BL/6J and C3H/HeJ have also been mapped (228). Interestingly, not all spinal BMD QTL corresponded to femoral BMD QTL. Important spinal BMD QTL on Chr 7 and 9 have no femoral counterparts, suggesting that genetic regulation of BMD is, in part, dependent upon anatomical site. QTL for several vertebral microstructure phenotypes from the C57BL/6J and C3H/HeJ cross are at loci that do not correspond to femoral BMD QTL (227).

One of the clear advantages of using rodents for genetic studies is the availability of the bones for direct measurement of bone biomechanical properties including strength and

fragility. Fundamental biomechanical properties include force to failure (a measure of strength), stiffness, and work to failure (a measure of overall fragility). Biomechanical properties can be assessed at several sites including femoral midshaft, femoral neck, and vertebra (229). Preliminary studies of femoral strength in the C57BL/6J and C3H/HeJ cross have identified several important QTL, some of which overlap femoral BMD QTL (Chr 1, 4, 6, and 18) and others which do not (Chr 8, 11, and 13) (230). As with BMD, it is likely that genetic regulation of bone strength is site specific and that femoral and vertebral strength segregate somewhat independently (231).

BMD and skeletal biomechanical properties are polygenic traits. Consequently, there may be numerous interactions among genes contributing to these traits. To isolate gene effects, congenic strains in which a single QTL is moved from the donor strain to a recipient strain can be constructed. This is typically done with selective backcrossing for 6–10 generations. Congenic strains have been created by moving QTL for high bone mass donated by C3H/HeJ onto the low bone mass C57BL/6J line. C3H QTL caused significant differences in B6 BMD and femoral strength. QTL from mouse Chr 1, 4, and 18 increased BMD in recipient mice, whereas the donated QTL at Chr 6 reduced BMD (232, 233) indicating that genetic influences on bone structure can be isolated in congenic mice.

A second animal model that is available is the baboon (220). Colonies represent very large pedigrees that can be used for linkage analysis using many of the markers that are present in the human genome. Importantly, the size and shape of the baboon skeleton at the hip and vertebra approach those of the human much more closely than those of the mouse. Linkage studies performed in a large baboon colony have identified a QTL on baboon Chr 11 that influences BMD (234). Interestingly, this is the same region of Chr 11 identified in the three Mendelian bone-related disorders (9, 205, 212) as well as a large sample of premenopausal sister pairs (211).

More recently, the rat has been developed as a model for studying susceptibility genes of osteoporosis. The advantage of the rat is the extensive information on its physiology and skeletal biology that is available. Variability in femoral, vertebral, and femoral neck fragility among 11 inbred strains of rats has been reported (221). Fischer 344 and Lewis strains show the greatest variance in the vertebral fragility phenotype, and the Copenhagen 2331 and DA strains show the greatest variance in the femoral neck fragility phenotype, indicating that these strains will be useful for QTL analysis using the intercross strategy. As with mice, variation in skeletal fragility phenotypes in rats is dependent upon the anatomical site studied. Therefore, it is likely that rats will be useful for uncovering site-specific genetic influences on skeletal fragility.

Using these three animal models is likely to provide complementary information to the human. The plans to fully sequence the mouse and the rat genome within the next 3 yr will be a major factor in the rate at which the susceptibility genes for osteoporosis can be identified. Emerging evidence of site specificity of skeletal phenotypes and the findings in mice that bone biomechanical phenotypes do not always correspond to BMD highlight the importance of measuring multiple phenotypes relating to BMD, geometry, structure, and biomechanical properties at multiple skeletal sites where osteoporotic fracture is common, such as the proximal femur and the vertebra.

VII. Bioethics

As for all human genetic studies (16), the endeavor to identify the genes causing monogenetic forms of osteoporosis and the susceptibility genes underlying the common form of osteoporosis raises important legal and ethical issues. Key resources in this endeavor are the development of large repositories of human tissue, serum, and DNA and extensive data files containing essential phenotypic variables from healthy subjects and patients with osteoporotic diseases. In the United States, these resources are being developed in various centers depending mainly on the location of the researchers. However, very large national repositories are being developed in a number of countries, notably Iceland and UK (www.publications.parliament.UK). In order for researchers to access such resources, guidelines and policies need to be in place both to protect patient privacy and to ensure that the essentials of patient informed consent are maintained. In 1999, the National Bioethics Advisory Commission made recommendations on these issues to the US President on research involving human biological materials (www.bioethics.gov). However, this is an evolving area (235), and the policies will undoubtedly undergo modification in the future.

An equally important issue is the question of making available genetic testing (236, 237) for osteoporosis. Genetic testing is currently available for numerous single-gene disorders. Genetic testing is also being performed for Alzheimer's disease and breast cancer, both of which are disorders with complex genetic inheritance. In both instances, however, individuals appropriate for genetic counseling are typically those in whose families a mutation in a single gene has resulted in a disorder with autosomal dominant inheritance. The susceptibility genes for the common form of osteoporosis do not appear to include a single gene with major effect. Furthermore, it appears that the susceptibility genes interact with important environmental factors. Thus, genetic counseling will consist not only of genetic data but also of environmental information that modulate an individual's risk for reduced bone mass, reduced bone strength, and the risk of sustaining an osteoporotic fracture.

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References

1. **Horsman A, Marshall DH, Peacock M** 1985 A stochastic model of age-related bone loss and fractures. Clin Orthop 195:207–215
2. **Bertelloni S, Cinquanta L, Baroncelli GI, Simi P, Rossi S, Saggese G** 2000 Volumetric bone mineral density in young women with Turner's syndrome treated with estrogens or estrogens plus growth hormone. Horm Res 53:72–76
3. **Horowitz M, Wishart JM, O'Loughlin PD, Morris HA, Need AG, Nordin BEC** 1992 Osteoporosis and Klinefelter's syndrome. Clin Endocrinol (Oxf) 36:113–118
4. **Prockop DJ, Colige A, Helminen H, Khillan JS, Pereira R, Vandenberg P** 1993 Mutations in type 1 procollagen that cause osteogenesis imperfecta: effects of the mutations on the assembly of collagen into fibrils, the basis of phenotypic variations, and potential antisense therapies. J Bone Miner Res 8(Suppl 2):489–492
5. **Lubec B, Fang-Kircher S, Lubec T, Blom HJ, Boers GHJ** 1996 Evidence for McKusick's hypothesis of deficient collagen cross-linking in patients with homocystinuria. Biochim Biophys Acta Mol Basis Dis 1315:159–162
6. **Goto M** 1997 Hierarchial deterioration of body systems in Werner's syndrome. Mech Ageing Dev 98:239
7. **Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, Williams TC, Lubahn DB, Korach KS** 1994 Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. N Engl J Med 331:1056–1061
8. **Carani C, Qin K, Simoni M, Faustini-Fustini M, Serpente S, Boyd J, Korach KS, Simpson ER** 1997 Effect of testosterone and estradiol in a man with aromatase deficiency. N Engl J Med 337:91–95
9. **Gong Y, Slee RB, Fukai N, Rawadi G, Roman-Roman S, Reginato AM, Wang H, Cundy T, Glorieux FH, Lev D, Zacharin M, Oexle K, Marcelino J, Suwairi W, Heeger S, Sabatakos G, Apte S, Adkins WN, Allgrove J, Arslan-Kirchner M, Batch BA, Beighton P, Black GC, Boles RG, Boon LM, Borrone C, Brunner HG, Carle GF, Dallapiccola B, De Paepe A, Floege B, Halfhide ML, Hall B, Hennekam RC, Hirose T, Jans A, Juppner H, Kim CE, Keppler-Noreuil K, Kohlschuetter A, Lacombe D, Lambert M, Lemyre E, Letteboer T, Peltonen L, Ramesar R, Romanengo M, Somer H, Steichen-Gerdsorf E, Steinmann B, Sullivan B, Superti-Furga A, Swoboda W, Boogaard M, Van Hul W, Vikkula M, Votruba M, Zabel B, Garcia T, Baron R, Olsen BJ, Warman ML** 2001 LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. Cell 107:513–523
10. **Lander ES, Schork NJ** 1994 Genetic dissection of complex traits. Science 265:2037–2048
11. **Weeks DE, Lathrop GM** 1995 Polygenic disease: methods for mapping complex disease traits. Trends Genet 11:513–519
12. **International Human Genome Sequencing Consortium** 2001 Initial sequencing and analysis of the human genome. Nature 409: 860–921
13. **Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, Gocayne JD, Amanatides P, Ballew RM, Huson DH, Wortman JR, Zhang Q, Kodira CD, Zheng XH, Chen L, Skupski M, Subramanian G, Thomas PD, Zhang J, Gabor Miklos GL, Nelson C, Broder S, Clark AG, Nadeau J, McKusick VA, Zinder N, Levine AJ, Roberts RJ, Simon M, Slayman C, Hunkapiller M, Bolanos R, Delcher A, Dew I, Fasulo D, Flanigan M, Florea L, Halpern A, Hannenhalli S, Kravitz S, Levy S, Mobarry C, Reinert K, Remington K, Abu-Threideh J, Beasley E, Biddick K, Bonazzi V, Brandon R, Cargill M, Chandramouliswaran I, Charlab R, Chaturvedi K, Deng Z, Di Francesco V, Dunn P, Eilbeck K, Evangelista C, Gabrielian AE, Gan W, Ge W, Gong F, Gu Z, Guan P, Heiman TJ, Higgins ME, Ji RR, Ke Z, Ketchum KA, Lai Z, Lei Y, Li Z, Li J, Liang Y, Lin X, Lu F, Merkulov GV, Milshina N, Moore HM, Naik AK, Narayan VA, Neelam B, Nusskern D, Rusch DB, Salzberg S, Shao W, Shue B, Sun J, Wang ZY, Wang A, Wang X, Wang J, Wei MH, Wides R, Xiao C, Yan C, Yao A, Ye J, Zhan M, Zhang W, Zhang H, Zhao Q, Zheng L, Zhong F, Zhong W, Zhu SC, Zhao S, Gilbert D, Baumhueter S, Spier G, Carter C, Cravchik A, Woodage T, Ali F, An H, Awe A, Baldwin D, Baden H, Barnstead M, Barrow I, Beeson K, Busam D, Carver A, Center A, Cheng ML, Curry L, Danaher S, Davenport L, Desilets R, Dietz S, Dodson K, Doup L, Ferriera S, Garg N, Gluecksmann A, Hart B, Haynes J, Haynes C, Heiner C, Hladun S, Hostin D, Houck J, Howland T, Ibegwam C, Johnson J, Kalush F, Kline L, Koduru S, Love A, Mann F, May D, McCawley S, McIntosh T, McMullen I, Moy M, Moy L, Murphy B, Nelson K, Pfannkoch C, Pratts E, Puri V, Qureshi H, Reardon M, Rodriguez R, Rogers YH, Romblad D, Ruhfel B, Scott R, Sitter C, Smallwood M, Stewart E, Strong R, Suh E, Thomas R, Tint NN, Tse S, Vech C, Wang G, Wetter J, Williams S, Williams M, Windsor S, Winn-Deen E, Wolfe K, Zaveri J, Zaveri K, Abril JF, Guigo R, Campbell MJ, Sjolander KV, Karlak B, Kejariwal A, Mi H, Lazareva B, Hatton T, Narechania A, Diemer K, Muruganujan A, Guo N, Sato S, Bafna V, Istrail S, Lippert R, Schwartz R, Walenz B, Yooseph S, Allen D, Basu A, Baxendale J, Blick L, Caminha M, Carnes-Stine J, Caulk P, Chiang YH, Coyne M, Dahlke C, Mays AD, Dombroski M, Donnelly M, Ely D, Esparham S, Fosler C, Gire H, Glanowski S, Glasser K, Glodek A, Gorokhov M, Graham K, Gropman B, Harris M, Heil J, Henderson S, Hoover J, Jennings D, Jordan C, Jordan J, Kasha J, Kagan L, Kraft C, Levitsky A, Lewis M, Liu X, Lopez J, Ma D, Majoros W, McDaniel J, Murphy S, Newman M, Nguyen T, Nguyen N, Nodell M** 2001 The sequence of the human genome. Science 291:1304–1351
14. **The International SNP Map Working Group** 2001 A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. Nature 409:928–933
15. **DeBry RW, Seldin MF** 1996 Human/mouse homology relationships. Genomics 33:337–351
16. **Meslin EM, Thomson EJ, Boyer JT** 1997 The ethical, legal and social implications research program at the National Human Genome Research Institute. Kennedy Inst Ethics J 7:291
17. **Looker AC, Orwoll ES, Johnston Jr CC, Lindsay RL, Wahner HW, Dunn WL, Calvo MS, Harris TB, Heyse SP** 1997 Prevalence of low femoral bone density in older US adults from NHANES III. J Bone Miner Res 12:1761–1768
18. **Riis BJ, Hansen MA, Jensen AM, Overgaard K, Christiansen C** 1996 Low bone mass and fast rate of bone loss at menopause: equal risk factors for future fracture: a 15-year follow-up study. Bone 19:9–12
19. **Newton-John H, Morgan B** 1970 The loss of bone with age: osteoporosis and fractures. Clin Orthop 71:229–252
20. **Peacock M, Liu G, Carey M, Ambrosius W, Turner CH, Hui SL, Johnston Jr CC** 1998 Bone mass and structure at the hip in men and women over the age of 60 years. Osteoporos Int 8:231–239
21. **Lu Y, Genant HK, Shepherd J, Zhao SJ, Mathur A, Fuerst TP, Cummings SR** 2001 Classification of osteoporosis based on bone mineral densities. J Bone Miner Res 16:901–910
22. **Atkinson PJ** 1967 Variation in trabecular structure of vertebrae with age. Calcif Tissue Res 1:24–32
23. **Aaron JE, Makins NB, Sagreiya K** 1987 The microanatomy of trabecular bone loss in normal aging men and women. Clin Orthop 215:260–271
24. **Garn SM, Wagner B, Rohmann CG, Ascoli W** 1968 Further evidence for continuing bone expansion. Am J Phys Anthropol 28: 219–221
25. **Cauley JA, Gutai JP, Kuller LH, Scott J, Nevitt MC** 1994 Black-white differences in serum sex hormones and bone mineral density. Am J Epidemiol 139:1035–1046
26. **Kleerekoper M, Nelson DA, Flynn MJ, Pawluszka AS, Jacobsen G, Peterson EL** 1994 Comparison of radiographic absorptiometry with dual-energy x-ray absorptiometry and quantitative computed tomography in normal older white and black women. J Bone Miner Res 9:1745–1749
27. **Tobias JH, Cook DG, Chambers TJ, Dalzell N** 1994 A comparison of bone mineral density between Caucasian, Asian and Afro-Caribbean women. Clin Sci 87:587–591
28. **Ross PD, He YF, Yates AJ, Coupland C, Ravn P, McClung MR, Thompson D, Wasnich RD** 1996 Body size accounts for most differences in bone density between Asian and Caucasian women. Calcif Tissue Int 59:339–343
29. **Albright F** 1947 Osteoporosis. Ann Intern Med 27:861–882
30. **Nordin BEC** 1987 The definition and diagnosis of osteoporosis. Calcif Tissue Int 40:57–58
31. **NIH** 1993 NIH Consensus Development Conference: diagnosis, prophylaxis and treatment of osteoporosis. Am J Med 94:646–650

32. **Atkinson PJ** 1965 Changes in resorption spaces in femoral cortical bone with age. J Pathol Bacteriol 89:173–178
33. **Bousson V, Meunier A, Bergot C, Vicaut E, Rocha MA, Morais MH, Laval-Jeantet A-M, Laredo JD** 2001 Distribution of intracortical porosity in human midfemoral cortex by age and gender. J Bone Miner Res 16:1308–1317
34. **Aaron JE, Shore PA, Shore RC, Beneton M, Kanis JA** 2000 Trabecular architecture in women and men of similar bone mass with and without vertebral fracture. II. Three-dimensional histology. Bone 27:277–282
35. **Dempster DM** 2000 The contribution of trabecular architecture to cancellous bone quality. J Bone Miner Res 15:20–23
36. **Gluer CC, Cummings SR, Pressman A, Li J, Gluer K, Faulkner KG, Grampp S, Genant HK** 1994 Prediction of hip fractures from pelvic radiographs: the study of osteoporotic fractures. J Bone Miner Res 9:671–677
37. **Peacock M, Turner CH, Liu G, Manatunga AK, Timmerman L, Johnston Jr CC** 1995 Better discrimination of hip fracture using bone density, geometry and architecture. Osteoporos Int 5:167–173
38. **Faulkner KG, Cummings SR, Nevitt MC, Pressman A, Jergas M, Genant HK** 1995 Hip axis length and osteoporotic fractures. J Bone Miner Res 10:506–508 (letter)
39. **Karlsson KM, Sernbo I, Obrant KJ, Redlund-Johnell I, Johnell O** 1996 Femoral neck geometry and radiographic signs of osteoporosis as predictors of hip fracture. Bone 18:327–330
40. **Rodino MA, Shane E** 1998 Osteoporosis after organ transplantation. Am J Med 104:459–469
41. **Van Staa TP, Leufkens HGM, Abenhaim L, Zhang B, Cooper C** 2000 Use of oral corticosteroids and risk of fractures. J Bone Miner Res 15:993–1000
42. **Schwartz AV, Sellmeyer DE, Ensrud KE, Cauley JA, Tabor HK, Schreiner PJ, Jamal SA, Black DM, Cummings SR** 2001 Older women with diabetes have an increased risk of fracture: a prospective study. J Clin Endocrinol Metab 86:32–38
43. **Riggs BL, Hodson SF, O'Fallon WM, Chao EY, Wahner HW, Muhs JM, Cedel SL, Melton III LJ** 1990 Effect of fluoride treatment on the fracture rate in postmenopausal women with osteoporosis. N Engl J Med 322:802–809
44. **Bollerslev J** 1989 Autosomal dominant osteopetrosis: bone metabolism and epidemiological, clinical and hormonal aspects. Endocr Rev 10:45–67
45. **Genant HK, Lang TF, Engelke K, Fuerst T, Glüer CC, Majumdar S, Jergas M** 1996 Advances in the noninvasive assessment of bone density, quality, and structure. Calcif Tissue Int 59(Suppl 1): S10–S15
46. **Hangartner TN, Gilsanz V** 1996 Evaluation of cortical bone by computed tomography. J Bone Miner Res 11:1518–1525
47. **World Health Organization (WHO)** 1994 Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: report of a WHO study group. WHO Technical Report Series. Geneva, Switzerland: World Health Organization; report 843
48. **Faulkner KG, Stetten EV, Miller P** 1999 Discordance in patient classification using T-scores. J Clin Densitomet 2:343–350
49. **Orwoll E** 2000 Assessing bone density in men. J Bone Miner Res 15:1867–1870
50. **Marshall D, Johnell O, Wedel H** 1996 Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures. Br Med J 312:1254–1259
51. **Melton III LJ, Atkinson EJ, O'Fallon WM, Wahner HW, Riggs BL** 1993 Long-term fracture prediction by bone mineral assessed at different skeletal sites. J Bone Miner Res 8:1227–1233
52. **Bolotin HH, Sievänen H** 2001 Inaccuracies inherent in dual-energy X-ray absorptiometry *in vivo* bone mineral density can seriously mislead diagnostic/prognostic interpretations of patient-specific bone fragility. J Bone Miner Res 16:799–805
53. **Genant HK, Gordon C, Jiang YB, Link TM, Hans D, Majumdar S, Lang TF** 2000 Advanced imaging of the macrostructure and microstructure of bone. Horm Res 54(Suppl):24–30
54. **Genant HK, Jiao L, Chun JW, Shepard JA** 2000 Vertebral fractures in osteoporosis. J Clin Densitomet 3:281–290
55. **Martin RB, Burr DB** 1984 Non-invasive measurement of long bone cross-sectional moment of inertia by photon absorptiometry. J Biomechanics 17:195–201
56. **Beck TJ, Looker AC, Ruff CB, Sievanen H, Wahner HW** 2000 Structural trends in the aging femoral neck and proximal shaft: analysis of the Third National Health and Nutrition Examination Survey dual-energy X-ray absorptiometry data. J Bone Miner Res 15:2297–2304
57. **Turner CH, Peacock M, Timmerman L, Neal JM, Johnston Jr CC** 1995 Calcaneal ultrasonic measurements discriminate hip fracture independently of bone mass. Osteoporos Int 5:130–135
58. **Bauer DC, Gluer CC, Cauley JA, Vogt TM, Ensrud KE, Genant HK, Black DM** 1997 Broadband ultrasound attenuation predicts fractures strongly and independently of densitometry in older women: a prospective study. Arch Intern Med 157:629–634
59. **Hong J, Hipp JA, Mulkern RV, Jaramillo D, Snyder BD** 2000 Magnetic resonance imaging measurements of bone density and cross-sectional geometry. Calcif Tissue Int 66:74–78
60. **Garnero P, Sornay-Rendu E, Claustrat B, Delmas PD** 2000 Biochemical markers of bone turnover, endogenous hormones and the risk of fractures in postmenopausal women: the OFELY study. J Bone Miner Res 15:1526–1536
61. **Alffram P** 1964 An epidemiological study of cervical and trochanteric fractures of the femur in an urban population. Acta Orthop Scand Suppl 65:1–109
62. **Grisso JA, Kelsey JL, Strom BL, Chiu GY, Maislin G, O'Brien LA, Hoffman S, Kaplan F, Northeast Hip Fracture Study Group** 1991 Risk factors for falls as a cause of hip fracture in women. N Engl J Med 324:1326–1331
63. **Cumming RG, Klineberg RJ** 1994 Fall frequency and characteristics and the risk of hip fractures. J Am Geriatr Soc 42:774–778
64. **Hayes WC, Myers ER, Morris JN, Gerhart TN, Yett HS, Lipsitz LA** 1993 Impact near the hip dominates fracture risk in elderly nursing home residents who fall. Calcif Tissue Int 52:192–198
65. **Nordin BEC, Peacock M, Aaron J, Crilly RG, Heyburn PJ, Horsman A, Marshall DH** 1980 Osteoporosis and osteomalacia. Clin Endocrinol Metab 9:177–205
66. **Slemenda CW, Turner CH, Peacock M, Christian JC, Sorbel J, Hui SL, Johnston CC** 1996 The genetics of proximal femur geometry, distribution of bone mass and bone mineral density. Osteoporos Int 6:178–182
67. **Frost HM** 1992 The role of changes in mechanical usage set points in the pathogenesis of osteoporosis. J Bone Miner Res 7:253–261
68. **Melton LJ, III** 2090 1997 Epidemiology of spinal osteoporosis. Spine 22(Suppl):2S–11S
69. **Kannus P, Parkkari J, Sievänen H, Heinonen A, Vuori I, Järvinen M** 1996 Epidemiology of hip fractures. Bone 18(Suppl):57S–63S
70. **Silverman SL, Madison RE** 1988 Decreased incidence of hip fracture in Hispanics, Asians, and Blacks: California hospital discharge data. Am J Public Health 78:1482–1483
71. **Kellie SE, Brody JA** 1990 Sex-specific and race-specific hip fracture rates. Am J Public Health 80:326–328
72. **Tinetti ME, Speechley M, Ginter SF** 1988 Risk factors for falls among elderly persons living in the community. N Engl J Med 319:1701–1707
73. **Snedecor GW, Cochran WG** 1967 Statistical methods. Ames, IA: The Iowa State University Press
74. **Pocock NA, Eisman JA, Hopper JL, Yeates MG, Sambrook PN, Ebert S** 1987 Genetic determinants of bone mass in adults: a twin study. J Clin Invest 80:706–710
75. **Slemenda CW, Christian JC, Williams CJ, Norton JA, Johnston Jr CC** 1991 Genetic determinants of bone mass in adult women: a reevaluation of the twin model and the potential importance of gene interaction on heritability estimates. J Bone Miner Res 6: 561–567
76. **Hustmyer FG, Peacock M, Hui S, Johnston CC, Christian J** 1994 Bone mineral density in relation to polymorphism at the vitamin D receptor gene locus. J Clin Invest 94:2130–2134
77. **Koller DL, Peacock M, Conneally PM, Liu G, Hui SL, McClintock R, Christian JC, Johnston CC, Econs MJ, Foroud T** 1998 Heritability of bone-related phenotypes in Caucasian and African-American sister pairs. Am J Hum Genet 63:A214 (Abstract)
78. **Smith DM, Nance WE, Kang KW, Christian JC, Johnston Jr CC** 1973 Genetic factors in determining bone mass. J Clin Invest 52: 2800–2808
79. **Moller M, Horsman A, Harvald B, Hauge M, Henningsen K,**

Nordin BEC 1978 Metacarpal morphometry in monozygotic and dizygotic elderly twins. Calcif Tiss Res 25:197–201
80. **Dequeker J, Nijs N, Verstraeten A, Geusens P, Gevers G** 1987 Genetic determinants of bone mineral content at the spine and radius: a twin study. Bone 8:207–209
81. **Sowers MR, Burns TL, Wallace RB** 1986 Familial resemblance of bone mass in adult women. Genet Epidemiol 3(Suppl):85–93
82. **Lutz J** 1986 Bone mineral, serum calcium, and dietary intakes of mother/daughter pairs. Am J Clin Nutr 44:99–106
83. **Tylavsky FA, Bortz AD, Hancock RL, Anderson JJ** 1989 Familial resemblance of radial bone mass between premenopausal mothers and their college-age daughters. Calcif Tissue Int 45:265–272
84. **Lutz J, Tesar R** 1990 Mother-daughter pairs: spinal and femoral bone densities and dietary intakes. Am J Clin Nutr 52:872–877
85. **Sowers MR, Boehnke M, Jannausch ML, Crutchfield M, Corton G, Burns TL** 1992 Familiality and partitioning the variability of femoral bone mineral density in women of child-bearing age. Calcif Tissue Int 50:110–114
86. **Gueguen R, Jouanny P, Guillemin F, Kuntz C, Pourel J, Siest G** 1995 Segregation analysis and variance components analysis of bone mineral density in healthy families. J Bone Miner Res 10: 2017–2022
87. **Koller DL, Econs MJ, Morin PA, Christian JC, Hui SL, Parry P, Curran ME, Rodriguez LA, Conneally PM, Joslyn G, Peacock M, Johnston CC, Foroud T** 2000 Genome screen for QTLs contributing to normal variation in bone mineral density and osteoporosis. J Clin Endocrinol Metab 85:3116–3120
88. **Seeman E, Hopper JL, Back LA, Cooper ME, Parkinson E, McKay J, Jermus G** 1989 Reduced bone mass in daughters of women with osteoporosis. N Engl J Med 320:554–558
89. **Seeman E, Tsalamandris C, Formica C, Hopper JL, McKay J** 1994 Reduced femoral neck bone density in the daughters of women with hip fractures: the role of low peak bone density in the pathogenesis of osteoporosis. J Bone Miner Res 9:739–743
90. **Danielson ME, Cauley JA, Baker CE, Newman AB, Dorman JS, Towers JD, Kuller LH** 1999 Familial resemblance of bone mineral density (BMD) and calcaneal ultrasound attenuation: the BMD in mothers and daughters study. J Bone Miner Res 14:102–110
91. **Lonzer MD, Imrie R, Rogers D, Worley D, Licata A, Secic M** 1996 Effects of heredity, age, weight, puberty, activity, and calcium intake on bone mineral density in children. Clin Pediatr (Phila) 35:185–189
92. **Ferrari S, Rizzoli R, Slosman D, Bonjour JP** 1998 Familial resemblance for bone mineral mass is expressed before puberty. J Clin Endocrinol Metab 83:358–361
93. **Orwoll ES, Belknap JK, Klein RF** 2001 Gender specificity in the genetic determinants of peak bone mass. J Bone Miner Res 16: 1962–1971
94. **Holzinger KJ** 1929 The relative effect of nature and nurture influences on twin differences. J Educ Psychol 20:241–248
95. **Clark PJ** 1956 The heritability of certain anthropometric characters as ascertained from measurements of twins. Am J Hum Genet 8:49–54
96. **Feinleib M, Garrison RJ, Fabsitz R, Christian JC, Hrubec Z, Borhani NO, Kannel WB, Rosenman R, Schwartz JT, Wagner JO** 1977 The NHLBI Twin Study of Cardiovascular Disease Risk Factors: methodology and summary of results. Am J Epidemiol 106: 284–295
97. **Arden NK, Baker J, Hogg C, Baan K, Spetor TD** 1996 The heritability of bone mineral density, ultrasound of the calcaneus and hip axis length: a study of postmenopausal twins. J Bone Miner Res 11:530–534
98. **Koller DL, Liu GD, Econs MJ, Hui SL, Morin PA, Joslyn G, Rodriguez LA, Conneally PM, Christian JC, Johnston Jr CC, Foroud T, Peacock M** 2001 Genome screen for quantitative trait loci underlying normal variation in femoral structure. J Bone Miner Res 16:985–991
99. **Koller DL, Liu G, Econs MJ, Hui SL, Conneally PM, Johnston Jr CC, Foroud T, Peacock M** 2001 Genome screen for QTLs underlying normal variation in vertebral structure. J Bone Miner Res 16(Suppl):S155
100. **Christian JC, Yu P-L, Slemenda CW, Johnston Jr CC** 1989 Heritability of bone mass: a longitudinal study in aging male twins. Am J Hum Genet 44:429–433
101. **Kelly PJ, Nguyen T, Hopper J, Pocock N, Sambrook P, Eisman J** 1993 Changes in axial bone density with age: a twin study. J Bone Miner Res 8:11–17
102. **Delmas PD** 1993 Biochemical markers of bone turnover. J Bone Miner Res 8(Suppl 2):S549–S555
103. **Weaver CM, Peacock M, Martin BR, McCabe GP, Zhao J, Smith DL, Wastney ME** 1997 Quantification of biochemical markers of bone turnover by kinetic measures of bone formation and resorption in young healthy females. J Bone Miner Res 12:1714–1720
104. **Kleerekoper M, Nelson DA, Peterson EL, Flynn MJ, Pawluszka AS, Jacobsen G, Wilson P** 1994 Reference data for bone mass, calciotropic hormones, and biochemical markers of bone remodeling in older (55–75) postmenopausal white and black women. J Bone Miner Res 9:1267–1276
105. **Greenspan SL, Parker RA, Ferguson L, Rosen HN, Maitland-Ramsey L, Karpf DB** 1998 Early changes in biochemical markers of bone turnover predict the long-term response to alendronate therapy in representative elderly women: a randomized clinical trial. J Bone Miner Res 13:1431–1438
106. **Bauer DC, Sklarin P, Stone KL, Black DM, Nevitt MC, Ensrud KE, Arnaud CD, Genant HK, Garnero P, Delmas PD, Lawaetz H, Cummings SR** 1999 Biochemical markers of bone turnover and prediction of hip bone loss in older women: the study of osteoporotic fractures. J Bone Miner Res 14:1404–1410
107. **Ross PD, Knowlton W** 1998 Rapid bone loss is associated with increased levels of biochemical markers. J Bone Miner Res 13: 297–302
108. **Kelly PJ, Hopper JL, Macaskill GT, Pocock NA, Sambrook PN, Eisman JA** 1991 Genetic factors in bone turnover. J Clin Endocrinol Metab 72:808–813
109. **Livshits G, Yakovenko C, Kobyliansky E** 2000 Quantitative genetic analysis of circulating levels of biochemical markers of bone formation. Am J Med Genet 94:324–331
110. **Cummings SR, Nevitt MC, Browner WS, Stone K, Fox KM, Ensrud KE, Cauley J, Black D, Vogt TM** 1995 Risk factors for hip fracture in white women. N Engl J Med 332:767–773
111. **Kannus P, Palvanen M, Kaprio J, Parkkari J, Koskenvuo M** 1999 Genetic factors and osteoporotic fracture in elderly people: prospective 25 year follow up of a nationwide cohort of elderly Finnish twins. Br Med J 319:1334–1337
112. **MacGregor A, Sneider H, Spector TD** 2000 Genetic factors and osteoporotic fractures in elderly people: twin data support genetic contribution to risk of fracture. Br Med J 320:1669
113. **Deng HW, Chen WM, Recker S, Stegman MR, Li JL, Davies KM, Zhou Y, Deng HY, Heaney R, Recker RR** 2000 Genetic determination of Colles' fracture and differential bone mass in women with and without Colles' fracture. J Bone Miner Res 15:1243–1252
114. **Carmelli D, Kelly-Hayes M, Wolf PA, Swan GE, Jack LM, Reed T, Guralnik JM** 2000 The contribution of genetic influences to measures of lower-extremity function in older male twins. J Gerontol [A] 55A:B49–B53
115. **Wark JD, Hill K, Cassano AM, El Haber N, MacInnis R** 2001 Genetic effects on falls risk may help explain why fractures run in families: a twin study. Bone 28(Suppl):S72 (Abstract)
116. **Sheldon JH** 1960 On the natural history of falls in old age. Br Med J 1685–1690
117. **Tinetti ME, Williams CS** 1997 Falls, injuries due to falls, and the risk of admission to a nursing home. N Engl J Med 337:1279–1284
118. **Campbell A, Reinken J, Allan B, Martinez G** 1981 Falls in old age: a study of frequency and related clinical factors. Age Ageing 10: 264–270
119. **Lauritzen JB, Petersen MM, Lund B** 1993 Effect of external hip protectors on hip fractures. Lancet 341:11–13
120. **Rubenstein L** 2000 Hip protectors—a breakthrough in fracture prevention. N Engl J Med 343:1562–1563
121. **Rubenstein LZ, Robbins AS, Schulman BL, Rosado J, Osterweil D, Josephson KR** 1988 Falls and instability in the elderly. J Am Geriatr Soc 36:266–278
122. **Kelsey JL, Hoffman S** 1987 Risk factors for hip fracture. N Engl J Med 316:404–406
123. **Nevitt MC, Cummings SR, Kidd S, Black D** 1989 Risk factors for

recurrent nonsyncopal falls: a prospective study. JAMA 261:2663–2668
124. **Ho NC, Libin J, Driscoll CC, Gutter EM, Francomano CA** 2000 A skeletal gene database. J Bone Miner Res 15:2095–2122
125. **Risch N, Merikangas K** 1996 The future of genetic studies of complex human diseases. Science 273:1516–1517
126. **Spielman RS, Ewens WJ** 1996 The TDT and other family-based tests for linkage disequilibrium and association. Am J Hum Genet 59:983–989
127. **Pritchard JK, Rosenberg NA** 1999 Use of unlinked genetic markers to detect population stratification in association studies. Am J Hum Genet 65:220–228
128. **Pritchard JK, Stephens M, Rosenberg NA, Donnelly P** 2000 Association mapping in structured populations. Am J Hum Genet 67:170–181
129. **Rioux JD, Daly MJ, Silverberg MS, Lindblad K, Steinhart H, Cohen Z, Delmonte T, Kocher K, Miller K, Guschwan S, Kulbokas EJ, O'Leary S, Winchester E, Dewar K, Green T, Stone V, Chow C, Cohen A, Langelier D, Lapointe G, Gaudet D, Faith J, Branco N, Bull SB, McLeod RS, Griffiths AM, Bitton A, Greenberg GR, Lander ES, Siminovitch KA, Hudson TJ** 2001 Genetic variation in the 5q31 cytokine gene cluster confers susceptibility to Crohn disease. Nat Genet 29:223–228
130. **Daly MJ, Rioux JD, Schaffner SF, Hudson TJ, Lander ES** 2001 High-resolution haplotype structure in the human genome. Nat Genet 29:229–232
131. **Johnson GC, Esposito L, Barratt BJ, Smith AN, Heward J, Di Genova G, Ueda H, Cordell HJ, Eaves IA, Dudbridge F, Twells RC, Payne F, Hughes W, Nutland S, Stevens H, Carr P, Tuomilehto-Wolf E, Tuomilehto J, Gough SC, Clayton DG, Todd JA** 2001 Haplotype tagging for the identification of common disease genes. Nat Genet 29:233–237
132. **Cooper GS, Umbach DM** 1996 Are vitamin D receptor polymorphisms associated with bone mineral density? A meta-analysis. J Bone Miner Res 11:1841–1849
133. **Barger-Lux MJ, Heaney RP, Hayes J, DeLuca HF, Johnson ML, Gong G** 1995 Vitamin D receptor gene polymorphism, bone mass, body size, and vitamin D receptor density. Calcif Tissue Int 57: 161–162
134. **Lorentzon M, Lorentzon R, Nordström P** 2000 Vitamin D receptor gene polymorphism is associated with birth height, growth to adolescence, and adult stature in healthy Caucasian men: a cross-sectional and longitudinal study. J Clin Endocrinol Metab 85:1666–1670
135. **Keen RW, Egger P, Fall C, Major PJ, Lanchbury JS, Spector TD, Cooper C** 1997 Polymorphisms of the vitamin D receptor, infant growth, and adult bone mass. Calcif Tissue Int 60:233–235
136. **Kitagawa I, Kitagawa Y, Kawase Y, Nagaya T, Tokudome S** 1998 Advanced onset of menarche and higher bone mineral density depending on vitamin D receptor gene polymorphism. Eur J Endocrinol 139:522–527
137. **Geusens P, Vandevyver C, Vanhoof J, Cassiman JJ, Boonen S, Raus J** 1997 Quadriceps and grip strength are related to vitamin D receptor genotype in elderly nonobese women. J Bone Miner Res 12:2082–2088
138. **Gennari L, Becherini L, Masi L, Gonnelli S, Cepollaro C, Martini S, Mansani R, Brandi ML** 1997 Vitamin D receptor genotypes and intestinal calcium absorption in postmenopausal women. Calcif Tissue Int 61:460–463
139. **Kiel DP, Myers RH, Cupples LA, Kong XF, Zhu XH, Ordovas J, Schaefer EJ, Felson DT, Rush D, Wilson PW, Eisman JA, Holick MF** 1997 The *Bsm*I vitamin D receptor restriction fragment length polymorphism (bb) influences the effect of calcium intake on bone mineral density. J Bone Miner Res 12:1049–1057
140. **Ongphiphadhanakul B, Rajatanavin R, Chanprasertyothin S, Chailurkit L, Piaseu N, Teerarungsikul K, Sirisriro R, Komindr S, Puavilai G** 1997 Vitamin D receptor gene polymorphism is associated with urinary calcium excretion but not with bone mineral density in postmenopausal women. J Endocrinol Invest 20: 592–596
141. **Schwartz BS, Lee BK, Lee GS, Stewart WF, Simon D, Kelsey K, Todd AC** 2000 Associations of blood lead, dimercaptosuccinic acid-chelatable lead, and tibia lead with polymorphisms in the vitamin D receptor and δ-aminolevulinic acid dehydratase genes. Environ Health Perspect 108:949–954
142. **Lee BK, Lee GS, Stewart WF, Ahn KD, Simon D, Kelsey KT, Todd AC, Schwartz BS** 2001 Associations of blood pressure and hypertension with lead dose measures and polymorphisms in the vitamin D receptor and δ-aminolevulinic acid dehydratase genes. Environ Health Perspect 109:383–389
143. **Carling T, Ridefelt P, Hellman P, Juhlin C, Lundgren E, Åkerström G, Rastad J** 1998 Vitamin D receptor gene polymorphism and parathyroid calcium sensor protein (CAS/gp330) expression in primary hyperparathyroidism. World J Surg 22:700–707
144. **Chang TJ, Lei HH, Yeh JI, Chiu KC, Lee KC, Chen MC, Tai TY, Chuang LM** 2000 Vitamin D receptor gene polymorphisms influence susceptibility to type 1 diabetes mellitus in the Taiwanese population. Clin Endocrinol (Oxf) 52:575–580
145. **Fernández E, Fibla J, Betriu A, Piulats JM, Almirall J, Montoliu J** 1997 Association between vitamin D receptor gene polymorphism and relative hypoparathyroidism in patients with chronic renal failure. J Am Soc Nephrol 8:1546–1552
146. **Fischer PR, Thacher TD, Pettifor JM, Jorde LB, Eccleshall TR, Feldman D** 2000 Vitamin D receptor polymorphisms and nutritional rickets in Nigerian children. J Bone Miner Res 15:2206–2210
147. **Ortlepp JR, Hoffmann R, Ohme F, Lauscher J, Bleckmann F, Hanrath P** 2001 The vitamin D receptor genotype predisposes to the development of calcific aortic valve stenosis. Heart 85:635–638
148. **Fukazawa T, Yabe I, Kikuchi S, Sasaki H, Hamada T, Miyasaka K, Tashiro K** 1999 Association of vitamin D receptor gene polymorphism with multiple sclerosis in Japanese. J Neurol Sci 166: 47–52
149. **Ban Y, Taniyama M** 2000 Vitamin D receptor gene polymorphism is associated with Graves' disease in the Japanese population. J Clin Endocrinol Metab 85:4639–4643
150. **Videman T, Leppävuori J, Kaprio J, Battié MC, Gibbons LE, Peltonen L, Koskenvuo M** 1998 1998 Volvo Award winner in basic science studies—intragenic polymorphisms of the vitamin D receptor gene associated with intervertebral disc degeneration. Spine 23:2477–2485
151. **Uitterlinden AG, Burger H, Huang QJ, Odding E, Van Duijn CM, Hofman A, Birkenhäger JC, Van Leeuwen JP, Pols HA** 1997 Vitamin D receptor genotype is associated with radiographic osteoarthritis at the knee. J Clin Invest 100:259–263
152. **Halmos B, Szalay F, Cserniczky T, Nemesanszky E, Lakatos P, Barlage S, Schmitz G, Romics L, Csaszar A** 2000 Association of primary biliary cirrhosis with vitamin D receptor BsmI genotype polymorphism in a Hungarian population. Dig Dis Sci 45:1091–1095
153. **Bretherton-Watt D, Given-Wilson R, Mansi JL, Thomas V, Carter N, Colston KW** 2001 Vitamin D receptor gene polymorphisms are associated with breast cancer risk in a UK Caucasian population. Br J Cancer 85:171–175
154. **Garcia-Lozano JR, Gonzalez-Escribano MF, Valenzuela A, Garcia A, Núñez-Roldán A** 2001 Association of vitamin D receptor genotypes with early onset rheumatoid arthritis. Eur J Immunogenet 28:89–93
155. **Habuchi T, Suzuki T, Sasaki R, Wang LZ, Sato K, Satoh S, Akao T, Tsuchiya N, Shimoda N, Wada Y, Koizumi A, Chihara J, Osamu OA, Kato T** 2000 Association of *vitamin D receptor* gene polymorphism with prostate cancer and benign prostatic hyperplasia in a Japanese population. Cancer Res 60:305–308
156. **Bellamy R** 2000 Identifying genetic susceptibility factors for tuberculosis in Africans: a combined approach using a candidate gene study and a genome-wide screen. Clin Sci 98:245–250
157. **Eichner JE, Friedrich CA, Cauley JA, Kamboh MI, Gutai JP, Kuller LH, Ferrell RE** 1990 α_2-HS glycoprotein phenotypes and quantitative hormone and bone measures in postmenopausal women. Calcif Tissue Int 47:345–349
158. **Dickson IR, Gwilliam R, Arora M, Murphy S, Khaw K-T, Phillips C, Lincoln P** 1994 Lumbar vertebral and femoral neck bone mineral density are higher in postmenopausal women with the α_2HS-glycoprotein 2 phenotype. Bone Miner 24:181–188
159. **Dickson IR, Poole AR, Veis A** 1975 Localization of plasma α HS-glycoprotein in mineralizing human bone. Nature 256:430–432

160. **Malone JD, Richards M** 1987 α2 HS-glycoprotein is chemotactic for mononuclear phagocytes. J Cell Physiol 132:118–124
161. **Haussler MR, Haussler CA, Jurutka PW, Thompson PD, Hsieh JC, Remus LS, Selznick SH, Whitfield GK** 1997 The vitamin D hormone and its nuclear receptor: molecular actions and disease states. J Endocrinol 154(Suppl):S57–S73
162. **Hughes MR, Malloy PJ, Kieback DG, Kesterson RA, Pike JW, Feldman D, O'Malley BW** 1988 Point mutations in the human vitamin D receptor gene associated with hypocalcemic rickets. Science 242:1702–1705
163. **Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, Sambrook PN, Eisman JA** 1994 Prediction of bone density from vitamin D receptor alleles. Nature 367:284–287
164. **Peacock M, Hustmyer FG, Hui S, Johnston CC, Christian J** 1995 Vitamin D receptor genotype and bone mineral density—evidence conflicts on link. Br Med J 311:874–875
165. **Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, Sambrook PN, Eisman JA** 1997 Prediction of bone density from vitamin D receptor alleles: corrections. Nature 387:106
166. **Zee RYL, Myers RH, Hannan MT, Wilson PWF, Ordovas JM, Schaefer EJ, Lindpaintner K, Kiel DP** 2000 Absence of linkage for bone mineral density to chromosome 12q12–14 in the region of the vitamin D receptor gene. Calcif Tissue Int 67:434–439
167. **Parker MG, Arbuckle N, Dauvois S, Danielian P, White R** 1993 Structure and function of the estrogen receptor. Ann NY Acad Sci 684:119–126
168. **Kuiper GGJM, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson J-A** 1996 Cloning of a novel estrogen receptor expressed in rat prostate and ovary. Proc Natl Acad Sci USA 93:5925–5930
169. **MacGillivray MH, Morishima A, Conte F, Grumbach M, Smith EP** 1998 Pediatric endocrinology update: an overview—the essential roles of estrogens in pubertal growth, epiphyseal fusion and bone turnover: lessons from mutations in the genes for aromatase and the estrogen receptor. Horm Res 49:2–8
170. **Lindsay R** 1998 The role of estrogen in the prevention of osteoporosis. Endocrinol Metabol Clin North Am 27:399–409
171. **Sano M, Inoue S, Hosoi T, Ouchi Y, Emi M, Shiraki M, Orimo H** 1995 Association of estrogen receptor dinucleotide repeat polymorphism with osteroporosis. Biochem Biophys Res Commun 217: 378–383
172. **Kobayashi S, Inoue S, Hosoi T, Ouchi Y, Shiraki M, Orimo H** 1996 Association of bone mineral density with polymorphism of the estrogen receptor gene. J Bone Miner Res 11:306–311
173. **Han KO, Moon IG, Kang YS, Chung HY, Min HK, Han IK** 1997 Nonassociation of estrogen receptor genotypes with bone mineral density and estrogen responsiveness to hormone replacement therapy in Korean postmenopausal women. J Clin Endocrinol Metab 82:991–995
174. **Bagger YZ, Jorgensen HL, Heegaard AM, Bayer L, Hansen L, Hassager C** 2000 No major effect of estrogen receptor gene polymorphisms on bone mineral density or bone loss in postmenopausal Danish women. Bone 26:111–116
175. **Ogawa S, Hosoi T, Shiraki M, Orimo H, Emi M, Muramatsu M, Ouchi Y, Inoue S** 2000 Association of estrogen receptor β gene polymorphism with bone mineral density. Biochem Biophys Res Commun 269:537–541
176. **Grant SF, Reid DM, Blake G, Herd R, Fogelman I, Ralston SH** 1996 Reduced bone density and osteoporosis associated with a polymorphic Sp1 binding site in the collagen type I α 1 gene. Nat Genet 14:203–205
177. **Garnero P, Borel O, Grant SF, Ralston SH, Delmas PD** 1998 Collagen Iα1 Sp1 polymorphism, bone mass, and bone turnover in healthy French premenopausal women: the OFELY study. J Bone Miner Res 13:813–817
178. **Hustmyer FG, Liu G, Johnston CC, Christian J, Peacock M** 1999 Polymorphism at an Sp1 binding site of COL1A1 and bone mineral density in premenopausal female twins and elderly fracture patients. Osteoporos Int 9:346–350
179. **Willing MC, Torner JC, Burns TL, Segar ET, Werner JR** 1997 Determinants of bone mineral density in postmenopausal white Iowans. J Gerontol [A] 52A:M337–M342
180. **Tsuji S, Munkhbat B, Hagihara M, Tsuritani I, Abe H, Tsuji K** 1998 HLA-A-A*24-B*07-DRB1*01 haplotype implicated with genetic disposition of peak bone mass in healthy young Japanese women. Hum Immunol 59:243–249
181. **Shiraki M, Shiraki W, Aoki C, Hosoi T, Inoue S, Kaneki M, Ouchi Y** 1997 Association of bone mineral density with apolipoprotein E phenotype. J Bone Miner Res 12:1438–1445
182. **Heikkinen AM, Kröger H, Niskanen L, Komulainen MH, Ryynänen M, Parviainen MT, Tuppurainen MT, Honkanen R, Saarikoski S** 2000 Does apolipoprotein E genotype relate to BMD and bone markers in postmenopausal women? Maturitas 34:33–41
183. **Salamone LM, Cauley JA, Zmuda J, Pasagian-Macaulay A, Epstein RS, Ferrell RE, Black DM, Kuller LH** 2000 Apolipoprotein E gene polymorphism and bone loss: estrogen status modifies the influence of apolipoprotein E on bone loss. J Bone Miner Res 15: 308–314
184. **Duncan EL, Brown MA, Sinsheimer J, Bell J, Carr AJ, Wordsworth BP, Wass JAH** 1999 Suggestive linkage of the parathyroid receptor type 1 to osteoporosis. J Bone Miner Res 14: 1993–1999
185. **Ota N, Hunt SC, Nakajima T, Suzuki T, Hosoi T, Orimo H, Shirai Y, Emi M** 1999 Linkage of interleukin 6 locus to human osteopenia by sibling pair analysis. Hum Genet 105:253–257
186. **Ota N, Hunt SC, Nakajima T, Suzuki T, Hosoi T, Shirai Y, Emi M** 2000 Linkage of human tumor necrosis factor-α to human osteoporosis by sib pair analysis. Genes Immunity 1:260–264
187. **Spielman RS, McGinnis RE, Ewens WJ** 1993 Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). Am J Hum Genet 52:506–516
188. **McGinnis RE, Ewens WJ, Spielman RS** 1995 The TDT reveals linkage and linkage disequilibrium in a rare disease. Genet Epidemiol 12:637–640
189. **Spielman RS, Ewens WJ** 1998 A sibship test for linkage in the presence of association: the sib transmission/disequilibrium test. Am J Hum Genet 62:450–458
190. **Boehnke M, Langefeld CD** 1998 Genetic association mapping based on discordant sib pairs: the discordant-allele test. Am J Hum Genet 62:950–961
191. **Curtis D** 1997 Use of siblings as controls in case-control association studies. Ann Hum Genet 61:319–333
192. **Martin ER, Monks SA, Warren LL, Kaplan NL** 2000 A test for linkage and association in general pedigrees: the pedigree disequilibrium test. Am J Hum Genet 67:146–154
193. **Allison DB** 1997 Transmission-disequilibrium tests for quantitative traits. Am J Hum Genet 60:676–690
194. **Allison DB, Heo M, Kaplan N, Martin ER** 1999 Sibling-based tests of linkage and association for quantitative traits. Am J Hum Genet 64:1754–1763
195. **Sun FZ, Flanders WD, Yang QH, Zhao HY** 2000 Transmission/disequilibrium tests for quantitative traits. Ann Hum Genet 64: 555–565
196. **Zhu X, Elston RC** 2001 Transmission/disequilibrium tests for quantitative traits. Genet Epidemiol 20:57–74
197. **Schaffer AA, Gupta SK, Shriram K, Cottingham Jr RW** 1994 Avoiding recomputation in linkage analysis. Hum Hered 44: 225–237
198. **O'Connell JR, Weeks DE** 1995 The VITESSE algorithm for rapid exact multilocus linkage analysis via genotype set-recoding and fuzzy inheritance. Nat Genet 11:402–408
199. **Sham PC, Lin MW, Zhao JH, Curtis D** 2001 Power comparison of parametric and nonparametric linkage test in small pedigrees. Am J Hum Genet 66:1661–1668
200. **Kruglyak L, Lander ES** 1995 Complete multipoint sib-pair analysis of qualitative and quantitative traits. Am J Hum Genet 57:439–454
201. **Almasy L, Blangero J** 1998 Multipoint quantitative-trait linkage analysis in general pedigrees. Am J Hum Genet 62:1198–1211
202. **Johnson ML, Gong GD, Kimberling W, Recker SM, Kimmel DB, Recker RR** 1997 Linkage of a gene causing high bone mass to human chromosome 11 (11q12–13). Am J Hum Genet 60:1326–1332
203. **Recker RR, Johnson ML, Davies KM, Recker SM, Heaney RP** 2001 Autosomal dominant high bone mass: the phenotype. J Bone Miner Res 16(Suppl):S470 (Abstract)
204. **Whyte MP** 1996 Sclerosing bone dysplasias. In: Favus MJ, ed. Primer on the metabolic bone diseases and disorders of mineral metabolism. Philadelphia: Lippincott-Raven; 363–379

205. **Little RD, Carulli JP, Del Mastro RG, Dupuis J, Osborne M, Folz C, Manning SP, Swain PM, Zhao S-C, Eustace B, Lappe MM, Spitzer L, Zweier S, Braunschweiger K, Benchekroun Y, Hu X, Adair R, Chee L, FitzGerald MG, Tulig C, Caruso A, Tzellas N, Bawa A, Franklin B, McGuire S, Nogues X, Gong G, Allen KM, Anisowicz A, Morales AJ, Lomedico PT, Recker SM, Eerdewegh PV, Recker RR, Johnson ML** 2001 A mutation in the LDL receptor-related protein 5 gene results in the autosomal dominant high-bone mass trait. Am J Hum Genet 70:11–19
206. **Tamai K, Semenov M, Kato Y, Spokony R, Liu CM, Katsuyama Y, Hess F, Saint-Jeannet JP, He X** 2000 LDL-receptor-related proteins in Wnt signal transduction. Nature 407:530–535
207. **Wehrli M, Dougan ST, Caldwell K, O'Keefe L, Schwartz S, Vaizel-Ohayon D, Schejter E, Tomlinson A, DiNardo S** 2000 Arrow encodes an LDL-receptor-related protein essential for Wingless signalling. Nature 407:527–530
208. **Spotila LD, Caminis J, Devoto M, Shimoya K, Sereda L, Ott J, Whyte MP, Tenenhouse A, Prockop DJ** 1996 Osteopenia in 37 members of seven families: analysis based on a model of dominant inheritance. Mol Med 2:313–324
209. **Devoto M, Shimoya K, Caminis J, Ott J, Tenenhouse A, Whyte MP, Sereda L, Hall S, Considine E, Williams CJ, Tromp G, Kuivaniemi H, Ala-Kokko L, Prockop DJ, Spotila LD** 1998 First-stage autosomal genome screen in extended pedigrees suggests genes predisposing to low bone mineral density on chromosomes 1p, 2p and 4q. Eur J Hum Genet 6:151–157
210. **Cardon LR, Garner C, Bennett ST, MacKay IJ, Edwards RM, Cornish J, Hegde M, Murray MAF, Reid IR, Cundy T** 2000 Evidence for a major gene for bone mineral density in idiopathic osteoporotic families. J Bone Miner Res 15:1132–1137
211. **Koller DL, Rodriguez LA, Christian JC, Slemenda CW, Econs MJ, Hui SL, Morin P, Conneally PM, Joslyn G, Curran ME, Peacock M, Johnston CC, Foroud T** 1998 Linkage of a QTL contributing to normal variation in bone mineral density to chromosome 11q12–13. J Bone Miner Res 13:1903–1908
212. **Frattini A, Orchard PJ, Sobacchi C, Giliani S, Abinun M, Mattsson JP, Keeling DJ, Andersson AK, Wallbrandt P, Zecca L, Notarangelo LD, Vezzoni P, Villa A** 2000 Defects in TCIRG1 subunit of the vacuolar proton pump are responsible for a subset of human autosomal recessive osteopetrosis. Nat Genet 25:343–346
213. **Deng HW, Xu FH, Conway T, Deng XT, Li JL, Davies KM, Deng HY, Johnson M, Recker RR** 2001 Is population bone mineral density variation linked to the marker D11S987 on chromosome 11q12–13? J Clin Endocrinol Metab 86:3735–3741
214. **Niu TH, Chen CZ, Cordell H, Yang JH, Wang BY, Wang ZX, Fang Z, Schork NJ, Rosen CJ, Xu XP** 1999 A genome-wide scan for loci linked to forearm bone mineral density. Hum Genet 104:226–233
215. **Feakes R, Sawcer S, Chataway J, Coraddu F, Broadley S, Gray J, Jones HB, Clayton D, Goodfellow PN, Compston A** 1999 Exploring the dense mapping of a region of potential linkage in complex disease: an example in multiple sclerosis. Genet Epidemiol 17: 51–63
216. **Francis F, Hennig S, Korn B, Reinhardt R, De Jong P, Poustka A, Lehrach H, Rowe PSN, Goulding JN, Summerfield T, Mountford R, Read AP, Popowska E, Pronicka E, Davies KE, O'Riordan JLH, Econs MJ, Nesbitt T, Drezner MK, Oudet C, Pannetier S, Hanauer A, Strom TM, Meindl A** 1995 A gene (*PEX*) with homologies to endopeptidases is mutated in patients with X-linked hypophosphatemic rickets. Nat Genet 11:130–136
217. **Marchuk DA** 1998 Laboratory approaches toward gene identification. In: Haines JL, Pericak-Vance MA, eds. Approaches to gene mapping in complex human diseases. New York: Wiley-Liss; 351–378
218. **Rowe PSN** 2000 Finding mutations in disease genes. In: Econs MJ, ed. The genetics of osteoporosis and metabolic bone disease. Totowa, NJ: Humana Press; 431–446
219. **Beamer WG, Donahue LR, Rosen CJ, Baylink DJ** 1996 Genetic variability in adult bone density among inbred strains of mice. Bone 18:397–403
220. **Rogers J, Hixson JE** 1997 Insights from model systems: baboons as an animal model for genetic studies of common human disease. Am J Hum Genet 61:489–493
221. **Turner CH, Roeder RK, Wieczorek A, Foroud T, Liu G, Peacock M** 2001 Variability in skeletal mass, structure, and biomechanical properties between inbred strains of rats. J Bone Miner Res 16: 1532–1539
222. **Silver LM** 1995 Mouse genetics. New York: Oxford University Press
223. **Klein RF, Mitchell SR, Phillips TJ, Belknap JK, Orwoll ES** 1998 Quantitative trait loci affecting peak bone mineral density in mice. J Bone Miner Res 13:1648–1656
224. **Beamer WG, Shultz KL, Churchill GA, Frankel WN, Baylink DJ, Rosen CJ, Donahue LR** 1999 Quantitative trait loci for bone density in C57BL/6J and CAST/EiJ inbred mice. Mamm Genome 10:1043–1049
225. **Shimizu M, Higuchi K, Bennett B, Xia C, Tsuboyama T, Kasai S, Chiba T, Fujisawa H, Kogishi K, Kitado H, Kimoto M, Takeda N, Matsushita M, Okumura H, Serikawa T, Nakamura T, Johnson TE, Hosokawa M** 1999 Identification of peak bone mass QTL in a spontaneously osteoporotic mouse strain. Mamm Genome 10:81–87
226. **Benes H, Weinstein RS, Zheng WH, Thaden JJ, Jilka RL, Manolagas SC, Reis RJS** 2000 Chromosomal mapping of osteopenia-associated quantitative trait loci using closely related mouse strains. J Bone Miner Res 15:626–633
227. **Bouxsein ML, Uchiyama T, Mytar J, Beamer WG, Donahue LR, Rosen CJ, Turner CH, Mueller R** 2001 Chromosomal location of genes that contribute to vertebral trabecular bone density and microarchitecture in mice. Bone 28(Suppl):S72–S73
228. **Beamer WG, Shultz KL, Donahue LR, Churchill GA, Sen S, Wergedal JR, Baylink DJ, Rosen CJ** 2001 Quantitative trait loci for femoral and lumbar vertebral bone mineral density in C57BL/6J and C3H/HeJ inbred strains of mice. J Bone Miner Res 16:1195–1206
229. **Turner CH, Hsieh YF, Müller R, Bouxsein ML, Baylink DJ, Rosen CJ, Grynpas MD, Donahue LR, Beamer WG** 2000 Genetic regulation of cortical and trabecular bone strength and microstructure in inbred strains of mice. J Bone Miner Res 15:1126–1131
230. **Turner CH, Sun Q, Bouxsein ML, Rosen CJ, Donahue LR, Shultz KL, Beamer WG** 2001 Major genetic influence on femoral stiffness and strength identified on mouse chromosome 4. J Bone Miner Res 16(Suppl):S298
231. **Turner CH, Hsieh YF, Müller R, Bouxsein ML, Rosen CJ, McCrann ME, Donahue LR, Beamer WG** 2001 Variation in bone biomechanical properties, microstructure, and density in BXH recombinant inbred mice. J Bone Miner Res 16:206–213
232. **Beamer WG, Donahue LR, Shultz KL, Rosen CJ, Churchill GA, Baylink DJ** 2000 Genetic regulation of BMD in low density C57BL/6J mice carrying donated QTLs from high density C3H/HeJ mice. J Bone Miner Res 15(Suppl):S186
233. **Turner CH, Sun Q, Bouxsein ML, Rosen CJ, Donahue LR, Shultz KL, Beamer WG** 2001 Improved bone strength in low bone density C57BL/6J mice carrying donated QTLs from C3H/HeJ mice. Bone 28(Suppl):S85
234. **Mahaney MC, Morin P, Rodriguez LA, Newman DE, Rogers J** 1997 A quantitative trait locus on chromosome 11 may influence bone mineral density at several sites: linkage analyses in pedigreed baboons. J Bone Miner Res 12(Suppl):S118
235. **Roche PA, Annas GJ** 2001 Protecting genetic privacy. Nat Rev Genet 2:392–396
236. **Korf B** 1995 Molecular diagnosis (1). N Engl J Med 332:1218–1220
237. **Korf B** 1995 Molecular diagnosis (2). N Engl J Med 332:1499–1502
238. **Nordin BEC** 1997 Calcium and osteoporosis. Nutrition 13:664–686
239. **Peacock M, Liu G, Carey M, McClintock R, Ambrosius W, Hui S, Johnston CC** 2000 Effect of calcium or 25OH vitamin D_3 dietary supplementation on bone loss at the hip in men and women over the age of 60. J Clin Endocrinol Metab 85:3011–3019
240. **Peacock M** 1998 Effects of calcium and vitamin D insufficiency on the skeleton. Osteoporos Int 8(Suppl):S45–S51
241. **Hall SL, Greendale GA** 1998 The relation of dietary vitamin C intake to bone mineral density: results from the PEPI study. Calcif Tissue Int 63:183–189
242. **Morton DJ, Barrett-Connor EL, Schneider DL** 2001 Vitamin C supplement use and bone mineral density in postmenopausal women. J Bone Miner Res 16:135–140
243. **Hannan MT, Tucker KL, Dawson-Hughes B, Cupples LA, Felson**

DT, Kiel DP 2000 Effect of dietary protein on bone loss in elderly men and women: the Framingham osteoporosis study. J Bone Miner Res 15:2504–2512
244. **Sellmeyer DE, Stone KL, Sebastian A, Cummings SR** 2001 A high ratio of dietary animal to vegetable protein increases the rate of bone loss and the risk of fracture in postmenopausal women. Study Osteoporotic Fractures Research Group. Am J Clin Nutr 73:118–122
245. **Kelley GA, Kelley KS, Tran ZV** 2000 Exercise and bone mineral density in men: a meta-analysis. J Appl Physiol 88:1730–1736
246. **Bailey DA, McKay HA, Mirwald RL, Crocker PRE, Faulkner RA** 1999 A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: the University of Saskatchewan bone mineral accrual study. J Bone Miner Res 14:1672–1679
247. **Hermann AP, Brot C, Gram J, Kolthoff N, Mosekilde L** 2000 Premenopausal smoking and bone density in 2015 perimenopausal women. J Bone Miner Res 15:780–787
248. **Ward KD, Klesges RC** 2001 A meta-analysis of the effects of cigarette smoking on bone mineral density. Calcif Tissue Int 68: 259–270
249. **Law MR, Hackshaw AK** 1997 A meta-analysis of cigarette smoking, bone mineral density and risk of hip fracture: recognition of a major effect. Br Med J 315:841–846
250. **Ganry O, Baudoin C, Fardellone P** 2000 Effect of alcohol intake on bone mineral density in elderly women—the EPIDOS study. Am J Epidemiol 151:773–780
251. **Smith R, Athanasou NA, Ostlere SJ, Vipond SE** 1995 Pregnancy-associated osteoporosis. QJM 88:865–878
252. **Soyka LA, Grinspoon S, Levitsky LL, Herzog DB, Klibanski A** 1999 The effects of anorexia nervosa on bone metabolism in female adolescents. J Clin Endocrinol Metab 84:4489–4496
253. **Bush TL, Wells HB, James MK, Barrett-Connor E, Marcus R, Greendale G, Hunsberger S, McGowan J** 1996 Effects of hormone therapy on bone mineral density—results from the postmenopausal estrogen/progestin interventions (PEPI) trial. JAMA 276: 1389–1396
254. **Greendale GA, Edelstein S, Barrett-Connor E** 1997 Endogenous sex steroids and bone mineral density in older women and men: the Rancho Bernardo study. J Bone Miner Res 12:1833–1843
255. **Francis RM, Peacock M, Aaron JE** 1986 Osteoporosis in hypogonadal men: role of decreased plasma 1,25 dihydroxyvitamin D, calcium absorption and low bone formation. Bone 7:261–268
256. **Godang K, Ueland T, Bollerslev J** 1999 Decreased bone area, bone mineral content, formative markers, and increased bone resorptive markers in endogenous Cushing's syndrome. Eur J Endocrinol 141:126–131
257. **Howland WH, Pugh DH, Sprague RG** 1958 Roentgenologic changes of the skeletal system in Cushing's syndrome. Radiology 71:69–78
258. **Peacock M** 1991 Interpretation of bone mass determinations as they relate to fracture: implications for asymptomatic primary hyperparathyroidism. J Bone Miner Res 6(Suppl 2):S77–S82
259. **Nakaoka D, Sugimoto T, Kobayashi T, Yamaguchi T, Kobayashi A, Chihara K** 2000 Prediction of bone mass change after parathyroidectomy in patients with primary hyperparathyroidism. J Clin Endocrinol Metab 85:1901–1907
260. **Rosen CJ, Adler RA** 1992 Longitudinal changes in lumbar bone density among thyrotoxic patients after attainment of euthyroidism. J Clin Endocrinol Metab 75:1531–1534
261. **Wüster C, Abs R, Bengtsson B, Bennmarker H, Feldt-Rasmussen U, Hernberg-Ståhl E, Monson JP, Westberg B, Wilton P, on behalf of the Kims Study Group and the Kims International Board** 2001 The infuence of growth hormone deficiency, growth hormone replacement therapy, and other aspects of hypopituitarism on fracture rate and bone mineral density. J Bone Miner Res 16:398–405
262. **De Boer H, Blok GJ, Van Lingen A, Teule GJJ, Lips P, Van der Veen EA** 1994 Consequences of childhood-onset growth hormone deficiency for adult bone mass. J Bone Miner Res 9:1319–1326
263. **Adachi Y, Shiota E, Matsumata T, Iso Y, Yoh R, Kitano S** 2000 Osteoporosis after gastrectomy: bone mineral density of lumbar spine assessed by dual-energy X-ray absorptiometry. Calcif Tissue Int 66:119–122
264. **Heiskanen JT, Kröger H, Pääkkönen M, Parviainen MT, Lamberg-Allardt C, Alhava E** 2001 Bone mineral metabolism after total gastrectomy. Bone 28:123–127
265. **Tjellesen L, Staun M, Rannem T, Nielsen PK, Jarnum S** 1996 Body composition in patients on home parenteral nutrition. Scand J Clin Lab Invest 56:295–303
266. **Mautalen C, Gonzalez D, Mazure R, Vazquez H, Lorenzetti MP, Maurino E, Niveloni S, Pedreira S, Smecuol E, Boerr LA, Bai JC** 1997 Effect of treatment on bone mass, mineral metabolism, and body composition in untreated celiac disease patients. Am J Gastroenterol 92:313–318
267. **Mora S, Barera G, Ricotti A, Weber G, Bianchi C, Chiumello G** 1998 Reversal of low bone density with a gluten-free diet in children and adolescents with celiac disease. Am J Clin Nutr 67:477–481
268. **Gokhale R, Favus MJ, Karrison T, Sutton MM, Rich B, Kirschner BS** 1998 Bone mineral density assessment in children with inflammatory bowel disease. Gastroenterology 114:902–911
269. **Ghosh S, Cowen S, Hannan WJ, Ferguson A** 1994 Low bone mineral density in Crohn's disease, but not in ulcerative colitis, at diagnosis. Gastroenterology 107:1031–1039
270. **Bhudhikanok GS, Lim J, Marcus R, Harkins A, Moss RB, Bachrach LK** 1996 Correlates of osteopenia in patients with cystic fibrosis. Pediatrics 97:103–111
271. **Lecouvet FE, Vande B, Maldague BE, Michaux L, Laterre E, Michaux JL, Ferrant A, Malghem J** 1997 Vertebral compression fractures in multiple myeloma. I. Distribution and appearance at MR imaging. Radiology 204:195–199
272. **Abildgaard N, Brixen K, Kristensen JE, Vejlgaard T, Charles P, Nielsen JL** 1996 Assessment of bone involvement in patients with multiple myeloma using bone densitometry. Eur J Haematol 57: 370–376
273. **Marshall A, Kavanagh RT, Crisp AJ** 1997 The effect of pamidronate on lumbar spine bone density and pain in osteoporosis secondary to systemic mastocytosis. Br J Rheumatol 36:393–396
274. **Gough A, Sambrook P, Devlin J, Huissoon A, Njeh C, Robbins S, Nguyen T, Emery P** 1998 Osteoclastic activation is the principal mechanism leading to secondary osteoporosis in rheumatoid arthritis. J Rheumatol 25:1282–1289
275. **Gilboe IM, Kvien TK, Haugeberg G, Husby G** 2000 Bone mineral density in systemic lupus erythematosus: comparison with rheumatoid arthritis and healthy controls. Ann Rheum Dis 59:110–115
276. **Fairfield WP, Finkelstein JS, Klibanski A, Grinspoon SK** 2001 Osteopenia in eugonadal men with acquired immune deficiency syndrome wasting syndrome. J Clin Endocrinol Metab 86:2020–2026
277. **Cizza G, Ravn P, Chrousos GP, Gold PW** 2001 Depression: a major, unrecognized risk factor for osteoporosis? Trends Endocrinol Metab 12:198–203
278. **Eastell R, Reid DM, Compston J, Cooper C, Fogelman I, Francis RM, Hosking DJ, Purdie DW, Ralston SH, Reeve J, Russell RGG, Stevenson JC, Torgerson DJ** 1998 A UK consensus group on management of glucocorticoid-induced osteoporosis: an update. J Intern Med 244:271–292
279. **Feldkamp J, Becker A, Witte OW, Scharff D, Scherbaum WA** 2000 Long-term anticonvulsant therapy leads to low bone mineral density—evidence for direct drug effects of phenytoin and carbamazepine on human osteoblast-like cells. Exp Clin Endocrinol Diabetes 108:37–43
280. **Shane E, Epstein S** 1994 Immunosuppressive therapy and the skeleton. Trends Endocrinol Metab 5:169–175
281. **Kaste SC, Chesney RW, Hudson MM, Lustig RH, Rose SR, Carbone LD** 1999 Bone mineral status during and after therapy of childhood cancer: an increasing population with multiple risk factors for impaired bone health. J Bone Miner Res 14:2010–2014
282. **Uzzan B, Campos J, Cucherat M, Nony P, Boissel JP, Perret GY** 1996 Effects on bone mass of long term treatment with thyroid hormones: a meta-analysis. J Clin Endocrinol Metab 81:4278–4289
283. **De Swiet M, Dorrington Ward P, Fidler J, Horsman A, Katz D, Letsky E, Peacock M, Wise PH** 1983 Prolonged heparin therapy in pregnancy causes bone demineralization. Br J Obstet Gynaecol 90:1129–1134
284. **Koller DL, Foroud T, Slemenda CW, Conneally PM, Christian JC, Peacock M, Johnston Jr CC** 1997 Heritability of bone mineral

density in Caucasian and African-American sister pairs. J Bone Miner Res 12(Suppl):S556
285. **Murray RE, McGuigan F, Grant SF, Reid DM, Ralston SH** 1997 Polymorphisms of the interleukin-6 gene are associated with bone mineral density. Bone 21:89–92
286. **Takacs I, Koller DL, Peacock M, Christian JC, Evans WE, Hui SL, Conneally PM, Johnston CC, Foroud T, Econs MJ** 2000 Sib pair linkage and association studies between bone mineral density and the interleukin-6 gene locus. Bone 27:169–173
287. **Yamada Y, Miyauchi A, Goto J, Takagi Y, Okuizumi H, Kanematsu M, Hase M, Takai H, Harada A, Ikeda K** 1998 Association of a polymorphism of the transforming growth factor-β1 gene with genetic susceptibility to osteoporosis in postmenopausal Japanese women. J Bone Miner Res 13:1569–1576
288. **Keen RW, Snieder H, Molloy H, Daniels J, Chiano M, Gibson F, Fairbairn L, Smith P, MacGregor AJ, Gewert D, Spector TD** 2001 Evidence of association and linkage disequilibrium between a novel polymorphism in the transforming growth factor β1 gene and hip bone mineral density: a study of female twins. Rheumatology 40:48–54
289. **Langdahl B, Knudsen JY, Jensen HK, Gregersen N, Eriksen EF** 1997 A sequence variation: 713–8delC in the transforming growth factor-β 1 gene has higher prevalence in osteoporotic women than in normal women and is associated with very low bone mass in osteoporotic women and increased bone turnover in both osteoporotic and normal women. Bone 20:289–294
290. **Braga V, Mottes M, Mirandola S, Lisi V, Malerba G, Sartori L, Bianchi G, Gatti D, Rossini M, Bianchini D, Adami S** 2000 Association of CTR and COLIA1 alleles with BMD values in peri- and postmenopausal women. Calcif Tissue Int 67:361–366
291. **Masi L, Becherini L, Colli E, Gennari L, Mansani R, Falchetti A, Becorpi AM, Cepollaro C, Gonnelli S, Tanini A, Brandi ML** 1998 Polymorphisms of the calcitonin receptor gene are associated with bone mineral density in postmenopausal Italian women. Biochem Biophys Res Commun 248:190–195
292. **Rosen CJ, Kurland ES, Vereault D, Adler RA, Rackoff PJ, Craig WY, Witte S, Rogers J, Bilezikian JP** 1998 Association between serum insulin growth factor-I (IGF-I) and a simple sequence repeat in IGF-I gene: implications for genetic studies of bone mineral density. J Clin Endocrinol Metab 83:2286–2290
293. **Miyao M, Hosoi T, Inoue S, Hoshino S, Shiraki M, Orimo H, Ouchi Y** 1998 Polymorphism of insulin-like growth factor I gene and bone mineral density. Calcif Tissue Int 63:306–311
294. **Takacs I, Koller DL, Peacock M, Christian JC, Hui SL, Conneally PM, Johnston Jr CC, Foroud T, Econs MJ** 1999 Sibling pair linkage and association studies between bone mineral density and the insulin-like growth factor I gene locus. J Clin Endocrinol Metab 84:4467–4471
295. **Dohi Y, Iki M, Ohgushi H, Gojo S, Tabata S, Kajita E, Nishino H, Yonemasu K** 1998 A novel polymorphism in the promoter region for the human osteocalcin gene: the possibility of a correlation with bone mineral density in postmenopausal Japanese women. J Bone Miner Res 13:1633–1639
296. **Raymond MH, Schutte BC, Torner JC, Burns TL, Willing MC** 1999 Osteocalcin: genetic and physical mapping of the human gene BGLAP and its potential role in postmenopausal osteoporosis. Genomics 60:210–217
297. **Miyao M, Morita H, Hosoi T, Kurihara H, Inoue S, Hoshino S, Shiraki M, Yazaki Y, Ouchi Y** 2000 Association of methylenetetrahydrofolate reductase (MTHFR) polymorphism with bone mineral density in postmenopausal Japanese women. Calcif Tissue Int 66:190–194
298. **Keen RW, Woodford-Richens KL, Lanchbury JS, Spector TD** 1998 Allelic variation at the interleukin-1 receptor antagonist gene is associated with early postmenopausal bone loss at the spine. Bone 23:367–371
299. **Langdahl BL, Lokke E, Carstens M, Stenkjær LL, Eriksen EF** 2000 Osteoporotic fractures are associated with an 86-base pair repeat polymorphism in the interleukin-1-receptor antagonist gene but not with polymorphisms in the interleukin-1β gene. J Bone Miner Res 15:402–414
300. **Takács I, Vargha P, Speer G, Nagy Z, Lakatos P** 2000 Lack of association between interleukin-1 receptor antagonist protein gene polymorphism and bone mineral density in Hungarian postmenopausal women. Bone 27:559–562
301. **Spotila LD, Rodriguez H, Koch M, Adams K, Caminis J, Tenenhouse HS, Tenenhouse A** 2000 Association of a polymorphism in the *TNFR2* gene with low bone mineral density. J Bone Miner Res 15:1376–1383
302. **Tsukamoto K, Orimo H, Hosoi T, Miyao M, Ota N, Nakajima T, Yoshida H, Watanabe S, Suzuki T, Emi M** 2000 Association of bone mineral density with polymorphism of the human calcium-sensing receptor locus. Calcif Tissue Int 66:181–183
303. **Masi L, Becherini L, Gennari L, Amedei A, Colli E, Falchetti A, Farci M, Silvestri S, Gonnelli S, Brandi ML** 2001 Polymorphism of the aromatase gene in postmenopausal Italian women: distribution and correlation with bone mass and fracture risk. J Clin Endocrinol Metab 86:2263–2269
304. **Urano T, Hosoi T, Shiraki M, Toyoshima H, Ouchi Y, Inoue S** 2000 Possible involvement of the $p57^{Kip2}$ gene in bone metabolism. Biochem Biophys Res Commun 269:422–426
305. **Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C, Scully S, Tan HL, Xu WL, Lacey DL, Boyle WJ, Simonet WS** 1998 *osteoprotegerin*-Deficient mice develop early onset osteoporosis and arterial calcification. Genes Dev 12:1260–1268
306. **Erlebacher A, Derynck R** 1996 Increased expression of TGF-β2 in osteoblasts results in an osteoporosis-like phenotype. J Cell Biol 132:195–210
307. **Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, Ohyama Y, Kurabayashi M, Kaname T, Kume E, Iwasaki H, Iida A, Shiraki-Iida T, Nishikawa S, Nagai R, Nabeshima YI** 1997 Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature 390:45–51
308. **Li B, Boast S, de los SK, Schieren I, Quiroz M, Teitelbaum SL, Tondravi MM, Goff SP** 2000 Mice deficient in Abl are osteoporotic and have defects in osteoblast maturation. Nat Genet 24:304–308
309. **Pereira R, Khillan JS, Helminen HJ, Hume EL, Prockop DJ** 1993 Transgenic mice expressing a partially deleted gene for type I procollagen (COL1A1). A breeding line with a phenotype of spontaneous fractures and decreased bone collagen and mineral. J Clin Invest 91:709–716
310. **Bonadio J, Saunders TL, Tsai E, Goldstein SA, Morris-Wiman J, Brinkley L, Dolan DF, Altschuler RA, Hawkins Jr JE, Bateman JF, Mascara T, Jaenisch R** 1990 Transgenic mouse model of the mild dominant form of osteogenesis imperfecta. Proc Natl Acad Sci USA 87:7145–7149
311. **Forlino A, Porter FD, Lee EJ, Westphal H, Marini JC** 1999 Use of the Cre/lox recombination system to develop a non-lethal knock-in murine model for osteogenesis imperfecta with an α1(I) G349C substitution. Variability in phenotype in BrtlIV mice. J Biol Chem 274:37923–37931
312. **Chipman SD, Sweet HO, McBride Jr DJ, Davisson MT, Marks Jr SC, Shuldiner AR, Wenstrup RJ, Rowe DW, Shapiro JR** 1993 Defective pro α 2(I) collagen synthesis in a recessive mutation in mice: a model of human osteogenesis imperfecta. Proc Natl Acad Sci USA 90:1701–1705
313. **Levasseur R, Kato M, Patel MS, Chan L, Karsenty G** 2001 Low bone mass, low body weight and abnormal eye vascularization in mice deficient in Lrp5, the gene mutated in human osteoporosis pseudoglioma syndrome (OPS). J Bone Miner Res 16:S152 (Abstract)
314. **Geiser AG, Zeng QQ, Sato M, Helvering LM, Hirano T, Turner CH** 1998 Decreased bone mass and bone elasticity in mice lacking the transforming growth factor-β1 gene. Bone 23:87–93
315. **Aguirre J, Buttery L, O'Shaughnessy M, Afzal F, De Marticorena IF, Hukkanen M, Huang P, MacIntyre I, Polak J** 2001 Endothelial nitric oxide synthase gene-deficient mice demonstrate marked retardation in postnatal bone formation, reduced bone volume, and defects in osteoblast maturation and activity. Am J Pathol 158: 247–257
316. **Delany AM, Amling M, Priemel M, Howe C, Baron R, Canalis E** 2000 Osteopenia and decreased bone formation in osteonectin-deficient mice. J Clin Invest 105:915–923
317. **Xu T, Bianco P, Fisher LW, Longenecker G, Smith E, Goldstein S, Bonadio J, Boskey A, Heegaard AM, Sommer B, Satomura K, Dominguez P, Zhao C, Kulkarni AB, Robey PG, Young MF** 1998

Targeted disruption of the biglycan gene leads to an osteoporosis-like phenotype in mice. Nat Genet 20:78–82

318. **Holmbeck K, Bianco P, Caterina J, Yamada S, Kromer M, Kuznetsov SA, Mankani M, Robey PG, Poole AR, Pidoux I, Ward JM, Birkedal-Hansen H** 1999 MT1-MMP-deficient mice develop dwarfism, osteopenia, arthritis, and connective tissue disease due to inadequate collagen turnover. Cell 99:81–92
319. **Zhao G, Monier-Faugere MC, Langub MC, Geng Z, Nakayama T, Pike JW, Chernausek SD, Rosen CJ, Donahue LR, Malluche HH, Fagin JA, Clemens TL** 2000 Targeted overexpression of insulin-like growth factor I to osteoblasts of transgenic mice: increased trabecular bone volume without increased osteoblast proliferation. Endocrinology 141:2674–2682
320. **Filvaroff E, Erlebacher A, Ye JQ, Gitelman SE, Lotz J, Heillman M, Derynck R** 1999 Inhibition of TGF-β receptor signaling in osteoblasts leads to decreased bone remodeling and increased trabecular bone mass. Development 126:4267–4279
321. **Gardiner EM, Baldock PA, Thomas GP, Sims NA, Henderson NK, Hollis B, White CP, Sunn KL, Morrison NA, Walsh WR, Eisman JA** 2000 Increased formation and decreased resorption of bone in mice with elevated vitamin D receptor in mature cells of the osteoblastic lineage. FASEB J 14:1908–1916
322. **Ducy P, Amling M, Takeda S, Priemel M, Schilling AF, Beil FT, Shen JH, Vinson C, Rueger JM, Karsenty G** 2000 Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. Cell 100:197–207
323. **Chellaiah M, Kizer N, Silva M, Alvarez U, Kwiatkowski D, Hruska KA** 2000 Gelsolin deficiency blocks podosome assembly and produces increased bone mass and strength. J Cell Biol 148: 665–678
324. **Jochum W, David JP, Elliott C, Wutz A, Plenk Jr H, Matsuo K, Wagner EF** 2000 Increased bone formation and osteosclerosis in mice overexpressing the transcription factor Fra-l. Nat Med 6: 980–984
325. **Sabatakos G, Sims NA, Chen J, Aoki K, Kelz MB, Amling M, Bouali Y, Mukhopadhyay K, Ford K, Nestler EJ, Baron R** 2000 Overexpression of DeltaFosB transcription factor(s) increases bone formation and inhibits adipogenesis. Nat Med 6:985–990
326. **Naito A, Azuma S, Tanaka S, Miyazaki T, Takaki S, Takatsu K, Nakao K, Nakamura K, Katsuki M, Yamamoto T, Inoue J** 1999 Severe osteopetrosis, defective interleukin-1 signalling and lymph node organogenesis in TRAF6-deficient mice. Genes Cells 4: 353–362
327. **Lomaga MA, Yeh WC, Sarosi I, Duncan GS, Furlonger C, Ho A, Morony S, Capparelli C, Van G, Kaufman S, van der HA, Itie A, Wakeham A, Khoo W, Sasaki T, Cao Z, Penninger JM, Paige CJ, Lacey DL, Dunstan CR, Boyle WJ, Goeddel DV, Mak TW** 1999 TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling. Genes Dev 13:1015–1024
328. **Saftig P, Hunziker E, Wehmeyer O, Jones S, Boyde A, Rommerskirch W, Moritz JD, Schu P, Von Figura K** 1998 Impaired osteoclastic bone resorption leads to osteopetrosis in cathepsin-K-deficient mice. Proc Natl Acad Sci USA 95:13453–13458
329. **McHugh KP, Hodivala-Dilke K, Zheng MH, Namba N, Lam J, Novack D, Feng X, Ross FP, Hynes RO, Teitelbaum SL** 2000 Mice lacking β3 integrins are osteosclerotic because of dysfunctional osteoclasts. J Clin Invest 105:433–440
330. **Wang Z-Q, Ovitt C, Grigoriadis AE, Möhle-Steinlein U, Rüther U, Wagner EF** 1992 Bone and haematopoietic defects in mice lacking c-*fos*. Nature 360:741–745
331. **Li J, Sarosi I, Yan XQ, Morony S, Capparelli C, Tan HL, McCabe S, Elliott R, Scully S, Van G, Kaufman S, Juan SC, Sun Y, Tarpley J, Martin L, Christensen K, McCabe J, Kostenuik P, Hsu H, Fletcher F, Dunstan CR, Lacey DL, Boyle WJ** 2000 RANK is the intrinsic hematopoietic cell surface receptor that controls osteoclastogenesis and regulation of bone mass and calcium metabolism. Proc Natl Acad Sci USA 97:1566–1571
332. **Li YP, Chen W, Liang Y, Li E, Stashenko P** 1999 Atp6i-deficient mice exhibit severe osteopetrosis due to loss of osteoclast-mediated extracellular acidification. Nat Genet 23:447–451
333. **Iotsova V, Caamano J, Loy J, Yang Y, Lewin A, Bravo R** 1997 Osteopetrosis in mice lacking NF-κB1 and NF-κB2. Nat Med 3:1285–1289
334. **Soriano P, Montgomery C, Geske R, Bradley A** 1991 Targeted disruption of the c-*src* proto-oncogene leads to osteopetrosis in mice. Cell 64:693–702
335. **Yoshida H, Hayashi S, Kunisada T, Ogawa M, Nishikawa S, Okamura H, Sudo T, Shultz LD** 1990 The murine mutation osteopetrosis is in the coding region of the macrophage colony stimulating factor gene. Nature 345:442–444
336. **Tondravi MM, McKercher SR, Anderson K, Erdmann JM, Quiroz M, Maki R, Teitelbaum SL** 1997 Osteopetrosis in mice lacking haematopoietic transcription factor PU.1. Nature 386:81–84
337. **Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Lüthy R, Nguyen HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan HL, Trail G, Sullivan J, Davy E, Bucay N, Renshaw-Gegg L, Hughes TM, Hill D, Pattison W, Campbell P** 1997 Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 89:309–319
338. **Roth DE, Venta PJ, Tashian RE, Sly WS** 1992 Molecular basis of human carbonic anhydrase II deficiency. Proc Natl Acad Sci USA 89:1804–1808
339. **Orimo H, Hosoi T, Nakamura T, Ouchi Y, Shiraki M** 1990 Epidemiology of fractures in Asia. In: Christiansen C, Overgaard K, eds. Osteoporosis. Copenhagen: Osteopress; 55–61