Introduction

Osteoporosis is a complex, multifactorial disorder characterized by a loss of bone mass and bone quality and an increased risk of fragility fracture. Although the etiology of osteoporotic fractures is multifactorial, low bone mineral density (BMD) is an important predictive risk factor. BMD measurements in NHANES III revealed that 5-8 million Americans aged 50 years and older have osteoporosis, and that another 21-40 million have osteopenia (low BMD) and are at increased risk of osteoporosis and fracture. The prevalence of osteoporosis has major implications for the health of older adults. For instance, the estimated lifetime risk of an osteoporosis-related hip, spine or forearm fracture is 40% for Caucasian American women and 13% for Caucasian American men. The majority of this risk comes after age 65, when fracture rates increase exponentially. In the US alone, about 1.5 million fractures annually have been attributed to osteoporosis. Osteoporosis and fractures are associated with considerable morbidity and mortality. Osteoporotic fractures cause pain, long-lasting disability and dependency; few individuals fully return to their normal pre-fracture level of activity. Health care utilization and medical expenditures associated with osteoporotic fractures are also considerable, and represented an estimated 14 billion in the US alone in 1995, exceeding expenditures for breast and gynaecological cancers combined. Compounding the problem, the number of affected individuals is expected to double in the next 25 years, primarily due to increases in the elderly population. These demographic trends make the need to elucidate the causes of osteoporosis an urgent priority.

The importance of genetics

Research over the past two to three decades has unequivocally established a strong hereditary influence on osteoporosis and its associated fractures. For example, BMD is significantly related to genetics.
reduced in first-degree relatives of osteoporotic individuals, an effect that has been quite strong in some studies\textsuperscript{20-28}. For instance, a son had nearly a 4 times higher risk of having low whole body BMD if his father had low BMD, and a daughter 5 times greater risk if her mother had low BMD in one study\textsuperscript{28}. High recurrence risks ($\lambda>5$) for low BMD have also been reported among siblings of probands with low BMD\textsuperscript{29}. This familial influence on BMD may be evident very early in life\textsuperscript{30,31}.

There is also very convincing evidence of a strong genetic influence (e.g., heritability $>50\%$) on most osteoporosis-related traits from family and twin studies (e.g., reviewed in\textsuperscript{12}). A high heritability of bone-related traits has been demonstrated into the eighth decade of life\textsuperscript{33,34}. Heritability estimates for BMD and related traits may differ between men and women\textsuperscript{29,35,36} and between anatomical regions\textsuperscript{36}. Segregation analysis supports a major gene effect on bone related phenotypes in some families\textsuperscript{37-42} whereas in others a polygenic model has been supported\textsuperscript{40,43}. Although less well studied, there is also evidence of a heritable component for age-related bone loss\textsuperscript{44}. A substantial heritability of bone

<table>
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*Only QTLs with a LOD score $\geq 2.0$ are shown in the Table.

Table 1. Chromosomal regions implicated by genome-wide linkage scans of BMD.*
resorption and formation indices among older adults\textsuperscript{45-48} and the hormones regulating these processes\textsuperscript{7,49} also indicates that age-related bone loss has genetic foundations.

Susceptibility to clinically important fractures also appears to have a hereditary foundation\textsuperscript{50-57}. For example, a parental history of hip fracture bestows a 2-fold increased risk of hip fracture that is independent of BMD\textsuperscript{53}. The heritability of wrist fracture has been estimated to be between 23\% to 54\% and appears to be independent of BMD\textsuperscript{54,55}. A familial and heritable influence on fracture independent of BMD suggests that genetic factors may contribute to fracture risk through mechanisms other than bone mass. Such factors might include skeletal characteristics like the rate of bone remodeling, accumulation of microdamage, bone size and shape, cortical porosity, trabecular microarchitecture, and osteocyte cell function that may not be well captured by BMD measurements alone. A familial influence on fracture may also operate through non-skeletal related factors such as neuromuscular dysfunction, balance and gait impairments and/or poor muscular strength\textsuperscript{56}.

The high heritability of BMD and related traits in family and twin studies, increased risk of fracture among individuals with a positive family history of fracture and evidence for a major gene effect on bone-related traits in families have motivated the search for genes and allelic variants contributing to osteoporosis. There are two broadly defined strategies to map the genetic loci for complex diseases in humans: genome-wide and candidate gene approaches. We review the relative merits and limitations of each approach and the progress in identifying osteoporosis susceptibility genes using these approaches.

**Strategies for defining genetic factors in osteoporosis**

**Genome-wide linkage mapping**

Genome-wide linkage mapping follows the segregation of chromosomal regions marked by random genetic variants in families in the search for regions of the genome that co-segregate with the disease or phenotype. Families may range from simple sibling pairs to large, complex extended pedigrees. Genome-wide linkage mapping has great potential for advancing our understanding of osteoporosis because previously unknown genes and protein products may be discovered with this strategy.

Genome-wide linkage mapping has provided valuable information about the likely chromosomal locations of genes involved in the regulation of BMD (Table 1)\textsuperscript{57,67}. However, the specific genes contributing to the linkage signals at the majority of the chromosomal sites remain to be identified. The most convincing evidence of a gene influencing BMD comes from a report by Johnson and co-workers, where a single large family with very high BMD (Z-score>5) was shown to have a locus on chromosome 11q12-13\textsuperscript{68}. The gene was subsequently identified as the low density lipoprotein receptor-related protein 5 (\textit{LRP5}), a co-receptor for the wingless-type family of growth factors, and a single gain-of-function mutation was shown to segregate with the high BMD phenotype\textsuperscript{69,70}. Additional mutations in \textit{LRP5} have been discovered, predisposing to osteoporosis-pseudoglioma syndrome - an autosomal recessive condition associated with low BMD\textsuperscript{71}. Follow-up studies have shown that high frequency \textit{LRP5} polymorphisms may also contribute to normal variation in BMD in the general population\textsuperscript{72,73}.

It is important to note that the biological function of \textit{LRP5} in bone was unknown prior to its discovery in the genome-wide linkage mapping study\textsuperscript{74}, and the pharmaceutical industry is currently developing agents targeted at this newly discovered pathway. Given that \textit{LRP5} was not on \textit{a priori} grounds a good candidate gene for causing low or high BMD, it is probable that this gene would not have been identified using a candidate gene approach. The discovery of \textit{LRP5} as a genetic element in BMD regulation illustrates the power and promise of the genome-wide linkage mapping approach.

Only a single other positional candidate gene for BMD or related traits has emerged from genome-wide linkage mapping in humans to date: bone morphogenetic protein 2 (\textit{BMP2})\textsuperscript{75}. This result has not been replicated yet, and together variation in \textit{LRP5} and \textit{BMP2} is only likely to explain a small fraction of the known heritability in the genetic risk of osteoporosis. As with many complex disease traits, there has been limited overlap between the positive linkage mapping results of the different studies (Table 1). Moreover, the evidence for linkage in most linkage mapping studies to date is not statistically significant at a genome-wide significance level (i.e., genomic \textit{p} value >0.05). These observations may be due in part to genetic heterogeneity (multiple genetic loci responsible for disease risk, each with only a weak effect) or to the small family size and limited power of most studies to detect what are likely to be very modest locus-specific risks for osteoporosis. For instance, extended pedigrees are known to be more informative than sib-pair or nuclear families in terms of statistical power to detect linkage\textsuperscript{76,77} and accuracy of gene localization\textsuperscript{76,77}. The difference in power to map loci is particularly marked and likely related to the additional meioses in large pedigrees and increased ability to determine marker phase\textsuperscript{76}. With some exceptions\textsuperscript{64}, there have been only a few genome-wide linkage mapping studies for osteoporosis-related traits in large, extended multigenerational family sets.

Genome-wide linkage mapping in rare families, such as that reported by Johnson et al.\textsuperscript{74}, and sib-pairs or other relative pairs\textsuperscript{62,63,67,78-82}, will only likely be able to identify loci with a major influence on variation in bone-related traits. The involvement of many gene products in the regulation of bone metabolism and the modulating effects of numerous environmental factors suggests that many genes and environmental factors contribute to osteoporosis, and that the contribution of some biologically important genes to variation in risk in the general population may be too small to detect by standard linkage mapping approaches. Additional strategies will clearly be needed to identify all of the genetic risk factors for osteoporosis.
Genome-wide linkage disequilibrium (LD) mapping

Genome-wide LD mapping with single nucleotide polymorphisms (SNPs) in unrelated individuals has been proposed as a potentially powerful strategy for localizing disease genes in unrelated individuals\(^{3,85}\) and may be a promising strategy for identifying osteoporosis susceptibility genes. This approach tests for differences in allele frequencies between cases compared with controls on a genome-wide basis to find variants that are associated with a disease-related phenotype. Genome-wide LD mapping has greater power to discover the contributions of genes of small effect size than linkage mapping, but many more markers must be tested to scan the genome using this method.

Genome-wide LD mapping requires that the genetic variant being tested is either the causal disease allele or is in LD with the disease causing allele. Linkage disequilibrium refers to the excess co-occurrence of two alleles over that expected if the two alleles occurred independently. The size of the genomic region in which genetic variants are in LD with the disease allele is thus a key factor in the success of LD mapping\(^{36}\). Recent surveys of the human genome have revealed that much of the genome may indeed fall into segments of strong LD, where variants within these segments are strongly correlated with each other\(^{27,90}\). Genotypes of one SNP in these genomic regions are highly correlated with neighboring SNPs such that one SNP can serve as a proxy for many others in an LD mapping screen. Nonetheless, searching the entire genome in an LD mapping screen is expected to require genotyping >100,000 SNPs and limitations in high-throughput genotyping methods have until only recently prevented the use of genome-wide LD mapping to identify disease genes. However, recent advances in high-throughput genotyping methods\(^{95-94}\) and tremendous increases in the number of validated SNPs in the human genome\(^{95,96}\) have now made genome-wide LD mapping feasible. The potential utility of this strategy for identifying complex disease genes was recently illustrated by the report of a genome-wide screen of cases and controls for polymorphisms associated with age-related macular degeneration\(^{97}\). Genome-wide LD screens have not yet been completed for osteoporosis related traits, but are likely to provide additional insight on the genetic factors involved.

Genome-wide admixture mapping

Admixture between genetically different populations creates linkage disequilibrium, which in recently admixed populations can extend for megabases because the genome has not undergone recombination since population mixing began. Admixture linkage disequilibrium can be used to map genes that underlie ethnic differences in disease risk\(^{98}\). If admixture (defined as the proportion of an individual's genome that has ancestry from the high-risk population) varies among individuals in the admixed population, the risk of disease will vary with the degree of admixture. The frequency of alleles that have ancestry from the high-risk population therefore will be higher in affected than in unaffected individuals sampled from the population of mixed ancestry. A major advantage of admixture mapping is that many fewer genetic markers are needed to scan the genome compared with genome-wide LD mapping. Admixture mapping was first proposed nearly two decades ago\(^{99}\), but only recently became feasible with the development of genetic marker maps with known large allele frequency differences between populations\(^{99}\).

Admixture linkage disequilibrium exists in African Americans\(^{98,105}\), and can be used to map disease genes\(^{98,101,106}\). For instance, genome-wide admixture mapping has recently been used to identify genomic regions harboring loci for hypertension among African Americans supporting the utility of this approach\(^{99}\). African Americans are known to have higher BMD and lower osteoporosis risk than Caucasian Europeans. We\(^{107}\) and others\(^{108}\) have recently provided evidence for an inverse correlation between the extent of European ancestry and BMD among African Americans. Admixture mapping should thus be another viable strategy for identifying osteoporosis susceptibility genes.

Candidate gene association studies

In contrast to linkage studies, candidate gene association studies rely on traditional epidemiologic methodology, such as case-control and cohort study designs, and therefore do not require kinship information. A primary limitation of candidate gene association studies is that many important biological candidates that regulate bone metabolism may be unknown. Nonetheless, a variety of candidate genes for BMD and related traits have been suggested\(^{32,109-114}\) and association studies support the hypothesis that polymorphisms at several biologically plausible candidate genes influence bone related traits. The most widely studied candidate genes have been the vitamin D receptor (VDR), estrogen receptor alpha (ESR1) and type I collagen (COL1A1) genes, and there is evidence of a small but significant influence of variation at each of these loci on BMD and/or fracture risk\(^{32,109-114}\). Polymorphisms in other candidate genes including those encoding bone matrix proteins, receptors for steroid and calcitropic hormones, and structural and functional loci for cytokine and growth factor related genes have also been associated with bone-related traits or fracture risk in recent years, but these loci are less well studied and associations remain to be robustly demonstrated across studies\(^{32,109-114}\). The inconsistency of results across studies thus far is not unique to the osteoporosis field\(^{115-117}\) and illustrates the limitations of the candidate gene association strategy and the need for additional approaches.

As noted by others\(^{105,118}\), the small sample size and insufficient power of most studies to detect what is likely to be a modest effect size may be a major explanation for the limited success of the candidate gene association approach to date. For example, Liu et al.\(^{114}\) recently conducted an extensive review of over 200 candidate gene association studies of osteoporosis related traits. The vast majority of the studies reviewed included less than 500 subjects with >50% having fewer than 200 subjects. Given that the effect size of any indi-
vidual genetic variant is likely to be modest and explain 5% to 10% or less of the phenotypic variation, these studies are likely to have been grossly underpowered. For instance, 1000 subjects would be required to detect a locus that occurs at 5% frequency in the population and that explains 5% of the phenotypic variance in a quantitative trait like BMD\textsuperscript{138}. Greater than 1500 would be needed if the same locus explained 2% of the phenotypic variance\textsuperscript{118}. Clearly, much larger sample sizes will be needed to achieve more robust findings for variants with a modest effect size and to better define the magnitude of association between genetic variation and the relative, absolute and attributable risks of disease.

Another important limitation of candidate gene association studies of osteoporosis phenotypes to date has been that most studies have genotyped only a few variants within a gene, usually without prior knowledge of the functional significance of the variant tested or its LD with the functional variant(s). Recent surveys of LD in human populations indicate that most of the genome lies within segments of strong LD, within which genetic variants are in strong LD\textsuperscript{137,140}. In these regions, one or a few variants can serve as a proxy for many others essentially capturing or “tagging” most of the genetic variation in an association test of a phenotype. However, these regions of strong LD are separated by discrete regions of low LD, wherein neighbors of variants are only very poorly correlated with each other\textsuperscript{119}. Without prior knowledge of these patterns of LD to guide the selection of variants for genotyping, studies that select a single or only a few genetic variants at random in a candidate gene are unlikely to be successful or to yield robust association results. A recent comprehensive survey of 100 candidate genes indicated that an average of 7 SNPs across a gene may be needed to resolve 80% of all haplotypes in European Caucasians with some genes requiring greater than 20 SNPs\textsuperscript{120}. Fortunately, major efforts are currently underway to create a publicly available catalogue of SNPs and to characterize the extent of LD throughout the human genome in several populations. This project, known as the International Haplotype Map (HapMap) Project (www.hapmap.org), is expected to provide a valuable resource for the design of candidate gene and genome-wide association studies.

There are also a number of sources of potential confounding inherent in candidate gene association studies in outbred populations, particularly if genetic heterogeneity in the population is not accounted for. For instance, spurious associations of alleles with disease phenotypes may be obtained or true associations overlooked when allele frequencies differ among subpopulations that are not represented equally among cases and controls. Population stratification has been argued to be a major source of false positive associations and the inability to reproduce candidate gene associations\textsuperscript{121,122}. Even in well designed candidate gene association studies, small amounts of population stratification can apparently lead to false positive associations\textsuperscript{123-125}. Family-based tests of association can avoid this problem, but such studies are often more expensive and may be impractical for late-onset diseases such as osteoporosis or fracture. Statistical methods have also been developed to assess and control for population stratification in case-control studies of unrelated individuals\textsuperscript{126-133}, but these procedures have not generally been applied in candidate gene association studies of osteoporosis phenotypes.

**Genetics of osteoporosis: What have we learned from animal models?**

Genetic segregation analyses in inbred animal strains provide a powerful tool for dissecting the genetics of complex diseases and phenotypes. This strategy has yielded valuable insight on the genetic architecture of osteoporosis related traits\textsuperscript{134-148}. For example, studies in inbred rodents have shown that the genetic component to bone related traits is complex in that there are likely to be anatomical, bone compartment, and gender-specific genetic effects\textsuperscript{134,135,139,142,143,149,150}. The recent discovery of the 12/15-lipoxygenase locus (ALOX15) as a novel genetic element in the control of BMD using genetic segregation analyses in inbred mouse strains\textsuperscript{151} and subsequent confirmation of this finding in humans\textsuperscript{152} illustrates the power and promise of this strategy.

Studies in inbred animal strains indicate that different quantitative trait loci (QTL) regulate bone mass and the biomechanical properties of the femur and spine\textsuperscript{153,155}. Moreover, not only is bone mass under genetic control, the distribution of bone mineral into its trabecular and cortical compartments also appears to be under genetic regulation\textsuperscript{155}. Others have shown that QTL for vertebral trabecular microarchitecture may be distinct from those for total vertebral BMD\textsuperscript{156}. Separate QTL also appear to regulate bone length and width\textsuperscript{144} and bone size and BMD\textsuperscript{135}. Finally, studies in inbred rodents suggest that some QTL may have pleiotropic effects on body weight and bone mass and size\textsuperscript{150,157}. Thus, these studies indicate that the genetic architecture of bone strength is complex and also illustrate the importance of deconvoluting the “bone strength phenotype” into separate measures of trabecular and cortical bone mass, bone size and skeletal geometry at multiple anatomical regions. Searches for osteoporosis susceptibility genes in humans will therefore need to carefully measure the skeletal phenotype.

Studies of inbred rodents have also provided insight on the potential distribution of phenotypic effect size of genetic variants. These studies have revealed that the genetic component to bone related traits likely results from multiple loci each with small to moderate individual effects\textsuperscript{142,150,154}, and from additive or nonadditive interactions of unlinked loci\textsuperscript{142,150}. For example, Masinde et al. recently identified 9 quantitative trait loci (QTL) contributing to femoral shaft bone size variation independent of body weight among F\textsubscript{2} MRL/SJL mice\textsuperscript{150}. The vast majority of these individual loci explained ≤5% of the total phenotypic variation in femoral size, but these QTLs collectively explained 39% of the phenotypic variation\textsuperscript{150}. In addition, there were 4 significant loci interactions each contributing 8-10% of the phenotypic variance in femoral size, suggest-
ing that gene-gene interactions play an equally if not more important role as single genes in the regulation of bone size. A polygenic determination of BMD is also supported by the work of Beamer and colleagues[139] who identified 12 QTLs which collectively explained 35% and 24% of variation in femoral and vertebral BMD, respectively, in crosses between inbred strains of mice. Each individual QTL explained 1-10% of the total variation in BMD. Work to failure, a measure of the maximum energy that bone can absorb before breaking, is also a polygenic trait that appears to be regulated by multiple loci, with each locus explaining <5% of the phenotypic variance and locus-locus interactions explaining about 50% of the genetic variance[141]. These individual loci with small to moderate individual effects and potentially strong interactive effects may be more likely to be identified in humans by association studies in large, well-characterized population samples.

The role of gene interactions and environmental exposures

Genes and proteins rarely act alone but rather operate in networks of interactions. Recent work in model organisms has indeed revealed the importance of genetic interactions where the effects of a given gene on a biological trait are masked or enhanced by one or more other genes[158-160]. The physiologic pathways that regulate normal bone formation and resorption involve many gene products that are known to interact biologically (e.g., ligands and their receptors). Thus, it is likely that variation in genes within these pathways will have interactive effects on bone related traits and the risk of osteoporosis. As discussed already, studies in inbred strains of mice emphasize the importance of gene interactions in the regulation of bone size and mass, and the need to consider such interactions when dissecting the genetic architecture of osteoporosis phenotypes[142,150]. However, gene interactions have only rarely been investigated in studies of osteoporosis related traits in humans[164-165]. A complete understanding of the genetic architecture of osteoporosis and related phenotypes will require a systematic investigation of candidate pathways involved in bone modeling and remodeling and mineral homeostasis rather than single genes within a given pathway. A more refined understanding of the genetic networks modulating the likelihood of osteoporosis may reveal novel biological relationships among gene products, insights into the pathogenesis of disease and ultimately suggest effective new therapeutic targets.

Normal skeletal integrity is also influenced by numerous environmental factors. The combination of genetic susceptibility and environmental exposure (e.g., age, obesity, dietary calcium, etc.) is therefore also likely to be important in determining phenotypic variability over and above the independent contribution of single genes or environments. However, with some exceptions[166-169], most candidate gene association studies in humans have investigated candidate genes without regard to environmental context and often with insufficient sample size and statistical power to elucidate important gene interactions. The genetic dissection of osteoporosis and fracture will require much larger sample sizes to disentangle the complex web of interactions among genes, environmental factors and phenotype. A better understanding of gene-environment interactions may suggest novel ways of preventing osteoporosis and its clinical outcomes.

Summary

Genetic factors play an important role in the development of osteoporosis and osteoporosis related fractures. The genes and mutations conferring osteoporotic risk remain largely undefined. Studies employing candidate gene association and genome-wide linkage analyses in the past several years have begun to make progress towards the identification of genetic factors contributing to BMD and osteoporotic risk in the general population and within families. More rapid progress can be expected in the near future as studies employing newer ultra-high-throughput genotyping technologies and high density genome-wide SNP maps are completed. Ongoing studies in baboons[180] and inbred rodents[139,141,157] should also reveal additional genes whose human homologs contribute to osteoporotic risk. The more comprehensive understanding of the genetic predisposition to osteoporosis that will likely emerge from this ongoing research should suggest novel approaches to the diagnosis, treatment and prevention of this common and disabling condition.

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