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Role of Genetics in Osteoporosis

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Osteoporosis is a disease characterized by fragile bones and high susceptibility to low-trauma fractures. It is a serious health problem, especially in elderly women. Bone mineral density (BMD) has been employed most commonly as the index for defining and studying osteoporosis. BMD has high genetic determination, with heritability ranging from 50 to 90%. Various gene-mapping approaches have been applied to identify specific genes underlying osteoporosis, largely using BMD as the study phenotype. We review here the genetic determination of osteoporosis as defined by BMD and discuss a fundamental issue we encounter in genetic research in osteoporosis: the choice of phenotype(s) to study. We briefly summarize and discuss advantages and disadvantages of various approaches used in genetic studies of osteoporosis. Finally, we review and discuss the current status for mapping and identification of genes for osteoporosis. We focus on linkage studies in humans and quantitative trait loci mapping in mice to supplement the already extensive reviews of association studies made by many investigators for candidate genes.

Key Words: Bone mineral density; gene mapping; genetics; osteoporosis; osteoporotic fractures.

Introduction

A Definition of Osteoporosis

Osteoporosis is characterized by an excessively fragile skeleton and susceptibility to fractures *(1)*. Low-trauma fractures owing to osteoporosis are a major public health problem among the elderly *(2–6)*, especially among women. The social, personal, financial, and other costs of the worldwide epidemic of fractures is enormous *(7)*. The most important measurable feature of the excess skeletal fragility of osteoporosis is reduced bone mass *(3,8,9)*, as measured in a variety of ways, most commonly by dual X-ray absorptiometry (DXA). The most common expression of the DXA measurement is bone mineral density (BMD). However, there are other features interacting with BMD, most notably, microarchitectural deterioration in which bone loss in vulnerable locations (trabecular cross connections) exaggerates the fragility. There may also be deterioration of intrinsic mechanical properties of bone tissue. Other etiologic factors might include muscle weakness, loss of neuromuscular coordination, loss of balance, and a host of factors that remain hypothetical.

Measurement of bone mass has dominated our thinking about the etiology of osteoporosis *(10)*. Early investigators and clinicians recognized low skeletal mass and density in elderly patients with fractures from the radiographic appearance of the skeleton. In the past two decades, photon absorptiometry, DXA, has developed to the point that we can now measure the amount of bone in a person's skeleton quite accurately. DXA measurements yield a very strong estimate of the risk of future fracture, and thus much effort and attention has been devoted to these measurements. In the process, we have gone from the clinical observation that osteoporosis with fracture "runs in families," to recognizing that bone mass, however measured, is a complex trait that is determined by multiple genetic and environmental factors *(11–14)*. It is among the strongest of genetically determined complex traits with a heritability (h^2) of >50% *(12, 14–19)*. Thus, genetic studies are important to gain a comprehensive understanding of factors underlying the majority of the variation in bone mass.

While measurement of bone mass has dominated our thinking about the etiology of osteoporosis, there are limitations in this paradigm. Bone mass, no matter how it is measured, by DXA, quantitative computed tomography (QCT), peripheral QCT, ultrasound, and any expressions of these measurements intended to expand the information they give on bone strength or resistance to fracture, explain only part of the determination of fracture. Thus, as discussed subsequently, some attention must be given to the heritability and the genetic basis of fracture *per se*.

Purpose of Genetic Studies

Many studies have been conducted with the explicit or implicit purpose of finding genes and/or genomic regions that contribute to the risk of osteoporotic fractures, even if they focus on bone mass rather than fracture *per se*. Identifying such loci is the first step in discovering functional mutations responsible for variation in risk of osteoporotic

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fractures. This will guide research into, and discovery of, the functional mechanisms of distinct allele products and will help focus and direct basic research on the mechanisms underlying risk of osteoporotic fracture. One general goal in studying the genetics of osteoporosis is to create shortcuts to the development of new tools, markers, and therapies for diagnosis and treatment. The genes and genomic regions thus identified will also form the basis for launching studies of the interaction between genotype and environmental factors (GxE interaction) and gene-by-gene interactions (epistasis) that affect risk of osteoporotic fracture. While it seems unlikely (though not impossible) that the outcome of this research will lead to "gene therapy" for osteoporosis, the results may nevertheless be useful in devising genotype-specific interventions to reduce fracture rates.

This article focuses on methods and paradigms involved in the search for genes and/or genomic regions underlying the risk of osteoporosis. Several approaches employed in the past have generated both progress and controversies. The controversies have centered on questions regarding the robustness of the results and are mostly owing to lack of understanding of the strengths and weaknesses of the various approaches used. Several reviews in the field of bone genetics *(20–25)* have focused mainly on the results of candidate gene studies. Some reviews *(24,26)* have described approaches that are standard for the search of genes underlying complex traits in general. Others have extensively reviewed issues involving choice of study samples and populations *(27–35)*. None have addressed the problems and controversies in applying these methods in bone genetics. Because of inconsistent results from the population association approach, other methods (i.e., linkage analyses in humans and quantitative trait loci [QTL] mapping in mice) tend to be regarded as panaceas in solving the problems encountered in identifying QTLs for osteoporotic fracture and/or BMD *(24,36)*.

We try to summarize here the various approaches and assess the status of each in genetic studies of osteoporosis. We outline advantages and disadvantages of various gene-mapping approaches in the context of our own studies and those of others in osteoporosis genetics. We begin with a fundamental issue, the goal of genetic studies in osteoporosis, and the phenotype(s) that should be studied in order to reach this goal.

Heritability and Choice of Study Phenotypes

It should be emphasized that the goal of these studies is to locate genes underlying variation in susceptibility to osteoporotic fracture. This is true whether osteoporotic fracture *per se* is the phenotype under study, or risk factor(s) (such as BMD, bone size, ultrasound, and volumetric density) are used as surrogate phenotypes. This goal has been explicit or implicit, and it has not been otherwise stated or challenged *(26,36,37)*. All the other goals *(37)* are derived from this major goal.

Continuously distributed complex quantitative traits are commonly studied as surrogate phenotypes in searching for genes underlying the etiology of complex diseases. An example of this in another field is blood lipid measurements as surrogate phenotypes for cardiovascular disease. This strategy is based on two conditions: (1) the underlying risk factor is phenotypically correlated with the disease; that is, low values confer low risk and high values confer high risk of disease; and (2) dividing a complex disease into its underlying risk factors facilitates gene search because the risk factors are less complex and thus provide simplified phenotypes for study.

The validity of using condition 1 is problematic, as shown later in our findings on the genetic correlation between BMD and osteoporotic fracture *(38)*. Condition 2 has never been substantiated, at least in the bone field. Manifestly, underlying risk factors of complex diseases are themselves usually also complex quantitative traits determined by multiple genetic and environmental factors, often in addition to their interactions. Thus, the genetic determination of their variation may not be less complex, since they have peculiar determining factors, both genetic and environmental, that are not relevant to the risk of disease.

BMD variation is under strong genetic control with heritability (h^2) estimates ranging from 0.5 to 0.9 *(13–16,18,19)*. Further, most of the segregation analyses *(18,34–37,39–42)* suggest that there is at least one major gene for population BMD variation. Although BMD and osteoporotic fracture are *phenotypically* correlated, so that lower BMD values are associated with higher risk of osteoporotic fracture *(2, 6)*, it is not known whether they are *genetically* correlated, and neither is known for the genetic determination of osteoporotic fracture. Thus, it is not clear generally how relevant the genes determining BMD variation to the genetic determination of osteoporotic fracture susceptibility are. It is well known from the theory of quantitative genetics *(43)* that for two complex traits, such as osteoporotic fracture and BMD, phenotypic correlation may be caused by both genetic and environmental factors. However, significant phenotypic correlation between them does not necessarily imply that they are genetically correlated (see Appendix) or, in the case of osteoporosis, that the heritability of osteoporotic fracture is nonzero.

Deng et al. *(38)* demonstrated a moderately high genetic determination for Colles fractures in a US Midwestern population (heritability 0.254, ±SE 0.118). This fracture is relatively common, symptomatic, and relatively easy to ascertain and confirm. Although the heritability of Colles fracture does not appear to be high compared with BMD, the gene(s) that might be identified using Colles fracture as a study phenotype is directly relevant to our focus on the underlying genetic susceptibility to osteoporotic fracture.

That this study design represents a practical approach to determining linkage is demonstrated by the fact that in this report, Deng et al. *(38)* identified about 40 affected sib pairs with Colles fracture over a period of about 3 mo by a part-time research recruiter. It is worth pointing out that the statistical power for linkage analyses or candidate gene studies depends more on the genetic effects of individual loci rather than on the overall magnitude of genetic determination of a complex trait *(44,45)*. Hence, the relatively low heritability of Colles fracture compared to BMD is not sufficient reason to justify eliminating osteoporotic fracture as a study phenotype to identify genes for osteoporotic fracture, as argued by Blank *(36)*.

In another study, Deng et al. *(46)* investigated the relevance of genetic determination of BMD variation to that of differential risk of osteoporotic fracture. This relevance is characterized by genetic correlation (ρ_G) between BMD and osteoporotic fracture. For 50 Caucasian pedigrees with 703 subjects, we estimated that h^2 at the hip is 0.64 ($p < 0.0001$) for BMD and 0.52 ($p < 0.05$) for osteoporotic fracture; however, ρ_G between BMD and osteoporotic fracture is nonsignificant ($p > 0.45$), and <1% of additive genetic variance is shared between them. Hence, most genes found important for BMD may not be relevant to osteoporotic fracture at the hip. The phenotypic correlation (ρ_P) between high BMD and low risk of osteoporotic fracture at the hip (~–0.30) is therefore largely owing to an *environmental* correlation ($\rho_E = -0.73$, $p < 0.0001$), which is the result of *nongenetic* factors. Gene search for osteoporotic fracture should start with a significant h^2 for osteoporotic fracture and may include risk factors (besides BMD) that are *genetically* correlated with osteoporotic fracture. Ideally, employing osteoporotic fracture *per se* as a direct phenotype for gene hunting and testing can ensure the importance and direct relevance of the genes found for the risk of osteoporotic fracture.

The limitations of gene mapping for underlying risk factors rather than the complex disease for which they are markers for risk can be demonstrated in other fields. For example, in the Finland–U.S. genetic studies of non-insulin-dependent diabetes mellitus, potential susceptibility genes were detected, in order of the significance of their supporting evidence, on chromosomes 20, 11, 2, 10, and 6, respectively *(47)*. However, studies of a suite of diabetes-related risk factors (quantitative traits) *(48)* by the same research team in the same population revealed potential QTLs on chromosomes 3 (fasting C-peptide/glucose), 10 (acute insulin response), 13 (body mass index and 2-h insulin), 17, and 19 (empirical insulin-resistance index), respectively. Except for chromosome 10, genes detected for diabetes were found located on chromosomes different from those for various underlying risk factors. Even on chromosome 10, the genomic region important for type 2 diabetes peaked at 75.0 cM from pter with a weighted maximum logarithm of odds (LOD) score (MLS) of 0.77, whereas that for acute insulin response peaked at 21.0 cM from pter with an MLS of 3.11. Hence, it seems that none of the putative genomic regions found to be important for underlying various risk factors were important enough to be detected by direct linkage studies for diabetes. In addition, none of the putative genomic regions found important for diabetes were important enough to be detected in linkage studies for several important underlying risk factors. These studies clearly indicate that a gene found for a risk factor may not be important or even relevant for a disease, and vice versa. Further, a disease and its underlying risk factors may have genes peculiar each to their own risk or variation even if they may share some common genes.

To pursue and fulfill the major goal of bone genetics, i.e., to identify genes underlying risk to osteoporotic fracture, osteoporotic fracture *per se* may be the ideal study phenotype. If we study risk factors to fulfill our major goal *(26,36)*, these factors ideally should first be shown to be genetically determined and, more important, to be genetically correlated with osteoporotic fracture. At least the genes identified as important for an underlying risk factor should be tested for their relevance to osteoporotic fracture. Searching for genes underlying a suite of risk factors may turn out to be less efficient and economic than studying osteoporotic fracture directly to reach our major goal. This is simply because there are many known risk factors to study and many other risk factors that may not yet be identified, since no combination of known risk factors can predict osteoporotic fracture with high confidence. In addition, osteoporotic fracture must eventually be studied as a phenotype in order to confirm and test the relevance and importance of genes found for risk factors of osteoporotic fracture.

The preceding argument does not necessarily mean that all the extensive efforts, including many of our own *(49–55)*, for gene identification for BMD are futile and meaningless. Studying the genetic basis for variation in BMD is significant for many reasons. For example, because the heritability of BMD is quite high, identification of genes for BMD may help us to understand genetic architecture of complex traits in general just like the effort for gene identification for height *(56)*; to pave the way for studying gene-by-environment (including drugs) interaction for BMD variation, thus identifying effective pathways to reduce osteoporotic fracture risk; and by facilitating the understanding of the biology of bone mass regulation, to hasten the development of drugs that increase bone mass and thereby reduce risk of fracture. This is because a considerable proportion (10–50%) of BMD variation is owing to environmental factors and the environmental correlation between BMD and osteoporotic fracture is high *(46)*. In addition, although the genetic correlation between osteoporotic fracture and BMD cannot be established in the first sample *(46)* examined, larger samples and more studies are needed to assess the

genetic correlation between osteoporotic fracture and BMD given the importance of the issue.

Since the vast majority of genetic studies of osteoporosis have used BMD, a quantitative trait, as a surrogate phenotype, we address the strengths and weaknesses of various gene identification approaches currently employed in BMD research as well as the approaches applicable in research on osteoporotic fracture. The strengths and weaknesses of an approach would be shared when the same approach is applied to gene identification for a complex disease (e.g., osteoporotic fracture) or quantitative trait (e.g., BMD).

Molecular Genetic Approaches to Identify Genes Underlying Complex Traits

Three approaches have been employed in humans: population association studies, linkage studies, and transmission disequilibrium tests (TDTs). Population association studies test whether particular alleles or genotypes are associated with a higher risk or a larger trait value, usually in unrelated population samples. An association usually reflects statistical nonindependence (linkage disequilibrium) between a marker allele(s) and a functional mutation(s) underlying the risk or variation of a trait and does not necessarily imply causality. Linkage tests whether there is cosegregation or coinheritance between alleles of a gene and a phenotype under study in pedigrees or affected relative pairs. Linkage here refers to close physical location of such a gene to a chromosomal locus (found by the study) that is linked (through its alleles) to variation in the trait. Utilizing families or discordant relative pairs, a TDT tests both linkage and association between marker alleles and a functional mutation underlying the study trait and is significant if, and only if, both linkage and association exists. Figure 1 illustrates the principles of the three approaches intuitively.

The three approaches are all useful tools and may complement each other in searching for genes underlying complex traits. The three approaches have been compared or reviewed in several excellent articles on genetics regarding their statistical properties and practical applications *(27–35,57)*. We outline here some of their most salient features, as well as strengths and weaknesses of each in order to compare them regarding practical applications, statistical power, and interpretation.

Association Studies

Population association studies in which candidate genes have been identified *a priori* based on known biologic functions of gene products are generally statistically much more powerful and the samples are much easier to recruit than is the case for linkage studies *(58,59)*. However, association depends on linkage disequilibrium (statistical association) of markers employed with functional mutations in or near the candidate genes. It is vulnerable to population admixture/stratification in often yielding not only false positive results *(12,60,61)* but also false negative ones *(62)*. Population stratification/admixture is extremely difficult to detect, even with large samples, and its damaging effects on association studies are very significant *(61,62)*. More important, the association approach tests *only association*, which may not be relevant to causation; thus, the association approach

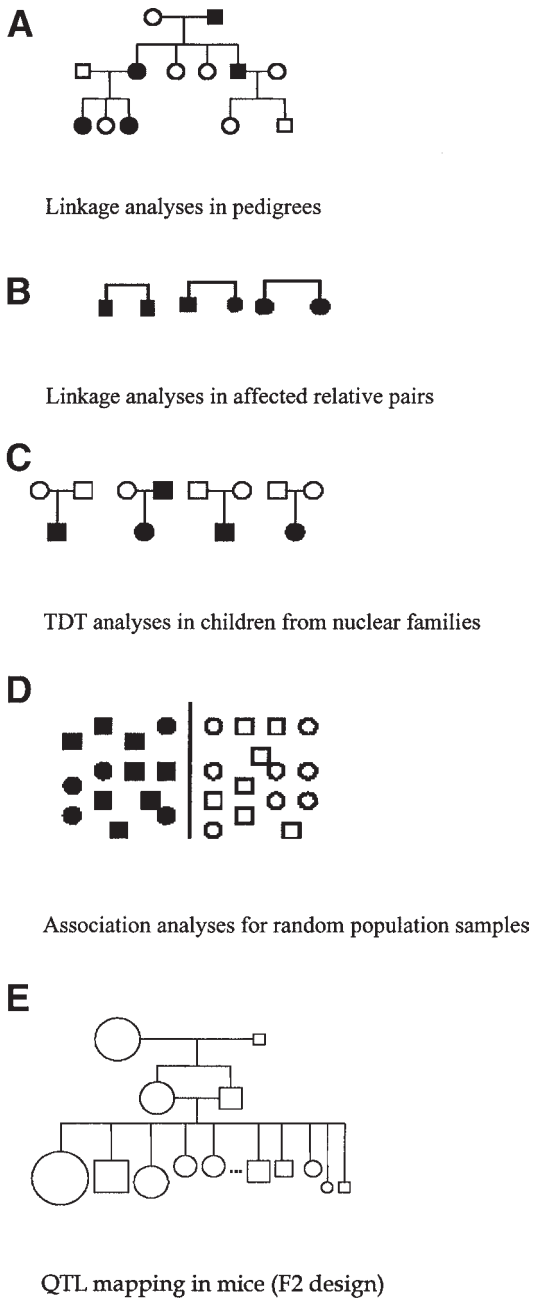

Fig. 1. Different approaches of mapping and identification of genes for complex disorders. Linkage analyses generally require pedigrees with multiple affected and nonaffected individuals or affected relative pairs. TDT analyses typically require at least one affected child from nuclear families with both parents (whose phenotypes may be unknown), and at least one parent needs to be heterozygous at the test marker. Association analyses generally require unrelated affected cases and unaffected controls. In the demonstration for the F2 QTL mapping approach, the size of the symbols may represent different individual phenotypic values.

alone has an inherent difficulty in unambiguously identifying causative genes underlying complex traits.

Linkage Studies

Linkage studies test *only linkage* and can search for *any* genomic region (without any prior knowledge) contributing relatively large variation in complex traits. The linkage approach does not depend on the presence of linkage disequilibrium among genes or markers in adjacent genomic locations and is robust to population admixture/stratification. However, even if significant linkage results are found, extensive fine-mapping efforts *(63,64)*, which generally do depend on linkage disequilibrium, are needed in order to pinpoint a QTL to a small genomic region (~1 cM). Physical mapping may be feasible to identify a specific QTL only after the large genomic region (~30 cM) found in a linkage study has been reduced. Linkage studies are very powerful in searching for genes underlying simple genetic traits determined by single genes, i.e., Mendelian inherited genetic traits *(65)*. However, using the linkage approach to search for genes underlying complex traits generally requires very large samples to be screened and/or genotyped *(57,66, 67)*, unless the genetic effect of the locus is large.

Transmission Dissociation Tests

The TDT *(68)* and its variants *(61,66,69)* can be employed to test specific candidate genes simultaneously for *both linkage and association* and is robust to population admixture/stratification *(70,71)*. Depending on the distribution of linkage disequilibrium in human genomes and the development of highly automatic single nucleotide polymorphism genotyping, the TDT can also be employed for whole-genome scan studies *(57)*. The TDT requires existence of linkage disequilibrium of adjacent genes (or markers with functional mutations) to a trait, and utilization of a much denser distribution of genetic markers as compared to conventional linkage studies *(57)*. Because the TDT depends on population linkage disequilibrium, and because it tests for both linkage and association, significant results (if any) can pinpoint a small region containing causative genes with functional mutations (alleles) *(72)*. Therefore, the TDT approach is ideal for testing candidate genes (with functional relevance) or candidate genomic regions that have shown evidence for association or linkage. Carefully designed population sampling schemes in practice can substantially increase the power of linkage and TDT studies *(30)*.

When testing specific candidate genes, regardless of the approach employed (association, linkage, or TDT), one can utilize the enormous body of knowledge obtained from other fields of bone biology regarding the pathophysiology of osteoporosis. Additionally, because genes are only investigated when there is *a priori* evidence of their potential involvement in the trait concerned, the chance of false positive findings using the candidate gene approach may be lower than with whole-genome screens *(73)*. On the other hand, whole-genome scans can identify genomic regions that are important but would otherwise be unknown.

The TDT has far greater power than the conventional linkage approach for testing or identifying candidate genes using functional markers inside the genes (e.g., [*57,66,67]*). Often, the numbers of families required to detect a complex trait locus may be several orders of magnitude less for the TDT than for the conventional sib-pair linkage approach *(57,66,67)*. Take the example of a marker locus that has functional mutations relevant to complex diseases (such as osteoporotic fracture). To identify such a gene causing a genotypic relative risk of 4 under multiplicative effects, and to reach a power of 80% at the significance level of 10^{-4} when the allele frequency is 0.8, one needs 2013 affected sib pairs and their parents for conventional affected sib-pair linkage analyses *(57)*. However, with the TDT approach, only 222 families each including both parents and one affected child are necessary *(57)*. Another example can be given for continuously distributed quantitative traits (such as BMD). To identify a QTL that accounts for 10% of phenotypic variation under the additive model with 80% power at a significance level of 10^{-4}, one requires at least 800,000 randomly selected sib pairs or 1647 extremely discordant sib pairs (one belonging to the top 10% and the other to the bottom 10% of the population distribution). To identify 1647 such extremely discordant sib pairs, 19,120 sib pairs need to be phenotypically screened. By contrast, only 1194 randomly ascertained nuclear families each with two parents and two children are necessary for the TDT method *(67)*.

The TDT is increasingly being employed *(74)* with some success *(71)* in resolving controversies regarding results obtained from association and linkage studies and with great expectations *(57)* in the searches for unknown and significant genes underlying complex traits in whole-genome screen studies.

All three approaches have merits and disadvantages when employed in isolation. However, they may complement each other with their respective strengths in the search for genes underlying complex traits by testing and identifying different aspects of importance for a gene or genomic region. Since these three approaches test different hypotheses regarding association, or linkage, or linkage and association, and their power may differ to a large extent, it should come as no surprise that the results from these approaches may differ when applied to the same gene or genomic regions. Even for the TDT, different results from different populations also may not be too surprising. While the TDT is robust to population admixture, other factors such as genotype-by-environment interaction, population differentiation, and epistasis together or alone may yield inconsistent TDT results across populations *(62)*.

QTL mapping in experimental or model organisms such as mice may help identify homologous human genes, but there are also advantages as well as disadvantages *(28,35)*. The advantages include short life spans of many experimen-

tal organisms, flexibility for controlled breeding and environment, and elimination of genetic heterogeneity. One difficulty is the construction of models that truly mirror etiology of human disease. It is difficult to construct experimental lines with variation that is representative and significant in wild populations of experimental organisms, and the relevance of the variation in the experimental lines to those in humans is even more evasive. For example, a mutation in the mouse *OB* (leptin) gene leads to severe obesity in mice *(75)*; however, few obese people carry the mutation in the human homologue of OB *(76)*. On the other hand, there are egregious examples of disease mutations in humans that may be relatively benign in experimental organisms *(35)*. Hence, it is not trivial to establish the importance and relevance to humans of the genes found important in experimental organisms.

Molecular Genetic Studies to Search for Genes Underlying BMD

Association Studies of Candidate Genes

Identification of genes responsible for risk of osteoporosis began with a reported association between the vitamin D receptor (VDR) gene and spine and hip BMD *(77)*. Since then, numerous articles have been published on the association of molecular markers of a number of candidate genes with BMD variation. Decades of studies of various disciplines (e.g., molecular biology, cell biology) of bone have suggested an increasingly long list of genes that are of potential importance in skeletal biology *(78)*. The functional importance of prominent candidate genes thought to be relevant to the human skeleton and results of association studies have been comprehensively reviewed and summarized *(20,22–25)*; thus, a comparable review is not attempted here.

Given the weaknesses in association analyses already mentioned, we would first like to assert that lack of significance in association analyses is not evidence against the importance of a candidate gene under test. In this regard, data from population association analyses can be used as an important tool when subjected to exclusion analyses that can formally test *against* the importance of candidate genes. Deng et al. *(79)* developed an exclusion analysis approach for both complex diseases and quantitative traits and applied these approaches to VDR and estrogen receptor genes in relation to BMD and osteoporotic fracture. In addition, the presence or absence of significance for a given association may both be spurious owing to population admixture. Population admixture may, in fact, result in reversal of allelic effects in VDR and BMD association studies *(62)*.

Linkage Studies of Chromosome 11q12-13

In human pedigrees, significant linkage to chromosome 11q12-13 has been reported for three distinct Mendelian inherited traits that are BMD related. The first is the autosomal recessive syndrome, osteoporosis-pseudoglioma, which is characterized by severe juvenile-onset osteoporosis and congenital or juvenile-onset blindness. It was linked to chromosome 11q12-13 with a maximum LOD score of 5.99 achieved at marker D11S987 *(80)*. The second is autosomal recessive osteopetrosis that is characterized by abnormally dense bones, as well as acrocephaly, anemia, and progressive deafness and blindness that are the result of a failure of osteoclast-mediated bone resorption. It is linked to chromosome 11q12-13 with a maximum LOD score of 5.9 achieved *(81)* in two consanguineous kindreds. In addition, mutations for the gene responsible for subsets of autosomal recessive osteopetrosis have been identified *(82,83)*. The third is an autosomal dominant trait characterized by high bone mass (HBM) that is not accompanied by other clinical manifestations. Our group reported it to be linked to chromosome 11q12-13 with a maximum LOD score of 5.74 achieved at the marker D11S987 *(48)*.

Because of these results, chromosome 11q12-13 has been investigated for its linkage to normal BMD variation *(84, 85)*. A sib-pair study with 374 sibships reported that for seven microsatellite markers typed in chromosome 11q12-13, a maximum LOD score of 3.50 was achieved near D11S987 for linkage to femoral neck BMD variation *(84)*. However, a subsequent linkage study *(86)* by the same group with an expanded sample size of a total of 595 sib pairs demonstrated much reduced significance for linkage to the same site, with an LOD score achieved at D11S987 of <2.2 (indeterminate for linkage). (LOD scores >3.6 and >2.2 are, respectively, considered as indicative of significant or suggestive linkage for a sib-pair whole-genome scan to identify genes for complex traits *[28]*). The *p*-value corresponding to an LOD score of 2.2 is 0.0015 and to an LOD score of 3.6 is 0.000059 *(28)*. These reports illustrate that sib-pair linkage studies have limited power and thus give less robust results in identification of QTLs *(66,67)*.

We *(52)* genotyped five markers in a genomic region of ~27 cM centering on D11S987 for 635 individuals from 53 human pedigrees. Each of these pedigrees was ascertained through a proband with a BMD Z-score of <–1.28 at the hip or spine. These pedigrees contain more than 11,000 informative relative pairs, including 1249 sibling pairs. We did not find evidence of linkage of these five markers to BMD at the spine, hip, wrist, and total body bone mineral content. The maximum LOD score at these five markers was 0.25 and at D11S987 was 0.15. In fact, we (unpublished data) have three markers genotyped covering a 3-cM region centering on the putative *HBM* gene, and still, we did not find evidence suggesting that the region is important for population BMD variation. We *(52)* offered explanations for the inconsistent results concerning the linkage of D11S987 to BMD (discussed later). Thus, it remains uncertain whether the *HBM* gene determines any of the variation in BMD in the general population. It would not necessarily be impossible for a mutation (such as *HBM*) to cause a skeletal phe-

notype and the wild-type gene not influence skeletal phenotype in the general population.

Current Status of the HBM *Studies*

While the sib-pair linkage studies discussed *(84–86)* were a bit disappointing regarding linkage of chromosome 11q12-13 to normal BMD variation, clearly the kindreds with osteoporosis-pseudoglioma and *HBM* demonstrated profound BMD phenotypes linked to chromosome 11q12-13. Subsequent studies of these syndromes have demonstrated that both syndromes are the result of point mutations in a gene on chromosome 11 *(100)*. Our group has determined that the *HBM* gene codes for the low-density lipoprotein receptor–related protein-5 *(100)*. Mutation detection uncovered an amino acid change in a predicted β-propeller module of the LRP-5 protein. The osteoporosis pseudoglioma mutation involves a mutation in the intracytoplasmic component of LRP-5. This receptor is a regulator of the Wnt signaling pathway. Currently, work is under way to determine the mechanism whereby this receptor and the Wnt pathway can regulate bone mass. The most interesting feature of the phenotype is that the skeletons of affected individuals were normally shaped and increased in mass starting in childhood and remained so throughout adult life. There was no instance of marrow insufficiency or nerve root compression. This means that the mutation functioned by rendering the skeleton overly sensitive to normal physiologic loading. Thus, normal skeletal loads led to an overadapted skeleton that was increased in mass by about 50%, and resistant to fracture. Details of the skeletal function of LRP-5, both the wild type and the mutations, should be forthcoming given the intensive interest and research now under way as a result of these discoveries.

Other Linkage Studies

Linkage studies, particularly those for the whole genome, are rare in the field of bone genetics, largely because of the high cost involved. Devoto et al. *(87)* genotyped 149 subjects from seven pedigrees recruited through probands having low BMD or osteoporotic fracture using 330 DNA markers distributed on autosomes. These samples contain 74 sib pairs. Niu et al. *(88)* genotyped 367 markers throughout the genome for 218 subjects (153 sib pairs) from 96 Chinese nuclear families. Duncan et al. *(73)* tested markers within or near the genomic regions of 23 candidate genes for 614 subjects from 150 families identified via probands with extremely low BMD values. Koller et al. *(85)* genotyped 270 markers throughout the human autosomes for randomly ascertained 429 Caucasian sister pairs. Genomic regions with LOD scores >1.85 were further tested in an expanded sample of 595 sister pairs (464 Caucasians and 131 African Americans) randomly ascertained. Deng et al. *(53)* genotyped 400 markers throughout the whole human genome for 635 individuals from 50 large pedigrees identified via probands with extremely low BMD values. This sample contained more than 11,000 informative relative pairs for linkage tests, including 1249 sib pairs. While the full report of Deng et al. *(54)* will be published later, the potentially significant linkage results identified in all the early linkage studies are summarized and compared against those found in the same region in our study in Table 1. Whole-genome linkage studies are also performed for bone size for 309 Caucasian sister pairs *(86)* and more than 11,000 informative relative pairs that include 1249 sib pairs *(14)*. Bone size also has strong genetic determination *(89)*, is a component of BMD, correlates with BMD, and is a risk factor for osteoporotic fracture *(90)*.

It can be seen that although only a few linkage studies have been reported, the results so far have demonstrated that, as with the association studies in bone genetic research, significant genomic regions revealed by different groups are different. This is owing partially to the limited power of the conventional linkage approach in searching for genes underlying complex traits that are determined by multiple genetic and environmental factors. Thus, when there are multiple genes underlying variation in a complex trait, even if the power to detect linkage for each locus is low, the power to detect one (any one) of the genes can still be high. Nevertheless, the power to repeat the linkage to this previously found locus is again very low. As a demonstration, suppose that there are 10 QTLs each accounting for 8% of peak bone mass variation; with a sample of *N* sib pairs, for a fixed significance level, the power to detect each of the 10 genes is only 10%. Then it is straightforward from probability that the power to detect a single QTL (any QTL) of the 10 QTLs in the whole-genome scan will be ~66%. That is, there is substantial power to detect one QTL of the 10 QTLs even if the power to detect a particular one of them is low. However, with another sample of the same sample size, —*N* sib pairs from the same population, the power to detect *the* gene detected in the previous genome scan will be 10%. The power is so low that it is unlikely that the linkage finding of the QTL found in the first genome scan will be repeatable in a following linkage study.

The low power of the conventional linkage study approach and the high false positive rate with a whole-genome scan are among the major causes underlying many inconsistent linkage results for complex traits. The inconsistency of the linkage studies of complex traits can be demonstrated by the studies of obesity *(91)* and insulin-dependent diabetes mellitus *(47,68)*. The robustness of linkage results and the power of linkage studies for complex traits generally require sample sizes far larger than those currently employed in the few early linkage studies in bone genetics *(85,87,88)*.

TDT Analyses

The TDT has seldom been applied in bone genetic studies. In the bone field, association studies of candidate genes have been reported, but the results are largely inconsistent,

Table 1
Summary and Comparison of Early Linkage Results Against Those of Deng et al. *(53)* [a]

Reference	BMD	Marker or genomic regions	LOD score or MLS (authors)	Deng *(53)* LOD score			Closest marker (cM)
				Hip	Spine	Wrist	
87	Hip	1p36	2.29	0.00	0.30	0.15	
		D1S450	3.51	0.21	0.01	0.01	
		D1S214	2.62	0.04	0.00	0.00	
		4q32-34	2.28	3.08		2.26	
		D4S1535	2.74	0.00	0.01	0.00	
		D7S558	2.99	0.00	0.14	0.05	D7S657 (1.23)
		D17S261	2.34	1.58	0.76	0.08	D17S1857 (1.9)
		D18S42	2.58				
		D18S70	2.14	0.07	0.00	0..00	
		CD3D	>1.9				
	Spine	2p23-24	2.25	0.20	0.21	0.01	
		D2S149	2.07	0.11	0.00	0.19	D2S305 (4.83)
		D2S144	1.49	0.00	0.00	0.00	D2S165 (2.13)
		D2S71	1.72	0.00	0.00	0.00	D2S112 (2.9)
		D4S1539	2.95	0.00	0.00	0.38	
88	Proximal	2p21.1-24	2.15	0.20	0.21	0.01	D2S168 (1.33)
	and distal	D2S1400		0.14	0.33	0.1	
	forearm	D2S405		0.00	0.00	0.00	D2S165 (0.54)
	Distal	13q21-34	1.67	2.43	0.32	0.89	
	forearm	13S788		0.00	0.24	0.00	D13S153 (0)
		D13S800		0.00	0.47	0.00	D13S156 (0.54)
84	Spine	1q21-23 (D1S484)	3.86	0.00	0.00	0.01	
		6p11-12 (D6S462)	2.13	0.13	0.09	0.56	D6S257 (19.1)
		11q12-13 (D11S987)	1.65	0.00	0.47	0.00	
		22q12-13 (D22S423)	0.99	0.00	0.29	0.02	
	Femoral neck	5q33-35 (D5S422)	2.23	0.00	0.00	0.05	
		11q12-13 (D11S935)	2.16	0.44	0.11	0.00	

[a]For those markers that were reported by the other studies but not genotyped in ref. *53*, we reported the LOD scores for the ref. *53* markers (the identity given) that are closest to them and give the genetic distance (found through the database "Comprehensive human genetic maps of Marshfield" at http://research.marshfieldclinic.org/genetics/Map_Markers/maps/indexmap.html) between the markers in other studies and the closest markers of ours in parentheses. Markers D18S42 and CD3D were not mapped onto the Genethon and Marshfield human genetic linkage maps (http://www.ncbi.nlm.nih.gov/PMGifs/Genomes/humansearch.html), and, hence, we are not able to identify our closest markers to them. MLS denotes maximum LOD scores in the genomic regions.

and the few available linkage studies have failed to establish linkage of these candidate genes with BMD variation. In addition, whole-genome scans in humans and QTL mapping in mice have suggested genomic regions and new candidate genes for confirmation and replication. Therefore, we have an ideal situation in which to apply the TDT to genetic studies of bone mass. It will be built on our extensive work in population association, linkage, and QTL-mapping studies, to identify and test genes underlying osteoporosis using various approaches.

Keen et al. *(92)* tested association and transmission disequilibrium in 1706 Caucasian female dizygotic twins and support the importance of the transforming growth factor-β1 (TGF-β1) gene underlying hip BMD variation. In a previous linkage study, Duncan et al. *(73)* did not find evidence for linkage for the TGF-β1 gene, possibly owing to higher power of the TDT relative to the linkage test for candidate genes.

Deng et al. *(54)* simultaneously tested linkage and/or association of the genes for VDR, osteocalcin, and parathyroid hormone (PTH) with BMD in 630 subjects from 53 human pedigrees. Each of these pedigrees was ascertained through a proband with an extreme BMD value at the hip or spine (Z-score ≤−1.28). This sample contained more than 11,000 informative relative pairs for linkage tests, including 1249 sib pairs. The investigators performed tests for

Table 2
Putative QTLs Underlying BMD Variation in Mouse

Chr	Marker or gene	MGD map position (cm)[a]	LOD scores or *p* values	Bone site	Human homologous regions	Reference
Chr 1	cfh	74.1	0.0093	Whole body	1q25;1q31-41	*93*
	Mit14	81.6	24.4;14	Femur, vertebral	1q23-31	*98*
	Mit15	87.9	<<0.01	Femur	1q21-25	*95*
Chr 2	Mit312	1	0.003	Spine	10p15	*96*
	Mit119	5	<0.0001	Spine	10p13-15	*96*
	Il2ra	6.4	0.0071	Whole body	10p11-15	*93*
	Mit464	9.5	<0.0001	Spine	10p11-15;2q14;9q34	*96*
	Mit296	18	<0.0001	Spine	9q33-34	*96*
	Mit413	84.2	3.8–3.9	Femur	20p11-q12	*97*
	Iapls2-4	86	0.002	Whole body	20p11-q12	*93*
	Mit456	86.3	3.14	Femur	20p11-q12	*98*
Chr 3	Mit14	64.1	2.5;3.3	Femur	1p13;4q24-27	*97*
Chr 4	Mit124	57.4	16.3;14.85	Femur, vertebral	1p31-35	*98*
Chr 5	Mit112	42	0.01	Femur	4p12-14;4q11-13;4q21	*95*
Chr 6	Mit150	51	4.56	Femur	3p25-26;3q21-24;19q13;10q11	*98*
	Mit198	67	2.3;2.7	Femur	12p12;12q23-24	*97*
Chr 7	Mit210	11	<0.001	Spine	19q12—13	*96*
	Mit227	16	0.001	Spine	19q12—13	*96*
	Mit80	18	2.04;2.37	Femur	19q12-13	*97*
	Mit234	44	0.0007	Whole body	6p24-25;15q23-25;11q13-22;11p15	*93*
	Mit332	65.6	5.01	Vertebral	10q24-26	*98*
Chr 9	Mit196	48	5.12	Vertebral	6q12-16;15q24-25;3q21-24	*98*
Chr 11	Mit242	31	6.76;2.98	Femur,vertebral	5q31-32;17p11-12	*98*
	Mit59,90	42-58.5	10.8	Femur	17p11-13;17q11-13;17q21-23;1p36	*94*
	Mit284	52	<0.0001	Spine	17q21-23	*96*
	Mit14	57	0.0104	Whole body	17q11-12;17q21-23	*93*
	Mit160	58	<0.0001	Spine	3p21;17q11-12;17q21-23	*96*
Chr1 2	Mit215	2	2.89	Femur	2p22-25	*98*
Chr1 3	Mit174,135	9–10	5.8	Femur	7p13-15;6p21-23;9q22	*94*
	Mit16	10	<0.1	Femur	7p13-15;6p22;9q22	*95*
	Mit13	35	7.73	Femur	5q22-32;9q22	*98*
	Mit20	35	0.001	Spine	5q22-32;9q22	*96*
Chr 14	Ptprg	2	0.0007	Whole body	3p14;3p24;10q21-24;8p23	*93*
	Mit160	40	4.3;4.48	Femur,vertebral	8p21-22;13q14-21	*98*
Chr 15	Mit13	6.7	2.97;3.21	Femur	5p12-14;5q31	*97*
	Mit206	17.2	3.19-4.73	Femur	8q22-23	*97*
	Mit29	42.8	<0.01	Femur	8q21;8q24;22q12-13	*95*
	Atf4	43.3	0.0099	Whole body	8q21;8q24;22q12-14	*93*
Chr 16	Mit100	8.5	0.02	Spine	8q11;22q11	*96*
	Hmg1-rs7	19	0.0055	Whole body	3q13;3q21-22;3q25;3q27-29	*93*
	Mit12	27.6	4.07	Femur	3q13-21;3q25;3q29	*98*
	Mit39	29.1	0.001	Spine	3q13-21;3q28-29	*96*
Chr 18	Mit36	24	13.67;8.35	Femur,vertebral	5q21-33	*98*
	Ncvs23	48	0.0094	Whole body	18q21	*93*
Chr 19	Ncvs21	53	0.0093	Whole body	10q24-26	*93*

[a]MDG denotes Mouse Genome Database available at http://www.informatics.jax.org. Human homologous regions correspond to ± 3 cM of published marker or gene location identified at MDG's Web site.

linkage alone, association alone, then for both linkage and association by the TDT. We found evidence for association and/or linkage for spine BMD at the VDR gene. Significant results were also found for association and/or linkage for the osteocalcin gene with hip BMD. Our data support the VDR gene as a QTL underlying spine BMD variation and the osteocalcin gene as a QTL underlying hip BMD variation. However, our data do not support the PTH gene as a QTL underlying hip or spine BMD variation. This may be the first study in the broad field of bone genetics that tests

candidate genes as QTLs for BMD by testing simultaneously for association alone, for linkage alone, and for association and linkage (via the TDT).

QTL Mapping in Mice

Using 24 recombinant inbred mouse lines derived from a cross between C57BL/6 (of low BMD) and DBA/2 (of high BMD) progenitors, Klein et al. *(93)* reported 10 QTLs mapped for peak bone mass. Using F2 populations derived from crosses of various inbred mouse lines of high or low BMD values, Shimizu et al. *(94)*, Beamer et al. *(95)*, Benes *(96)*, Drake et al. *(97)*, and Beamer et al. *(95)* reported dozens of locations that may harbor QTLs for BMD variation. While ignoring the technical aspects of the measurement of BMD in mice, the current findings of QTL genomic regions, and the associated human homologous regions are summarized in Table 2. Sorting out the genomic regions that contain important QTLs for BMD variation in natural mouse populations among these dozens of regions reported from different laboratories is a challenge for mouse geneticists. It may be even more challenging to establish and confirm the relevance of these regions to human population BMD variation given the problems already existent in the human genetic studies of osteoporosis. Given that the natural selection challenge for mouse populations is not the same as for human populations and given that osteoporotic fracture may not be a fitness problem in mice as in humans, the genomic regions identified and confirmed for mice may not be of the same importance for humans. This may also be true for other reasons such as the genetic differentiation of mice from humans during the long time history of separate evolution.

Conclusion

With the extensive molecular genetic research initiated recently in the bone field, we are making steady progress toward mapping and identifying of genes for osteoporosis. This is true as witnessed not only by the interesting, albeit somewhat debatable, results reviewed earlier *(20–25)* and here, but also by the problems and challenges surfacing during our progress. These problems and challenges may sometimes be specific to the given approach used and sometimes may be shared by all the approaches as outlined here. By continuously confronting these problems and challenges utilizing the multiple approaches outlined here, as well as new approaches such as DNA microarrays for gene expression analyses *(99,100)* that may complement and confirm each other, we are on our way to unraveling the tangle of the genetic variation in osteoporosis.

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Appendix

It is well known from the theory of quantitative genetics *(43)* that for two complex traits, such as osteoporotic fracture and BMD, phenotypic correlation (ρ_P) may be caused by both genetic and environmental factors as reflected, respectively, by genetic (ρ_G) and environmental (ρ_E) correlations, and

$$\rho_P = \sqrt{h_1^2}\sqrt{h_2^2}\,\rho_G + \sqrt{(1 - h_1^2)}\sqrt{(1 - h_2^2)}\,\rho_E \qquad (1)$$

in which h_1^2 and h_2^2 may be here the heritabilities for the BMD and osteoporotic fracture, respectively. Obviously from Equation 1, significant ρ_P between BMD and osteoporotic fracture does not necessarily imply significant ρ_G and thus significantly shared genetic determination by them. Moreover, significant ρ_P between BMD and osteoporotic fracture and significant h_1^2 do not even imply that h_2^2, the heritability of osteoporotic fracture, is nonzero.

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