Genome Medicine

Review

# How genomics has informed our understanding of the pathogenesis of osteoporosis

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**Abstract**

Osteoporosis is a skeletal disorder characterized by compromised bone strength that predisposes a person to an increased risk of fracture. Osteoporosis is a complex trait that involves multiple genes, environmental factors, and gene-gene and gene-environment interactions. Twin and family studies have indicated that between 25% and 85% of the variation in bone mass and other skeletal phenotypes is heritable, but our knowledge of the underlying genes is limited. Bone mineral density is the most common assessment for diagnosing osteoporosis and is the most often used quantitative value in the design of genetic studies. In recent years, our understanding of the pathophysiology of osteoporosis has been greatly facilitated by advances brought about by the Human Genome Project. Genetic approaches ranging from family studies of monogenic traits to association studies with candidate genes, to whole-genome scans in both humans and animals have identified a small number of genes that contribute to the heritability of bone mass. Studies with transgenic and knockout mouse models have revealed major new insights into the biology of many of these identified genes, but much more needs to be learned. Ultimately, we hope that by revealing the underlying genetics and biology driving the pathophysiology of osteoporosis, new and effective treatment can be developed to combat and possibly cure this devastating disease. Here we review the rapidly evolving field of the genomics of osteoporosis with a focus on important gene discoveries, new biological/physiological paradigms that are emerging, and many of the unanswered questions and hurdles yet to be overcome in the field.

## Introduction

The past decade has been witness to one of the greatest scientific efforts and achievements in recorded history, the Human Genome Project (HGP). The impact of this effort in terms of our understanding of the genetic basis of disease will be manifest in multiple ways for many decades, even centuries, to come. In the field of osteoporosis, as in all diseases that have a genetic component, the sequencing and cataloguing of the repertoire of genes in the human genome and other species, the development of high-density single nucleotide polymorphism (SNP) maps, high-throughput sequencing along with other genomic technologies, advances in statistical approaches for the analysis of the derived genetic information, and the ability to generate transgenic and knockout mouse models to assess the contribution of individual genes to bone mass regulation have propelled research in the field at an incredible pace.

As in all genetic studies, the choice of phenotype ultimately determines the end-product of the genetic analysis, that is, the genes that may eventually be identified. In the simplest terms, osteoporosis has been defined by The National Institutes of Health (NIH) Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy as a '...skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture' [1]. However, this definition offers little phenotypic guidance for the design of studies to dissect out the underlying genetics or for identifying the causal genes of the disease. The World Health Organization Study Group suggested an operational definition for osteoporosis of a bone mineral density (BMD) of 2.5 standard deviations below the mean for young healthy adult women [2]. This definition is widely used today, but does not take into account bone microarchitecture and/or bone quality, which are much more difficult to define. BMD does provide a quantitative variable that can serve as a surrogate for 'osteoporosis' and thus is useful for genetic studies. As such, BMD has been one of the primary phenotypes used in genetic studies of osteoporosis, although as will be briefly referenced in this review there are several other surrogate phenotypes that have also been used in this regard.

## Lessons from 'the early days'

In order to fully appreciate how genomics has informed our understanding of the pathogenesis of osteoporosis, a

BAC, bacterial artificial chromosome; BMD, bone mineral density; HBM, high bone mass; HGP, Human Genome Project; IL, interleukin; LRP5, low density lipoprotein-related protein 5; NIH, National Institutes of Health; OPG, osteoprotegerin; OPPG, osteoporosis pseudoglia syndrome; QTL, quantitative trait locus; PTH, parathyroid hormone; RANKL, receptor activator of nuclear factor κB ligand; SNP, single nucleotide polymorphism; TGF, transforming growth factor; VDR, vitamin D receptor.

short historical review to provide perspective seems warranted. Osteoporosis is a complex trait involving multiple genes, strong environmental influences and, between individuals, a variable mix of gene-gene and gene-environment interactions. Evidence from twin and family studies has suggested that the heritability of BMD and other skeletal phenotypes ranges between 25% and 85% [3,4]. Given the high heritability of some of these bone traits (as surrogates for osteoporosis), there is reason to be optimistic that the underlying disease-causing genes can be identified.

### Candidate genes

The entrée of the bone field into the identification of genes involved in 'osteoporosis' was launched by Eisman and colleagues in 1992, with their seminal work on vitamin D receptor gene (*VDR*) polymorphisms [5]. This report was quickly followed by a number of studies (now totaling over 400) on *VDR* polymorphisms in relation to BMD and several other phenotypes. Not surprisingly, some studies showed positive associations [6-8], negative correlations [9-11] and some positive or negative correlations depending on phenotype [12,13]. While on the surface this might seem paradoxical, what these studies demonstrated with this single candidate gene approach is that differences in skeletal site, ethnicity, sex, age, menopausal status and diet all have effects that may account for why a candidate gene could have a positive association in one study and not in another. Even more important, these studies illustrated the need for careful study design, including the choice of phenotype, consideration of sample size, choice of study population and statistical methods of analysis, in order to be able to tease out the underlying genetic basis of osteoporosis.

Following the first report on *VDR* gene polymorphisms, several other favorite candidate gene studies (estrogen receptor (*ESR1*, *ESR2*), collagen type 1 (*COL1A1)*, parathyroid hormone (*PTH*), calcitonin (*CALCA*), transforming growth factor-β (*TGFB1*), and so on) began to appear, again with variable positive and negative correlations [14]. These studies all began in the pre-HGP later half of the decade of the 1990s and mainly used polymerase chain reaction (PCR) to amplify gene fragments with known restriction enzyme-based polymorphisms in candidate genes that were chosen based on their known role in bone biology. As the field evolved from a candidate gene focus towards more genome-wide approaches, the hope remained that the number of genes would be limited to a few with a 'large effect size', as the technology and statistical methods had not yet reached a capability of dealing with a large number of genes each accounting for less than 5% of the phenotypic variation. As we have moved into the post-HGP era, both technology and statistical methods of analysis have reached a level of sophistication that allows us to begin to identify these small-effect-size genes.

### Monogenic traits

In terms of understanding the pathophysiology of osteoporosis, there were major advances during this pre-HGP period that came out of studies of human families with monogenic traits. Advances in our understanding of osteoclast biology were a direct consequence of the identification of genes causing osteopetrosis [15,16], and osteoblast biology was greatly advanced from studies of both human kindreds and mouse model systems [17-19]. Another major advance in our understanding of bone mass regulation resulted from studies of decreased and increased bone mass conditions as in the case of osteoporosis pseudoglioma syndrome (OPPG) [20] and high bone mass (HBM) [21], respectively. Both were initially mapped to human chromosome 11q12-13 [22,23] using standard linkage mapping approaches. In the case of the HBM family, the genome linkage study after the initial definition of the linkage interval was accomplished in one month because of newly available (at that time) dinucleotide repeat-based human linkage mapping panels that used fluorescence-based detection methods on a DNA sequencing platform [23]. The causal mutations for these two conditions were subsequently shown to lie in the low-density lipoprotein receptor-related protein 5 (*LRP5*) gene [24,25]. Subsequently, several other groups have found mutations in *LRP5* that give rise to conditions of low and high bone mass [26-28]. LRP5 is a co-receptor along with the frizzled family of proteins for Wnt proteins, and this discovery introduced the importance of Wnt/β-catenin signaling [29] in the regulation of bone mass and, since those initial published reports, it has become one of the most heavily studied areas in bone biology. The discovery of *LRP5* also illustrates a major limitation of candidate gene studies; they are based on some knowledge for the selection of genes tested, and the linkage interval containing *LRP5* had no previous identified genes with a known role in bone biology. Similarly, whole-genome scans have produced a number of potential regions or quantitative trait loci (QTLs); many do not harbor any gene with a known role in bone biology. While this makes actual gene identification potentially more difficult, it also increases the likelihood of revealing unsuspected and dramatic new insights [30-34].

*LRP5* polymorphisms have subsequently been investigated for a role in bone mass variation, and the majority of studies report positive associations, but on average only approximately 2 to 3% of the variance can be explained, depending upon the SNPs and phenotypes examined [35-38]. Thus, while specific mutations in *LRP5* can have a dramatic effect on bone mass, more common variants of the gene appear to only contribute modestly to changes in BMD and consequently the pathophysiology of osteoporosis. LRP5 and the Wnt/β-catenin signaling pathway have been suggested as major targets for the development of new therapies to treat osteoporosis, although this has been the subject of some debate in the literature [39-41].

Another important family-based gene discovery that also relates to the Wnt/β-catenin signaling pathway was the identification of the *SOST* gene and its role in the pathogenesis of sclerosteosis [42-44] and van Buchem's disease [45,46]. The protein product of the *Sost* gene, sclerostin, inhibits Wnt/β-catenin signaling by binding to LRP5 and preventing Wnt from binding [47,48]. Sclerostin is thought to be exclusively produced by osteocytes [49,50] and functions on osteoblasts to regulate the pathway. Currently, an antibody to sclerostin is under development as a bone anabolic therapeutic for diseases of low bone mass such as osteoporosis [51].

### Animal studies

Additional advances have came from mouse mapping studies that employed a combination of genetic and genomic approaches to identify the *Alox15* gene as an important negative regulator of bone mass in mice [52]. Subsequent studies in humans suggested that variants in *ALOX12* but not *ALOX15* accounted for approximately 3% of the variation in human bone mass [53,54]. The 12/15-lipoxygenase pathway might therefore be another therapeutic target for osteoporosis [52]. Other genes that have appeared recently from various mouse mapping studies include the Duffy antigen receptor for chemokines (*Darc*) [55], endothelin-converting enzyme 1 (*Ece1*) [56] and secreted frizzled related-protein 4 (*sFRP4*) [57]. Whether these genes will contribute to bone mass variation in humans has not yet been fully investigated.

Several QTLs in the mouse have been identified [58-60] and it appears that the locations of these loci vary between inbred lines of mice. This can partially be explained by the known differences in geometry and BMD that is known to exist between strains [58]. One advantage of the mouse models besides the ability to generate specific breeding crosses is the ability to perform functional testing that cannot be carried out in humans, and thereby identify a different set of genes underlying these phenotypes [56,61,62]. Completion of the mouse genome DNA sequence and development of dense marker maps will greatly facilitate the identification of the genes underlying these mouse QTLs, in a similar way to the advances made in human studies.

Mapping studies in other animal model systems such as the baboon [63-65], chicken [66] and cow [67] have also been reported in the literature. Because of its close genetic relatedness to humans, the baboon offers an excellent model system and has been used in an integrative approach using chromosomal syntenic overlaps to define QTLs that are shared between species [63,68].

## Recent advances

In recent years, there has been a literal explosion in the number of published reports based on SNP-based candidate gene studies and whole-genome scans employing different phenotypes and study designs [30,31,69-71]. This can largely be attributed to the development of physical maps, cataloguing of genes and development of high-density SNP maps along with high-throughput technology platforms and statistical advances that came out of the HGP. A comprehensive description of these published reports is not within the scope of this review and so we will highlight just a few of the studies. What is perhaps the most exciting aspect of the more recent studies is that the first genes associated with these various traits are beginning to be identified, and this holds great promise for advancing our understanding of the pathophysiology of osteoporosis.

### Genome scans identify numerous QTLs

A recent review by Ferrari [4] summarizes the location of BMD-related QTLs that have been reported for the human genome and those that have been confirmed by meta-analysis. Interestingly, QTLs have been found on every human autosome, but only a subset has been confirmed by meta-analysis. The first of the genes to be identified from a whole-genome scan was *BMP2*, which came out of an analysis of extended osteoporotic families in Iceland and a subsequent follow-up association analysis [33]. Other genes that have been associated with BMD and/or fractures include *VDR*, estrogen receptor alpha (*ESR1*) and beta (*ESR2*), collagen type 1α (*COL1A1*), *LRP5*, TGF-β (*TGFB1*), interleukin-6 (*IL6*), osteoprotegerin or OPG (*TNFRSF11b*) and receptor activator of nuclear factor κB ligand or RANKL (*TNFSF11*). Recently *LTBP2* [72] and signal transducer and activator of transcription (*STAT1*) [73] have also been proposed to be genes involved in osteoporotic phenotypes. *STAT1* is an interesting candidate, as the *Stat1*$^{-/-}$ mouse has increased bone mass and has been shown to function as an attenuator of Runx2-mediated osteoblast differentiation [74]. Given the large number of candidate intervals identified from the whole-genome scan studies, and the potential large number of genes within those intervals, the next step towards underlying gene identification will be refinement of the mapped intervals. Based on the Icelandic population study [75], refinement of established genome-wide significant loci resulted in the identification of prime candidate genes MAP/microtubule affinity-regulating kinase 3 (*MARK3*) and *SOST*. Another approach is to combine data from multiple genome-wide studies and perform meta-analysis [4] to focus initial efforts for fine mapping of intervals. Interestingly, new intervals have appeared from this type of analysis [76], likely due to the sample size and power to detect small effect loci that is often the case with any one study. Added into this mix is a consideration of the relative contribution that one particular gene may have in one ethnic group versus another population. Also, in addition to choice of phenotype, which can yield different QTLs and genes, overlapping and different QTLs in males versus females have been identified in several of the published studies [30,34,69,71,76,77]. Clearly the gene discovery process is far from over at this point.

### Mouse genomic/genetic approaches

While the identification of an underlying trait gene within one of the QTLs is an important first step, understanding how this gene relates to pathophysiology of osteoporosis is even more important. In this regard, advances in genomics have played another critical role. Bacterial artificial chromosome (BAC) recombineering [78,79] has become a mainline approach in the bone field, and the pace with which these genetic mouse models can be generated is no longer a rate-limiting step for understanding gene function. The ability to create transgenic and knockout mice to explore the gene function has become a main investigative tool for bone biologists. The development of various Cre recombinase lines of mice in which Cre expression is driven by bone cell-specific promoter elements has propelled the field forward rapidly in the past few years. Crossing these mice with a mouse line containing floxed alleles of the particular gene of interest has provided us with the means to examine the role of any gene in just about any tissue or cell type for a role in bone biology. These conditional knockout mice represent an alternative approach to overcome the lethality of gene deletion that can be encountered in a traditional global knockout approach. In the bone field, mice such as the human osteocalcin-Cre [80], rat 3.6 kb Col1-Cre and 2.3 kb Col1-Cre [81] and the mouse α1(I)-Col-Cre [82] are commonly used for selective deletion of genes in osteoblastic cells; the DMP-1 Cre mouse is used for deletion of genes in osteocytes [83] and the Trap-Cre and Cathepsin-Cre [84] mice can be used for deletion of genes in osteoclasts. A common refinement of these Cre approaches is to include an inducible control element in the promoter construct [85] to overcome lethality problems that can sometimes be encountered even with conditional knockout crosses. However, all of these Cre approaches are not without limitations. As different promoters are used to drive Cre, the cell/tissue specificity and extent of gene deletion in each model system/cross needs to be carefully determined, especially with respect to the developmental pattern and timing of expression as well as the extent to which potential cells downstream in a differentiation pathway are affected, in order to fully interpret the results.

Nonetheless, considerable advances in our understanding of bone biology have resulted from the use of these mouse genetic approaches. For example, we now appreciate that bone plays a key role in several endocrine loops. Recent studies have suggested that bone mass is partially regulated by a fat-brain-bone axis involving leptin and the sympathetic nervous system [86,87]. Also, a key role for the skeleton in the regulation of energy metabolism involving osteoblast-produced osteocalcin has been shown [88]. Finally, bone mass regulation by a gut-brain-bone axis involving serotonin [89] has been proposed. The serotonin story is also interesting from the perspective that Lrp5 expression in the gut rather than the osteoblast has been proposed to be the critical site for Lrp5-mediated bone mass regulation. While this finding will require independent confirmation, it potentially adds another level of complexity to bone mass regulation and raises an important question of what tissue key genes need to be expressed in. Obviously these proposed endocrine loop systems are complicated and there are published data to suggest that there may be different effects on trabecular versus cortical bone [90,91], which adds an additional level of complexity. Regardless of how these evolving stories ultimately turn out, what they clearly illustrate is that bone mass regulation is also dependent on other organ systems besides the classical parathyroid-kidney-gut level of regulation that controls calcium and phosphate homeostasis. These new endocrine loop systems point out the complex nature of the regulation of bone mass and the need to not restrict genomic and functional study efforts solely to genes expressed in bone.

### Other approaches and advances

Gene-expression studies in the bone field have yet to be fully integrated into the genomic effort. There are many reasons for this lack of integration, mainly relating to the difficulty of obtaining appropriate human bone or other tissue samples (besides blood) for conducting expression profiling. One success in this regard was the combination of mapping and gene expression microarray analysis to identify Alox15 as an important regulator of bone mass in mice [52]. A recent report combined gene expression profiling in isolated human peripheral monocytes with a genome-wide association study to identify the *STAT1* gene [73], but human expression profiling relating to osteoporosis has lagged far behind other diseases.

Another important recent development is related to our understanding of the multifaceted roles played by the osteocyte. This cell is the most abundant cell type in bone. It is embedded within the mineralized matrix and through the lacunar canalicular system forms a communication network via its dendritic processes with other osteocytes and cells on the bone surfaces and within the vascular beds of bone. The osteocyte has long been thought to be the mechanosensory cell within bone, and as such plays a central role in controlling bone mass in response to changes in load environment. Recent studies have now shown that the osteocyte also plays a key role in regulating phosphate metabolism and mineral homeostasis, and orchestrating the activity of the bone-forming osteoblasts and bone-resorbing osteoclasts through autocrine, paracrine and endocrine mechanisms [92]. Osteocytes also appear to be the only cells producing sclerostin, the product of the *SOST* gene, which modulates the activity of Lrp5/6 and the Wnt signaling pathway. What all of these recent findings illustrate is that bone and bone cells do not exist in their own sequestered environment. Bone mass regulation over an individual's lifetime is finely tuned by a number of factors produced

**Table 1**

**List of the most prominent genes associated with osteoporosis phenotypes confirmed by multiple study designs**

| Gene | Signaling pathway | Study design | References |
|---|---|---|---|
| *LRP5* | Wnt/β-catenin | Human family linkage, GWAS, CGS, mouse genetics | [25-27,70,77,107-109] |
| *SOST* | Wnt/β-catenin | Human family linkage, GWAS | [43,44,46,75] |
| *sFRP4* | Wnt/β-catenin | GWAS, CGS, mouse genomics and genetics | [57,110,111] |
| *ALOX 12/15* | Arachidonic acid metabolism | Mouse genomics and genetics, GWAS, CGS | [52-54] |
| *BMP2* | Bone morphogenetic protein signaling | GWAS, CGS, mouse genomics and genetics | [33,112,113] |
| *VDR* | Transcriptional regulation | CGS, GWAS | [5,32,107] |
| *ESR1/ESR2* | Transcriptional regulation | CGS, GWAS | [4,72,107] |
| *STAT1* | Transcriptional regulation | GEP, CGS, mouse genetics | [73,74] |
| *Col1A1* | Extracellular matrix | CGS, GWAS | [3,32] |

CGS, candidate gene study; GEP, gene expression profiling; GWAS, genome-wide association study. For additional references see article and reviews cited therein.

from both outside and within bone. As such, there is still a great amount of bone biology that we do not yet know, and a combination of genomic, proteomic and other approaches will be needed to fully understand the pathophysiology of bone diseases like osteoporosis.

## Conclusions

Advances in genomics have propelled discovery in the bone field forward at an ever-accelerating pace, but there is still much to be learned. How many genes will ultimately be involved in explaining variation in BMD or other bone phenotypes is clearly an open question at this time. Given the new insights recently achieved regarding the complexities of bone mass regulation, it seems likely that many of the genes that will be discovered in the near future will be expressed in tissues other than bone, and understanding this will be an important aspect of future genomic efforts.

Given the pace of research, it seems almost certain that we will eventually catalogue the entire repertoire of genes that contribute to the pathophysiology of osteoporosis. It is not unreasonable to believe that in the near future, technology platforms will evolve to the point where sequencing one's entire genome will become feasible. New approaches to high-throughput sequencing are currently being developed, with real potential to make this a reality [93-95]. This implies that individual whole-genome analysis is not that far away. However, one major lesson from previous research is that within individuals, or groups of individuals, only a subset of genes may play a predominant role. Furthermore, in the case of osteoporosis, which is mainly a disease of older age and involves not only genetic, but also environmental, gene-gene and gene-environment interactions, determining lifetime risk will be much more complicated. One of the great challenges going forward will be not only identifying the genes, but also understanding how these genes are influenced by other factors.

Ultimately we hope to fully understand the pathophysiology of osteoporosis so that effective treatment can be achieved. As shown in Table 1, several genes have now been identified that contribute to the variation in bone density or other phenotypes. Three of the strongest candidate genes that have currently been identified are components of the Wnt/β-catenin signaling pathway. An antibody to sclerostin is already being developed; it is an anabolic agent and initial studies in rats have demonstrated a significant increase in BMD and show great promise [51]. Also, a series of sulfonamide derivatives have been shown to modulate Wnt signaling through inhibition of sFRP-1 [96], suggesting another class of compounds that might be developed into a therapeutic agent to increase bone mass. Other targets such as GSK-3β [97,98] and Dickkopf-1 [99,100] have been described in the literature. A major concern with targeting the Wnt pathway is its known role in tumor formation and whether manipulating the pathway in bone will increase the risk of tumor formation in other tissues [39,41,101-106]. Manipulating sclerostin appears to circumvent this concern because it is produced by osteocytes and therefore potentially restricts the target to bone, although the extent to which sclerostin circulates could be a potential unknown issue that will need to be resolved. While it appears that a new generation of anabolic agents is now on the horizon, clearly our understanding of the underlying genetics of osteoporosis has yet to reveal all there is to be discovered. Looking further ahead as new agents for treating osteoporosis are developed, studies will be needed to understand how any given individual's response to different therapeutic approaches may vary as a function of their specific genetic background. Clearly, advances in genomics will also play a major role in addressing and solving these and many other future questions.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

The authors shared equally in the writing of this review and agree to its content.

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