Genetic Determinants of Bone Density and Fracture Risk—State of the Art and Future Directions

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Context: Osteoporosis is a common, highly heritable condition that causes substantial morbidity and mortality, the etiopathogenesis of which is poorly understood. Genetic studies are making increasingly rapid progress in identifying the genes involved.

Evidence Acquisition and Synthesis: In this review, we will summarize the current understanding of the genetics of osteoporosis based on publications from PubMed from the year 1987 onward.

Conclusions: Most genes involved in osteoporosis identified to date encode components of known pathways involved in bone synthesis or resorption, but as the field progresses, new pathways are being identified. Only a small proportion of the total genetic variation involved in osteoporosis has been identified, and new approaches will be required to identify most of the remaining genes. (J Clin Endocrinol Metab 95: 2576–2587, 2010)

The application of genomewide association study (GWAS) approaches in sufficiently large cohorts has initiated a wave of discoveries of genes involved in common diseases, including osteoporosis and fracture. This review aims to discuss the underlying assumptions, strengths, and weaknesses of various genetic approaches to date; to outline those genes that have been identified in GWAS of bone mineral density (BMD) and fracture, and what is known of their function; and to consider how genetic studies in osteoporosis may be improved in the future.

Genetic Epidemiology of Bone Phenotypes

Segregation studies in twins and families and the findings of gene-mapping studies to date all show that a substantial fraction of the variation in the bone phenotypes studied is polygenic. BMD and height are the most widely studied bone phenotypes in the general population. Twin and family studies of BMD confirm high heritability, with 60–90% of BMD variation estimated to be due to genetic factors (1–7). High heritability of BMD has been confirmed for all the major sites at which BMD has been measured, including hip, spine, and distal forearm (3, 8, 9). Similar data are also available for variation in height.

Most studies of bone BMD and height are cross-sectional, and thus heritability of longitudinal variation in BMD and of growth have not been well defined. At this point, we do not know in humans how early familiality of BMD, height, and skeletal growth are apparent or, importantly for planning gene-mapping experiments, are maximal. Monozygotic twin concordance of BMD reduces with age, suggesting that heritability may be highest at the youngest age, but this has not been extensively studied. The age of peak BMD heritability has been estimated in a family study at 26 yr, but the study concerned had at best modest power to define this tightly (10). Heritability has been shown to be higher in premenopausal than postmenopausal women (9, 11), although this has not been an entirely consistent finding, and heritability of BMD has been shown to be high even in elderly cohorts (12). In a study of mothers and prepubertal children (age 8 yr), significant mother-child correlation was evident, and moderate heritability was observed, indicating that heritable...
genetic effects are seen even at this early age (13). This study and others (6) have also raised the possibility of gender specificity of genetic effects, with greater mother-daughter correlation observed than mother-son, particularly at the hip. Site and gender specificity of BMD in family and twin studies (6, 14) indicate that it is likely that there will be differences observed in genetic associations with males and females, warranting studies specifically in each gender.

It is clear that the main clinical trait of interest in osteoporosis (i.e., fracture) is less heritable than intermediate heritable phenotypes such as BMD, skeletal size (e.g., height, hip axis length, etc.), and bone turnover markers. The familiality of fracture is low, ranging in first-degree relatives from 1.3–2.4 (15, 16). Heritability studies of fracture have generally shown limited support for shared genetic factors, with the possible exception of hip fracture in younger cohorts (age <69 yr) (summarized in Table 1).

Studies that have examined both BMD and fracture heritability have suggested limited shared genetics between the two traits (17, 18). This suggestion is of critical importance to the design of gene-mapping studies; if it is true, then studies investigating BMD as an intermediate trait aiming to then use BMD-associated genes to inform focused fracture-genetics studies are unlikely to succeed. Against this, premenopausal daughters of mothers with osteoporotic fracture have low BMD, suggesting that the factors that lead to osteoporotic fracture are cofamilial with low BMD (19–21). Given the high heritability of BMD itself, it is likely that this cofamiliality is caused by shared heritable, genetic factors. Furthermore, thus far, all genes that have been found to be associated with fracture are also associated with BMD, although the converse has not been established, potentially because of the lack of power of studies to date to identify fracture genes.

Segregation studies in families have universally found that a significant fraction of the heritability of BMD variation is polygenic. Collectively though, gene-mapping studies have to date only explained a tiny part of the estimated heritability of BMD. Moreover, those genes that have been identified have small effects, individually contributing less than 1% of the genetic variance of BMD. However, it is not true that just because a gene has a modest effect upon contribution to total variance at a population level, it is therefore proportionately only that important in the development of the phenotype. This is a common misconception. As an example, Osterix (Sp7) has been identified at a GWAS level as a significant determinant of BMD and growth (22). Although the total amount of variation explained by this gene is very small (likely <1%), Osterix itself plays an absolutely critical role in bone; homozygous Osx-null mice have no bone formation (23).

Although it is the case that most genes only contribute a small amount to BMD variation, it is also clear that BMD variation can occur due to more deleterious (but less frequent) mutations, as evidenced by the occurrence of monogenic conditions associated with major variation in skeletal strength and BMD. Studies of several genes associated with monogenic skeletal disease have shown that common variants of those genes are associated with BMD in the general population. *LRP5* is one such example in which loss of function mutations cause osteoporosis pseudoglioma syndrome (24), whereas gain of function results in high bone mass syndrome (25). Common variants, presumably of less major functional effect, were shown to influence general population BMD well before the GWAS era (26–28). Similarly, loss of function mutations of *SOST* cause van Buchem disease (29), whereas polymorphisms are associated with population variation in BMD (30, 31).

Genes identified by tag-single nucleotide polymorphism (SNP) association studies of common variants may also harbor rare variants that have more significant effects upon the phenotype of interest than the identified tagSNP. The rare variants can contribute significantly to overall population disease risk and may be able to confirm involvement of genes in a disease where tagSNP studies have not been clear-cut. They may also be very informative regarding the true disease-associated region in a gene be-

### TABLE 1. Genetic epidemiology studies of fracture risk

<table>
<thead>
<tr>
<th>First author (Ref.)</th>
<th>Ethnicity</th>
<th>No. of relative pairs</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kannus (88)</td>
<td>Finnish</td>
<td>15,098 twins</td>
<td>No increase in MZ concordance for fracture. Reanalysis by MacGregor suggested 35% heritability (89). Heritability of Colles’ fracture, 54%</td>
</tr>
<tr>
<td>Andrew (17)</td>
<td>British</td>
<td>6,750 twins</td>
<td>Heritability of any fracture of 16%, osteoporotic fractures of 27%, and hip fracture of 46%. Heritability of fracture 68% if age at fracture &lt;69 yr, but only 3% if age &gt;79 yr.</td>
</tr>
<tr>
<td>Michaelsson (90)</td>
<td>Swedish</td>
<td>33,432 twins</td>
<td>Heritability of Colles’ fracture, 25.4%</td>
</tr>
<tr>
<td>Deng (16)</td>
<td>American</td>
<td>6,274 sisters or mothers</td>
<td>Hip fracture heritability, 53%, but marginally significant (<em>P</em> = 0.048)</td>
</tr>
<tr>
<td>Deng (18)</td>
<td>American</td>
<td>50 families</td>
<td></td>
</tr>
</tbody>
</table>

MZ, Monozygotic twin.
cause the rare variants have lower surrounding linkage disequilibrium (LD), and thus provide much better resolution than common variants.

Identifying such variants will remain challenging, however, particularly for genes not already identified by tag-SNP studies, until whole genome sequencing becomes a practical reality. New sequencing technologies have extended our capacity to study monogenic conditions, reducing the number of families that are needed for recombination mapping by increasing the intervals that can reasonably be sequenced. Recently, it has been demonstrated that a monogenic condition could be mapped by sequencing the exome using next-generation sequencers, then filtering the variants identified according to the likely functional consequence and existence in public databases (32). As a consequence, it may be that monogenic diseases can be mapped using only a few affected individuals, rather than requiring the collection of Mendelian families with sufficient informative meioses.

Gene-gene interactions (epistasis) have been studied extensively in animals and have been shown to be important in skeletal phenotypes in mice (33), rats (34), chickens (35), and pigs (36). Genetic studies in humans to date have focused on individual rather than interactive gene effects, at least partly because most of our gene-mapping methods are poor at identifying interactive effects, particularly where the effects of the individual loci (the “marginal” effects) are at the limit of our capacity to identify them.

The difficulties that epistasis may cause in gene identification are well demonstrated in congeneric mapping studies (37, 38). Breeding programs aimed at isolating linked regions to map the involved genes remove existing epistatic effects, often leading to loss or substantial modification of the phenotypes of interest. In most human diseases studied to date by adequately powered GWAS, marker-by-marker analysis has been successful in identifying some genes, whereas few examples exist of robust, confirmed epistasis. This is a problem for all common human diseases, not just osteoporosis, and until solved, it is likely that at least some genes, whose marginal effects are too small to identify without accounting for epistasis, may not be found.

Gene-environment interaction has also been a tricky field. Environmental factors are notoriously difficult to measure and to model in analysis. It is usually not clear at what age the environmental effect is relevant, the number of potential environmental effects is larger than the potential number of genetic effects, and quantitation of those environmental factors is more difficult than genotyping. Environmental effects are often closely correlated, and thus distinguishing the true environmental factor involved is often problematic. However, it may be possible to study some well-defined environmental effects more easily, such as cigarette smoking, where gene-environment interaction is well established in other diseases (39). To date, environmental effects have only been modeled in limited candidate gene studies in osteoporosis, and never in a GWAS.

Genetic Approaches

Linkage

Classical Mendelian monogenic diseases can be mapped with a relatively small number of affected pedigrees using linkage (the cosegregation of genetic markers with disease phenotype within families). Skeletal monogenic diseases mapped by linkage include osteogenesis imperfecta type 1 (40), osteoporosis pseudoglioma (41), high bone mass syndromes (42), autosomal dominant osteopetroses (43), hypophosphatasia (44), sclerosteosis (45), and Van Buchem disease (46).

These same linkage approaches proved much less successful in quantitative traits, such as BMD and fracture risk. Despite valiant efforts of large individual centers and consortia, including studies in unselected populations (47–50) and in populations identified through extreme truncate proband ascertainment (51, 52), it would be difficult to argue that any osteoporosis-quantitative trait locus was definitively identified through linkage. In retrospect, this was not surprising, considering the vast numbers of sib-pairs and/or families that would be needed for adequate power to identify even those quantitative trait loci contributing a moderate-to-large effect upon variance (53).

BMP2 [bone morphogenetic protein 2; a member of the TGF-β family, a critical inducer of bone and cartilage formation (OMIM 112261)] was identified through linkage studies of BMD in the unselected Icelandic population (50). Other larger studies did not replicate these results or found association with different SNPs (54, 55), and thus BMP2 cannot really be said to be “established” as a BMD gene.

Candidate gene association studies

Multiple small, generally underpowered, candidate gene association studies had been performed in osteoporosis, as in many other diseases. Few unequivocal associations were established, most findings had borderline levels of significance, and contradictory results were frequent. Large consortia were established to attempt sufficiently powered association studies, but generally these were not particularly informative because in most studies, only a small proportion of the genetic variation in the candidate gene of interest was studied. Because the true dis-
ease-associated variant was unknown for these genes, their negative results did not exclude association of the gene concerned (56).

Improved study design and genotyping technology and lower genotyping cost have enabled more recent studies to perform more comprehensive studies of the candidate genes they target. A recent large candidate gene study used data from five cohorts with GWAS data (overall population, 19,195 individuals of European origin) and selected for study 36,016 SNPs either within, or in close proximity to, 150 candidate genes of interest (57). Association was confirmed with nine genes (ESR1, LRP4, ITGA1, LRP5, SOST, SPP1, TNFRSF11A, TNFRSF11B, TNFSF11) associated with lumbar spine BMD; SPP1, SOST, LRP5, and TNFRSF11A/RANK were associated with fracture, although the study was underpowered for this end point.

GWAS

Improved genotyping technology, statistical and study design developments, and the acceptance by investigators that large, comprehensive, hypothesis-free studies were the way forward led to the development of GWAS, which have revolutionized common disease genetics over the past few years. A landmark publication in 2007 by the Wellcome Trust Case Control Consortium (WTCCC) provided the proof-of-principle of these studies, identifying dozens of genes that were associated with development of common diseases such as type 1 diabetes mellitus and inflammatory bowel disease (58). The WTCCC also made all the genotype data from this study freely available to bona fide researchers, a model that has largely, but unfortunately not universally, been followed in the field since then.

Current thresholds for reporting significant association (P < 5 × 10−8) have proven fairly robust, but even at this level, not all associations are replicated, and false positives occur. For associations to be considered definite, this level of significance and convincing replication with the same SNP in the same direction are required.

Most individual GWASs remain underpowered. True disease-associated SNPs therefore often only achieve suggestive levels of association in GWAS studies. Identifying these SNPs requires either very deep replication studies or some mechanism of prioritizing SNPs for replication based on other data. Bioinformatic approaches including gene-ontology searches, expression findings in disease and health, expression quantitative trait loci studies, and keyword sharing statistical approaches using PubMed abstracts [e.g. Gene Relationships Among Implicated Loci (GRAIL) (59)] are being developed for these purposes. Meta-analysis has also proven to be a useful tool to identify associations that individually underpowered studies were not able to detect.

GWAS in Osteoporosis

An early screen published by the Framingham group had insufficient marker density to be called a true genomewide screen and was underpowered; not surprisingly, no findings of genomewide significance were observed. However, modest evidence of association was seen at several “osteoporosis” candidate genes, including MTHFR, ESR1, COL1A1, LRP5, VDR, PPARG, and CYP19 (60). Subsequently, two back-to-back adequately powered GWAS in osteoporosis (examining BMD and fracture in unselected populations) were published (reviewed in Ref. 61), followed by further GWAS in Koreans (62) and in Chinese (63), focusing mainly on BMD. The findings of these studies and of other genomewide significant “definite” osteoporosis associations are summarized in Table 2.

An osteoporosis GWAS by deCODE Genetics tested approximately 300,000 SNPs in 5,861 men and women, with 74 SNPs taken forward in a replication cohort of nearly 8,000 men and women from Iceland, Australia, and Denmark (64). Five regions were reported to be associated with BMD at genomewide significance, most notably SNPs in LD blocks with the genes RANKL and OPG (discussed below). Several markers nearby ESR1 were reported to show association with BMD, including one within an intron of a splice variant; all the others were upstream and in closer LD to an open reading frame C6orf97. This area was replicated in a more recent meta-analysis (65), suggesting that there is a true BMD locus on chromosome 6q25. Other areas included 1p36, attributed to ZBTB40 (see comments later) and the major histocompatibility complex (MHC) on 6p25. Association with fracture was also tested, but only results of suggestive statistical significance were obtained [with 1p36, MHC, RANK, and two novel areas including 2p16 and 11p11 (the latter locus containing LRP4)]. Commendably, deCODE made summary statistics for all SNPs publicly available for both hip and spine BMD phenotypes.

The second GWAS in the Twins UK and Rotterdam cohorts (66), involving 2094 cases in the discovery cohort and a two-phase replication study involving 6463 individuals, found LRP5 and OPG to be significantly associated with BMD and marginally associated with fracture. Disappointingly, neither summary statistics nor genotype data are publicly available from this study.

Cho et al. (62) reported a GWAS of eight quantitative traits in 8842 unselected Koreans, including BMD measured at the radius and tibia. Replication was performed with measurement at a different site (the heel), which, given the likely site specificity of a significant component of osteoporosis genes, is of uncertain relevance. Despite the large sample size, this study showed weak or absent
replication of established BMD loci (RANKL, \( P = 0.012 \); no significant replication reported at LRP5 or ZBTB40/ Wnt4). This may reflect the sites studied, the genotyping chip used (the older generation Affymetrix 500K chip), or ethnic-specific effects. One gene was identified at genomewide significance (FAM3C), but the study had no replication cohort and has not been reported in other GWAS studies to date. Near genomewide significance was noted at chromosome 7p14.1 near sFRP4 (minimum \( P = 1.4 \times 10^{-7} \)), near a genomewide significant finding in the GEFOS meta-analysis discussed below (65), although the specific associated marker (rs1721400) showed no association in the GEFOS study and is also of uncertain significance. Comendably, the authors of this study have made the genotype data publicly available, although disappointingly not the phenotype or analyzed summary data.

These studies were of unselected populations, which raises issues about the efficiency of these designs. Despite moderately large cohorts, little was found at a GWAS level of significance. Options to improve power would be to use...
a more efficient study design and/or increasing sample size. As discussed below, much larger cohorts are likely to be required, particularly if unselected population cohorts are used for studies.

One GWAS using selected samples has been reported, involving 800 unrelated southern Chinese women with a two-phase replication study in 18,098 subjects of white European and Asian descent (63). The discovery cohort included only cases with BMD in either the bottom 10% of the population or the top 16%. This study identified and confirmed one new osteoporosis gene (JAG1, discussed below), but the study power was limited because the cohort was of modest size, and degree of selection was only moderate. Weak association was also observed with fracture for the key JAG1 SNP. Disappointingly, neither summary statistics nor genotype data are publicly available from this study also.

Because individual studies are generally underpowered to identify osteoporosis genes, investigators have employed meta-analysis to combine results and improve study power. A recent meta-analysis of BMD GWASs combined GWAS data from 19,195 subjects of white European descent and achieved genomewide significance at 13 novel and seven previously reported loci. MARK3, SOST, and the MHC were also reported to be strongly associated with BMD, although not at genomewide-significant levels. There loci are discussed below (65).

The deCODE group has also adopted the “bigger is better” approach, increasing the size of the discovery cohort by 20% and replication cohorts by 40% (67). This extended study found four new genomewide significant loci compared with their original study, including SOST, SP7, TNFRSF11A (RANK) (all discussed above) and MARK3, with suggestive results at LRP4, ADAM19, IBSP, and C17orf53. Of note, the three SNPs in SOST associated with BMD also had modest effect upon fracture (odds ratios, 1.07–1.1). Some of these genes had been identified elsewhere. SOST had previously been associated with BMD in candidate gene studies (30, 31). SP7, which encodes Osterix, was identified in parallel by British and Australian investigators and was confirmed to influence both height and BMD in children, as well as BMD in adults (22).

Lastly, a GWAS of BMD and fracture has been published, using a discovery set of unselected 1000 white Americans (68), with five replication cohorts of different ethnicity, with meta-analysis for the data from all the cohorts for five SNPs of interest. Two to five SNPs in the two genes of interest were then studied in several replication cohorts, with results pooled and meta-analyzed in both white-only and all-ethnicity analyses. The investigators in this study made good use of the publicly available Framingham osteoporosis GWAS for replication, but the data from their own discovery set have not yet been made publicly available. Hopefully, this will be corrected in the near future. Three SNPs in ADAMTS18 achieved genomewide significance overall (most significant rs16945612, hip BMD $P = 2.13 \times 10^{-8}$), whereas SNPs in TGFBR3 achieved suggestive association (peak association rs1713547, $P = 1.49 \times 10^{-6}$).

Although tagSNP mapping has better resolution than linkage, in many cases the peak of association does not lie in a coding region of a gene, and the gene reported to be underlying the observed association is in most cases putative only. For example, the 11p12 locus, nominally identified as ARHGAP1, also contains LRP4, arguably a better candidate gene to explain the observed association. It is also possible that the associated variants influence transcription of remote genes, as is likely the case for associations involving intergenic regions. To take these observations forward requires much further work to determine the true disease-associated gene.

Nonetheless, it is apparent from even a cursory glance at the loci identified through GWAS to date (Table 2) that at least two major bone pathways are overrepresented: the RANK (TNFRSF11A)/RANKL (TNFSF11)/OPG (TNFSF11B) pathway and the Wnt/LRP5 pathway.

The RANK/RANKL/OPG pathway (recently reviewed in Ref. 69) is critical in the remodeling cycle of bone. Binding of RANKL to RANK stimulates osteoclast formation from precursors, osteoclast differentiation and activation, bone resorption, and osteoclast survival. Soluble OPG binds to RANKL through interaction of its TNF-receptor with TNF domains of RANKL and prevents RANKL interaction with RANK. Thus, OPG “protects bone” by preventing osteoclast activity. In contrast with many biological systems, there is little redundancy in this system: RANKL-null mice develop osteopetrosis and high bone mass, with absence of osteoclasts; and RANK-null mice have an almost identical phenotype. Macrophage colony-stimulating factor (M-CSF) is also important in osteoclast proliferation, differentiation, function, bone resorption, and survival; however, m-CSF-deficient mice, although osteopetrotic, have a paucity rather than absolute deficiency of osteoclasts.

It is therefore of great interest that SNPs in proximity to each of these genes—RANK (TNFRSF11A), RANKL (TNFSF11), and OPG (TNFSF11B)—have been found to be associated with BMD at a population level. As discussed above, the total amount of BMD variance explained by these genes remains small, yet this is obviously a pathway of critical importance in the development and maintenance of the skeleton.

The Wnt/LRP5 pathway is a, if not the, major anabolic pathway in bone (recently reviewed in Ref. 70). Wnt proteins are a family of secreted signaling proteins critical for
normal embryogenesis and morphogenic signaling including limb development. Wntless is a highly conserved membrane protein that promotes secretion of Wnt proteins from signaling cells (71). GPR177, which is BMD associated at genomewide significant levels, is also known as Wntless homolog. In bone, activation of the canonical Wnt pathway by a complex of Wnt, LRP5 or LRP6, and membrane bound Frizzled results in inhibition of glycogen synthase kinase-3β and subsequent stabilization of β-catenin, which then translocates to the nucleus to affect gene transcription. In the absence of Wnt signaling, β-catenin is phosphorylated by glycogen synthase kinase-3β, resulting in its degradation.

The major effect of Wnt activation in bone is to promote bone anabolism. This includes differentiation of mesenchymal precursors into osteoblasts, osteoblast proliferation, increased osteoblast synthesis, bone mineralization, and avoidance of osteoblast apoptosis, and inhibition of osteoclastogenesis through effects on expression of OPG and RANKL.

Inhibitors of this pathway include dickkopfs, secreted frizzled-related proteins, and sclerostin (SOST). SOST is expressed exclusively by osteoclasts, and its action is facilitated and regulated by LRP4 (72), which is itself BMD associated at genomewide significance.

LRP5, LRP6, SOST, GPR177 (discussed above), and CTNNB1 all have been associated with BMD at genomewide significance. CTNNB1 codes for catenin (cadherin-associated protein) β1. Catenins are involved in the intracellular component of canonical Wnt signaling as described above. Catenins are also part of protein complexes constituting adherens junctions, regulating cell growth and cellular adhesion. CTNNB1 also anchors actin cytoskeleton and may be an inhibitory signal to cellular proliferation resulting from cellular contact inhibition.

The 1p36 locus, which is significantly associated with BMD, contains another Wnt pathway gene, Wnt4. The role of Wnt4 in bone, however, is not clear. Wnt4 plays a role in sex determination, antagonizing testis-determining factor to prevent testes formation and control female development. However, the other gene of interest in the 1p36 locus is EPHA8. Ephrins are a family of proteins involved in signaling in the nervous system. However, ephrins are also involved in signaling in bone and are involved in coupling of osteoblastogenesis with osteoclastogenesis. EphrinB2 is expressed in bone (both in osteoblasts and osteoclasts) under regulation by PTH and PTHrP (73) and binds to its receptor EphB4. This family has also been implicated in dental development (74) and myeloma bone disease (75). EPHA8 codes for a receptor that interacts with ephrins A2, A3, and A5, guiding axonal development. The gene currently attributed to the association at 1p36, ZBTB40, is without known function in bone.

At the 11p12 locus, ARHGAPI1 is one of several genes in LD with the two significantly associated SNPs. One SNP is within the promoter region for ARHGAPI1; the other lies in a coagulation factor 2 gene (F2). However, LRP4 also lies within the same disease-associated LD block, and it is therefore difficult to attribute the observed association to any one gene. ARHGAPI1 codes for a Rho GTPase-activating protein 1, also known as Cdc42 GTPase-activating protein, part of a family of cytoskeleton regulators. CDC42GAP-null mice develop a senescence phenotype including osteoporosis (76). F2 codes for thrombin, one of the vitamin K-dependent clotting factors. A number of coagulation factors have been reported to be associated with skeletal disorders. These include skeletal defects associated with warfarin exposure in utero, and Ghosal hematodiaphyseal dysplasia, caused by thromboxane synthase mutations, which is associated with high bone mass. As mentioned above, LRP4, under the influence of PTH, negatively regulates SOST, itself an inhibitor of the Wnt/LRP5/frizzled pathway in bone.

A host of bone-related genes are encoded at the 4q21.1 locus, including MEPE (matrix extracellular phosphoglycoprotein, osteoblast/osteocyte factor 4S), IBSP (integrin binding sialoprotein), and SPP1 (secreted phosphoprotein, osteopontin). MEPE was first identified as an upregulated gene in oncogenic osteomalacia. It is expressed mainly in osteocytes (77) and is thought to act as a mineralization inhibitor (78). SPP1 is the principal noncollagenous phosphorylated glycoprotein in bone. IBSP is also one of the most abundantly expressed proteins in bone matrix. Both osteopontin and IBSP are thought to mediate cell–matrix interaction, binding through a common cell attachment consensus sequence, to affect binding of osteoblasts and osteoclasts to matrix, and to influence angiogenesis. Both have extremely high affinity to calcium and hydroxyapatite through a polyacidic amino acid sequence, and their roles include regulation of bone mineralization. SPP1 is thought to be an inhibitor of bone mineralization, whereas IBSP is thought to promote mineralization. IBSP is expressed by all bone cells and by hypertrophic cartilage and trophoblasts. SPP1 is expressed more widely both in calcified tissues and in other tissues. All of these genes make good candidates underlying the observed association at this locus, and it is not clear which gene is principally involved at this locus.

SOX6 [Sry (sex-determining region Y) box] has now been reported to be BMD associated at genomewide significance in two studies (65, 79). Sox6 is a gene transcription factor expressed in cartilage, with high homology to L-Sox5. Along with Sox9, all three transcription factors
are expressed in embryonic chondrogenic sites and activate COL2A1. Thus, Sox6 has an essential role in chondrocyte differentiation, cartilage formation, and hence endochondral bone formation (80). Sox5/−/− and Sox6/−/− mice have a relatively mild skeletal phenotype. However, double knockout mice have a lethal severe generalized chondrodysplasia, due to deficient differentiation and function of chondroblasts, demonstrating the critical role of this path in normal cartilage and hence endochondral bone formation (81). In humans, a child with craniosynostosis has been reported to have a balanced translocation that disrupts SOX6 (82).

MEF2C (myocyte enhancer factor 2C) codes for a muscle transcription activator that binds to the MEF2 regulatory region present in many muscle-specific genes, interacting with histone deacetylase 4, 7, and 9. Initially, its role was thought to be mainly related to cardiac morphogenesis and vascular and muscle development. However, Rivadeneira et al. (65) found MEF2C to be most strongly associated at the hip, a site where BMD is more strongly influenced by muscle action than at the lumbar spine, and postulated that the effect of this gene upon bone and BMD could be mediated through its effects on muscle. Arnold et al. (83) reported that MEF2C is responsible for controlling bone development, although activating chondrocyte hypertrophy. MEF2C overexpression results in precocious chondrocyte hypertrophy, ossification of growth plates, and dwarfism, whereas MEF2C-null mice or mice with a dominant-negative mutant MEF2C had decreased chondrocyte hypertrophy, angiogenesis, ossification, and longitudinal bone growth. They also noted the critical balance between MEF2C and HDAC4, such that double mutants in both genes resulted in a rescuing of the phenotype of either mutant alone (83). MEF2C also activates SOST and COL10A1 transcription, and thus may influence BMD by effects on the transcription of these genes.

HDAC5 (at locus 17q21) was also associated more strongly with BMD at hip compared with lumbar spine. Histone deacetylators play a critical role in histone acetylation and deacetylation, important epigenetic modifiers for gene activation and transcription. HDAC5 is another class II HDAC, like HDAC4, and is known to interact with MEF2 proteins in muscle, inhibiting transcription and retaining muscle maturation. Other histone-deacetylators have been shown to accelerate matrix mineralization and osteoblast maturation, with increased expression of osteocalcin, alkaline phosphatase, and Wnt receptor genes (84). Histone deacetylase enzymes repress the expression of MEF2 target genes. This repression is in turn controlled by calcium/calmodulin-dependent protein kinases. Perturbation of this control pathway seems likely to provide a molecular explanation for the genetic associations observed with MEF2C and HDAC5.

STARD3NL codes for an endosomal protein that may mediate cholesterol transport; as such, a putative role in bone could include an effect upon steroidogenesis.

CHR1 codes for corticotrophin-releasing factor receptor 1, the main receptor modulating the effect of CRH, resulting in secretion of ACTH and thence cortisol, with its many known effects upon the skeleton. Polymorphisms of CHR1 have a pharmacogenomic effect upon asthmatic response to inhaled corticosteroids (85).

The 16q24.3 locus contains several FOX (fork-head box) genes in the LD block, including FOXF1, FOXL1, and FOXC2, as well as MTHFSD. FOX proteins are a...
large family of transcription factors, and FOXC2 is thought to be involved in mesenchymal differentiation, in particular regulating adipocyte metabolism. This locus is of special interest, therefore, given recent discoveries of the close interregulation of bone and fat tissue (86). Furthermore, FOXC2 expression is important for early chondrogenesis in mesenchymal tissues, and in skeletal precursors FOXC2 expression is regulated by BMPs (87).

SPTBN1 codes for the β-subunit of Spectrin, a tetrameric protein essential in cell scaffolding. Spectrin’s roles include determination of cell shape, resilience of membranes to mechanical stress, positioning of transmembrane proteins, and organization of organelles and molecular traffic. β-Subunits, such as SPTBN1, are responsible for most of the spectrin binding activity. Its role in bone pathophysiology is unclear.

MARK3 (MAP/microtubule affinity-regulating kinase 3) codes for an ubiquitously expressed protein kinase involved in microtubular phosphorylation (specifically MAPT, MAP2, and MAP4). Microtubules play a role in cell polarity and in cell cycle regulation.

ADAM19 codes for a member of the disintegrin and metalloprotease domain family, which are membrane-anchored proteins thought to have a role in cell-cell and cell-matrix interactions. This particular protein is a marker for dendritic cell differentiation. Its relevance to bone is not known.

Similarly to the ADAM family, ADAMTS18 encodes a member of another family of proteins anchored to the extracellular matrix, but through interactions involving one or more thrombospondin type 1 motifs. A specific role in bone biology is not known.

Mutations of JAG1 cause Alagille syndrome, an autosomal dominant multisystem developmental disorder associated with vertebral deformities and shortened vertebral height. Jagged 1 belongs to the Delta/Serrate domain family of proteins, which are Notch ligands. Notch receptors have important effects on osteoclastogenesis and in osteoblast proliferation.

ESR1 codes for the estrogen receptor type 1, also known as ER-α. Estrogen receptors in humans have two isoforms (α and β) that have distinct tissue and cell patterns of expression. Estrogen is well known to inhibit bone resorption through both direct and indirect actions on osteoclasts, and it is a major anabolic steroid in bone, particularly evident in the establishment of peak bone mass.

AKAP11 encodes a member of the A-kinase anchor protein family. It lies close to RANK, and therefore it is not clear how much weight to put on this gene independently because RANK seems a more likely candidate at this locus. AKAP11 codes for a protein expressed at high levels through spermatogenesis and in mature sperm that binds to protein kinase A.

Conclusion

Currently, the emphasis in osteoporosis genetics is on bigger and more powerful GWASs. This is clearly required particularly for fracture, given the low power such studies currently have for what is a lower heritability, high heterogeneity phenotype. We have plotted the required sample sizes to identify genes of particular effect sizes at the current accepted threshold for a definite genetic finding ($P = 5 \times 10^{-8}$) (Fig. 1). These sample sizes are difficult to achieve, and alternate approaches will have value in the search for osteoporosis genes (reviewed in Ref. 90). It is hoped that with improved power and sophistication of study design, genetic studies in BMD and fracture will result in fresh insights into pathways that influence BMD in the general population and, ultimately, new paradigms in the treatment of osteoporosis.

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