Genetics of Osteoporosis

Stuart H. Ralston and André G. Uitterlinden

Rheumatic Diseases Unit Molecular Medicine Centre (S.H.R.), Institute of Genetics and Molecular Medicine, University of Edinburgh, Western General Hospital Edinburgh EH4 2XU, United Kingdom; and Department of Internal Medicine and Epidemiology (A.G.U.), Erasmus Medical Centre, 3075 EA Rotterdam, The Netherlands

Osteoporosis is a common disease with a strong genetic component characterized by reduced bone mass, defects in the microarchitecture of bone tissue, and an increased risk of fragility fractures. Twin and family studies have shown high heritability of bone mineral density (BMD) and other determinants of fracture risk such as ultrasound properties of bone, skeletal geometry, and bone turnover. Osteoporotic fractures also have a heritable component, but this reduces with age as environmental factors such as risk of falling come into play. Susceptibility to osteoporosis is governed by many different genetic variants and their interaction with environmental factors such as diet and exercise. Notable successes in identification of genes that regulate BMD have come from the study of rare Mendelian bone diseases characterized by major abnormalities of bone mass where variants of large effect size are operative. Genome-wide association studies have also identified common genetic variants of small effect size that contribute to regulation of BMD and fracture risk in the general population. In many cases, the loci and genes identified by these studies had not previously been suspected to play a role in bone metabolism. Although there has been extensive progress in identifying the genes and loci that contribute to the regulation of BMD and fracture over the past 15 yr, most of the genetic variants that regulate these phenotypes remain to be discovered. **(*Endocrine Reviews* 31: 629–662, 2010)**

I. Introduction

Osteoporosis is a common disease characterized by low bone mass and defects in the microarchitecture of bone tissue, which impairs bone strength and leads to an increased risk of fragility fractures (1). Osteoporosis is defined to exist when bone mineral density (BMD) values at the lumbar spine or hip fall at least 2.5 SD values below

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Abbreviations: BMD, Bone mineral density; BMP, bone morphogenic protein; CNV, copy number variant; GWAS, genome-wide association study/studies; HRT, hormone replacement therapy; LRP5, lipoprotein receptor-related protein 5; OPG, osteoprotegerin; QTL, quantitative trait loci; RANK, receptor activator of nuclear factor κB; RANKL, RANK ligand; SNP, single nucleotide polymorphism; TDT, transmission disequilibrium test.

the population average in young healthy individuals (BMD T-score of −2.5 or less). The term osteopenia is used to describe the situation whereby the BMD T-score is above −2.5, but below −1.0, whereas subjects with BMD T-score values of greater than −1.0 and less than +2.5 are said to have normal BMD. Although the risk of fracture increases with decreasing levels of BMD, it is important to note that many patients with osteoporosis do not go on to have a fracture and that most fractures in the general population occur in patients without osteoporosis (2). Many factors influence the risk of osteoporosis, including diet, physical activity, medication use, and coexisting diseases, but one of the most important clinical risk factors is a positive family history, emphasizing the importance of genetics in the pathogenesis of the disease (3, 4).

In this article, we first review the evidence for a genetic contribution to osteoporosis and related phenotypes. We then discuss the approaches that have been used to find the underlying genes and review the role that specific genetic variants play in regulating susceptibility to osteoporosis.

II. Regulation of Bone Mass and Bone Turnover

In recent years, several key regulators of bone resorption, bone formation, and bone mass that act in a paracrine or autocrine manner to regulate bone cell activity, under the control of circulating calcium-regulating hormones, have been identified. The relevance of this to the present review is that inherited variations (polymorphisms or mutations) in the genes that encode many of these factors have been implicated as genetic determinants of susceptibility to osteoporosis (see *Section V*). Bone resorption is primarily regulated by the receptor activator of nuclear factor κB (RANK) signaling pathway, which plays a central role in osteoclast differentiation and function (5). The RANK receptor is expressed on cells of the osteoclast lineage and is activated by RANK ligand (RANKL), which causes osteoclast activation by up-regulating nuclear factor κB and other intracellular signaling pathways. This process is blocked by osteoprotegerin (OPG), which acts as a decoy receptor for RANKL. Bone formation is regulated by many factors, including PTH, TGFβ, bone morphogenic proteins (BMPs), and the *Wnt* signaling pathway. Members of the *Wnt* family of proteins bind to and activate lipoprotein receptor-related protein 5 (LRP5) to regulate bone formation, bone resorption, and bone mass (6, 7). There are at least 19 *Wnt* family members, and it remains to be determined which are most important in regulating bone metabolism, but current evidence suggests that *Wnt7b* and *Wnt10b* are both involved (8). A variety of inhibitors of LRP5 signaling have also been identified, including soluble frizzled-related proteins (sFRP), Dickkopf1 (*Dkk1*), and sclerostin (*SOST*), and it is likely that regulation of bone formation depends on the balance between levels of the stimulatory *Wnt* molecules and levels of the inhibitors such as sFRP and *SOST*. Sclerostin is of particular interest because it is produced by osteocytes in response to mechanical loading and probably plays a key role in mechano-transduction (9). Recent research has highlighted the fact that neuronal pathways also play a role in regulating bone turnover. These include the sympathetic nervous system through production of catecholamines (10), nitric oxide (11), and the endocannabinoid system (12–14). In view of this, it can be appreciated that genetic variation in a very wide number of candidate genes might be expected to influence bone metabolism, including some that are not expressed in bone.

III. Pathogenesis of Fractures

The clinical and economic importance of osteoporosis lies in its association with fracture. Although the risk of fracture increases as BMD values fall, about two thirds of individuals who suffer a fracture do not have osteoporosis as defined on the basis of BMD values (2, 15). Going along with this, the age-related increase in fracture is largely independent of changes in BMD (16). The most probable reason for this is an increased risk of falling with aging due to factors such as reduced muscle power, postural instability, and reduced visual acuity. Other factors also affect the risk of fracture by mechanisms that are independent of BMD. For example, biochemical markers of bone turnover including the bone resorption markers, urinary C-telopeptide cross-links of collagen type I and free urinary deoxypyridinoline, and the bone formation marker undercarboxylated osteocalcin have been shown to predict fractures independently of BMD (17, 18). Similarly, various aspects of femoral neck geometry including hip axis length have also been shown to act as predictors of fracture, particularly hip fracture (19–21). Indeed, it has been suggested that differences in femoral neck geometry may explain, in part, differences in the rate of hip fractures between Caucasians and some other ethnic groups (22). In view of the above, it can be appreciated that fracture is a very complex phenotype that is quite challenging to address by genetic analysis.

IV. Heritability of Osteoporosis-Related Traits

A. Bone mineral density

Twin and family studies have shown that between 50 and 85% of the variance in peak BMD is genetically de-

termined (23–26). Studies in twins have generally yielded higher estimates for heritability than family-based studies (27–29) where individuals have been compared across generations (26, 30), presumably because of nongenetic influences on rates of bone loss. In most studies, heritability of BMD at axial sites such as the spine and hip has been higher than at the forearm (27, 28), but this has not always been the case (29).

B. Bone loss

Twin studies have confirmed that there is a heritable component to age-related bone loss, but the genetic contribution seems to be weaker than for peak bone mass. The largest study performed to date is that by Makovey *et al.* (31) who analyzed bone loss over a 5-yr period in 724 postmenopausal female twins. This showed that about 40% of the variance in bone loss at the wrist and lumbar spine was genetically determined, although no significant heritable component was found for bone loss at the femoral neck. This supports the results of an earlier study involving about 40 pairs of predominantly female twins where a significant genetic contribution to bone loss at the spine and Ward's triangle was identified, whereas bone loss at the femoral neck was found to be nonheritable (32). In another family-based study, Shaffer *et al.* (33) reported a significant heritable component to bone loss at the spine (heritability, 0.42), total hip (0.44), and distal radius (0.25) in Mexican-American families. Gender-specific analysis in this study revealed similar heritability estimates in women, but the small sample size precluded a meaningful analysis in men (33). One of the most important determinants of bone loss in women is estrogen deficiency after the menopause, and twin studies have indicated that age at menopause is genetically determined (34), providing further support for the concept that genetic factors play a role in determining bone loss, at least in women. In keeping with this, several genetic variants have been recently identified as being associated with age at menopause (35). Heritability of age-related bone loss in men has been studied relatively little, but in an analysis of 50 male twins, no evidence for a heritable component to bone loss at the wrist was observed over a 16-yr follow-up period (36).

C. Fracture

Conflicting results have been reported with regard to the heritability of fracture, which is not surprising given the complexity of the phenotype and the difficulty in collecting sufficiently powered datasets. A family history of fracture has been shown in several studies to be a risk factor for fractures independently of BMD (4, 37). In keeping with this, several investigators have reported that fracture may have a heritable component. For example, studies of postmenopausal women and their first-degree relatives from the United States (38) showed that the heritability of wrist fracture was about 25%, whereas similar studies in a cohort of female twins from the United Kingdom suggested that heritability of wrist fracture may be as much as 54% (39). Interestingly, the heritable component to wrist fracture in both of these studies seemed largely independent of BMD, suggesting that predisposition may have been mediated through genetic influences on other factors such as bone turnover, bone geometry, or nonskeletal factors such as cognition and neuromuscular control, which influence the risk of falling. In contrast to this work, however, another heritability study of elderly twins from Finland provided little evidence to suggest that fractures were heritable (40). These divergent results are probably explained by the fact that the heritability of fracture decreases with age as environmental factors become more important. This was elegantly demonstrated in a large study of Swedish twins that showed the heritability of hip fracture was high among those under the age of 65 (approximately 68%) but dropped off rapidly with age to reach a value of almost zero by the eighth decade (41).

D. Other phenotypes

Heritability studies have also shown evidence of significant genetic effects on other key determinants of osteoporotic fracture risk such as quantitative ultrasound properties of bone (28), femoral neck geometry (28), and composite bone phenotypes derived from geometrical variables such as bucking ratio and section modulus (42). Other osteoporosis-related traits that are heritable include muscle strength (43), body mass index (44), circulating levels of calciotropic hormones (45), and biochemical markers of bone turnover (45, 46). The largest and most comprehensive study of biochemical markers is that of Hunter *et al.* (45) who reported that the heritability of circulating levels of 1,25 dihydroxyvitamin D and PTH was 60 and 65%, respectively, compared with 74% for bone-specific alkaline phosphatase and 58% for urinary deoxypyridinoline/creatinine ratio.

V. Genetic Architecture of Osteoporosis

The genetic architecture of osteoporosis is typical of a complex disease with contributions from several genes, most of which have small effects, but a few of which have large effects as illustrated in Fig. 1. Before discussing details of the genetic architecture of osteoporosis, we will briefly discuss the different types of genetic variants that exist in the human genome. In simple terms, genetic variants can be divided into two broad classes, based on their

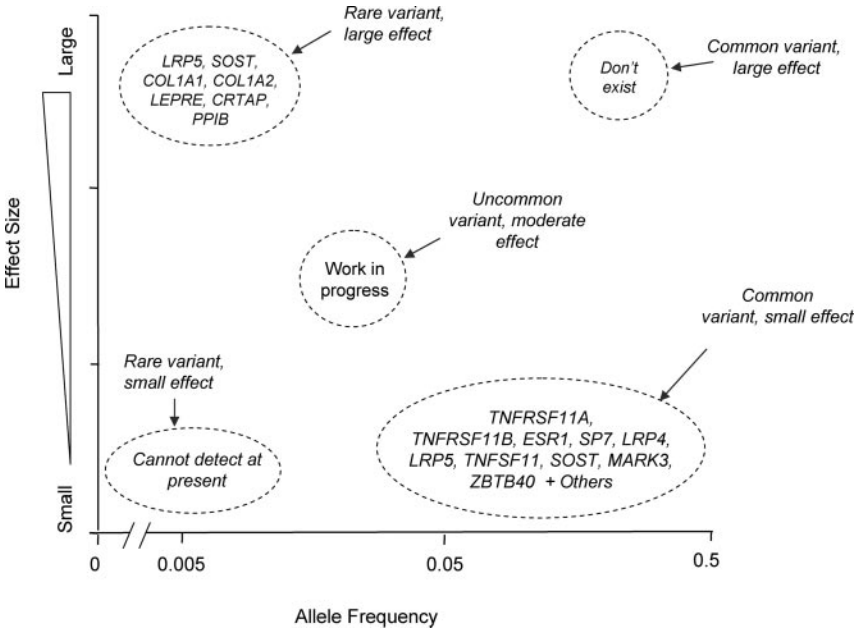

FIG. 1. Allelic architecture of susceptibility to osteoporosis. Alleles that are known to contribute to regulation of BMD and fracture comprise rare variants of large effect (*top left*) and common variants of small effect (*bottom right*). Common variants of large effect have not been identified and are unlikely to exist. Rare variants of small effect may exist but cannot be detected or validated at present. It is possible that uncommon variants of moderately large effect (*circle*) might contribute to osteoporosis, and this is an area of active investigation.

frequency in the general population and their functional effects on the target gene. The term "polymorphism" is used to describe common genetic variants that occur frequently (>1%) in the population. The most common type is a single nucleotide polymorphism (SNP) in which one nucleotide in DNA is substituted for another but deletions and duplications also occur. Another category of polymorphism is the variable number tandem repeat, which were previously used for linkage analysis in families. Deletions and duplications of large segments of DNA (typically 10 kb to 1 million bp) are also known to occur throughout the genome, and these are referred to as copy number variants (CNVs). Current estimates suggest that there are about 20 million polymorphisms in the human genome. Only a tiny fraction of these have so far been investigated to determine whether they have functional effects. Those polymorphisms that have been studied generally have been found to have modest effects on gene function either by altering the protein structure of the gene product or by altering gene expression. Although the resulting changes in expression or function of an individual gene are small, it is thought that common diseases like osteoporosis are attributable to a substantial extent to the combined effects of many hundreds to thousands of these polymorphisms. The term "mutation" is used to describe a rare genetic variant (frequency much less than 1%) that has a major effect on gene function. Most mutations directly affect the protein coding sequence of the target gene, causing profound changes in protein structure and function, but some act by regulating gene expression. Mutations typically cause monogenic "Mendelian" disorders that segregate in pedigrees according to a predictable pattern such as cystic fibrosis and osteogenesis imperfecta.

Segregation analysis in families has shown that regulation of BMD and other osteoporosis-related phenotypes is primarily determined by the effects of polymorphisms in multiple genes, each with relatively small effects, rather than the effects of mutations in a few genes (25). This notion is strongly supported by the findings of the recent genome-wide association studies (GWAS) where small effects from dozens of common variants in or near genes were observed. It has been suggested that in some populations and families, variants may exist that have larger effects, but these remain to be identified (47, 48). Severe osteoporosis, bone fragility, or abnormally high bone mass may also be inherited as the result of rare mutations in single genes. Single gene disorders of relevance to osteoporosis are discussed in more detail in *Section VI*, whereas the more common genetic variants are discussed in later sections.

VI. Single Gene Disorders of Relevance to Osteoporosis

Several rare diseases have been identified where profound effects on bone mass, bone fragility, and bone turnover occur as the result of mutations in single genes (Table 1). These diseases have provided important insights into the molecular pathways that regulate bone mass, bone cell function, and bone quality.

A. Osteogenesis imperfecta

Osteogenesis imperfecta is characterized by low bone mass and a marked increase in bone fragility. The disease is most often caused by mutations in the *COL1A1* and *COL1A2* genes (49), but recent work has shown that mutations in the *CRTAP*, *LEPRE*, and *PPIB* genes, which form a protein complex necessary for prolyl-3-hydroxylation of collagen, can cause recessive forms of osteogenesis imperfecta (50–52).

TABLE 1. Monogenic bone diseases associated with abnormal bone mass

Disease	Phenotype	Genes	Function
Osteogenesis imperfecta	Low BMD, fractures	*COL1A1*	Major protein of bone
		COL1A2	Major protein of bone
		CRTAP	Prolyl hydroxylation of collagen
		LEPRE	Prolyl hydroxylation of collagen
		PPIB	Prolyl hydroxylation of collagen
Osteopetrosis	High bone mass, fractures, bone marrow failure, blindness, osteoarthritis, osteomyelitis	*CLCN7*	Osteoclast chloride channel
		TCIRG1	Osteoclast proton pump
		CATK	Degrades bone matrix
		OSTM1	Vesicular trafficking
		RANKL	Essential for osteoclast differentiation
		RANK	Essential for osteoclast differentiation
High bone mass syndrome	High bone mass, torus palatinus	*LRP5*[a]	Increases bone formation and inhibits bone resorption by regulating OPG production by osteoblasts
Osteoporosis pseudoglioma syndrome	Low bone mass, fractures	*LRP5*[b]	Increases bone formation and inhibits bone resorption by regulating OPG production by osteoblasts
Sclerosteosis, van Buchem disease	High bone mass, bone overgrowth, nerve compression syndromes	*SOST*	Inhibits LRP5 signaling
Aromatase deficiency	Osteoporosis	*CYP17*	Converts androgens to estrogen in peripheral tissues
Estrogen receptor deficiency	Osteoporosis, tall stature	*ESR1*	Required for signal transduction by estrogen

[a] Gain of function mutations.

[b] Loss of function mutations.

B. Mendelian osteoporosis syndromes

The osteoporosis-pseudoglioma syndrome is a rare recessive disorder characterized by low bone mass and increased bone fragility that has been found to be caused by inactivating mutations in the *LRP5* gene (53, 54). Severe osteoporosis in males can also form part of the phenotype in patients with inactivating mutations in the *CYP17* gene encoding aromatase (55) and the *ESR1* gene encoding the estrogen receptor α (56), conditions that both illustrate the importance of estrogen in the regulation of bone mass in men.

C. High bone mass syndromes

Other rare syndromes have been described in which affected individuals have unusually high bone mass and are protected against osteoporotic fractures. Examples are the various autosomal dominant high bone mass syndromes associated with activating mutations in the *LRP5* gene (57–60) and the recessive syndromes of sclerosteosis and Van Buchem disease that are caused by inactivating mutations in the sclerostin (*SOST*) gene (61–64). Interestingly, individuals who are heterozygous for disease-causing mutations in *SOST* have elevated bone mass, indicating that some instances of unusually high BMD in the normal population may be due to heterozygosity for *SOST* mutations (65).

D. Osteopetrosis

Osteopetrosis is the name given to a group of syndromes characterized by failure of osteoclastic bone resorption. Osteopetrosis most often occurs as the result of defects in osteoclast function (osteoclast-rich osteopetrosis), but it can occasionally be caused by defects in osteoclast differentiation (osteoclast-poor osteopetrosis) (66). Osteoclast-poor osteopetrosis is caused in many cases by inactivating mutations in the *TNFRSF11A* gene that encodes RANK or the *TNFSF11* gene that encodes RANKL (67, 68). Many different gene mutations have been identified in osteoclast-rich osteopetrosis, all of which impair the ability of osteoclasts to resorb bone (66, 69).

E. Camurati-Engelmann disease

Camurati-Engelmann disease is a rare disorder characterized by increased bone turnover, bone pain, and osteosclerosis mainly affecting the diaphysis of long bones (70, 71). It is caused by mutations that cluster in the latency-associated peptide region of TGFβ1, which prevent or inhibit binding of latency-associated peptide to the mature TGFβ1 molecule (72). The effect of this is to increase levels of bioactive TGFβ1, which presumably is the cause of the increased bone turnover that is characteristic of the disease (73).

In all of the examples listed above, the consequences of the gene mutation on bone cell function or bone matrix are so profound as to overwhelm the effects of the many other genes that contribute to regulation of bone fragility and bone mass. Although the above disorders are caused by rare mutations with large effects, common polymorphic variations in some of these genes have also been described

that regulate BMD in the normal population, albeit with much smaller effects.

VII. Methods for Identifying Osteoporosis Susceptibility Genes

Several approaches have been used to identify the genes responsible for the more common form of osteoporosis, and the principles underlying these approaches are discussed below.

A. Linkage analysis

Linkage analysis is the classical approach for gene discovery in an inherited monogenic Mendelian human disease. There are two main subtypes of linkage analysis: parametric linkage analysis and nonparametric linkage.

Parametric linkage analysis involves specifying a model of inheritance for the disease within a family (such as dominant or recessive) and looking for evidence of segregation of the disease within a family according to that model. Linkage studies are usually carried out on a genome-wide basis, which classically involves genotyping between 400 and 800 microsatellite markers spread at 5- to 10-cM intervals across the genome. In recent years, however, higher density panels of SNP markers have become the preferred method for genome-wide linkage scans (74).

Nonparametric linkage has been more widely used for analysis of complex traits. In this case, no model of inheritance is specified except to assume that there will be sharing of inherited alleles in relation to sharing of the disease phenotype. For quantitative traits, variance component methods (75) or regression-based methods (76) can be employed to estimate the proportion of genetic covariance between relatives as a function of identity by descent relationships at a marker, assuming that the marker is tightly linked to the disease-causing mutation. Variance component methods of linkage analysis can be further broken down into "univariate" and "bivariate" subtypes. In univariate analysis, a single phenotype is analyzed at a time, and this is the approach that has been most widely used. In bivariate analysis, two related traits are examined simultaneously, such as BMD values at two different skeletal sites. Bivariate linkage analysis can theoretically increase power to detect linkage of related traits to a common quantitative trait loci (QTL) because it exploits the additional information contained in the correlation pattern between the two traits. In the studies published so far, however, bivariate analysis of different skeletal sites has yielded results broadly similar to those of univariate analysis (77). Bivariate linkage analysis has also been used to try and identify loci that underlie related traits such as BMD and obesity (78), but so far no genes have been identified through this route. The results of linkage studies are typically expressed as lodscores, which are defined as the logarithm of the odds that the disease locus and marker locus are linked. In the case of parametric analysis, linkage is considered significant when the lodscore is above +3.3, whereas linkage is considered to be "suggestive" when the lodscore is above +1.9. Conversely, linkage can be excluded when the lodscore is below −2.0. For nonparametric analysis, significant linkage is defined by a lodscore of above approximately +3.6 and suggestive linkage by a lodscore above +2.2 (79). It is not possible to exclude linkage by nonparametric analysis. The weakness of these approaches is that they rely on the presence of a single mutation of very strong effect causing the disease (highly penetrant variants). Although linkage analysis has been very successful in identifying gene mutations underlying monogenic bone diseases, it has largely failed to identify genes involved in common forms of osteoporosis as seen in the general population.

Linkage studies in animal models provide another possible way of identifying genes that regulate BMD and other phenotypes relevant to the pathogenesis of osteoporosis. These approaches rely on the assumption that at least some of the genes that regulate BMD in animals will be the same as those in humans. Animal studies in the osteoporosis field have mostly involved crossing inbred laboratory strains of mice with low and high bone density. By interbreeding offspring from the first generation (F1), a second generation (F2) of mice can be established with varying levels of BMD because of segregation of the alleles that regulate BMD in the F2 offspring. A genome-wide search is then performed in the F2 generation and inheritance of alleles related to levels of BMD in the offspring. There are several advantages of these studies; environment can be carefully controlled, thus minimizing the influence of confounding factors, and large numbers of progeny can be generated, giving excellent statistical power. Fine mapping of loci identified is challenging but can be achieved by backcrossing mice that inherit a locus for regulation of BMD into the background strain and selecting offspring that retain the phenotype. However, this can be a time-consuming process because the loci identified by linkage studies in inbred strains of mice are large (20–40 cM), and many generations of backcrossing need to be performed to narrow the critical interval to manageable proportions. To circumvent this problem, other strategies have been proposed, such as performing genetic mapping in outbred mice of known ancestry (80). This takes advantage of the more limited linkage disequilibrium that exists in outbred strains to immediately obtain a narrow region of interest. The above-mentioned approach has been successfully applied to several quantitative traits (81) but has not yet

been applied to the field of bone disease. Another approach involves the use of *in silico* analysis to identify genetic differences between different mouse strains and relate these to phenotypic differences with the aim of uncovering candidate loci responsible for the phenotype under study (82).

B. Candidate gene association studies

Candidate gene association studies have been widely used in the field of osteoporosis and in the genetics of other complex diseases. They involve analyzing polymorphic variants in candidate genes with a role in bone biology and relating carriage of a specific allele (or haplotype) to a quantitative trait or disease of interest. In addition to studying single candidate genes, some investigators have employed "pathway" analysis in which several candidate genes in a signaling pathway are studied simultaneously (83, 84).

Case-control study designs are used for categorical traits such as fracture where allele frequencies are compared in the two groups. For quantitative traits such as BMD, the mean values are calculated according to genotype or allele at the chosen polymorphic site, and differences are assessed by ANOVA, usually with inclusion of confounding factors in the statistical model (such as age, body weight, and menopausal status). Association studies are straightforward in design and relatively easy to perform and can be powered to detect small effects of alleles. However, when executed carelessly, they are prone to give spurious results, due to factors such as small sample size, lack of standardized phenotyping and genotyping, and population stratification when insufficient care has been paid to matching cases and controls. Another drawback of the candidate gene association studies performed so far has been the fact that only a very limited number of variants have been assessed across a gene of interest. However, we now know that most genes contain hundreds of common polymorphisms as well as many rare variants. Because it is unknown *a priori* which of these is most likely to be involved in osteoporosis, it is important that analysis of candidate genes should be as comprehensive as possible. Until recently, this was challenging, but the prospects for comprehensive coverage of candidate genes have improved with advances in genotyping techniques.

The transmission disequilibrium test (TDT) is a special type of association study performed in related individuals, which is less susceptible to confounding than a standard association study. Before the introduction of GWAS, this technique was widely used to confirm the results obtained from population-based association studies (85). The TDT tests the hypothesis that a polymorphism or allele contributes to disease by analyzing the frequency with which affected individuals inherit the allele from a heterozygous parent. If the allele contributes to the trait or disease of interest, then the probability that an affected person has inherited the allele from a heterozygous parent should vary from the expected Mendelian ratio of 50:50. Because the transmitted allele acts as the "case" and the nontransmitted allele acts as the "control," the TDT is unaffected by confounding due to population stratification. Although TDT is a valuable technique, one important disadvantage is that only heterozygous individuals are informative, which can reduce the effective sample size available for study and limit statistical power.

Most of the problems of candidate gene association studies can be circumvented by careful study design, including the assembly of cohorts of adequate sample size and statistical correction for confounding factors (86). Many of these issues are being addressed by the creation of large consortia to address the genetic contribution to various complex diseases (87). For example, within the osteoporosis field, the GENOMOS (www.genomos.eu) and GEFOS (www.gefos.org) consortia have been established to address the role of common genetic variants in the pathogenesis of osteoporosis. The GENOMOS consortium has focused on testing known candidate gene polymorphisms in a large-scale setting involving approximately 45,000 subjects, whereas the GEFOS consortium focuses on performing meta-analysis of GWAS datasets from about 20,000 subjects.

C. Genome-wide association studies (GWAS)

Advances in genotyping technologies have now made it possible to perform association studies on a genome-wide basis by genotyping large numbers (100,000 to 1,000,000) of SNPs spread at close intervals across the genome, rather than focusing on a specific candidate gene. GWAS have been successfully applied to the study of many complex diseases and in less than 3 yr have identified more than 500 loci that predispose to several diseases and quantitative traits (see www.genome.gov/gwastudies), including osteoporosis (88–93). A major advantage of GWAS over candidate gene studies is that they offer the possibility of ranking the importance of several association signals across the genome and of identifying novel pathways that contribute to the trait that is being studied. Disadvantages include the fact that currently available marker sets are designed to identify common alleles and are not well suited to study the effects of rare polymorphisms (<1–5% population frequency) within a gene of interest.

The resulting dataset also allows one to assess, in a comprehensive way, common variants across a large number of candidate genes. The statistical thresholds for significance in GWAS are stringent ($P < \sim 1 \times 10^{-7}$ or $< 5 \times 10^{-8}$ when using imputed data) due to the large number of

tests performed. In view of this, many polymorphisms that truly contribute to a trait but with a small effect size may be missed by individual GWAS, particularly if the size of the discovery sample is limited. To circumvent this problem, researchers are trying to increase power by combining results from different GWAS (see *Section VII.E*) and exploring the approach of entering SNPs that are below the threshold of genome-wide significance, yet above the threshold likely for false-positive results, into statistical models to determine whether these can enhance prediction of phenotypes of interest (94).

D. Genome-wide sequencing

Sequencing technology has now advanced to a stage where it is possible to generate a complete catalog of all variants present within a given DNA sequence rather than having to rely on markers and patterns of linkage disequilibrium. These sequencing techniques are currently being used for analysis of selected areas such as candidate loci that have emerged from GWAS. It is likely that these techniques will soon provide the complete human genome sequence in large collections of samples, and this forms the basis of the 1000 genomes project (www.1000genomes.org). The aim of this project is to fully sequence the genome of 1000 individuals and to use this information as the basis for inferring (imputing) genetic variants in subjects who have been genotyped for a less dense set of markers. This will result in a second surge of genetic association studies generating comprehensive collections of sequence variations, both common and rare, including *de novo* events in individuals.

E. Meta-analysis

The technique of meta-analysis is increasingly being used in the field of osteoporosis genetics (95–97). Meta-analysis can be done retrospectively (based on published studies) or prospectively (with new and unpublished data). Retrospective meta-analysis involves combining data from several different published studies to enhance sample size and obtain a more accurate estimate of the effect size of individual genetic variants than can be achieved by analysis of single studies. It is applicable to a variety of study designs, from family-based linkage studies and population-based association studies to genome-wide linkage scans and GWAS. By combining relevant evidence from many studies, statistical power is increased and more precise estimates of effect size can be obtained than is possible with single studies. Prospective meta-analysis seeks the same increase in power by combining datasets, but it uses unpublished datasets in which *de novo* genotyping has been performed. This approach is more robust than retrospective meta-analysis because it circumvents the problem of publication bias that can inflate the estimates derived from retrospective meta-analysis. A limitation of meta-analysis is that there is an assumption that the effect and direction of effect for a given genetic variant are the same in all groups included in the meta-analysis. This seems to hold true for the most part, but some instances have been recorded among complex traits where a susceptibility allele in one population is a protective allele in another (98).

F. Functional studies

When an allelic association has been identified and replicated, the next step is to try and define the mechanisms that underlie the association. For Mendelian diseases, functional analyses are usually straightforward because the causal mutation(s) can easily be identified since they segregate with the disease in families and usually have a major effect on the protein coding region of the gene. The effects of the mutation on function of the target protein can then be defined by *in vitro* studies of the abnormal protein or by generating an animal model in which the disease-causing mutation has been knocked into the germ line of a model organism. It is much more difficult to define functional mechanisms for alleles of small effect, partly because the causal variant is difficult to identify. It is possible to gain insights into the mechanisms by which alleles of small effect regulate phenotype, however, by performing a deletion of the gene in question in an animal model. This was highly successful in the case of the *FTO* gene that was initially identified as susceptibility gene for type 2 diabetes but was then found to act by regulating body weight (99). At the time of its original discovery, the function of *FTO* was unknown, but targeted deletion of the gene in mice demonstrated that it protected against obesity by affecting energy homeostasis (100). Similar experiments can now be contemplated for the novel genes that have emerged as determinants of osteoporosis through GWAS. Susceptibility alleles for common diseases usually do not have a large enough effect to determine whether they segregate with the trait of interest in families. Furthermore, alleles associated with complex traits usually cluster together with a large number of related variants that are in linkage disequilibrium and that also could be responsible for the effects observed. Approaches that can be used to identify the causal variants are summarized in Table 2.

VIII. Human Linkage Studies

Most linkage studies in the field of osteoporosis have focused on BMD as the phenotypic trait of interest, but other phenotypes have also been investigated including femoral neck geometry (101–104), ultrasound properties of bone

TABLE 2. Approaches to identify causal variants in genetic association studies

1) Bioinformatic studies to identify:
Transcription factor binding sites
MicroRNA coding sites
Conservation across species
Protein coding changes
Alterations in splicing
2) Refinement of linkage disequilibrium blocks by studies in different ethnic groups
3) EMSAs and promoter-reporter assays
4) Cell biology-based studies:
Cell culture from subjects of different genotype
Expression of different variants *in vitro*
"Knock-in" or ethylnitrosourea-based studies of model organisms with variant alleles
5) Studies on the effect of alleles on gene expression *in vivo*:
Levels of mRNA expression
Allele-specific transcription in heterozygotes

(105, 106), and bone loss (33). In one study, a composite phenotype was also investigated in which patients were categorized as "affected" on the basis that they had BMD below a certain cutoff or were being treated with antiosteoporosis medications or had suffered a fracture (107). Given what we now know about the genetic architecture of osteoporosis and the low power of linkage analysis to detect common variants of small effect size, it is not surprising that linkage studies have met with little success in identifying osteoporosis susceptibility genes. Linkage studies are no longer being widely pursued for the study of osteoporosis, but family-based studies might become useful again with the advent of high throughout sequencing technology to identify the effect of rare variants if they have sufficient penetrance and to study parent-of-origin effects.

Several genome-wide linkage scans in humans have detected loci that exceeded the threshold for genome-wide significance for BMD, but there has been limited replication between studies (108–111). For example, a meta-analysis of nine genome-wide scans performed up until 2006 involving over 11,842 subjects failed to detect evidence of genome-wide significance for any locus (97). This mirrors experience in other complex traits and diseases (112) and probably reflects the fact that genes which regulate BMD have modest effects that are difficult to detect reproducibly by conventional linkage analysis. Indeed, to date only one candidate gene for osteoporosis has been detected by genome-wide linkage scan. This is *BMP2*, which was identified as a susceptibility gene for osteoporosis in the population isolate of Iceland (107). The investigators identified a nonsynonymous serine to alanine coding change at codon 37 in BMP2 that was associated with osteoporosis in the Icelandic and Danish populations (107). However, this gene was already known from studies of bone biology, and the association could not be replicated in a large and well-powered study in the Dutch population (113).

Several genome-wide linkage scans in humans have been performed to detect loci that regulate femoral neck geometry. Significant evidence of linkage to some chromosomal regions has been detected, but as in the case of BMD there has been limited replication of peaks between studies (101, 104, 114) and gender-specific effects have been observed (101). Two genome-wide scans have been carried out in relation to ultrasound properties of bone, with differing results (105, 106). Neither study detected QTL that reached genome-wide significance, although several suggestive linkage peaks were detected. One genome-wide scan for bone loss has been performed, and this showed that change in femoral neck BMD in young Mexican-American families was significantly linked to a locus on chromosome 1q23 (115). This finding has not yet been replicated in other cohorts.

Although several examples of gender-specific, age-specific, and site-specific effects have been reported in human linkage studies, stratified analyses such as these need to be interpreted with caution because they can yield false-positive results due to multiple testing artifacts.

IX. Linkage Studies in Model Organisms

Linkage studies in mice (116, 117), rats (118), and primates (119) have resulted in the identification of several QTL that regulate BMD. Linkage analysis has also been used to localize QTL for other osteoporosis-related phenotypes such as bone structure, bone shape, bone strength (120, 121) and circulating levels of IGF-I (122). Loci for regulation of BMD have now been identified on almost all mouse chromosomes (http://www.informatics.jax.org) and almost all rat chromosomes (http://rgd.mcw.edu/). In some cases, there has been replication of QTL across different strains, and replication of some human BMD QTL (118). These studies have also shown that the genes which regulate BMD in mice have effects that are site-specific and gender-specific (116, 123).

The first notable success to emerge from linkage studies in mice was the identification of the *alox15* gene as a regulator of bone mass. This gene, located on mouse chromosome 11, was identified by Klein *et al.* (124) by linkage in a cross of DBA/2 and C57BL/6 mice. Although the chromosome 11 linkage peak was very large, microarray analysis showed that the parental DBA2 strain of mice (low BMD) had 20-fold increased expression of the *Alox15* mRNA when compared with C57BL/6 (high BMD) mice. From this observation, the authors suspected that *Alox15* might act as a negative regulator of bone mass and confirmed this hypothesis by finding that *Alox15* knockout

mice had increased BMD and that inhibition of *Alox15* protected against ovariectomy-induced bone loss. The mechanism by which *Alox15* reduces BMD is unclear, but the gene encodes a lipoxygenase enzyme that converts arachidonic and linoleic acids into ligands for the transcription factor peroxisome proliferator-activated receptor γ, which is thought to regulate differentiation of mesenchymal cells into adipocytes and osteoblasts. A recent association study showed that genetic variation in *Alox12*, the human homolog of *Alox15*, was associated with spine BMD in humans (125).

A second gene to be identified as a regulator of bone mass through linkage studies in mice is that encoding the duffy antigen receptor for chemokines (*Darc*). Edderkaoui *et al.* (126) showed that the *Darc* gene lay in a BMD QTL identified on mouse chromosome 1 in a cross of Cast CAST/EiJ and C57BL/6 mice. Congenic mice carrying the chromosomal segment containing *Darc* from the CAST/EiJ strain were found to have high BMD, and mice with targeted inactivation of *Darc* were found to have low BMD, confirming the importance of *Darc* as a regulator of bone mass. Further studies showed that *Darc* mRNA expression was 6-fold increased in the congenic mice carrying the CAST/EiJ chromosomal segment containing *Darc*. Several SNPs were identified within *Darc* that differed between the two background strains, and six of these were conserved in the CAST/EiJ strain and another high bone mass mouse strain (C3H). Bioinfomatic analysis revealed that one of these SNPs, encoding a glycine or arginine at codon 65, was predicted to reduce the ability of the CAST/EiJ isoform of *Darc* to bind chemokines. This was confirmed experimentally using bone marrow cells from the congenic mice that bound several chemokines less well than C57BL/6 marrow cells. Finally, bone marrow cells cultured from *Darc* knockout mice and congenic mice carrying the CAST/EiJ chromosomal segment containing *Darc* had a reduced capacity to differentiate into osteoclasts as compared with control mice. Taken together, these data provide convincing evidence that *Darc* regulates bone mass probably by modulating chemokine-induced osteoclast formation.

Two loci derived from animal studies have been found to be associated with BMD in human studies. One is a locus on chromosome X that was linked to postmaturity change of BMD in mice (127). Synteny mapping of this locus in humans using a DNA pooling strategy showed evidence of an association between two polymorphisms in the *PIRIN* gene and lumbar spine BMD (127), although this has not yet been replicated in other populations. A second was identified through *in silico* genome-wide haplotype association mapping in 30 inbred strains of mice (128). Among 22 different regions identified as being associated with BMD, the investigators focused on a region of mouse chromosome 4 containing the *Cer1* gene, which is a cysteine knot protein that acts as an antagonist of BMP signaling. They found that a methionine to isoleucine polymorphism at codon 232 of *Cer1* was associated with BMD in mice and that two noncoding polymorphisms of the human *CER1* gene were associated with BMD and vertebral fracture in southern Chinese women. This result was of borderline significance, however, and this finding has not yet been replicated in other populations.

X. Candidate Genes and GWAS for Osteoporosis

Over the past decade, approximately 150 candidate genes have been investigated in at least one study for their relationship with BMD or fractures in human population studies [details of these genes can be found on the HUgeNet web site (http://www.hugenavigator.net/)]. Most have been investigated in less than five studies, and most individual studies have been underpowered. Accordingly, the results of the vast majority of the candidate gene studies performed to date must be treated with great caution, given what we know about the true effect size of common variants on phenotypes like BMD and fracture. In a comprehensive candidate gene study, Richards *et al.* (129) systematically evaluated common genetic variants in 150 genes previously implicated in the pathogenesis of osteoporosis in a cohort of about 19,000 individuals where GWAS data were available within the framework of the GEFOS consortium (www.gefos.org). Here, SNPs within the gene of interest and in the 200 kb of flanking sequence on either side were analyzed. Only nine of the 150 genes analyzed were found to be significantly associated with BMD. These included SNPs within or close to the *ITGA1*, *LRP5*, *SOST*, *SPP1*, *TNFRSF11A*, *TNFRSF11B*, and *TNFSF11* genes, but for most candidate genes there were no significant associations. The effect size for SNPs that were associated with BMD was small, ranging from 0.04 to 0.18 SD change in BMD per allele. Variants within or close to the *LRP5*, *SOST*, *OPN*, and *TNFRSF11A* genes were also significantly associated with fracture risk, with odds ratios ranging between 1.13 and 1.43 per allele. The association with fracture remained significant after correction for BMD for the *OPN* and *SOST* loci. This indicates that susceptibility to fracture for these genes might be mediated by effects on bone quality or other BMD-independent predictors of fracture.

It should be noted, however, that the absence of a signal in such a study does not fully exclude a candidate gene from involvement because the efficiency by which potentially causal polymorphisms are captured by GWAS varies, and these were not studied in detail for all genes. For

example, the Sp1 binding site polymorphism of *COL1A1* (rs1800012), which has been extensively studied in osteoporosis (130), has no validated proxy in HapMap and could not be analyzed in this study. The same might apply to SNPs in other genes that have previously been studied in relation to osteoporosis. In the following section, therefore, discussion will be restricted to candidate genes where the association with BMD or fracture reached genome-wide significance and those that have been investigated in large-scale studies involving more than 5000 participants. At the time of this writing, six GWAS and one meta-analysis of GWAS have been carried out to try and identify genes and loci that predispose to osteoporosis. Details of these are summarized in Table 3. The genes and loci that have been identified as being significantly associated with osteoporosis with *P* values exceeding the threshold for genome-wide significance for BMD are summarized in Table 4, including some summary statistics. About half of the loci identified contain genes that were not previously known to play a role in bone metabolism. In addition, site-specific analysis showed that roughly one third of the loci had genome-wide significant effects at both spine and hip, indicating that the causal genetic variants have generalized effects on bone mass. Although gender-specific effects were not observed, there was limited power to detect such effects due to the relatively small number of men studied. For several loci, there was evidence of an association with fracture, but none of these associations attained genome-wide significance.

A. Loci and genes with significant evidence for association with BMD

The genes and loci that have attained genome-wide significant evidence for association with BMD are discussed in alphabetical order below.

1. ADAMTS18

The *ADAMTS18* gene was identified as a candidate for osteoporosis susceptibility by a GWAS performed by Xiong *et al.* (92). In this study, several SNPs within the *ADAMTS18* locus were identified that were suggestively associated with BMD in Caucasian subjects, but none reached the threshold for genome-wide significance. Three of these SNPs (rs16945612, rs11859065, and rs11864477) were studied for evidence of association with BMD in other cohorts of subjects from the United States, China, and Tobago, and the threshold for genome-wide significance was attained. One of these SNPs (rs16945612) was found to generate a binding site for the transcription factor TEL2. EMSAs confirmed that oligonucleotides containing the T allele of rs16945612 bound TEL2, whereas the C allele did not. The authors speculated that this might down-regulate *ADAMTS18* expression, but effects on transcription were not studied. The *ADAMTS18* gene is one of a large family of genes containing disintegrin and metalloprotease domains with thrombospondin motifs and has been suggested to be a tumor suppressor (131). Its role in bone metabolism is unclear at present.

2. CRHR1

The *CRHR1* gene, which encodes corticotropin-releasing factor receptor, emerged as a candidate for regulation of BMD by the GEFOS meta-analysis (96). The rs9303521 SNP, located about 56 kb from the gene on chromosome 17q21, was significantly associated with spine BMD. Corticotropin-releasing factor plays an important role in regulating ACTH release from the pituitary, and this in turn is involved in regulating cortisol release from the adrenal glands. Although glucocorticoids have important effects on bone turnover, further studies will be required to determine the mechanisms by which polymorphisms in the region regulate BMD.

3. CTNNB1

The *CTNNB1* gene encodes β-catenin, which is a transcription factor that plays a key role in osteoblast differentiation from mesenchymal stem cells. A polymorphism situated about 100 kb upstream of this gene was found to be significantly associated with femoral neck BMD by the GEFOS meta-analysis (96). β-Catenin is an extremely good candidate for BMD regulation, given that deletion of the gene in osteoblasts results in osteopenia, and stabilization results in high bone mass (7). The mechanisms by which genetic variation at the *CTNNB1* locus regulates BMD in humans remain to be explored.

4. DCDC5 and DCDC1

The *DCDC5* and *DCDC1* genes, situated on chromosome 11, emerged as possible candidates for regulation of lumbar spine BMD by the GEFOS meta-analysis (96). This showed an association with the rs16921914 located 62 kb downstream of the *doublecortin domain containing 1* (*DCDC1*) and 73 kb upstream of the *DCDC5* gene. Doublecortin domains are found in a wide variety of genes and are involved in mediating protein-protein interactions (132). Genes that contain these domains are highly expressed in the central nervous system, and mutations in some members of this gene family have been associated with neurological disorders. The genes do not appear to be highly expressed in bone, and the mechanisms by which these genes might regulate BMD remain unclear at present.

5. ESR1

Estrogen, by interacting with its receptors in bone and other tissues, plays an important role in regulating skeletal

TABLE 3. Characteristics of GWAS for osteoporosis

Study	FOS	TwinsUK	deCODE 1	deCODE 2	Caucasian USA	KARE	GEFOS
Reference	91	88	90	89	92	93	96
In GEFOS study?	Yes	Yes	Yes	Yes	No	No	
Platform	Affymetrix 100K	Illumina 317K	Illumina 317K	Illumina 317K	Affymetrix 500K	Affymetrix 500K	Illumina 317K & 550K; Affymetrix 550K
Bone phenotyping	DEXA	DEXA	DEXA	DEXA	DEXA	Ultrasound	DEXA
Discovery sample	Caucasians USA (n = 1,141)	Caucasian Twins (n = 2,094)	Caucasian Icelanders (n = 5,861)	Caucasian Icelanders (n = 6,865)	Caucasians USA (n = 1,000)	Korean Asians (n = 8,842)	RS (n = 4,987), ERF (n = 1,228), TwinsUK (n = 2,734), deCODE (n = 6,743), FOS (n = 3,503)
Replication cohorts	N/A	RS (n = 4,081), British Twins (n = 1,692), British (n = 718)	Icelanders (n = 4,165), Danes (n = 2,269), Australians (n = 1,491)	Icelanders (n = 3,135), Danish (n = 3,884), Australian (n = 1,491)	Caucasians USA (n = 1,972), FOS (n = 2,953), Chinese (n = 1437), Chinese, hip fractures (n = 700), Tobagans (n = 908)	Korean Asians (n = 7,861)	N/A
Total sample	1,141	8,585	13,786	15,375	5,925	16,703	19,125
Genome-wide significant loci	0	2	5	4	1	2	20
Genes associated with fracture		*LRP5*	*ZFBT40, MHc, TNFRSF11B*	*SOST, MARK3, TNFRSF11A*	*ADAMTS18, TGFB3*		Allelic risk score for fracture generated from BMD loci
Explained variance BMD	N/A	0.6–1.0%	3%	4%	1.2–3.8%	N/A	1.9–2.9%

FOS, Framingham Osteoporosis Study; KARE, Korean Association Resource study; ERF, Erasmus Rucphen Family Study; RS, Rotterdam study; DEXA, dual-energy x-ray absorptiometry; N/A, Not applicable.

TABLE 4. Genes and loci with genome-wide significant evidence for association with BMD

No.	Gene(s)	Locus	Novel[a]	Spine BMD	Hip BMD	Fracture[b]	Mode of identification[c]
1	*ADAMTS18*	16q23.1	Yes	−	+	+	
2	*CRHR1*	17q21	Yes	+	−	−	GWAS meta-analysis
3	*CTNNB1*	3p22	No	−	+	−	GWAS meta-analysis
4	*DCDC1/DCDC5*	11p14.1	Yes	+	−	−	GWAS meta-analysis
5	*ESR1*	6q25	No	+	+	+	GWAS
6	*FLJ42280*	7q21.3	Yes	+	+	−	GWAS meta-analysis
7	*FOXL1/FOXC2*	16q24	No	+	−	−	GWAS meta-analysis
8	*GPR177*	1p31.3	Yes	+	+	−	GWAS meta-analysis
9	*HDAC5*	17q21	Yes	−	+	−	GWAS meta-analysis
10	*MARK3*	14q32	Yes	−	+	−	GWAS
11	*MEF2C*	5q14	No	−	+	−	GWAS meta-analysis
12	*LRP4/ARHGAP1/F2*	11p11.2	Yes	−	+	+	GWAS
13	*LRP5*	11q13.4	No	+	+	−	Candidate gene; GWAS; GWAS meta-analysis
14	*MEPE/IBSP/OPN*	4q21.1	No	+	−	+	GWAS meta-analysis
15	*MHC*	6p21	Yes	+	−	+	GWAS
16	*SOST*	17q21	No	−	−	+	GWAS
17	*SOX6*	11p15	Yes	−	+	−	GWAS meta-analysis
18	*SPTBN1*	2p16	Yes	+	−	+	GWAS meta-analysis
19	*SP7*	12q13	No	+	−	−	GWAS
20	*STARD3NL*	7p14	Yes	+	−	−	GWAS meta-analysis
21	*TNFRS11B*	8q24	No	+	+	+	GWAS
22	*TNFRS11A*	18q21	No	+	+	+	GWAS
23	*TNFSF11*	13q14	No	−	+	−	GWAS meta-analysis
24	*ZBTB40*	1p36	Yes	+	+	+	GWAS
	Total	24	12 (50%)	15 (63%)	15 (63%)	10 (42%)	

[a] Not previously known to play a role in bone metabolism.

[b] None of the genes shown demonstrate genome-significant evidence for an association with fracture.

[c] Primary route through which genome-wide significant association with BMD was attained.

growth and maintaining bone mass. The estrogen receptor type 1 gene (*ESR1*) is therefore a strong candidate for the genetic regulation of bone mass. The first report of an association between *ESR1* alleles and osteoporosis was by Sano *et al.* (133), who found a positive association between a TA repeat in the *ESR1* promoter and bone mass in a small study of Japanese women. Similar results were reported by groups in the United States and Italy (134, 135). Subsequently, other investigators reported positive associations between haplotypes defined by *Pvu*II and/or *Xba*I polymorphisms in the first intron of the *ESR1* gene and bone mass (134, 136–139) as well as age at menopause (140). In contrast, other studies in Korean (141), Belgian (142), and Italian (143) women found no association between the *Pvu*II polymorphism and bone mass.

Polymorphisms of *ESR1* have also been studied in relation to postmenopausal bone loss. In a longitudinal study of 322 Finnish women, increased rates of early postmenopausal bone loss were observed in women who carried the "P" allele at the *ESR1 Pvu*II polymorphism (144), but this was not confirmed by another study in the United States (145). In contrast, a relatively large-scale study involving 3054 women in the United Kingdom showed higher rates of bone loss, lower femoral neck BMD in postmenopausal women, and reduced calcaneal broadband ultrasound attenuation in women who carried the "px" haplotype (146).

A retrospective meta-analysis of published association studies performed up until 2002 involving 5834 participants showed no evidence of an association between BMD and fracture for the *Pvu*II polymorphism, but a positive association between BMD and fracture for the *Xba*I polymorphism, with a protective effect of the XX genotype (147). A prospective meta-analysis from the GENOMOS study involving 18,917 individuals showed no association between the TA repeat, *Pvu*II or *Xba*I polymorphism, and BMD, but a significant association between the *Pvu*II and *Xba*I polymorphisms and fracture was observed, which was independent of BMD (148).

The association between *ESR1* alleles and osteoporosis was confirmed by the deCODE GWAS, which showed a significant association with BMD and fracture (89, 90). Although *ESR1* did not emerge as a significant determinant of BMD in other individual GWAS (88, 91, 92), it has been confirmed to be a significant determinant of BMD by the GEFOS meta-analysis (96). This suggests that there is a small effect of *ESR1* promoter variation on BMD and/or fracture risk. Interestingly, GWAS of other phenotypes, such as breast cancer risk and height, have also found significant signals at the *ESR1* locus consistent with the

pleiotropic effects of this nuclear receptor on many physiological processes (149).

The molecular mechanism by which *ESR1* polymorphisms influence BMD and fracture are unclear, but there is evidence that the intronic polymorphisms may affect gene transcription. For example, the *Pvu*II polymorphism lies within consensus recognition sites for the AP4 and Myb transcription factors (139, 150), and promoter-reporter assays have shown that the *Pvu*II polymorphism influences Myb-driven transcription *in vitro* (150). Other studies have suggested that the *Xba*I and *Pvu*II polymorphisms influence reporter gene transcription *in vitro* (151). In this regard, it is of interest that the *Pvu*II and *Xba*I polymorphisms are located within a region that is 70–80% conserved in the human, mouse, and rat genomes, whereas the TA repeat polymorphism is not conserved to any significant extent across species, suggesting that the intron plays a role in regulating *ESR1* function.

6. FLJ42280

The *FLJ42280* gene encodes a hypothetical protein of unknown function, and several SNPs within this region on chromosome 7 were found to be significantly associated with both spine and hip BMD in the GEFOS meta-analysis (96). Although the most significant SNPs were closest to *FLJ42280*, there are several other genes within this region in a linkage disequilibrium block of about 480 kb, and it is unclear whether *FLJ42280* or other genes are responsible for the associations observed.

7. FOXC2 and FOXL1

The *FOXC2* and *FOXL1* genes were identified as possible determinants of spine BMD by the GEFOS meta-analysis, which showed that SNPs about 95 kb distant from these genes were associated with spine BMD (96). Mutations in *FOXC2* have been reported in the lymphedema-distichaisis syndrome, a disorder characterized by lymphedema of the limbs coupled with various other features (152). However, *FOXC2* has also been shown to play a role in osteoblast differentiation and preosteoblasts, probably by activating canonical Wnt-β-catenin signaling (153). Mice with deletion of *FOXL1* exhibit various intestinal abnormalities, aortic arch anomalies, craniofacial defects, and abnormalities of the vertebral column (154, 155). Although both genes therefore play a role in bone metabolism, *FOXC2* seems to be the best regional candidate gene because of its effects on osteoblast differentiation.

8. GPR177

The *GPR177* gene on chromosome 1p31 emerged as a candidate for regulation of bone mass, following the GEFOS meta-analysis (96). Two intronic SNPs within *GPR177* were significantly associated with lumbar spine and femoral neck BMD. The mechanism underlying the association remains to be explored, but it is relevant that *GPR177* is required for cell surface expression of *wnt3a* protein by HEK cells and was shown to be capable of activating nuclear factor κB when expressed in HEK cells (156).

9. HDAC5

The *HDAC5* gene on chromosome 17q21 was identified as a possible candidate gene for BMD regulation by the GEFOS meta-analysis (96). A significant association was observed with the rs228769 situated 8 kb upstream of the HDAC5 and 26 kb upstream of the C17orf53 gene. A nonsynonymous SNP (rs227584) coding for a threonine to proline substitution at codon 126 in the *C17orf53* gene was associated with hip BMD in the deCODE GWAS (90), but the result did not achieve genome-wide significance. At present, therefore, it is unclear whether the associations observed at this locus are mediated by variations within *HDAC5* or *C17orf53*. Although the function of *C17orf53* is unknown, HDAC5 is a class histone deacetylase II, which is ubiquitously expressed and involved in transcriptional regulation, cell cycle progression, and muscle differentiation.

10. LRP4

The lipoprotein receptor-related protein 4 gene (LRP4) on chromosome 11p11 was identified as a possible candidate for regulation of femoral neck BMD by the GEFOS meta-analysis (96), although in fact, the most strongly associated SNP lies within a region of high linkage disequilibrium containing several genes including the Rho GTPase-activating protein 1 (*ARHGAP1*) gene and the coagulation factor II (*F2*) gene. At the present time, it is difficult to determine which of these genes is responsible for the associations observed. Small GPTases such as Rho are known to play an important role in regulating bone cell activity, whereas *LRP4* is homologous to the *LRP5* gene, which is known to regulate BMD (see *Section X.A.11*), so both of these genes are good candidates. Further work will be required to investigate this genomic region in more detail to define the functional mechanisms underlying the associations that have been reported.

11. LRP5

The *LRP5* gene was discovered to be a key regulator of bone mass after linkage studies in the osteoporosis-pseudoglioma syndrome (53) and the high bone mass syndrome (57).

Early association studies showed that common variants in *LRP5* were associated with variation of BMD in the

general population (157–160). In a large study of 45,000 subjects from the GENOMOS consortium, van Meurs *et al.* (161) reported that common nonsynonymous coding variants in exons 9 and 18 of *LRP5* were significantly associated with BMD with *P* values exceeding the threshold for genome-wide significance. An association with fracture was also observed. Furthermore, the *LRP5* locus emerged as a significant determinant of BMD in the TwinsUK/Rotterdam GWAS (88) and in the GEFOS GWAS meta-analysis (96).

Functional studies of *LRP5* variants have mainly focused on rare mutations. Analysis of the bone from mice with targeted inactivation of *LRP5* has shown that the low bone mass is mainly a consequence of decreased bone formation rather than an increased bone resorption (162). The G171V mutation that is associated with high bone mass (57, 59) was found to cause increased bone mass when expressed in transgenic mice (163). In these studies, mineral apposition rate was increased, and the rate of osteoblast apoptosis was reduced, whereas eroded surface (reflecting bone resorption) was unaffected. There is evidence that the mutations of *LRP5* that cause high bone mass inhibit interactions between *LRP5* and *Dkk1*—an inhibitor of *Wnt* signaling. For example, studies by Boyden *et al.* (60) showed that the G171V mutation did not result in constitutive activation of *LRP5* signaling *in vitro*, but instead the mutation impaired the ability of *Dkk1* to inhibit *Wnt*-stimulated *LRP5* signaling. Another study reached the same conclusion for several high bone mass-associated mutants (G171V, G171R, A214T, A214V, A242T, T253I, and D111Y), showing that they were resistant to *Dkk1* inhibition and had lower affinity for *Dkk1* binding than wild-type *LRP5* (164).

Many common *LRP5* variants have been studied in association studies, but the most likely functional candidates are a valine to methionine variant in exon 9 at codon 667 (V667M) and an alanine to valine substitution at position 1330 (A1330V) in exon 18. Less functional work has been done on these polymorphisms, but promoter-reporter assays have indicated that different haplotypes for the V667M and A1330V polymorphisms differ in their ability to activate reporter gene transcription, indicating that they are also functional (165).

In conclusion, the data indicate that rare mutations in the *LRP5* gene can have a major effect on BMD, whereas more subtle polymorphisms seem also to regulate BMD and have an effect on fracture risk in the normal population, albeit with a smaller effect size.

12. MEF2C

The *MADS box transcription enhancer factor 2, polypeptide C* (*MEF2C*) gene on chromosome 5q14 emerged as a possible candidate for BMD regulation by the GEFOS meta-analysis on the observation that a SNP situated 197 kb upstream of the gene was associated with femoral neck BMD. *MEF2C* is a transcription factor that has been primarily implicated in muscle function, although recent studies indicate that it plays a key role in regulation of *SOST* gene expression by interacting with a conserved enhancer that is deleted in van Buchem disease (166).

13. MEPE

The matrix extracellular phosphoglycoprotein (*MEPE*) gene on chromosome 4q21 emerged as a candidate for regulation of spine BMD by the GEFOS meta-analysis, and in this study, the *P* value was close to genome-wide significant for hip BMD (96). The rs1471403 SNP, located 7 kb 3′ to MEPE, showed the strongest signal, but other genes within the region that might also explain the association include the integrin-binding sialoprotein (*IBSP*) gene (42 kb distant from rs1471403) and the osteopontin (*OPN*) gene (122 kb distant). All three genes are expressed in bone, and all exhibit a skeletal phenotype when deleted. For example, mice with targeted inactivation of *MEPE* have increased BMD (167), as do mice with deletion of *IBSP* (168). Mice with deletion of *OPN* are resistant to ovariectomy-induced bone loss (169). Alleles at the rs1471403 SNP were associated with levels of *IBSP* expression in osteoblasts, raising the possibility that functional variants driving the association might be situated in this gene, although further work will be required to investigate this locus in more detail and identify the causal variants.

14. MARK3

The *MARK3* gene encodes microtubule affinity-regulating kinase 3, a member of the AMP kinase superfamily of proteins (170). The *MARK3* gene on chromosome 14q32 was found to be significantly associated with total hip BMD in the deCODE GWAS (89), but just failed to reach genome-wide significance in the GEFOS meta-analysis (96). Members of this family have been implicated in a wide variety of cellular processes, and *MARK3* is known to play a role in regulating the cell cycle by phosphorylating the cdc25 protein. The mechanisms by which variations in this gene might affect BMD are unknown.

15. MHC locus

Genetic variations at the MHC locus on chromosome 6 are known to be associated with a wide variety of autoimmune diseases. Rather surprisingly, the rs3130340 SNP at this locus was found to be significantly associated with BMD and fracture in the deCODE GWAS (90), and the association was confirmed in an extended sample of this

study (89). However, this was not genome-wide significant for BMD in the GEFOS meta-analysis (96). This could indicate that the association is a spurious one due to population stratification for which this locus is quite sensitive, so its true contribution to BMD remains unclear. The possible mechanism responsible for the association is also unclear, except to note that there is a strong association between disorders of the immune system and susceptibility to osteoporosis (171).

16. SOST

The *SOST* gene on chromosome 17q21 encodes sclerostin, a protein that is produced almost exclusively by osteocytes and which inhibits bone formation, probably by preventing members of the *Wnt* family binding to the LRP5 receptor (172). Inactivating mutations of *SOST* cause the syndromes of sclerosteosis and van Buchem disease (*Section VI*), which makes the *SOST* gene an excellent candidate for genetic regulation of BMD. Polymorphisms of *SOST* were initially evaluated in relation to BMD in two candidate gene studies. In one study, no association between *SOST* polymorphisms and BMD was found in perimenopausal women using a case-control design (173), whereas in another study of older women, evidence of an association with BMD was observed in men and women, with effects that increased with age (174).

Three SNPs close to the *SOST* gene emerged as a significant determinant of total hip BMD in the deCODE GWAS (89). The *SOST* locus was also associated with BMD and fracture in a candidate gene meta-analysis reported by Richards *et al.* (129). The *SOST* locus was associated with BMD in the GEFOS meta-analysis, but the value did not reach genome-wide significance (96). On the basis of current evidence, it seems likely that polymorphic variation at the *SOST* locus does contribute to the genetic regulation of BMD, but the mechanisms responsible for the association remain to be fully explored.

17. SOX6

The *SRY* (*sex-determining region Y*)-*box 6* (*SOX6*) gene on chromosome 11p15 emerged as a candidate for regulation for BMD as a result of the GEFOS meta-analysis. In this study a significant association was found between the rs7117858 SNP situated 297 kb upstream from *SOX6* and femoral neck BMD. The *SOX6* gene encodes a transcription factor that, together with its homolog *SOX5*, plays an essential role in chondrocyte differentiation and endochondral ossification. This raises the possibility that variation in this gene might affect bone density by playing a role in skeletal development.

18. SP7

The *SP7* gene encodes osterix, a transcription factor that plays an essential role in osteoblast differentiation (175). The *SP7* gene on chromosome 12q13 emerged as a candidate for regulation of BMD by the extended deCODE GWAS (89), and SNPs from this region were also significant in the GEFOS meta-analysis (96). Further studies will now be required to investigate the mechanisms underlying this association.

19. SPTBN1

The spectrin, β, nonerythrocytic 1 (*SPTBN1*) gene on chromosome 2p16 encodes a cytoskeletal protein. The rs11898505 SNP, located within an intron of this gene, was found to be significantly associated with spine BMD in the GEFOS meta-analysis (96). This locus was also associated with fractures in the deCODE GWAS (90). The functional role of this gene in bone remains unclear, although targeted inactivation of the mouse homolog (embryonic liver fodrin) resulted in mid-gestational death with gastrointestinal, liver, neural, and heart defects, yielding a phenotype that was similar to double knockout of SMAD3 and SMAD4, downstream mediators of TGF-β signaling.

20. STARD3NL

The *STARD3 n-terminal like* (*STARD3NL*) gene on chromosome 7p14 encodes a cholesterol endosomal transporter that emerged as a candidate for BMD regulation after it was discovered that the rs1524058 approximately 81 kb upstream of the gene was associated with spine BMD in the GEFOS meta-analysis. The role that *STARD3NL* plays in bone metabolism remains unclear at present.

21. TNFRSF11A

The *TNFRSF11A* gene on chromosome 18q21 encodes the RANK, a member of the TNF superfamily of receptors. The RANK receptor is expressed on osteoclasts and osteoclast precursors and plays a critical role in regulating osteoclast differentiation and function (5). The *TNFRSF11A* gene has been the subject of several association studies. The first to be performed was that of Choi *et al.* (177), who found a significant association between BMD and an alanine to valine polymorphism at codon 192 of the RANK protein (A192V) in 650 Korean postmenopausal women. In a second study, Koh *et al.* (178) resequenced the gene, identified 25 SNPs, and studied 11 of these in a cohort of about 500 Korean postmenopausal women. Significant associations were reported for two intronic SNPs in relation to BMD. In another study of Chinese subjects, the A192V allele of RANK was associated with hip BMD in men but not women (179). Definite evidence for an association between RANK alleles and BMD came from the deCODE GWAS, which found an association between polymorphisms in the RANK

gene and fracture (90), as well as with BMD (89). This association has been confirmed by the GEFOS meta-analysis (96). The mechanisms by which polymorphisms at the *TNFRSF11A* locus regulate BMD remain to be investigated.

22. TNFRSF11B

The *TNFRSF11B* gene on chromosome 8 encodes OPG, which is an endogenously produced inhibitor of bone resorption. OPG plays a critical role in bone metabolism and has been the subject of several candidate gene association studies. These have focused on polymorphisms in the gene promoter and at codon 3, where the G1181C polymorphism introduces a nonsynonymous amino acid change from lysine to asparagine at codon 3 (L3K). One of the first association studies of *TNFRSF11B* was by Langdahl *et al.* (180), who found evidence of an association between the −163A/G, −245T/G, and 1181C/G polymorphisms of *TNFRSF11B* and vertebral fracture risk. Positive associations between *TNFRSF11B* polymorphisms and BMD or fracture were reported by some other groups (177, 181), whereas in a study by Ueland *et al.* (182), no association between *TNFRSF11B* alleles and BMD, ultrasound properties of bone, or fracture was found. Large-scale confirmation that *TNFRSF11B* is a true susceptibility gene for osteoporosis came from the observation that several SNPs at the *TNFRSF11B* locus were significantly associated with BMD in both the TwinsUK/Rotterdam GWAS and the deCODE GWAS (88–90). In the deCODE GWAS, *TNFRSF11B* alleles were also associated with an increased risk of fractures (90). The *TNFRSF11B* gene was also confirmed to be associated with BMD by the GEFOS meta-analysis (96). The functional mechanisms by which *TNFRSF11B* alleles predispose to osteoporosis are incompletely understood, but in the TwinsUK/Rotterdam GWAS (88), expression of the risk allele at the rs4355801 SNP was associated with reduced expression of *TNFRSF11B* in lymphoblastoid cell lines. This would be consistent with a model whereby the variants of *TNFRSF11B* that are associated with osteoporosis result in reduced gene expression, thereby increasing bone resorption and bone loss. This does not, of course, exclude the possibility that the L3K protein coding variant (or other variants still to be discovered) may also play a role in the pathogenesis of osteoporosis.

23. TNFSF11

The *TNFSF11* gene on chromosome 13 encodes RANKL, a member of the TNF superfamily that stimulates bone resorption by activating RANK signaling. The *TNFSF11* gene has been studied as a candidate for regulation of susceptibility to osteoporosis by Kim *et al.* (183) in Korean postmenopausal women and by Hsu *et al.* (179) in Chinese men and women. The study by Kim *et al.* (183) looked for evidence of an association between an intronic polymorphism of RANKL (rs2277438) and BMD in 385 Korean postmenopausal women. No association was found, but the rs2277438 SNP was reported to interact with the G1181C polymorphism of *TNFRSF11B* to affect BMD. The study by Hsu *et al.* (179) employed both a case-control study design and a family-based design. The case-control study involved about 1000 individuals, and the family-based study involved 200 individuals. Equal numbers of men and women were studied. The authors reported a significant association with a SNP (rs9594782) in the RANKL promoter and the likelihood of having low BMD (odds ratio, 2.1), but no association with BMD was found with the intronic polymorphism studied by Kim *et al.* (183). Confirmation that RANKL is a true susceptibility gene for osteoporosis came from the deCODE GWAS, which showed that several polymorphisms in *TNFSF11* were associated with lumbar spine BMD (89, 90). This association was subsequently confirmed to be present in the GEFOS meta-analysis (96). As is the case with RANK, the functional mechanisms by which polymorphisms of RANKL regulate BMD remains to be investigated.

24. ZBTB40

The *ZBTB40* gene on chromosome 1 was identified as a candidate for regulation of BMD by the deCODE GWAS (89, 90), and this association was confirmed to be present in the GEFOS meta-analysis (96) where two SNPs (rs7524102 and rs6696981) were found to be associated with hip and spine BMD. The gene is situated on chromosome 1p36 in a region previously implicated in the genetic regulation of BMD by linkage analysis in families (184, 185). However, the most strongly associated SNP identified in the deCODE GWAS was situated in a linkage disequilibrium block that does not contain other known genes. The *ZBTB40* gene encodes a protein of unknown function, which contains a zinc finger domain that likely confers the protein with DNA binding properties and a BT domain that is involved in protein-protein interactions. Many proteins with both of these domains act as transcription factors, and a similar function for *ZBTB40* seems likely. The *ZBTB40* mRNA is expressed in bone, but its function is as yet unknown.

B. Loci and genes with significant evidence for association with quantitative ultrasound

The loci and genes that have attained genome-wide significant evidence for association with ultrasound properties of bone are discussed below in alphabetical order.

1. FAM3C

An intronic polymorphism (rs7776725) in the *FAM3C* gene on chromosome 7q31 was identified as a significant determinant of ultrasound properties of bone (speed-of-sound) at the distal radius and tibia by the GWAS performed by Cho *et al.* (93) in Korean subjects. The *FAM3C* gene is widely expressed and belongs to a family of cytokine-like proteins comprising *FAM3A*, *FAM3B*, *FAM3C*, and *FAM3D*. These proteins were discovered by a homology search for four helix bundle motifs that are found in cytokines such as IL-2, IL-3, and IL-4, erythropoietin, and granulocyte-macrophage colony-stimulating factor. The *FAM3C* gene is known to be expressed by osteoblasts, but its role in regulating bone metabolism is as yet unclear.

2. SFRP4

A locus on 7p14.1 was identified as a predictor of ultrasound properties of bone (speed-of-sound) at the distal radius by a GWAS performed by Cho *et al.* (93) in Korean subjects. The association was strongest with the rs1721400 SNP (93). The most obvious candidate gene within this locus is *secreted frizzled related protein 4* (*SRFP4*) an antagonist of *Wnt* signaling. The *SFRP4* gene was previously implicated in the genetic regulation of bone mass by the study of Nakanishi *et al.* (186), who found that the mouse homolog lay within a QTL for regulation of peak bone mass in a cross of SAMP2 and SAMP6 mice. Moreover, levels of mRNA expression for *Sfrp4* were 40-fold higher in the SAMP6 parental strain than in the congenic strain carrying a 15-cM interval derived from the SAMP2 strain. This, taken together with the observation that Sfrp4 inhibited proliferation of MC3T3 osteoblast-like cells, led the authors to speculate that the cause of the low BMD in SAMP6 mice was overexpression of *Sfrp4*, which inhibited bone formation by inhibiting Wnt signaling. It should be noted, however, that other genes are also present in the 7p14.1 interval, including *TXNDC3* and *EPDR1*, although neither seems a strong candidate. The *TXNDC2* gene encodes a thioredoxin-containing protein involved in sperm maturation, which is specifically expressed in the testis. The EPDR1 gene encodes ependymin-related protein 1, a transmembrane protein that is thought to be involved in cell adhesion but has no known role in bone metabolism.

C. Other candidate genes for susceptibility to osteoporosis

Candidate genes that have been investigated in large-scale studies, but which have not as yet attained genome-wide significant evidence for association with osteoporosis, are discussed below in alphabetical order.

1. COL1A1

Type I collagen is the major protein of bone and is a heterotrimer consisting of $\alpha 1(1)$ and $\alpha 1(2)$ protein chains that are encoded by the *COL1A1* and *COL1A2* genes, respectively. Polymorphisms of the *COL1A1* gene have been studied extensively in relation to BMD and osteoporotic fracture. Most research has focused on a G/T polymorphism within intron 1 of the *COL1A1* gene that affects a binding site for the transcription factor Sp1 (187). The "T" allele of this polymorphism has been associated with BMD and/or osteoporotic fractures in several studies (188–198), but negative results have also been reported (199–202). Nonetheless, a retrospective meta-analysis of published studies showed that the *COL1A1* Sp1 polymorphism was significantly associated with osteoporotic fractures (203) and bone density (204, 205) with evidence of an allele dose effect. In the GENOMOS study of 20,786 subjects, *COL1A1* Sp1 alleles were found to be associated with spine and hip BMD with a recessive model of inheritance and to be associated with vertebral fractures in women with evidence of an allele dose effect (130).

The population prevalence of the *COL1A1* Sp1 polymorphism differs markedly in different ethnic groups. The osteoporosis associated "T" allele is relatively common in Caucasians, but it is rare in the African subcontinent and seems to be virtually absent from Asian populations (206–208). This has led to the suggestion that differences in population prevalence of *COL1A1* Sp1 alleles might contribute to ethnic differences in fracture risk (206), but this remains speculative.

Extensive studies have been performed on the molecular mechanism by which the Sp1 polymorphism predisposes to osteoporosis (209–212). These serve as an example of what is necessary to try to understand the molecular mechanisms underlying the observed associations of subtle effect. The osteoporosis-associated *COL1A1* "T"-allele has higher affinity for Sp1 protein binding than the wild-type "G" allele and that allele-specific transcription from the "T" allele has been found to be 3-fold higher than the "G" allele in heterozygotes. In keeping with this, cultured osteoblasts from subjects who are heterozygous for the G/T polymorphism produce increased amounts of collagen α 1 protein relative to α 2 *in vitro*, compared with "GG" homozygotes, and also express increased amounts of *COL1A1* mRNA relative to *COL1A2* mRNA. Biomechanical studies have shown that bone cores from G/T heterozygotes have significantly reduced bone strength *ex vivo* than those from GG homozygotes and also are less well mineralized (210, 211).

Corresponding with this, studies *in vitro* have also shown evidence of defective mineralization in bone cores cultured from Sp1 G/T heterozygotes compared with G/G homozygotes (209, 210). Overall, the data are consistent with a model whereby the "T" allele of the *COL1A1* Sp1 polymorphism increases *COL1A1* gene transcription, which leads to increased collagen α1 protein production, an abnormal ratio of α1 to α2 protein chains, a subtle defect in bone mineralization, and reduced bone strength, which might lead to an increased risk of fracture.

Polymorphisms have also been described in the promoter region of the *COL1A1* gene that are in linkage disequilibrium with the Sp1 polymorphism, including an insertion/deletion polymorphism in a polythymidine tract at position −1663 (−1663indelT) and a G/T polymorphism at position −1997 (−1997G/T). The −1997G/T polymorphism was found to be associated with BMD in Spanish postmenopausal women and to interact with the Sp1 polymorphism in regulating BMD (213). Similar findings were reported by another group of women from the United States (197). The largest study of these polymorphisms is that of Stewart *et al.* (214), who reported that haplotypes defined by all three polymorphisms regulated spine and hip BMD in women from the United Kingdom with effects that were stronger than those of the individual SNP. In keeping with this, haplotypes defined by the promoter and intron 1 polymorphisms were found to be associated with bone strength as assessed by biomechanical testing *ex vivo*, and a specific haplotype comprising the rare allele at each of the three sites was found to be enriched in a small study of patients with hip fracture (209). There is good evidence that the promoter polymorphisms are functional. The −1663indelT polymorphism is situated at a binding site for the transcription factor nuclear matrix protein 4, and promoter-reporter assays show that different promoter haplotypes differed in their ability to regulate reporter gene expression with high levels of transcription associated with the −1997G-1663delT haplotype (212, 215). In another study, all three polymorphisms interacted to regulate *COL1A1* transcription, and the −1663indelT polymorphism was found to be close to a binding site for osterix, a transcription factor encoded by the *SP7* gene (212). This is an established candidate gene for BMD regulation (see section X,A,18), which plays a critical role in osteoblast differentiation (175).

In summary, the studies that have been performed to date show that common allelic variants in intron 1 and the 5′ flank of the *COL1A1* gene might be associated with BMD and susceptibility to vertebral fractures, although the effects are modest and the associations reported so far fall somewhere short of genome-wide significance.

2. TGFB1

TGFβ1 encoded by the *TGFB1* gene is thought to act as a coupling factor between bone resorption and bone formation. A large number of studies have been performed on possible associations between polymorphisms in *TGFB1* and osteoporosis-related phenotypes. A rare C-deletion polymorphism in intron 4 of *TGFB1* has been associated with low BMD, increased bone turnover, and osteoporotic fracture in one study from Denmark (216), and very similar results were recently reported in another study from Italy (217). Although this polymorphism is close to the splice junction, it does not affect the splice acceptor site, and the functional effects on TGFβ1 function (if any) are unknown. Another polymorphism of the *TGFB1* coding region has been described that causes a leucine-proline substitution in the signal peptide region of TGFβ1 at amino acid 10. The C allele of the codon 10 polymorphism has been associated with high BMD and a reduced frequency of osteoporotic fractures in two Japanese populations (218), with BMD in Japanese adolescents (219), and with reduced rates of bone loss and improved response to treatment with alfacalcidol, an active metabolite of vitamin D (220). This polymorphism is associated with raised circulating levels of TGFβ1, suggesting that it may influence protein secretion or stability. However, two promoter polymorphisms of *TGFB1* have been described that are also associated with circulating TGFβ1 levels (221).

Large-scale studies have not shown evidence of a convincing association between *TGFB1* polymorphisms and osteoporosis-related phenotypes. The largest individual study is that of McGuigan *et al.* (222), who performed a comprehensive analysis of common polymorphisms in relation to BMD, bone loss, biochemical markers of bone turnover, and fracture in about 3000 Caucasian women. This study showed strong linkage disequilibrium between the polymorphisms, but no convincing association between BMD, bone loss, or fracture. Langdahl *et al.* (223) found no association between *TGFB1* polymorphisms and BMD or fracture in the GENOMOS study, which was a prospective meta-analysis involving more than 28,000 individuals. *TGFB1* did not emerge as a candidate gene for BMD regulation in the candidate gene meta-analysis performed by Richards *et al.* (129) from the GEFOS consortium. In view of this, it seems unlikely that common polymorphisms of *TGFB1* contribute substantially to the genetic regulation of BMD or fracture.

3. TGFB3

The *TGFB3* gene was identified as a possible regulator of susceptibility to osteoporosis in a GWAS performed by Xiong *et al.* (92). Although no individual SNP reached genome-wide significance, a haplotype of five SNPs containing the rs17131547 SNP was found to exceed the threshold for genome-wide significance ($P = 3.47 \times 10^{-8}$). However, replication analysis in other populations did not attain genome-wide significance for this SNP. The *TGFB3* gene encodes TGFβ3, a member of the BMP superfamily that is strongly expressed in the palate. In keeping with this, mice with deletion of *TGFB3* have been reported to have abnormal lung development and a cleft palate (224). The mechanisms by which *TGFB3* might regulate bone density are unclear.

4. VDR

The active metabolites of vitamin D play an important role in regulating bone cell function and maintenance of serum calcium homeostasis by binding to the vitamin D receptor (*VDR*), which regulates expression of various response genes. The *VDR* gene has been extensively studied as a potential candidate for regulating genetic susceptibility to osteoporosis. The first study was that of Morrison *et al.* (225), who found an association between polymorphisms affecting the 3′ region of *VDR* and circulating osteocalcin levels. In a subsequent study, the same group reported a significant association between a *Bsm*I polymorphism in intron 8 of *VDR* and BMD in a twin study and a population-based study, but this association was later found to be much weaker than originally reported due to genotyping errors (226).

A large number of association studies between *VDR* alleles and BMD and/or fracture were subsequently performed, but the results were conflicting, probably because none of the studies was adequately powered. A large-scale meta-analysis of *VDR* alleles in relation to BMD and fracture performed by the GENOMOS consortium involving 26,000 subjects failed to demonstrate any association between the *Bsm*I, *Apa*I, and *Taq*I 3′polymorphisms in relation to BMD or fracture. In addition, the candidate gene meta-analysis of the GEFOS dataset from 19,000 subjects showed no significant association between common *VDR* alleles on BMD or fracture (129). A common polymorphism has been described in exon 2 of the VDR gene that is a T-C transition, within exon 2 recognized by the *Fok*I restriction enzyme (227, 228). This transition introduces an alternative translational start codon that results in a shorter isoform of the *VDR* gene (227). Association studies between this polymorphism, BMD, and fracture have yielded conflicting results, and in the GENOMOS study of 26,000 subjects no evidence for an association between this SNP and either BMD or fracture was found. Another common G/A polymorphism affecting a binding site for the transcription factor Cdx2 in the VDR promoter was found to be associated with BMD in a cohort of 261 Japanese women, with lower bone mass in carriers of the "A" allele (229). This observation was confirmed in a large Dutch study and included a protective effect on fracture risk that is in line with the association with BMD reported by Fang *et al.* (230). An association between the Cdx2 polymorphism and BMD and fracture was also found in the GENOMOS study, although the *P* value did not reach genome-wide significance, and the effect sizes were modest (231).

A comprehensive single study of genetic variation across the *VDR* gene in relation to osteoporosis-related phenotypes was conducted by Fang *et al.* (232), who conducted a large-scale study of haplotype tagging of VDR in 6418 participants of the Rotterdam study. Although some effects on BMD and fracture risk were detected, this was on the basis of subgroup analysis, and the effect size was modest.

It has been suggested that the relation between *VDR* polymorphisms and BMD may be modified by environmental factors such as dietary calcium intake (233, 234) and vitamin D status (235), but this has not been investigated in properly powered studies. Intestinal calcium absorption has been associated with the *Bsm*I VDR polymorphism in some studies (236, 237), but the mechanism by which this occurs is unclear, and no association has been found between genotype and mucosal VDR density (228, 238). A positive association between the *Fok*I polymorphism and intestinal calcium absorption was reported in two studies (239, 240), but another study yielded negative results (241). The largest study of VDR alleles in relation to nutrient intake was that of Macdonald *et al.* (242), who in a population study of about 3000 British women found no association between VDR alleles and BMD. In this study, no evidence of an interaction between VDR alleles, dietary calcium intake, serum 25-hydroxyvitamin D levels, and BMD was observed (242). The only positive finding in this study was a weak association between the Cdx2 polymorphisms and bone loss, although this was not significant after correction for multiple testing.

Many investigators have conducted functional analyses of individual VDR polymorphisms and haplotypes. Reporter gene constructs prepared from the 3′ region of the VDR gene in different individuals have shown evidence of haplotype-specific differences in gene transcription, raising the possibility that polymorphisms in this region may be involved in regulating mRNA stability (243). In support of this view, cell lines that were heterozygous for the

*Taq*I polymorphism showed differences in allele-specific transcription of the VDR gene (244). In this study, however, transcripts from the "t" allele were 30% more abundant than the "T," which is the opposite from the result expected on the basis of Morrison's results (226). In another study, evidence of differences in allele-specific transcription were observed in relation to 3′ VDR haplotypes in bone samples from male subjects in the MrOS study (245). Specifically, carriage of haplotype 1 (baT) was associated with increased VDR mRNA abundance, and this haplotype was also associated with an increased risk of fracture in men. In a comprehensive analysis of several cell lines, Fang *et al.* (232) also demonstrated that the baT (haplotype 1) variants were associated with decreased VDR mRNA level. Other *in vitro* studies have shown no differences in allele-specific transcription, mRNA stability, or ligand binding in relation to the *Bsm*I polymorphism (246–248). Studies *in vitro* have shown that different VDR *Fok*I alleles differ in their ability to drive reporter gene expression (227, 249), and the polymorphic variant lacking three amino acids ("F") has also been found to interact with human basal transcription factor IIB more efficiently than the longer isoform ("f"). Finally, peripheral blood mononuclear cells from "FF" individuals were also found to be more sensitive to the growth inhibitory effects of calcitriol than peripheral blood mononuclear cells from "Ff" and "ff" individuals (250). Contrasting with these results, however, Gross *et al.* (228) found no evidence of functional differences between *Fok*I alleles in terms of ligand binding, DNA binding, or transactivation activity. There is good evidence that the Cdx2 polymorphism within the promoter of the VDR gene is functional. Arai *et al.* (229) noted that the G allele had reduced affinity for CDx2 protein binding and also had a 70% reduced ability to drive reporter gene expression compared with the A allele.

In summary, the studies that have been performed to date do not support the hypothesis that allelic variation at the *VDR* locus plays a major role in regulating bone mass or osteoporotic fracture. There is evidence that some of the polymorphisms described have functional effects, at least *in vitro*. For the Cdx2 polymorphism there is also, evidence to suggest that there may be an association with vertebral fracture risk, albeit modest.

XI. Gene-Gene Interactions

Several investigators have studied the relationship between combinations of candidate gene polymorphisms and BMD, although all of these studies were underpowered given what we now know about the strength of effects seen for common polymorphisms and osteoporosis-related phenotypes. Willing *et al.* (145) looked at the interaction between *VDR* and *ESR1* polymorphisms in predicting BMD in a series of 171 postmenopausal women and found that individuals with a combination of *ESR1 Pvu*II "PP" and VDR "bb" genotypes had very high average BMD values at all skeletal sites examined. Another study by Gennari *et al.* (143) in a population of postmenopausal Italian women showed that the combination of *VDR* and *ESR1* genotypes identified subgroups of individuals with very high and very low BMD. However, Vandevyver *et al.* (142) found no significant interaction between *VDR* and *ESR1* genotypes in predicting BMD in Belgian postmenopausal women.

Somewhat larger studies of candidate gene-gene interactions have been performed in the Rotterdam study. For example, Uitterlinden *et al.* (251) reported that *VDR* haplotypes and the *COL1A1* Sp1 polymorphism interacted to regulate susceptibility to fracture in 1004 women from this study. Carriers of the highest risk alleles for both genes had a 4.4-fold increase in fracture risk compared with the reference group. In another analysis of the Rotterdam population, Rivadeneira *et al.* (252) reported that alleles of *ESR1*, *ESR2*, and *IGF-I* all interacted to regulate susceptibility to osteoporotic fracture and other phenotypes, including BMD and aspects of femoral neck structure in 6363 subjects. The authors reported a significant interaction between these three genes and the phenotype studies in women, which persisted after correction for multiple testing, but no effects were observed in men.

The effects of combining information from several alleles that have been significantly associated with BMD in GWAS analysis have been explored in two studies. In the TwinsUK/Rotterdam GWAS (88), information from risk alleles at the *TNFRSF11B* and *LRP5* loci was combined to enhance prediction of those with fractures and to identify subgroups of subjects with very low or high BMD. A similar but more extensive analysis was done using the loci discovered in the GEFOS meta-analysis (96), where the combined effects of 20 risk alleles for BMD were investigated in one study sample where detailed phenotyping for both BMD and fracture were available (the Rotterdam Study). This resulted in the identification of subgroups of subjects (the bottom and top 5% of the population, respectively) with very low BMD (who carry many risk alleles) and those with high BMD (who carry few risk alleles) with a difference in BMD of up to 0.5 SD (for femoral neck BMD) and 0.7 SD (for lumbar spine BMD). Similarly, increased risk for fractures was observed for those subjects carrying more than 20 BMD-decreasing risk alleles, with odds ratios of 2 and 4 for nonvertebral and vertebral fractures, respectively.

XII. Genetic Determinants of Treatment Response

Uncovering the genetic determinants of response to therapeutic agents is a subject of increasing interest because it raises the prospect of being able to predict individual responses to drug treatment on the basis of genetic profiling (253). Several investigators have looked at associations between candidate gene polymorphisms and the response of BMD to antiosteoporotic treatments. Yet, given the modest effect we now know all of the polymorphisms exert, these studies should be interpreted with great caution because they are underpowered.

A. Calcium and vitamin D

The relationship between VDR alleles and bone loss was studied in 229 women who had participated in a controlled trial of calcium supplements in the prevention of postmenopausal bone loss (233). The calcium-supplemented group showed no relationship between VDR genotype and bone loss, whereas in the placebo group, bone loss was significantly greater in the BB group when compared with the other genotype groups. Graafmans *et al.* (235) studied the response to vitamin D supplementation in a series of 81 postmenopausal Dutch women who had taken part in a placebo-controlled trial of vitamin D supplementation on BMD and fracture incidence. These workers observed that the 2-yr change of BMD values in the vitamin D group relative to the placebo group was significantly higher in the "BB" and "Bb" genotypes when compared with the "bb" genotype group. This study is of interest in relation to another study by the same group that showed that the "bb" genotype also had low BMD in a population-based study. Yamada *et al.* (220) studied the relationship between the response of BMD to 1-α-hydroxyvitamin D in relation to a signal peptide polymorphism of the *TGFB1* gene. This study comprised 363 postmenopausal women who were treated with 1-α-hydroxyvitamin D (n = 117) or hormone replacement therapy (HRT) (n = 116) or who were untreated (n = 130). Individuals with the high BMD-associated "CC" genotype responded significantly better to vitamin D treatment than the other genotype groups. The same trend was observed in the HRT group, but the differences were not significant.

B. Hormone replacement therapy

Some information is available on the relationship between candidate gene polymorphisms and response to HRT. Ongphiphadhanakul *et al.* (254) studied the relationship between *ESR1* polymorphisms and 1-yr response to HRT treatment in 124 postmenopausal Thai women. Individuals with the "pp" genotype at the *ESR1 Pvu*II site were found to respond less well to HRT (+2.3% increase in BMD) than the other genotype groups (+6–7% increase in BMD). In a similar but larger study of 248 Korean women, however, Han *et al.* (255) found no association between *Xba*I or *Pvu*II polymorphisms and 1-yr response of BMD to HRT. Salmén *et al.* (144) similarly found no association between *ESR1* genotype and response to HRT in a study of 145 Finnish women. Taken together, these data do not support the view that these *ESR1* polymorphisms consistently predict response to HRT. Other candidate genes have also been studied in relation to HRT response. They include *TGFB1* (discussed above) and *APOE*, which was analyzed by Heikkinen *et al.* 256) in a study of 232 women who were treated with HRT and followed up after a 5-yr period. No association was observed in this study between *APOE* genotype and HRT responsiveness.

C. Bisphosphonates

Marc *et al.* (257) looked at the relationship between *VDR* genotype and response to bisphosphonate therapy in a small series of 24 postmenopausal women undergoing treatment with etidronate. The mean change in BMD over a 2-yr period was significantly greater in the BB *vs.* bb group with intermediate values in the heterozygotes. In another study, Qureshi *et al.* (258) looked at the association between *COL1A1* genotype and the response to treatment in a series of 48 early postmenopausal women who took part in a randomized controlled trial of etidronate in the prevention of postmenopausal bone loss. Although no difference was observed in response of spine BMD to etidronate treatment, those with the "s" allele responded significantly less well at the femoral neck when compared with "SS" homozygotes. These preliminary data are of some interest but need to be extended to much larger groups of patients.

XIII. Future Prospects and Clinical Implications

Studies on the genetic basis of osteoporosis have potential implications for clinical practice. Mapping and identification of genes that regulate BMD offer the prospect of identifying novel molecules that can serve as targets for drug design in the search for new treatments for bone diseases. This is exemplified by the studies of rare inherited bone diseases such as high bone mass syndrome, osteoporosis-pseudoglioma syndrome, and sclerosteosis that led to the identification of Wnt, LRP5, and SOST as key regulators of bone mass and bone turnover. The GWAS provide proof-of-concept that genetic studies of common complex diseases like osteoporosis can also bear fruit in identifying key regulatory pathways such as RANK, RANKL, OPG, LRP5, and osterix. Although these genes were already known to be involved in bone metabolism, many others were not and could represent potentially

novel mediators of bone mass and bone turnover. Although the effect of the identified variants is small, it could be that these genes identified or their downstream signaling pathways might play major roles in bone metabolism, as has been demonstrated for genes such as *TNFRSF11A*, *TNFRSF11B*, *LRP5*, and *osterix*. Accordingly, these pathways might form a focus for the design of new antiosteoporosis drugs that could be used in the prevention and treatment of osteoporosis and other bone diseases. Another potential application of osteoporosis genetics is in the field of diagnostics. Recent studies have shown that subgroups of patients with low and high BMD can be identified by combining the effects of risk alleles (88, 96). Despite this, the genetic markers for BMD and osteoporotic fractures lack the necessary sensitivity and specificity to be clinically useful, and it is clear that further studies are required to identify the many additional alleles that explain the heritability of BMD. Studies in other disease areas indicate that rare alleles of large effect may contribute significantly to the phenotype in common diseases (259, 260). Although this may be the case for osteoporosis, rare alleles with large effects on BMD that are distinct from the variants that cause monogenic bone disease remain to be identified. In addition, other types of genetic variation such as CNVs and variants in methylation pattern may play a role in regulating susceptibility to osteoporosis. In this regard, a CNV affecting the *UGT2B17* gene on chromosome 4q13.2 was reported to be associated with osteoporotic fracture and BMD (261), but this remains to be replicated in other studies. This is clearly an interesting area for further research, but further large-scale, well-designed studies most likely involving large consortia will be necessary to address the role of CNVs and methylation patents in the pathogenesis of osteoporosis.

Note Added in Proof

Since submission of this manuscript, Kung et al. (262) reported that SNP at the *Jagged 1* locus were significantly associated with lumbar spine BMD, following a GWAS study in a discovery cohort of 800 southern Chinese women, with replication in a further 18,098 subjects of European and Asian descent.

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Address all correspondence and requests for reprints to: Professor Stuart H. Ralston, M.D., FRSE, ARC Professor of Rheumatology, Rheumatic Diseases Unit, Molecular Medicine Centre, Western General Hospital, Edinburgh EH4 2XU, United Kingdom. E-mail: stuart.ralston@ed.ac.uk.

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References

1. **Kanis JA, Melton 3rd LJ, Christiansen C, Johnston CC, Khaltaev N** 1994 The diagnosis of osteoporosis. J Bone Miner Res 9:1137–1141
2. **Siris ES, Miller PD, Barrett-Connor E, Faulkner KG, Wehren LE, Abbott TA, Berger ML, Santora AC, Sherwood LM** 2001 Identification and fracture outcomes of undiagnosed low bone mineral density in postmenopausal women: results from the National Osteoporosis Risk Assessment. JAMA 286:2815–2822
3. **Sigurdsson G, Halldorsson BV, Styrkarsdottir U, Kristjansson K, Stefansson K** 2008 Impact of genetics on low bone mass in adults. J Bone Miner Res 23:1584–1590
4. **Torgerson DJ, Campbell MK, Thomas RE, Reid DM** 1996 Prediction of perimenopausal fractures by bone mineral density and other risk factors. J Bone Miner Res 11:293–297
5. **Boyce BF, Xing L** 2008 Functions of RANKL/RANK/OPG in bone modeling and remodeling. Arch Biochem Biophys 473:139–146
6. **Johnson ML, Harnish K, Nusse R, Van Hul W** 2004 LRP5 and Wnt signaling: a union made for bone. J Bone Miner Res 19:1749–1757
7. **Glass 2nd DA, Bialek P, Ahn JD, Starbuck M, Patel MS, Clevers H, Taketo MM, Long F, McMahon AP, Lang RA, Karsenty G** 2005 Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. Dev Cell 8:751–764
8. **Krishnan V, Bryant HU, Macdougald OA** 2006 Regulation of bone mass by Wnt signaling. J Clin Invest 116:1202–1209
9. **Robling AG, Niziolek PJ, Baldridge LA, Condon KW, Allen MR, Alam I, Mantila SM, Gluhak-Heinrich J, Bellido TM, Harris SE, Turner CH** 2008 Mechanical stimulation of bone in vivo reduces osteocyte expression of Sost/sclerostin. J Biol Chem 283:5866–5875
10. **Takeda S, Elefteriou F, Levasseur R, Liu X, Zhao L, Parker KL, Armstrong D, Ducy P, Karsenty G** 2002 Leptin regulates bone formation via the sympathetic nervous system. Cell 111:305–317
11. **van't Hof RJ, Macphee J, Libouban H, Helfrich MH, Ralston SH** 2004 Regulation of bone mass and bone turnover by neuronal nitric oxide synthase. Endocrinology 145:5068–5074
12. **Idris AI, van 't Hof RJ, Greig IR, Ridge SA, Baker D, Ross RA, Ralston SH** 2005 Regulation of bone mass, bone loss and osteoclast activity by cannabinoid receptors. Nat Med 11:774–779
13. **Ofek O, Karsak M, Leclerc N, Fogel M, Frenkel B, Wright K, Tam J, Attar-Namdar M, Kram V, Shohami E, Mechoulam R, Zimmer A, Bab I** 2006 Peripheral cannabinoid receptor, CB2, regulates bone mass. Proc Natl Acad Sci USA 103:696–701
14. **Idris AI, Sophocleous A, Landao-Bassonga E, Canals M, Milligan G, Baker D, van't Hof RJ, Ralston SH** 2009 Cannabinoid receptor type 1 protects against age-related os-

teoporosis by regulating osteoblast and adipocyte differentiation in marrow stromal cells. Cell Metab 10:139–147
15. **Ralston SH, de'Lara G, Farquhar DJ, Gallacher SJ, Hannan J, McLellan AR** 2009 NICE on osteoporosis. Women over 75 with fragility fractures should have DEXA. BMJ 338:b2340
16. **De Laet CE, van Hout BA, Burger H, Hofman A, Pols HA** 1997 Bone density and risk of hip fracture in men and women: cross sectional analysis [Erratum (1997) 315:916]. BMJ 315: 221–225
17. **Garnero P, Hausherr E, Chapuy MC, Marcelli C, Grandjean H, Muller C, Cormier C, Bréart G, Meunier PJ, Delmas PD** 1996 Markers of bone resorption predict hip fracture in elderly women: the EPIDOS Prospective Study. J Bone Miner Res 11:1531–1538
18. **Szulc P, Chapuy MC, Meunier PJ, Delmas PD** 1993 Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture in elderly women. J Clin Invest 91:1769–1774
19. **Faulkner KG, Cummings SR, Black D, Palermo L, Glüer CC, Genant HK** 1993 Simple measurement of femoral geometry predicts hip fracture: the study of osteoporotic fractures. J Bone Miner Res 8:1211–1217
20. **Leslie WD, Pahlavan PS, Tsang JF, Lix LM** 2009 Prediction of hip and other osteoporotic fractures from hip geometry in a large clinical cohort. Osteoporos Int 20:1767–1774
21. **El-Kaissi S, Pasco JA, Henry MJ, Panahi S, Nicholson JG, Nicholson GC, Kotowicz MA** 2005 Femoral neck geometry and hip fracture risk: the Geelong osteoporosis study. Osteoporos Int 16:1299–1303
22. **Cummings SR, Cauley JA, Palermo L, Ross PD, Wasnich RD, Black D, Faulkner KG** 1994 Racial differences in hip axis lengths might explain racial differences in rates of hip fracture. Study of Osteoporotic Fractures Research Group. Osteoporos Int 4:226–229
23. **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
24. **Smith DM, Nance WE, Kang KW, Christian JC, Johnston Jr CC** 1973 Genetic factors in determining bone mass. J Clin Invest 52:2800–2808
25. **Guéguen 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 12:2017–2022
26. **Krall EA, Dawson-Hughes B** 1993 Heritable and life-style determinants of bone mineral density. J Bone Miner Res 8:1–9
27. **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
28. **Arden NK, Baker J, Hogg C, Baan K, Spector 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
29. **Pocock NA, Eisman JA, Hopper JL, Yeates MG, Sambrook PN, Eberl S** 1987 Genetic determinants of bone mass in adults. A twin study. J Clin Invest 80:706–710
30. **Hansen MA, Hassager C, Jensen SB, Christiansen C** 1992 Is heritability a risk factor for postmenopausal osteoporosis? J Bone Miner Res 7:1037–1043
31. **Makovey J, Nguyen TV, Naganathan V, Wark JD, Sambrook PN** 2007 Genetic effects on bone loss in peri- and postmenopausal women: a longitudinal twin study. J Bone Miner Res 22:1773–1780
32. **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
33. **Shaffer JR, Kammerer CM, Bruder JM, Cole SA, Dyer TD, Almasy L, MacCluer JW, Blangero J, Bauer RL, Mitchell BD** 2008 Genetic influences on bone loss in the San Antonio Family Osteoporosis study. Osteoporos Int 19:1759–1767
34. **Snieder H, MacGregor AJ, Spector TD** 1998 Genes control the cessation of a woman's reproductive life: a twin study of hysterectomy and age at menopause. J Clin Endocrinol Metab 83:1875–1880
35. **Stolk L, Zhai G, van Meurs JB, Verbiest MM, Visser JA, Estrada K, Rivadeneira F, Williams FM, Cherkas L, Deloukas P, Soranzo N, de Keyzer JJ, Pop VJ, Lips P, Lebrun CE, van der Schouw YT, Grobbee DE, Witteman J, Hofman A, Pols HA, Laven JS, Spector TD, Uitterlinden AG** 2009 Loci at chromosomes 13, 19 and 20 influence age at natural menopause. Nat Genet 41:645–647
36. **Christian JC, Yu PL, Slemenda CW, Johnston Jr CC** 1989 Heritability of bone mass: a longitudinal study in aging male twins. Am J Hum Genet 44:429–433
37. **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. Study of Osteoporotic Fractures Research Group. N Engl J Med 332:767–773
38. **Deng HW, Chen WM, Recker S, Stegman MR, Li JL, Davies KM, Zhou Y, Deng H, 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
39. **Andrew T, Antioniades L, Scurrah KJ, Macgregor AJ, Spector TD** 2005 Risk of wrist fracture in women is heritable and is influenced by genes that are largely independent of those influencing BMD. J Bone Miner Res 20:67–74
40. **Kannus P, Palvanen M, Kaprio J, Parkkari J, Koskenvuo M** 1999 Genetic factors and osteoporotic fractures in elderly people: prospective 25 year follow up of a nationwide cohort of elderly Finnish twins. BMJ 319:1334–1337
41. **Michaëlsson K, Melhus H, Ferm H, Ahlbom A, Pedersen NL** 2005 Genetic liability to fractures in the elderly. Arch Intern Med 165:1825–1830
42. **Xiong DH, Shen H, Xiao P, Guo YF, Long JR, Zhao LJ, Liu YZ, Deng HY, Li JL, Recker RR, Deng HW** 2006 Genome-wide scan identified QTLs underlying femoral neck cross-sectional geometry that are novel studied risk factors of osteoporosis. J Bone Miner Res 21:424–437
43. **Arden NK, Spector TD** 1997 Genetic influences on muscle strength, lean body mass, and bone mineral density: a twin study. J Bone Miner Res 12:2076–2081
44. **Kaprio J, Rimpelä A, Winter T, Viken RJ, Rimpelä M, Rose RJ** 1995 Common genetic influences on BMI and age at menarche. Hum Biol 67:739–753
45. **Hunter D, De Lange M, Snieder H, MacGregor AJ, Swaminathan R, Thakker RV, Spector TD** 2001 Genetic contribution to bone metabolism, calcium excretion,

and vitamin D and parathyroid hormone regulation. J Bone Miner Res 16:371–378
46. **Garnero P, Arden NK, Griffiths G, Delmas PD, Spector TD** 1996 Genetic influence on bone turnover in postmenopausal twins. J Clin Endocrinol Metab 81:140–146
47. **Deng HW, Livshits G, Yakovenko K, Xu FH, Conway T, Davies KM, Deng H, Recker RR** 2002 Evidence for a major gene for bone mineral density/content in human pedigrees identified via probands with extreme bone mineral density. Ann Hum Genet 66:61–74
48. **Vidal C, Galea R, Brincat M, Anastasi AX** 2007 Linkage to chromosome 11p12 in two Maltese families with a highly penetrant form of osteoporosis. Eur J Hum Genet 15:800–809
49. **Marini JC, Forlino A, Cabral WA, Barnes AM, San Antonio JD, Milgrom S, Hyland JC, Körkkö J, Prockop DJ, De Paepe A, Coucke P, Symoens S, Glorieux FH, Roughley PJ, Lund AM, Kuurila-Svahn K, Hartikka H, Cohn DH, Krakow D, Mottes M, Schwarze U, Chen D, Yang K, Kuslich C, Troendle J, Dalgleish R, Byers PH** 2007 Consortium for osteogenesis imperfecta mutations in the helical domain of type I collagen: regions rich in lethal mutations align with collagen binding sites for integrins and proteoglycans. Hum Mutat 28:209–221
50. **Barnes AM, Chang W, Morello R, Cabral WA, Weis M, Eyre DR, Leikin S, Makareeva E, Kuznetsova N, Uveges TE, Ashok A, Flor AW, Mulvihill JJ, Wilson PL, Sundaram UT, Lee B, Marini JC** 2006 Deficiency of cartilage-associated protein in recessive lethal osteogenesis imperfecta. N Engl J Med 355:2757–2764
51. **Cabral WA, Chang W, Barnes AM, Weis M, Scott MA, Leikin S, Makareeva E, Kuznetsova NV, Rosenbaum KN, Tifft CJ, Bulas DI, Kozma C, Smith PA, Eyre DR, Marini JC** 2007 Prolyl 3-hydroxylase 1 deficiency causes a recessive metabolic bone disorder resembling lethal/severe osteogenesis imperfecta. Nat Genet 39:359–365
52. **Barnes AM, Carter EM, Cabral WA, Weis M, Chang W, Makareeva E, Leikin S, Rotimi CN, Eyre DR, Raggio CL, Marini JC** 2010 Lack of cyclophilin B in osteogenesis imperfecta with normal collagen folding. N Engl J Med 362:521–528
53. **Gong Y, Vikkula M, Boon L, Liu J, Beighton P, Ramesar R, Peltonen L, Somer H, Hirose T, Dallapiccola B, De Paepe A, Swoboda W, Zabel B, Superti-Furga A, Steinmann B, Brunner HG, Jans A, Boles RG, Adkins W, van den Boogaard MJ, Olsen BR, Warman ML** 1996 Osteoporosis-pseudoglioma syndrome, a disorder affecting skeletal strength and vision, is assigned to chromosome region 11q12–13. Am J Hum Genet 59:146–151
54. **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 JA, 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, Jüppner H, Kim CA, Keppler-Noreuil K, Kohlschuetter A, LaCombe D, Lambert M, Lemyre E, Letteboer T, Peltonen L, Ramesar RS, Romanengo M, Somer H, Steichen-Gersdorf E, Steinmann B, Sullivan B, Superti-Furga A, Swoboda W, van den Boogaard MJ, Van Hul W, Vikkula M, Votruba M, Zabel B, Garcia T, Baron R, Olsen BR, Warman ML** 2001 LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. Cell 107:513–523
55. **Morishima A, Grumbach MM, Simpson ER, Fisher C, Qin K** 1995 Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. J Clin Endocrinol Metab 80:3689–3698
56. **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
57. **Johnson ML, Gong G, Kimberling W, Reckér SM, Kimmel DB, Recker RB** 1997 Linkage of a gene causing high bone mass to human chromosome 11 (11q12–13). Am J Hum Genet 60:1326–1332
58. **Van Wesenbeeck L, Cleiren E, Gram J, Beals RK, Bénichou O, Scopelliti D, Key L, Renton T, Bartels C, Gong Y, Warman ML, De Vernejoul MC, Bollerslev J, Van Hul W** 2003 Six novel missense mutations in the LDL receptor-related protein 5 (LRP5) gene in different conditions with an increased bone density. Am J Hum Genet 72:763–771
59. **Little RD, Carulli JP, Del Mastro RG, Dupuis J, Osborne M, Folz C, Manning SP, Swain PM, Zhao SC, 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, Van Eerdewegh P, Recker RR, Johnson ML** 2002 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
60. **Boyden LM, Mao J, Belsky J, Mitzner L, Farhi A, Mitnick MA, Wu D, Insogna K, Lifton RP** 2002 High bone density due to a mutation in LDL-receptor-related protein 5. N Engl J Med 346:1513–1521
61. **Balemans W, Cleiren E, Siebers U, Horst J, Van Hul W** 2005 A generalized skeletal hyperostosis in two siblings caused by a novel mutation in the SOST gene. Bone 36:943–947
62. **Balemans W, Patel N, Ebeling M, Van Hul E, Wuyts W, Lacza C, Dioszegi M, Dikkers FG, Hildering P, Willems PJ, Verheij JB, Lindpaintner K, Vickery B, Foernzler D, Van Hul W** 2002 Identification of a 52 kb deletion downstream of the SOST gene in patients with van Buchem disease. J Med Genet 39:91–97
63. **Balemans W, Ebeling M, Patel N, Van Hul E, Olson P, Dioszegi M, Lacza C, Wuyts W, Van Den Ende J, Willems P, Paes-Alves AF, Hill S, Bueno M, Ramos FJ, Tacconi P, Dikkers FG, Stratakis C, Lindpaintner K, Vickery B, Foernzler D, Van Hul W** 2001 Increased bone density in sclerosteosis is due to the deficiency of a novel secreted protein (SOST). Hum Mol Genet 10:537–543
64. **Brunkow ME, Gardner JC, Van Ness J, Paeper BW, Kovacevich BR, Proll S, Skonier JE, Zhao L, Sabo PJ, Fu Y, Alisch RS, Gillett L, Colbert T, Tacconi P, Galas D, Hamersma H, Beighton P, Mulligan J** 2001 Bone dysplasia sclerosteosis results from loss of the sost gene product, a novel cystine knot-containing protein. Am J Hum Genet 68:577–589
65. **Gardner JC, van Bezooijen RL, Mervis B, Hamdy NA, Löwik CW, Hamersma H, Beighton P, Papapoulos SE**

2005 Bone mineral density in sclerosteosis; affected individuals and gene carriers. J Clin Endocrinol Metab 90: 6392–6395

66. **Villa A, Guerrini MM, Cassani B, Pangrazio A, Sobacchi C** 2009 Infantile malignant, autosomal recessive osteopetrosis: the rich and the poor. Calcif Tissue Int 84:1–12
67. **Guerrini MM, Sobacchi C, Cassani B, Abinun M, Kilic SS, Pangrazio A, Moratto D, Mazzolari E, Clayton-Smith J, Orchard P, Coxon FP, Helfrich MH, Crockett JC, Mellis D, Vellodi A, Tezcan I, Notarangelo LD, Rogers MJ, Vezzoni P, Villa A, Frattini A** 2008 Human osteoclast-poor osteopetrosis with hypogammaglobulinemia due to TNFRSF11A (RANK) mutations. Am J Hum Genet 83:64–76
68. **Sobacchi C, Frattini A, Guerrini MM, Abinun M, Pangrazio A, Susani L, Bredius R, Mancini G, Cant A, Bishop N, Grabowski P, Del Fattore A, Messina C, Errigo G, Coxon FP, Scott DI, Teti A, Rogers MJ, Vezzoni P, Villa A, Helfrich MH** 2007 Osteoclast-poor human osteopetrosis due to mutations in the gene encoding RANKL. Nat Genet 39:960–962
69. **Balemans W, Van Wesenbeeck L, Van Hul W** 2005 A clinical and molecular overview of the human osteopetroses. Calcif Tissue Int 77:263–274
70. **Janssens K, Gershoni-Baruch R, Guañabens N, Migone N, Ralston S, Bonduelle M, Lissens W, Van Maldergem L, Vanhoenacker F, Verbruggen L, Van Hul W** 2000 Mutations in the gene encoding the latency-associated peptide of TGF-β1 cause Camurati-Engelmann disease. Nat Genet 26:273–275
71. **Kinoshita A, Saito T, Tomita H, Makita Y, Yoshida K, Ghadami M, Yamada K, Kondo S, Ikegawa S, Nishimura G, Fukushima Y, Nakagomi T, Saito H, Sugimoto T, Kamegaya M, Hisa K, Murray JC, Taniguchi N, Niikawa N, Yoshiura K** 2000 Domain-specific mutations in TGFB1 result in Camurati-Engelmann disease. Nat Genet 26:19–20
72. **Janssens K, ten Dijke P, Ralston SH, Bergmann C, Van Hul W** 2003 Transforming growth factor β-1 mutations in Camurati-Engelmann disease lead to increased signaling by altering either activation or secretion of the mutant protein. J Biol Chem 278:7718–7724
73. **McGowan NW, MacPherson H, Janssens K, Van Hul W, Frith JC, Fraser WD, Ralston SH, Helfrich MH** 2003 A mutation affecting the latency-associated peptide of TGFβ1 in Camurati-Engelmann disease enhances osteoclast formation *in vitro*. J Clin Endocrinol Metab 88: 3321–3326
74. **Sawcer SJ, Maranian M, Singlehurst S, Yeo T, Compston A, Daly MJ, De Jager PL, Gabriel S, Hafler DA, Ivinson AJ, Lander ES, Rioux JD, Walsh E, Gregory SG, Schmidt S, Pericak-Vance MA, Barcellos L, Hauser SL, Oksenberg JR, Kenealy SJ, Haines JL** 2004 Enhancing linkage analysis of complex disorders: an evaluation of high-density genotyping. Hum Mol Genet 13:1943–1949
75. **Almasy L, Blangero J** 1998 Multipoint quantitative-trait linkage analysis in general pedigrees. Am J Hum Genet 62:1198–1211
76. **Abecasis GR, Cherny SS, Cookson WO, Cardon LR** 2002 Merlin–rapid analysis of dense genetic maps using sparse gene flow trees. Nat Genet 30:97–101
77. **Devoto M, Spotila LD, Stabley DL, Wharton GN, Rydbeck H, Korkko J, Kosich R, Prockop D, Tenenhouse A, Sol-Church K** 2005 Univariate and bivariate variance component linkage analysis of a whole-genome scan for loci contributing to bone mineral density. Eur J Hum Genet 13: 781–788
78. **Tang ZH, Xiao P, Lei SF, Deng FY, Zhao LJ, Deng HY, Tan LJ, Shen H, Xiong DH, Recker RR, Deng HW** 2007 A bivariate whole-genome linkage scan suggests several shared genomic regions for obesity and osteoporosis. J Clin Endocrinol Metab 92:2751–2757
79. **Nyholt DR** 2000 All LODs are not created equal. Am J Hum Genet 67:282–288
80. **Flint J, Valdar W, Shifman S, Mott R** 2005 Strategies for mapping and cloning quantitative trait genes in rodents. Nat Rev Genet 6:271–286
81. **Valdar W, Solberg LC, Gauguier D, Burnett S, Klenerman P, Cookson WO, Taylor MS, Rawlins JN, Mott R, Flint J** 2006 Genome-wide genetic association of complex traits in heterogeneous stock mice. Nat Genet 38:879–887
82. **Grupe A, Germer S, Usuka J, Aud D, Belknap JK, Klein RF, Ahluwalia MK, Higuchi R, Peltz G** 2001 In silico mapping of complex disease-related traits in mice. Science 292:1915–1918
83. **Velasco J, Zarrabeitia MT, Prieto JR, Perez-Castrillon JL, Perez-Aguilar MD, Perez-Nuñez MI, Sañudo C, Hernandez-Elena J, Calvo I, Ortiz F, Gonzalez-Macias J, Riancho JA** 2010 Wnt pathway genes in osteoporosis and osteoarthritis: differential expression and genetic association study. Osteoporos Int 21:109–118
84. **Tenne M, McGuigan F, Jansson L, Gerdhem P, Obrant KJ, Luthman H, Akesson K** 2008 Genetic variation in the PTH pathway and bone phenotypes in elderly women: evaluation of PTH, PTHLH, PTHR1 and PTHR2 genes. Bone 42:719–727
85. **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
86. **Hoggart CJ, Parra EJ, Shriver MD, Bonilla C, Kittles RA, Clayton DG, McKeigue PM** 2003 Control of confounding of genetic associations in stratified populations. Am J Hum Genet 72:1492–1504
87. **Ioannidis JP, Bernstein J, Boffetta P, Danesh J, Dolan S, Hartge P, Hunter D, Inskip P, Jarvelin MR, Little J, Maraganore DM, Bishop JA, O'Brien TR, Petersen G, Riboli E, Seminara D, Taioli E, Uitterlinden AG, Vineis P, Winn DM, Salanti G, Higgins JP, Khoury MJ** 2005 A network of investigator networks in human genome epidemiology. Am J Epidemiol 162:302–304
88. **Richards JB, Rivadeneira F, Inouye M, Pastinen TM, Soranzo N, Wilson SG, Andrew T, Falchi M, Gwilliam R, Ahmadi KR, Valdes AM, Arp P, Whittaker P, Verlaan DJ, Jhamai M, Kumanduri V, Moorhouse M, van Meurs JB, Hofman A, Pols HA, Hart D, Zhai G, Kato BS, Mullin BH, Zhang F, Deloukas P, Uitterlinden AG, Spector TD** 2008 Bone mineral density, osteoporosis, and osteoporotic fractures: a genome-wide association study. Lancet 371:1505–1512
89. **Styrkarsdottir U, Halldorsson BV, Gretarsdottir S, Gudbjartsson DF, Walters GB, Ingvarsson T, Jonsdottir T, Saemundsdottir J, Snorradóttir S, Center JR, Nguyen TV, Alexandersen P, Gulcher JR, Eisman JA, Christiansen C, Sigurdsson G, Kong A, Thorsteinsdottir U, Stefansson K**

2009 New sequence variants associated with bone mineral density. Nat Genet 41:15–17

90. **Styrkarsdottir U, Halldorsson BV, Gretarsdottir S, Gudbjartsson DF, Walters GB, Ingvarsson T, Jonsdottir T, Saemundsdottir J, Center JR, Nguyen TV, Bagger Y, Gulcher JR, Eisman JA, Christiansen C, Sigurdsson G, Kong A, Thorsteinsdottir U, Stefansson K** 2008 Multiple genetic loci for bone mineral density and fractures. N Engl J Med 358:2355–2365
91. **Kiel DP, Demissie S, Dupuis J, Lunetta KL, Murabito JM, Karasik D** 2007 Genome-wide association with bone mass and geometry in the Framingham Heart Study. BMC Med Genet 8(Suppl 1):S14
92. **Xiong DH, Liu XG, Guo YF, Tan LJ, Wang L, Sha BY, Tang ZH, Pan F, Yang TL, Chen XD, Lei SF, Yerges LM, Zhu XZ, Wheeler VW, Patrick AL, Bunker CH, Guo Y, Yan H, Pei YF, Zhang YP, Levy S, Papasian CJ, Xiao P, Lundberg YW, Recker RR, Liu YZ, Liu YJ, Zmuda JM, Deng HW** 2009 Genome-wide association and follow-up replication studies identified ADAMTS18 and TGFBR3 as bone mass candidate genes in different ethnic groups. Am J Hum Genet 84:388–398
93. **Cho YS, Go MJ, Kim YJ, Heo JY, Oh JH, Ban HJ, Yoon D, Lee MH, Kim DJ, Park M, Cha SH, Kim JW, Han BG, Min H, Ahn Y, Park MS, Han HR, Jang HY, Cho EY, Lee JE, Cho NH, Shin C, Park T, Park JW, Lee JK, Cardon L, Clarke G, McCarthy MI, Lee JY, Lee JK, Oh B, Kim HL** 2009 A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. Nat Genet 41:527–534
94. **Purcell SM, Wray NR, Stone JL, Visscher PM, O'Donovan MC, Sullivan PF, Sklar P** 2009 Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature 460:748–752
95. **Trikalinos TA, Salanti G, Zintzaras E, Ioannidis JP** 2008 Meta-analysis methods. Adv Genet 60:311–334
96. **Rivadeneira F, Styrkársdottir U, Estrada K, Halldórsson BV, Hsu YH, Richards JB, Zillikens MC, Kavvoura FK, Amin N, Aulchenko YS, Cupples LA, Deloukas P, Demissie S, Grundberg E, Hofman A, Kong A, Karasik D, van Meurs JB, Oostra B, Pastinen T, Pols HA, Sigurdsson G, Soranzo N, Thorleifsson G, Thorsteinsdottir U, Williams FM, Wilson SG, Zhou Y, Ralston SH, van Duijn CM, Spector T, Kiel DP, Stefansson K, Ioannidis JP, Uitterlinden AG** 2009 Twenty bone-mineral-density loci identified by large-scale meta-analysis of genome-wide association studies. Nat Genet 41:1199–1206
97. **Ioannidis JP, Ng MY, Sham PC, Zintzaras E, Lewis CM, Deng HW, Econs MJ, Karasik D, Devoto M, Kammerer CM, Spector T, Andrew T, Cupples LA, Duncan EL, Foroud T, Kiel DP, Koller D, Langdahl B, Mitchell BD, Peacock M, Recker R, Shen H, Sol-Church K, Spotila LD, Uitterlinden AG, Wilson SG, Kung AW, Ralston SH** 2007 Meta-analysis of genome-wide scans provides evidence for sex- and site-specific regulation of bone mass. J Bone Miner Res 22:173–183
98. **Ioannidis JP, Ntzani EE, Trikalinos TA** 2004 'Racial' differences in genetic effects for complex diseases. Nat Genet 36:1312–1318
99. **Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JR, Elliott KS, Lango H, Rayner NW, Shields B, Harries LW, Barrett JC, Ellard S, Groves CJ, Knight B, Patch AM, Ness AR, Ebrahim S, Lawlor DA, Ring SM, Ben-Shlomo Y, Jarvelin MR, Sovio U, Bennett AJ, Melzer D, Ferrucci L, Loos RJ, Barroso I, Wareham NJ, Karpe F, Owen KR, Cardon LR, Walker M, Hitman GA, Palmer CN, Doney AS, Morris AD, Smith GD, Hattersley AT, McCarthy MI** 2007 A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 316:889–894
100. **Fischer J, Koch L, Emmerling C, Vierkotten J, Peters T, Brüning JC, Rüther U** 2009 Inactivation of the Fto gene protects from obesity. Nature 458:894–898
101. **Peacock M, Koller DL, Lai D, Hui S, Foroud T, Econs MJ** 2005 Sex-specific quantitative trait loci contribute to normal variation in bone structure at the proximal femur in men. Bone 37:467–473
102. **Koller DL, White KE, Liu G, Hui SL, Conneally PM, Johnston CC, Econs MJ, Foroud T, Peacock M** 2003 Linkage of structure at the proximal femur to chromosomes 3, 7, 8, and 19. J Bone Miner Res 18:1057–1065
103. **Deng HW, Shen H, Xu FH, Deng H, Conway T, Liu YJ, Liu YZ, Li JL, Huang QY, Davies KM, Recker RR** 2003 Several genomic regions potentially containing QTLs for bone size variation were identified in a whole-genome linkage scan. Am J Med Genet 119A:121–131
104. **Demissie S, Dupuis J, Cupples LA, Beck TJ, Kiel DP, Karasik D** 2007 Proximal hip geometry is linked to several chromosomal regions: genome-wide linkage results from the Framingham Osteoporosis Study. Bone 40:743–750
105. **Wilson SG, Reed PW, Andrew T, Barber MJ, Lindersson M, Langdown M, Thompson D, Thompson E, Bailey M, Chiano M, Kleyn PW, Spector TD** 2004 A genome-screen of a large twin cohort reveals linkage for quantitative ultrasound of the calcaneus to 2q33–37 and 4q12–21. J Bone Miner Res 19:270–277
106. **Karasik D, Myers RH, Hannan MT, Gagnon D, McLean RR, Cupples LA, Kiel DP** 2002 Mapping of quantitative ultrasound of the calcaneus bone to chromosome 1 by genome-wide linkage analysis. Osteoporos Int 13:796–802
107. **Styrkarsdottir U, Cazier JB, Kong A, Rolfsson O, Larsen H, Bjarnadottir E, Johannsdottir VD, Sigurdardottir MS, Bagger Y, Christiansen C, Reynisdottir I, Grant SF, Jonasson K, Frigge ML, Gulcher JR, Sigurdsson G, Stefansson K** 2003 Linkage of osteoporosis to chromosome 20p12 and association to BMP2. PLoS Biol 1:E69
108. **Hsu YH, Xu X, Terwedow HA, Niu T, Hong X, Wu D, Wang L, Brain JD, Bouxsein ML, Cummings SR, Rosen CJ, Xu X** 2007 Large-scale genome-wide linkage analysis for loci linked to BMD at different skeletal sites in extreme selected sibships. J Bone Miner Res 22:184–194
109. **Xiao P, Shen H, Guo YF, Xiong DH, Liu YZ, Liu YJ, Zhao LJ, Long JR, Guo Y, Recker RR, Deng HW** 2006 Genomic regions identified for BMD in a large sample including epistatic interactions and gender-specific effects. J Bone Miner Res 21:1536–1544
110. **Peacock M, Koller DL, Lai D, Hui S, Foroud T, Econs MJ** 2009 Bone mineral density variation in men is influenced by sex-specific and non sex-specific quantitative trait loci. Bone 45:443–448
111. **Kaufman JM, Ostertag A, Saint-Pierre A, Cohen-Solal M, Boland A, Van Pottelbergh I, Toye K, de Vernejoul MC, Martinez M** 2008 Genome-wide linkage screen of bone

mineral density (BMD) in European pedigrees ascertained through a male relative with low BMD values: evidence for quantitative trait loci on 17q21-23, 11q12-13, 13q12-14, and 22q11. J Clin Endocrinol Metab 93:3755–3762

112. **Altmüller J, Palmer LJ, Fischer G, Scherb H, Wjst M** 2001 Genomewide scans of complex human diseases: true linkage is hard to find. Am J Hum Genet 69:936–950
113. **Medici M, van Meurs JB, Rivadeneira F, Zhao H, Arp PP, Hofman A, Pols HA, Uitterlinden AG** 2006 BMP-2 gene polymorphisms and osteoporosis: the Rotterdam Study. J Bone Miner Res 21:845–854
114. **Koller DL, Liu G, 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
115. **Shaffer JR, Kammerer CM, Bruder JM, Cole SA, Dyer TD, Almasy L, Maccluer JW, Blangero J, Bauer RL, Mitchell BD** 2009 Quantitative trait locus on chromosome 1q influences bone loss in young Mexican American adults. Calcif Tissue Int 84:75–84
116. **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
117. **Klein RF, Mitchell SR, Phillips TJ, Belknap JK, Orwoll ES** 1998 Genetic analysis of bone mass in mice. J Bone Miner Res 13:1648–1656
118. **Koller DL, Alam I, Sun Q, Liu L, Fishburn T, Carr LG, Econs MJ, Foroud T, Turner CH** 2005 Genome screen for bone mineral density phenotypes in Fisher 344 and Lewis rat strains. Mamm Genome 16:578–586
119. **Havill LM, Mahaney MC, Cox LA, Morin PA, Joslyn G, Rogers J** 2005 A quantitative trait locus for normal variation in forearm bone mineral density in pedigreed baboons maps to the ortholog of human chromosome 11q. J Clin Endocrinol Metab 90:3638–3645
120. **Turner CH, Sun Q, Schriefer J, Pitner N, Price R, Bouxsein ML, Rosen CJ, Donahue LR, Shultz KL, Beamer WG** 2003 Congenic mice reveal sex-specific genetic regulation of femoral structure and strength. Calcif Tissue Int 73:297–303
121. **Alam I, Sun Q, Liu L, Koller DL, Fishburn T, Carr LG, Econs MJ, Foroud T, Turner CH** 2005 Whole-genome scan for linkage to bone strength and structure in inbred Fischer 344 and Lewis rats. J Bone Miner Res 20:1589–1596
122. **Bouxsein ML, Rosen CJ, Turner CH, Ackert CL, Shultz KL, Donahue LR, Churchill G, Adamo ML, Powell DR, Turner RT, Muller R, Beamer WG** 2002 Generation of a new congenic mouse strain to test the relationships among serum insulin-like growth factor I, bone mineral density, and skeletal morphology in vivo. J Bone Miner Res 17:570–579
123. **Orwoll ES, Belknap JK, Klein RF** 2001 Gender specificity in the genetic determinants of peak bone mass. J Bone Miner Res 16:1962–1971
124. **Klein RF, Allard J, Avnur Z, Nikolcheva T, Rotstein D, Carlos AS, Shea M, Waters RV, Belknap JK, Peltz G, Orwoll ES** 2004 Regulation of bone mass in mice by the lipoxygenase gene Alox15. Science 303:229–232
125. **Ichikawa S, Koller DL, Johnson ML, Lai D, Xuei X, Edenberg HJ, Klein RF, Orwoll ES, Hui SL, Foroud TM, Peacock M, Econs MJ** 2006 Human ALOX12, but not ALOX15, is associated with BMD in white men and women. J Bone Miner Res 21:556–564
126. **Edderkaoui B, Baylink DJ, Beamer WG, Wergedal JE, Porte R, Chaudhuri A, Mohan S** 2007 Identification of mouse Duffy antigen receptor for chemokines (Darc) as a BMD QTL gene. Genome Res 17:577–585
127. **Parsons CA, Mroczkowski HJ, McGuigan FE, Albagha OM, Manolagas S, Reid DM, Ralston SH, Shmookler Reis RJ** 2005 Interspecies synteny mapping identifies a quantitative trait locus for bone mineral density on human chromosome Xp22. Hum Mol Genet 14:3141–3148
128. **Tang PL, Cheung CL, Sham PC, McClurg P, Lee B, Chan SY, Smith DK, Tanner JA, Su AI, Cheah KS, Kung AW, Song YQ** 2009 Genome-wide haplotype association mapping in mice identifies a genetic variant in CER1 associated with BMD and fracture in southern Chinese women. J Bone Miner Res 24:1013–1021
129. **Richards JB, Kavvoura FK, Rivadeneira F, Styrkársdóttir U, Estrada K, Halldórsson BV, Hsu YH, Zillikens MC, Wilson SG, Mullin BH, Amin N, Aulchenko YS, Cupples LA, Deloukas P, Demissie S, Hofman A, Kong A, Karasik D, van Meurs JB, Oostra BA, Pols HA, Sigurdsson G, Thorsteinsdottir U, Soranzo N, Williams FM, Zhou Y, Ralston SH, Thorleifsson G, van Duijn CM, Kiel DP, Stefansson K, Uitterlinden AG, Ioannidis JP, Spector TD** 2009 Collaborative meta-analysis: associations of 150 candidate genes with osteoporosis and osteoporotic fracture. Ann Intern Med 151:528–537
130. **Ralston SH, Uitterlinden AG, Brandi ML, Balcells S, Langdahl BL, Lips P, Lorenc R, Obermayer-Pietsch B, Scollen S, Bustamante M, Husted LB, Carey AH, Diez-Perez A, Dunning AM, Falchetti A, Karczmarewicz E, Kruk M, van Leeuwen JP, van Meurs JB, Mangion J, McGuigan FE, Mellibovsky L, del Monte F, Pols HA, Reeve J, Reid DM, Renner W, Rivadeneira F, van Schoor NM, Sherlock RE, Ioannidis JP** 2006 Large-scale evidence for the effect of the COLIA1 Sp1 polymorphism on osteoporosis outcomes: the GENOMOS Study. PLoS Med 3:e90
131. **Jin H, Wang X, Ying J, Wong AH, Li H, Lee KY, Srivastava G, Chan AT, Yeo W, Ma BB, Putti TC, Lung ML, Shen ZY, Xu LY, Langford C, Tao Q** 2007 Epigenetic identification of ADAMTS18 as a novel 16q23.1 tumor suppressor frequently silenced in esophageal, nasopharyngeal and multiple other carcinomas. Oncogene 26:7490–7498
132. **Reiner O, Coquelle FM, Peter B, Levy T, Kaplan A, Sapir T, Orr I, Barkai N, Eichele G, Bergmann S** 2006 The evolving doublecortin (DCX) superfamily. BMC Genomics 7:188
133. **Sano M, Inoue S, Hosoi T, Ouchi Y, Emi M, Shiraki M, Orimo H** 1995 Association of estrogen receptor dinucleotide repeat polymorphism with osteoporosis. Biochem Biophys Res Comm 217:378–383
134. **Sowers M, Willing M, Burns T, Deschenes S, Hollis B, Crutchfield M, Jannausch M** 1999 Genetic markers, bone mineral density and serum osteocalcin levels. J Bone Miner Res 14:1411–1419
135. **Becherini L, Gennari L, Masi L, Mansani R, Massart F, Morelli A, Falchetti A, Gonnelli S, Fiorelli G, Tanini A, Brandi ML** 2000 Evidence of a linkage disequilibrium be-

tween polymorphisms in the human estrogen receptor α gene and their relationship to bone mass variation in postmenopausal Italian women. Hum Mol Genet 9:2043–2050

136. **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
137. **Mizunuma H, Hosoi T, Okano H, Soda M, Tokizawa T, Kagami I, Miyamoto S, Ibuki Y, Inoue S, Shiraki M, Ouchi Y** 1997 Estrogen receptor gene polymorphism and bone mineral density at the lumbar spine of pre- and postmenopausal women. Bone 21:379–383
138. **Ongphiphadhanakul B, Rajatanavin R, Chanprasertyothin S, Piaseu N, Chailurkit L, Sirisriro R, Komindr S** 1998 Estrogen receptor gene polymorphism is associated with bone mineral density in premenopausal women but not in postmenopausal women. J Endocrinol Invest 21:487–493
139. **Albagha OM, McGuigan FE, Reid DM, Ralston SH** 2001 Estrogen receptor α gene polymorphisms and bone mineral density: haplotype analysis in women from the United Kingdom. J Bone Miner Res 16:128–134
140. **Weel AE, Uitterlinden AG, Westendorp IC, Burger H, Schuit SC, Hofman A, Helmerhorst TJ, van Leeuwen JP, Pols HA** 1999 Estrogen receptor polymorphism predicts the onset of natural and surgical menopause. J Clin Endocrinol Metab 84:3146–3150
141. **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 [see comments]. J Clin Endocrinol Metab 82:991–995
142. **Vandevyver C, Vanhoof J, Declerck K, Stinissen P, Vandervorst C, Michiels L, Cassiman JJ, Boonen S, Raus J, Geusens P** 1999 Lack of association between estrogen receptor genotypes and bone mineral density, fracture history, or muscle strength in elderly women. J Bone Miner Res 14:1576–1582
143. **Gennari L, Becherini L, Masi L, Mansani R, Gonnelli S, Cepollaro C, Martini S, Montagnani A, Lentini G, Becorpi AM, Brandi ML** 1998 Vitamin D and estrogen receptor allelic variants in Italian postmenopausal women: evidence of multiple gene contribution to bone mineral density. J Clin Endocrinol Metab 83:939–944
144. **Salmén T, Heikkinen AM, Mahonen A, Kröger H, Komulainen M, Saarikoski S, Honkanen R, Mäenpää PH** 2000 Early postmenopausal bone loss is associated with PvuII estrogen receptor gene polymorphism in Finnish women: effect of hormone replacement therapy. J Bone Miner Res 15:315–321
145. **Willing M, Sowers M, Aron D, Clark MK, Burns T, Bunten C, Crutchfield M, D'Agostino D, Jannausch M** 1998 Bone mineral density and its change in white women: estrogen and vitamin D receptor genotypes and their interaction. J Bone Miner Res 13:695–705
146. **Albagha OM, Pettersson U, Stewart A, McGuigan FE, MacDonald HM, Reid DM, Ralston SH** 2005 Association of oestrogen receptor α gene polymorphisms with postmenopausal bone loss, bone mass, and quantitative ultrasound properties of bone. J Med Genet 42:240–246
147. **Ioannidis JP, Stavrou I, Trikalinos TA, Zois C, Brandi ML, Gennari L, Albagha O, Ralston SH, Tsatsoulis A** 2002 Association of polymorphisms of the estrogen receptor α gene with bone mineral density and fracture risk in women: a meta-analysis. J Bone Miner Res 17:2048–2060
148. **Ioannidis JP, Ralston SH, Bennett ST, Brandi ML, Grinberg D, Karassa FB, Langdahl B, van Meurs JB, Mosekilde L, Scollen S, Albagha OM, Bustamante M, Carey AH, Dunning AM, Enjuanes A, van Leeuwen JP, Mavilia C, Masi L, McGuigan FE, Nogues X, Pols HA, Reid DM, Schuit SC, Sherlock RE, Uitterlinden AG** 2004 Differential genetic effects of ESR1 gene polymorphisms on osteoporosis outcomes. JAMA 292:2105–2114
149. **Dunning AM, Healey CS, Baynes C, Maia AT, Scollen S, Vega A, Rodríguez R, Barbosa-Morais NL, Ponder BA, Low YL, Bingham S, Haiman CA, Le Marchand L, Broeks A, Schmidt MK, Hopper J, Southey M, Beckmann MW, Fasching PA, Peto J, Johnson N, Bojesen SE, Nordestgaard B, Milne RL, Benitez J, Hamann U, Ko Y, Schmutzler RK, Burwinkel B, Schürmann P, Dörk T, Heikkinen T, Nevanlinna H, Lindblom A, Margolin S, Mannermaa A, Kosma VM, Chen X, Spurdle A, Change-Claude J, Flesch-Janys D, Couch FJ, Olson JE, Severi G, Baglietto L, Børresen-Dale AL, Kristensen V, Hunter DJ, Hankinson SE, Devilee P, Vreeswijk M, Lissowska J, Brinton L, Liu J, Hall P, Kang D, Yoo KY, Shen CY, Yu JC, Anton-Culver H, Ziogoas A, Sigurdson A, Struewing J, Easton DF, Garcia-Closas M, Humphreys MK, Morrison J, Pharoah PD, Pooley KA, Chenevix-Trench G** 2009 Association of ESR1 gene tagging SNPs with breast cancer risk. Hum Mol Genet 18:1131–1139
150. **Herrington DM, Howard TD, Brosnihan KB, McDonnell DP, Li X, Hawkins GA, Reboussin DM, Xu J, Zheng SL, Meyers DA, Bleecker ER** 2002 Common estrogen receptor polymorphism augments effects of hormone replacement therapy on E-selectin but not C-reactive protein. Circulation 105:1879–1882
151. **Maruyama H, Toji H, Harrington CR, Sasaki K, Izumi Y, Ohnuma T, Arai H, Yasuda M, Tanaka C, Emson PC, Nakamura S, Kawakami H** 2000 Lack of an association of estrogen receptor α gene polymorphisms and transcriptional activity with Alzheimer disease. Arch Neurol 57:236–240
152. **Mellor RH, Brice G, Stanton AW, French J, Smith A, Jeffery S, Levick JR, Burnand KG, Mortimer PS** 2007 Mutations in FOXC2 are strongly associated with primary valve failure in veins of the lower limb. Circulation 115:1912–1920
153. **Kim SH, Cho KW, Choi HS, Park SJ, Rhee Y, Jung HS, Lim SK** 2009 The forkhead transcription factor Foxc2 stimulates osteoblast differentiation. Biochem Biophys Res Commun 386:532–536
154. **Kaestner KH, Silberg DG, Traber PG, Schütz G** 1997 The mesenchymal winged helix transcription factor Fkh6 is required for the control of gastrointestinal proliferation and differentiation. Genes Dev 11:1583–1595
155. **Iida K, Koseki H, Kakinuma H, Kato N, Mizutani-Koseki Y, Ohuchi H, Yoshioka H, Noji S, Kawamura K, Kataoka Y, Ueno F, Taniguchi M, Yoshida N, Sugiyama T, Miura N** 1997 Essential roles of the winged helix transcription factor MFH-1 in aortic arch patterning and skeletogenesis. Development 124:4627–4638
156. **Bänziger C, Soldini D, Schütt C, Zipperlen P, Hausmann G, Basler K** 2006 Wntless, a conserved membrane protein

dedicated to the secretion of Wnt proteins from signaling cells. Cell 125:509–522

157. **Urano T, Shiraki M, Ezura Y, Fujita M, Sekine E, Hoshino S, Hosoi T, Orimo H, Emi M, Ouchi Y, Inoue S** 2004 Association of a single-nucleotide polymorphism in low-density lipoprotein receptor-related protein 5 gene with bone mineral density. J Bone Miner Metab 22:341–345
158. **Koh JM, Jung MH, Hong JS, Park HJ, Chang JS, Shin HD, Kim SY, Kim GS** 2004 Association between bone mineral density and LDL receptor-related protein 5 gene polymorphisms in young Korean men. J Korean Med Sci 19:407–412
159. **Ferrari SL, Deutsch S, Choudhury U, Chevalley T, Bonjour JP, Dermitzakis ET, Rizzoli R, Antonarakis SE** 2004 Polymorphisms in the low-density lipoprotein receptor-related protein 5 (LRP5) gene are associated with variation in vertebral bone mass, vertebral bone size, and stature in whites. Am J Hum Genet 74:866–875
160. **van Meurs JB, Rivadeneira F, Jhamai M, Hugens W, Hofman A, van Leeuwen JP, Pols HA, Uitterlinden AG** 2006 Common genetic variation of the low-density lipoprotein receptor-related protein 5 and 6 genes determines fracture risk in elderly white men. J Bone Miner Res 21:141–150
161. **van Meurs JB, Trikalinos TA, Ralston SH, Balcells S, Brandi ML, Brixen K, Kiel DP, Langdahl BL, Lips P, Ljunggren O, Lorenc R, Obermayer-Pietsch B, Ohlsson C, Pettersson U, Reid DM, Rousseau F, Scollen S, Van Hul W, Agueda L, Akesson K, Benevolenskaya LI, Ferrari SL, Hallmans G, Hofman A, Husted LB, Kruk M, Kaptoge S, Karasik D, Karlsson MK, Lorentzon M, Masi L, McGuigan FE, Mellström D, Mosekilde L, Nogues X, Pols HA, Reeve J, Renner W, Rivadeneira F, van Schoor NM, Weber K, Ioannidis JP, Uitterlinden AG** 2008 Large-scale analysis of association between LRP5 and LRP6 variants and osteoporosis. JAMA 299:1277–1290
162. **Kato M, Patel MS, Levasseur R, Lobov I, Chang BH, Glass 2nd DA, Hartmann C, Li L, Hwang TH, Brayton CF, Lang RA, Karsenty G, Chan L** 2002 Cbfa1-independent decrease in osteoblast proliferation, osteopenia, and persistent embryonic eye vascularization in mice deficient in Lrp5, a Wnt coreceptor. J Cell Biol 157:303–314
163. **Babij P, Zhao W, Small C, Kharode Y, Yaworsky PJ, Bouxsein ML, Reddy PS, Bodine PV, Robinson JA, Bhat B, Marzolf J, Moran RA, Bex F** 2003 High bone mass in mice expressing a mutant LRP5 gene. J Bone Miner Res 18:960–974
164. **Ai M, Holmen SL, Van Hul W, Williams BO, Warman ML** 2005 Reduced affinity to and inhibition by DKK1 form a common mechanism by which high bone mass-associated missense mutations in LRP5 affect canonical Wnt signaling. Mol Cell Biol 25:4946–4955
165. **Kiel DP, Ferrari SL, Cupples LA, Karasik D, Manen D, Imamovic A, Herbert AG, Dupuis J** 2007 Genetic variation at the low-density lipoprotein receptor-related protein 5 (LRP5) locus modulates Wnt signaling and the relationship of physical activity with bone mineral density in men. Bone 40:587–596
166. **Leupin O, Kramer I, Collette NM, Loots GG, Natt F, Kneissel M, Keller H** 2007 Control of the SOST bone enhancer by PTH using MEF2 transcription factors. J Bone Miner Res 22:1957–1967
167. **Gowen LC, Petersen DN, Mansolf AL, Qi H, Stock JL, Tkalcevic GT, Simmons HA, Crawford DT, Chidsey-Frink KL, Ke HZ, McNeish JD, Brown TA** 2003 Targeted disruption of the osteoblast/osteocyte factor 45 gene (OF45) results in increased bone formation and bone mass. J Biol Chem 278:1998–2007
168. **Malaval L, Wade-Guéye NM, Boudiffa M, Fei J, Zirngibl R, Chen F, Laroche N, Roux JP, Burt-Pichat B, Duboeuf F, Boivin G, Jurdic P, Lafage-Proust MH, Amédée J, Vico L, Rossant J, Aubin JE** 2008 Bone sialoprotein plays a functional role in bone formation and osteoclastogenesis. J Exp Med 205:1145–1153
169. **Yoshitake H, Rittling SR, Denhardt DT, Noda M** 1999 Osteopontin-deficient mice are resistant to ovariectomy-induced bone resorption. Proc Natl Acad Sci USA 96:8156–8160
170. **Bright NJ, Thornton C, Carling D** 2009 The regulation and function of mammalian AMPK-related kinases. Acta Physiol (Oxf) 196:15–26
171. **Riches PL, McRorie E, Fraser WD, Determann C, van't Hof R, Ralston SH** 2009 Osteoporosis associated with neutralizing autoantibodies against osteoprotegerin. N Engl J Med 361:1459–1465
172. **Balemans W, Piters E, Cleiren E, Ai M, Van Wesenbeeck L, Warman ML, Van Hul W** 2008 The binding between sclerostin and LRP5 is altered by DKK1 and by high-bone mass LRP5 mutations. Calcif Tissue Int 82:445–453
173. **Balemans W, Foernzler D, Parsons C, Ebeling M, Thompson A, Reid DM, Lindpaintner K, Ralston SH, Van Hul W** 2002 Lack of association between the SOST gene and bone mineral density in perimenopausal women: analysis of five polymorphisms. Bone 31:515–519
174. **Uitterlinden AG, Arp PP, Paeper BW, Charmley P, Proll S, Rivadeneira F, Fang Y, van Meurs JB, Britschgi TB, Latham JA, Schatzman RC, Pols HA, Brunkow ME** 2004 Polymorphisms in the sclerosteosis/van Buchem disease gene (SOST) region are associated with bone-mineral density in elderly whites. Am J Hum Genet 75:1032–1045
175. **Nakashima K, Zhou X, Kunkel G, Zhang Z, Deng JM, Behringer RR, de Crombrugghe B** 2002 The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. Cell 108:17–29
176. **Tang Y, Katuri V, Dillner A, Mishra B, Deng CX, Mishra L** 2003 Disruption of transforming growth factor-beta signaling in ELF beta-spectrin-deficient mice. Science 299:574–577
177. **Choi JY, Shin A, Park SK, Chung HW, Cho SI, Shin CS, Kim H, Lee KM, Lee KH, Kang C, Cho DY, Kang D** 2005 Genetic polymorphisms of OPG, RANK, and ESR1 and bone mineral density in Korean postmenopausal women. Calcif Tissue Int 77:152–159
178. **Koh JM, Park BL, Kim DJ, Kim GS, Cheong HS, Kim TH, Hong JM, Shin HI, Park EK, Kim SY, Shin HD** 2007 Identification of novel RANK polymorphisms and their putative association with low BMD among postmenopausal women. Osteoporos Int 18:323–331
179. **Hsu YH, Niu T, Terwedow HA, Xu X, Feng Y, Li Z, Brain JD, Rosen CJ, Laird N, Xu X** 2006 Variation in genes involved in the RANKL/RANK/OPG bone remodeling pathway are associated with bone mineral density at different skeletal sites in men. Hum Genet 118:568–577
180. **Langdahl BL, Carstens M, Stenkjaer L, Eriksen EF** 2002

Polymorphisms in the osteoprotegerin gene are associated with osteoporotic fractures. J Bone Miner Res 17:1245–1255

181. **Zhao HY, Liu JM, Ning G, Zhao YJ, Zhang LZ, Sun LH, Xu MY, Uitterlinden AG, Chen JL** 2005 The influence of Lys3Asn polymorphism in the osteoprotegerin gene on bone mineral density in Chinese postmenopausal women. Osteoporos Int 16:1519–1524
182. **Ueland T, Bollerslev J, Wilson SG, Dick IM, Islam FM, Mullin BH, Devine A, Prince RL** 2007 No associations between OPG gene polymorphisms or serum levels and measures of osteoporosis in elderly Australian women. Bone 40:175–181
183. **Kim JG, Kim JH, Kim JY, Ku SY, Jee BC, Suh CS, Kim SH, Choi YM** 2007 Association between osteoprotegerin (OPG), receptor activator of nuclear factor-κB (RANK), and RANK ligand (RANKL) gene polymorphisms and circulating OPG, soluble RANKL levels, and bone mineral density in Korean postmenopausal women. Menopause 14:913–918
184. **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
185. **Devoto M, Specchia C, Li HH, Caminis J, Tenenhouse A, Rodriguez H, Spotila LD** 2001 Variance component linkage analysis indicates a QTL for femoral neck bone mineral density on chromosome 1p36. Hum Mol Genet 10:2447–2452
186. **Nakanishi R, Shimizu M, Mori M, Akiyama H, Okudaira S, Otsuki B, Hashimoto M, Higuchi K, Hosokawa M, Tsuboyama T, Nakamura T** 2006 Secreted frizzled-related protein 4 is a negative regulator of peak BMD in SAMP6 mice. J Bone Miner Res 21:1713–1721
187. **Grant SF, Reid DM, Blake G, Herd R, Fogelman I, Ralston SH** 1996 Reduced bone density and osteoporosis associated with a polymorphic Sp1 site in the collagen type I α 1 gene. Nat Genet 14:203–205
188. **Garnero P, Borel O, Grant SF, Ralston SH, Delmas PD** 1998 Collagen I α 1 polymorphism, Bone mass and bone turnover in healthy French pre-menopausal women: the OFELY Study. J Bone Miner Res 13:813–817
189. **Langdahl BL, Ralston SH, Grant SF, Eriksen EF** 1998 An Sp1 binding site polymorphism in the COLIA1 gene predicts osteoporotic fractures in men and women. J Bone Miner Res 13:1384–1389
190. **Uitterlinden AG, Burger H, Huang Q, Yue F, McGuigan FE, Grant SF, Hofman A, van Leeuwen JP, Pols HA, Ralston SH** 1998 Relation of alleles of the collagen type I α 1 gene to bone density and risk of osteoporotic fractures in postmenopausal women. N Engl J Med 338:1016–1021
191. **Roux C, Dougados M, Abel L, Mercier G, Lucotte G** 1998 Association of a polymorphism in the collagen I α 1 gene with osteoporosis in French women. Arthritis Rheum 41:187–188
192. **Alvarez L, Oriola J, Jo J, Ferró T, Pons F, Peris P, Guañabens N, Durán M, Monegal A, Martínez de Osaba MJ, Rivera-Fillat F, Ballesta AM** 1999 Collagen type I α1 gene Sp1 polymorphism in premenopausal women with primary osteoporosis: improved detection of Sp1 binding site polymorphism in the collagen type 1 gene. Clin Chem 45:904–906
193. **Weichetová M, Stepán JJ, Michalská D, Haas T, Pols HA, Uitterlinden AG** 2000 COLIA1 polymorphism contributes to bone mineral density to assess prevalent wrist fractures. Bone 26:287–290
194. **McGuigan FE, Reid DM, Ralston SH** 2000 Susceptibility to osteoporotic fracture is determined by allelic variation at the Sp1 site, rather than other polymorphic sites at the COL1A1 locus. Osteoporos Int 11:338–343
195. **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
196. **Keen RW, Woodford-Richens KL, Grant SF, Ralston SH, Lanchbury JS, Spector TD** 1999 Association of polymorphism at the type I collagen (COL1A1) locus with reduced bone mineral density, increased fracture risk, and increased collagen turnover. Arthritis Rheum 42:285–290
197. **Liu PY, Lu Y, Long JR, Xu FH, Shen H, Recker RR, Deng HW** 2004 Common variants at the PCOL2 and Sp1 binding sites of the COL1A1 gene and their interactive effect influence bone mineral density in Caucasians. J Med Genet 41:752–757
198. **Efstathiadou Z, Kranas V, Ioannidis JP, Georgiou I, Tsatsoulis A** 2001 The Sp1 COLIA1 gene polymorphism, and not vitamin D receptor or estrogen receptor gene polymorphisms, determines bone mineral density in postmenopausal Greek women. Osteoporos Int 12:326–331
199. **Pluijm SM, van Essen HW, Bravenboer N, Uitterlinden AG, Smit JH, Pols HA, Lips P** 2004 Collagen type I α1 Sp1 polymorphism, osteoporosis, and intervertebral disc degeneration in older men and women. Ann Rheum Dis 63:71–77
200. **Ashford RU, Luchetti M, McCloskey EV, Gray RL, Pande KC, Dey A, Kayan K, Ralston SH, Kanis JA** 2001 Studies of bone density, quantitative ultrasound, and vertebral fractures in relation to collagen type I α 1 alleles in elderly women. Calcif Tissue Int 68:348–351
201. **Berg JP, Lehmann EH, Stakkestad JA, Haug E, Halse J** 2000 The Sp1 binding site polymorphism in the collagen type I α 1 (COLIA1) gene is not associated with bone mineral density in healthy children, adolescents, and young adults. Eur J Endocrinol 143:261–265
202. **Heegaard A, Jorgensen HL, Vestergaard AW, Hassager C, Ralston SH** 2000 Lack of influence of collagen type Iα1 Sp1 binding site polymorphism on the rate of bone loss in a cohort of postmenopausal Danish women followed for 18 years. Calcif Tissue Int 66:409–413
203. **Efstathiadou Z, Tsatsoulis A, Ioannidis JP** 2001 Association of collagen Iα 1 Sp1 polymorphism with the risk of prevalent fractures: a meta-analysis. J Bone Miner Res 16:1586–1592
204. **Mann V, Ralston SH** 2003 Meta-analysis of COL1A1 Sp1 polymorphism in relation to bone mineral density and osteoporotic fracture. Bone 32:711–717
205. **Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN** 2003 Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. Nat Genet 33:177–182
206. **Beavan S, Prentice A, Dibba B, Yan L, Cooper C, Ralston**

SH 1998 Polymorphism of the collagen type I α 1 gene and ethnic differences in hip-fracture rates. N Engl J Med 339: 351–352

207. **Nakajima T, Ota N, Shirai Y, Hata A, Yoshida H, Suzuki T, Hosoi T, Orimo H, Emi M** 1999 Ethnic difference in contribution of Sp1 site variation of COLIA1 gene in genetic predisposition to osteoporosis. Calcif Tissue Int 65: 352–353
208. **Lau EM, Choy DT, Li M, Woo J, Chung T, Sham A** 2004 The relationship between COLI A1 polymorphisms (Sp 1) and COLI A2 polymorphisms (Eco R1 and Puv II) with bone mineral density in Chinese men and women. Calcif Tissue Int 75:133–137
209. **Jin H, Stewart TL, Hof RV, Reid DM, Aspden RM, Ralston S** 2009 A rare haplotype in the upstream regulatory region of COL1A1 is associated with reduced bone quality and hip fracture. J Bone Miner Res 24:448–454
210. **Stewart TL, Roschger P, Misof BM, Mann V, Fratzl P, Klaushofer K, Aspden R, Ralston SH** 2005 Association of *COLIA1* Sp1 alleles with defective bone nodule formation *in vitro* and abnormal bone mineralisation *in vivo*. Calcif Tissue Int 77:113–118
211. **Mann V, Hobson EE, Li B, Stewart TL, Grant SF, Robins SP, Aspden RM, Ralston SH** 2001 A COL1A1 Sp1 binding site polymorphism predisposes to osteoporotic fracture by affecting bone density and quality. J Clin Invest 107:899–907
212. **Jin H, van't Hof RJ, Albagha OM, Ralston SH** 2009 Promoter and intron 1 polymorphisms of COL1A1 interact to regulate transcription and susceptibility to osteoporosis. Hum Mol Genet 18:2729–2738
213. **Garcia-Giralt N, Nogués X, Enjuanes A, Puig J, Mellibovsky L, Bay-Jensen A, Carreras R, Balcells S, Díez-Pérez A, Grinberg D** 2002 Two new single nucleotide polymorphisms in the COLIA1 upstream regulatory region and their relationship with bone mineral density. J Bone Miner Res 17:384–393
214. **Stewart TL, Jin H, McGuigan FE, Albagha OM, Garcia-Giralt N, Bassiti A, Grinberg D, Balcells S, Reid DM, Ralston SH** 2006 Haplotypes defined by promoter and intron 1 polymorphisms of the COLIA1 gene regulate bone mineral density in women. J Clin Endocrinol Metab 91: 3575–3583
215. **García-Giralt N, Enjuanes A, Bustamante M, Mellibovsky L, Nogués X, Carreras R, Díez-Pérez A, Grinberg D, Balcells S** 2005 In vitro functional assay of alleles and haplotypes of two COL1A1-promoter SNPs. Bone 36:902–908
216. **Langdahl BL, 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
217. **Bertoldo F, D'Agruma L, Furlan F, Colapietro F, Lorenzi MT, Maiorano N, Iolascon A, Zelante L, Locascio V, Gasparini P** 2000 Transforming growth factor-β1 gene polymorphism, bone turnover, and bone mass in Italian postmenopausal women. J Bone Miner Res 15:634–639
218. **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
219. **Yamada Y, Hosoi T, Makimoto F, Tanaka H, Seino Y, Ikeda K** 1999 Transforming growth factor β-1 gene polymorphism and bone mineral density in Japanese adolescents. Am J Med 106:477–479
220. **Yamada Y, Harada A, Hosoi T, Miyauchi A, Ikeda K, Ohta H, Shiraki M** 2000 Association of transforming growth factor β1 genotype with therapeutic response to active vitamin D for postmenopausal osteoporosis. J Bone Miner Res 15:415–420
221. **Grainger DJ, Heathcote K, Chiano M, Snieder H, Kemp PR, Metcalfe JC, Carter ND, Spector TD** 1999 Genetic control of the circulating concentration of transforming growth factor type β1. Hum Mol Genet 8:93–97
222. **McGuigan FE, Macdonald HM, Bassiti A, Farmer R, Bear S, Stewart A, Black A, Fraser WD, Welsh F, Reid DM, Ralston SH** 2007 Large-scale population-based study shows no association between common polymorphisms of the TGFB1 gene and BMD in women. J Bone Miner Res 22:195–202
223. **Langdahl BL, Uitterlinden AG, Ralston SH, Trikalinos TA, Balcells S, Brandi ML, Scollen S, Lips P, Lorenc R, Obermayer-Pietsch B, Reid DM, Armas JB, Arp PP, Bassiti A, Bustamante M, Husted LB, Carey AH, Pérez Cano R, Dobnig H, Dunning AM, Fahrleitner-Pammer A, Falchetti A, Karczmarewicz E, Kruk M, van Leeuwen JP, Masi L, van Meurs JB, Mangion J, McGuigan FE, Mellibovsky L, Mosekilde L, Nogués X, Pols HA, Reeve J, Renner W, Rivadeneira F, van Schoor NM, Ioannidis JP** 2008 Large-scale analysis of association between polymorphisms in the transforming growth factor β 1 gene (TGFB1) and osteoporosis: the GENOMOS study. Bone 42:969–981
224. **Kaartinen V, Voncken JW, Shuler C, Warburton D, Bu D, Heisterkamp N, Groffen J** 1995 Abnormal lung development and cleft palate in mice lacking TGF-β 3 indicates defects of epithelial-mesenchymal interaction. Nat Genet 11:415–421
225. **Morrison NA, Yeoman R, Kelly PJ, Eisman JA** 1992 Contribution of trans-acting factor alleles to normal physiological variability: vitamin D receptor gene polymorphisms and circulating osteocalcin. Proc Natl Acad Sci USA 89: 6665–6669
226. **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 [Erratum (1997) 387:106]. Nature 367:284–287
227. **Arai H, Miyamoto K, Taketani Y, Yamamoto H, Iemori Y, Morita K, Tonai T, Nishisho T, Mori S, Takeda E** 1997 A vitamin D receptor gene polymorphism in the translation initiation codon: effect on protein activity and relation to bone mineral density in Japanese women. J Bone Miner Res 12:915–921
228. **Gross C, Krishnan AV, Malloy PJ, Eccleshall TR, Zhao XY, Feldman D** 1998 The vitamin D receptor gene start codon polymorphism: a functional analysis of FokI variants. J Bone Miner Res 13:1691–1699
229. **Arai H, Miyamoto KI, Yoshida M, Yamamoto H, Taketani Y, Morita K, Kubota M, Yoshida S, Ikeda M, Watabe F, Kanemasa Y, Takeda E** 2001 The polymorphism in the

caudal-related homeodomain protein Cdx-2 binding element in the human vitamin D receptor gene. J Bone Miner Res 16:1256–1264

230. **Fang Y, van Meurs JB, Bergink AP, Hofman A, van Duijn CM, van Leeuwen JP, Pols HA, Uitterlinden AG** 2003 Cdx-2 polymorphism in the promoter region of the human vitamin D receptor gene determines susceptibility to fracture in the elderly. J Bone Miner Res 18:1632–1641
231. **Uitterlinden AG, Ralston SH, Brandi ML, Carey AH, Grinberg D, Langdahl BL, Lips P, Lorenc R, Obermayer-Pietsch B, Reeve J, Reid DM, Amedei A, Amidei A, Bassiti A, Bustamante M, Husted LB, Diez-Perez A, Dobnig H, Dunning AM, Enjuanes A, Fahrleitner-Pammer A, Fang Y, Karczmarewicz E, Kruk M, van Leeuwen JP, Mavilia C, van Meurs JB, Mangion J, McGuigan FE, Pols HA, Renner W, Rivadeneira F, van Schoor NM, Scollen S, Sherlock RE, Ioannidis JP** 2006 The association between common vitamin D receptor gene variations and osteoporosis: a participant-level meta-analysis. Ann Intern Med 145:255–264
232. **Fang Y, van Meurs JB, d'Alesio A, Jhamai M, Zhao H, Rivadeneira F, Hofman A, van Leeuwen JP, Jehan F, Pols HA, Uitterlinden AG** 2005 Promoter and 3′-untranslated-region haplotypes in the vitamin D receptor gene predispose to osteoporotic fracture: the Rotterdam study. Am J Hum Genet 77:807–823
233. **Krall EA, Parry P, Lichter JB, Dawson-Hughes B** 1995 Vitamin D receptor alleles and rates of bone loss: influence of years since menopause and calcium intake. J Bone Miner Res 10:978–984
234. **Ferrari S, Rizzoli R, Chevalley T, Slosman D, Eisman JA, Bonjour JP** 1995 Vitamin D receptor gene polymorphisms and change in lumbar spine bone mineral density. Lancet 345:423–424
235. **Graafmans WC, Lips P, Ooms ME, van Leeuwen JP, Pols HA, Uitterlinden AG** 1997 The effect of vitamin D supplementation on the bone mineral density of the femoral neck is associated with vitamin D receptor genotype. J Bone Miner Res 12:1241–1245
236. **Dawson-Hughes B, Harris SS, Finneran S** 1995 Calcium absorption on high and low calcium intakes in relation to vitamin D receptor genotype. J Clin Endocrinol Metab 80: 3657–3661
237. **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
238. **Barger-Lux MJ, Heaney RP, Hayes J, DeLuca HF, Johnson ML, Gong G** 1995 Vitamin D receptor gene polymorphism, bone mass, body size and mucosal VDR density. Calcif Tissue Int 57:161–162
239. **Ames SK, Ellis KJ, Gunn SK, Copeland KC, Abrams SA** 1999 Vitamin D receptor gene Fok1 polymorphism predicts calcium absorption and bone mineral density in children. J Bone Miner Res 14:740–746
240. **Abrams SA, Griffin IJ, Hawthorne KM, Chen Z, Gunn SK, Wilde M, Darlington G, Shypailo RJ, Ellis KJ** 2005 Vitamin D receptor Fok1 polymorphisms affect calcium absorption, kinetics, and bone mineralization rates during puberty. J Bone Miner Res 20:945–953
241. **Zmuda JM, Cauley JA, Danielson ME, Theobald TM, Ferrell RE** 1999 Vitamin D receptor translation initiation codon polymorphism and markers of osteoporotic risk in older African-American women. Osteoporos Int 9:214–219
242. **Macdonald HM, McGuigan FE, Stewart A, Black AJ, Fraser WD, Ralston S, Reid DM** 2006 Large-scale population-based study shows no evidence of association between common polymorphism of the VDR gene and BMD in British women. J Bone Miner Res 21:151–162
243. **Grundberg E, Brandstrom H, Ribom EL, Ljunggren O, Kindmark A, Mallmin H** 2003 A poly adenosine repeat in the human vitamin D receptor gene is associated with bone mineral density in young Swedish women. Calcif Tissue Int 73:455–462
244. **Verbeek W, Gombart AF, Shiohara M, Campbell M, Koeffler HP** 1997 Vitamin D receptor: no evidence for allele-specific mRNA stability in cells which are heterozygous for the Taq I restriction enzyme polymorphism. Biochem Biophys Res Comm 238:77–80
245. **Grundberg E, Lau EM, Pastinen T, Kindmark A, Nilsson O, Ljunggren O, Mellström D, Orwoll E, Redlund-Johnell I, Holmberg A, Gurd S, Leung PC, Kwok T, Ohlsson C, Mallmin H, Brändström H** 2007 Vitamin D receptor 3′ haplotypes are unequally expressed in primary human bone cells and associated with increased fracture risk: the MrOS Study in Sweden and Hong Kong. J Bone Miner Res 22:832–840
246. **Mocharla H, Butch AW, Pappas AA, Flick JT, Weinstein RS, De Togni P, Jilka RL, Roberson PK, Parfitt AM, Manolagas SC** 1997 Quantification of vitamin D receptor mRNA by competitive polymerase chain reaction in PBMC: lack of correspondence with common allelic variants. J Bone Miner Res 12:726–733
247. **Gross C, Musiol IM, Eccleshall TR, Malloy PJ, Feldman D** 1998 Vitamin D receptor gene polymorphisms: analysis of ligand binding and hormone responsiveness in cultured skin fibroblasts. Biochem Biophys Res Comm 242:467–473
248. **Durrin LK, Haile RW, Ingles SA, Coetzee GA** 1999 Vitamin D receptor 3′-untranslated region polymorphisms: lack of effect on mRNA stability. Biochim Biophys Acta 1453:311–320
249. **Jurutka PW, Remus LS, Whitfield GK, Thompson PD, Hsieh JC, Zitzer H, Tavakkoli P, Galligan MA, Dang HT, Haussler CA, Haussler MR** 2000 The polymorphic N terminus in human vitamin D receptor isoforms influences transcriptional activity by modulating interaction with transcription factor IIB. Mol Endocrinol 14:401–420
250. **Colin EM, Weel AE, Uitterlinden AG, Buurman CJ, Birkenhäger JC, Pols HA, van Leeuwen JP** 2000 Consequences of vitamin D receptor gene polymorphisms for growth inhibition of cultured human peripheral blood mononuclear cells by 1,25-dihydroxyvitamin D3. Clin Endocrinol (Oxf) 52:211–216
251. **Uitterlinden AG, Weel AE, Burger H, Fang Y, van Duijn CM, Hofman A, van Leeuwen JP, Pols HA** 2001 Interaction between the vitamin D receptor gene and collagen type Iα1 gene in susceptibility for fracture. J Bone Miner Res 16:379–385
252. **Rivadeneira F, van Meurs JB, Kant J, Zillikens MC, Stolk L, Beck TJ, Arp P, Schuit SC, Hofman A, Houwing-Duistermaat JJ, van Duijn CM, van Leeuwen JP, Pols HA, Uitterlinden AG** 2006 Estrogen receptor β (ESR2) polymorphisms in interaction with estrogen receptor α (ESR1)

and insulin-like growth factor I (IGF1) variants influence the risk of fracture in postmenopausal women. J Bone Miner Res 21:1443–1456

253. **Roses AD** 2000 Pharmacogenetics and the practice of medicine. Nature 405:857–865
254. **Ongphiphadhanakul B, Chanprasertyothin S, Payatikul P, Tung SS, Piaseu N, Chailurkit L, Chansirikarn S, Puavilai G, Rajatanavin R** 2000 Oestrogen-receptor-α gene polymorphism affects response in bone mineral density to oestrogen in post-menopausal women. Clin Endocrinol (Oxf) 52:581–585
255. **Han K, Choi J, Moon I, Yoon H, Han I, Min H, Kim Y, Choi Y** 1999 Non-association of estrogen receptor genotypes with bone mineral density and bone turnover in Korean pre-, peri-, and postmenopausal women. Osteoporos Int 9:290–295
256. **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
257. **Marc J, Prezelj J, Komel R, Kocijancic A** 1999 VDR genotype and response to etidronate therapy in late postmenopausal women. Osteoporos Int 10:303–306
258. **Qureshi AM, Herd RJ, Blake GM, Fogelman I, Ralston SH** 2002 COLIA1 Sp1 polymorphism predicts response of femoral neck bone density to cyclical etidronate therapy. Calcif Tissue Int 70:158–163
259. **Ji W, Foo JN, O'Roak BJ, Zhao H, Larson MG, Simon DB, Newton-Cheh C, State MW, Levy D, Lifton RP** 2008 Rare independent mutations in renal salt handling genes contribute to blood pressure variation. Nat Genet 40: 592–599
260. **Cohen JC, Kiss RS, Pertsemlidis A, Marcel YL, McPherson R, Hobbs HH** 2004 Multiple rare alleles contribute to low plasma levels of HDL cholesterol. Science 305:869–872
261. **Yang TL, Chen XD, Guo Y, Lei SF, Wang JT, Zhou Q, Pan F, Chen Y, Zhang ZX, Dong SS, Xu XH, Yan H, Liu X, Qiu C, Zhu XZ, Chen T, Li M, Zhang H, Zhang L, Drees BM, Hamilton JJ, Papasian CJ, Recker RR, Song XP, Cheng J, Deng HW** 2008 Genome-wide copy-number-variation study identified a susceptibility gene, UGT2B17, for osteoporosis. Am J Hum Genet 83:663–674
262. **Kung AW, Xiao SM, Cherny S, Li GH, Gao Y, Tso G, Lau KS, Luk KD, Liu JM, Cui B, Zhang MJ, Zhang ZL, He JW, Yue H, Xia WB, Luo LM, He SL, Kiel DP, Karasik D, Hsu YH, Cupples LA, Demissie S, Styrkarsdottir U, Halldorsson BV, Sigurdsson G, Thorsteinsdottir U, Stefansson K, Richards JB, Zhai G, Soranzo N, Valdes A, Spector TD, Sham PC** 2010 Association of JAG1 with bone mineral density and osteoporotic fractures: a genome-wide association study and follow-up replication studies. Am J Hum Genet 86:229–239