Series: Going Beyond the Sequence
Review

Dissecting the Genetics of Osteoporosis using Systems Approaches

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Osteoporosis is a condition characterized by low bone mineral density (BMD) and an increased risk of fracture. Traits contributing to osteoporotic fracture are highly heritable, indicating that a comprehensive understanding of bone requires a thorough understanding of the genetic basis of bone traits. Towards this goal, genome-wide association studies (GWASs) have identified over 500 loci associated with bone traits. However, few of the responsible genes have been identified, and little is known of how these genes work together to influence systems-level bone function. In this review, we describe how systems genetics approaches can be used to fill these knowledge gaps.

The Skeleton as a Dynamic System

The human skeleton is a dynamic, adaptive, and complex system impacting a wide array of physiological processes. It provides support and protection, enables locomotion, maintains hematopoiesis, serves as a reservoir for calcium and phosphorus, and has important endocrine functions [1–3]. Diseases of bone, however, inhibit the ability of the skeleton to carry out these functions. The most common disease of bone is **osteoporosis** (see Glossary), a condition of low **bone mineral density** (BMD) and an increased risk of fracture [4]. Osteoporosis affects over 12 million individuals in the US and over 200 million worldwide [5]. Osteoporotic fractures are a serious clinical outcome associated with increased morbidity and mortality, particularly in elderly people. In fact, of the ∼300 000 people in the US over the age of 50 years that suffer a hip fracture annually, one in five will die in the subsequent 12 months, and half of the survivors will not return to their prior independent living status [6]. Alarmingly, the incidence of fractures is expected to rise by 50% over the next decade, as the number of individuals over the age of 50 years increases [7].

One of the hallmarks of quantitative traits related to osteoporosis (BMD, bone size, etc.) is their high **heritability** ($h^2 = 0.5$–0.8) [8]. As a result, the development of a comprehensive understanding of bone biology necessitates a thorough understanding of the genetic factors underlying variation in bone traits. This not only includes defining the individual variants and genes contributing to osteoporosis, but also how they interact to impact molecular networks and systems-level function. Here, we discuss how **systems genetics** approaches are being used to accomplish these goals (Figure 1, Key Figure).

Current State of Osteoporosis Genetics

The genetic analysis of osteoporosis began in the early 1990s with candidate gene studies describing associations between polymorphisms in bone-relevant genes (e.g., vitamin D receptor and type I collagen) and BMD [9]. This was followed by a plethora of additional candidate gene investigations and linkage scans in families [10]. In retrospect, little information was gained from either approach [11,12]. In 2007, the tide began to turn with the first of many **genome-wide association studies** (GWASs) of BMD [13]. In a BMD GWAS, the genotypes

Highlights

Osteoporosis is a common, complex disease characterized by low bone mineral density (BMD). Quantitative bone traits influencing osteoporotic fracture, such as BMD, are highly heritable ($h^2 > 0.5$).

Genome-wide association studies (GWASs) have identified >300 loci for BMD. However, most causal genes, their mechanisms of action, and how they interact are unknown.

Systems genetics approaches can be used to integrate bone GWAS with other '-omics' data to identify causal variants and genes.

Network-based analysis approaches can provide insight into how genetic variation impacts molecular networks and how network dysfunction leads to osteoporosis.

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Key Figure

Overview of Systems Genetics
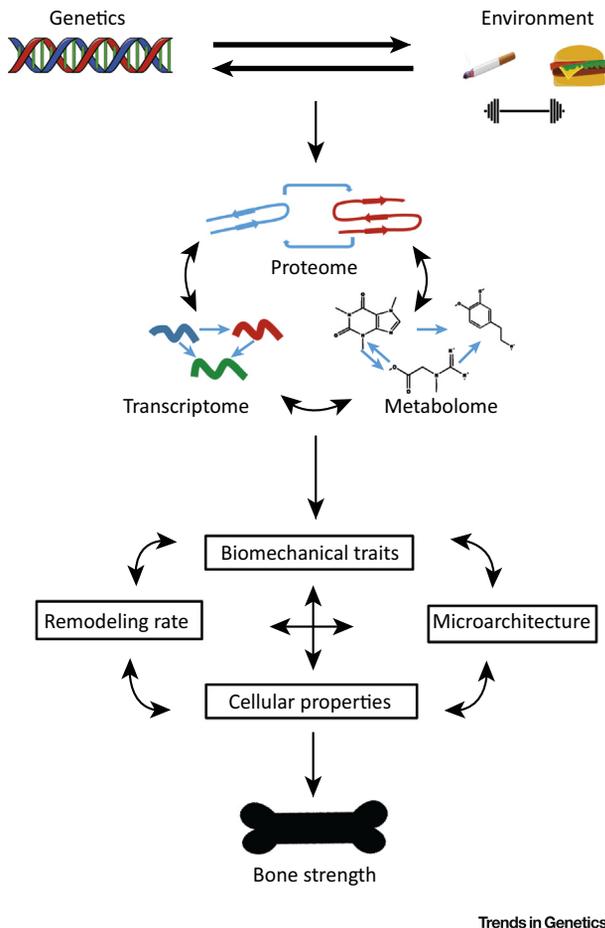

Trends in Genetics

Figure 1. Genetic and environmental factors, as well as their interactions, influence complex bone traits, such as bone strength. The influence of these factors is mediated through impacts on molecular intermediates (transcriptomes, proteomes, metabolomes, etc.) and can be assayed via appropriate -omics techniques. Biological information is propagated through complex molecular networks to affect bones traits and ultimately the risk of fracture.

Glossary

Acyclic graph: a graph that does not contain cycles. A cycle is a set of nodes and edges, where a node can be reachable from itself. For example, the graph with nodes A, B, and C with edges A→B, B→C, and C→A contains a cycle.

Bone mineral density (BMD): amount of mineral (hydroxyapatite) per volume of bone. BMD is the main diagnostic measure for osteoporosis and is one of the strongest predictors of fracture.

Edge: a connection between two nodes in a network.

Epigenetics: study of processes that affect gene expression or function but do not involve changes in DNA sequence. These include processes such as histone modifications (acetylation, phosphorylation, etc.) and DNA methylation.

Expression quantitative trait loci (eQTLs): genomic regions harboring genetic variation influencing RNA levels (via transcription, splicing, stability, etc.).

Genome-wide association studies (GWASs): identify genomic regions harboring genetic variation influencing a disease or quantitative trait.

Genetic architecture: for a given trait it refers to the number, mode of action, effect size and frequency of genetic variants that contribute to that trait in the population and their interactions with each other and the environment.

Heritability: fraction of variance in a trait that is attributable to genetic variance.

Machine learning: branch of artificial intelligence concerned with creating algorithms that can analyze data, and improve analytical performance, with minimal instruction. For example, machine learning algorithms can analyze data to uncover patterns that predict certain outcomes, while progressively improving metrics like the selectivity and sensitivity of predictions.

Node: along with edges, nodes are the basic units of a network. In coexpression networks, nodes are genes or transcripts.

Osteoblast: specialized bone-forming cells of mesenchymal origin.

of millions of **single nucleotide polymorphisms** (SNPs) across the genome are tested for an association with BMD in thousands, now often hundreds of thousands, of individuals [14]. To date, over 20 primary GWAS and GWAS meta-analyses have identified hundreds of associations for BMD [15–18]. The largest GWAS to date analyzed estimated BMD at the heel in 426 824 individuals and identified 1103 independent genome-wide significant associations in 518 loci (Table 1) [19]. BMD has been the primary target of GWASs, mainly because of its strong association with fracture, high heritability, and relative ease of assessment in large cohorts [20]. However, other traits such as bone size, bone geometry, and serum bone remodeling markers have been interrogated by GWAS [21–24]. Together, these studies have reinforced the

Table 1. Examples of Large GWASs and GWAS Meta-analyses for BMD Illustrates the Increase in Identified Loci as a Function of Sample Size.

Study	Phenotype	Sample size	Association count
Morris et al. (2018) [19]	Estimated heel BMD	426 824	1103 independent associations (518 loci[a], 301 novel)
Kemp et al. (2017) [17]	Estimated heel BMD	142 487	307 independent associations (203 loci, 153 novel)
Estrada et al. (2012) (meta-analysis) [16]	Lumbar spine and femoral neck BMD	83 894 (32 961 discovery, 50 933 replication)	64 independent associations (56 loci, 32 novel)
Rivadeneira et al. (2009) [40]	Lumbar spine and femoral neck BMD	19 195	20 independent associations (20 loci, 13 novel)

[a]Note that the most recent discovery of 518 loci encompassed nearly all of the previously discovered loci identified in prior studies.

importance of known genes and pathways (RANK–RANKL, WNT signaling, etc.) in human bone biology. More importantly, GWAS has provided a 'treasure trove' of loci containing only novel genes with the potential to revolutionize our understanding of the genetics, and more importantly, the biology of bone.

A limitation of current GWASs is that they have yet to fully uncover the **genetic architecture** of BMD [25]. In the heel estimated BMD study referenced above, the 518 independent associations explained only 20% of the phenotypic variance in estimated BMD [17]. These data suggest that BMD is highly polygenic, or even omnigenic (Box 1), and that much of the genetic basis of BMD remains to be discovered. GWASs are ideally suited to identify associations with common variants [minor allele frequency (MAF) >1%]. Therefore, it is possible that rare variants (MAF <1%) may explain part of the missing heritability [26]. In support of this hypothesis, recent whole genome-sequencing projects have identified rare variants with large effects on BMD [17,27–29]. It will likely require larger GWASs and rare variant studies to fully dissect the genetic architecture of BMD and other bone traits.

Box 1. Polygenicity of Complex Traits

Recently, an omnigenic model was proposed to explain the genetic architecture of complex traits [81]. This model is essentially a modernization of Fisher's infinitesimal model [82]. The omnigenic model states that any gene expressed in a disease-relevant cell type is likely genetically associated with the disease. The authors supported this model with data from large GWASs for multiple diseases/traits suggesting that hundreds of thousands of variants spread uniformly across the genome have nonzero genetic effects. They also provide data suggesting that disease heritability is not concentrated in biologically relevant processes. They postulate that this is due to the fact that genes expressed within a cell are members of a highly interconnected network made up of core (genes participating in a biological process directly influencing a disease) and peripheral (genes connected to core genes to varying extents) genes. In the omnigenic model, peripheral genes influence disease indirectly through subtle network interactions with core genes.

An opposing viewpoint to the classification of genes as core or peripheral argues that assuming only core genes directly affect disease is underestimating biological complexity [83]. Alternatively, it is possible that highly polygenic, complex diseases are affected by common variants not through their indirect effects on core genes, but by altering complex biological relationships across many genes. Thus, without the distinction between core and peripheral genes, the omnigenic model is no different than a polygenic model and it is evident that complex disease genes work together in complex networks. Thus, the polygenicity of complex disease reinforces the importance of systems genetics approaches.

Osteoclast: specialized bone-resorbing cells of hematopoietic origin.

Osteoporosis: metabolic bone disease characterized by decreased bone mass and increased risk of fracture.

Single nucleotide polymorphisms (SNPs): single base pair substitutions. SNPs are the most common type of variation in the human genome, occurring on average every 300 bases. SNPs can have many molecular consequences such as altering protein structure, gene regulation or splicing.

Structure learning: in BNs, this refers to the process of learning the structure of the directed acyclic graph. That is to say, learning the connections and directionality between nodes. Two main classes of algorithms are used for structure learning: score-based and constraint-based algorithms.

Systems genetics: integrative field that aims to understand how genetic information is transmitted and integrated through biological systems and networks.

By any standard, GWASs have been successful at identifying new loci; however, to date this information has done little to increase our understanding of bone biology or disease. Of the hundreds of loci impacting BMD and other bone traits, the genes responsible for nearly all of the associations are unknown. There are many reasons for this knowledge gap, including the fact that most associations are due to noncoding variation, the lack of bone-specific -omic resources, and the inherent difficulties in experimentally establishing causality between variants, genes, and traits.

Using Systems Genetics to Inform Bone GWAS

One approach that has the potential to increase our understanding of bone genetics is the emerging field of systems genetics [30,31]. Systems genetics integrates the principles of systems biology with genetics to determine how genetic variation affects molecular phenotypes and cellular networks [30]. In the context of GWASs, systems genetics approaches have proven useful for connecting associated variants with molecular functions (e.g., transcription). The layering of different -omics datasets (transcriptomics, metabolomics, proteomics, etc.) onto a set of GWAS loci is the most direct way to begin to identify the molecular consequences of disease-associated variants (Figure 2). Most importantly, it also serves to connect disease-associated variants to the genes they regulate.

One of the most widely used systems genetics approaches for informing GWASs is the identification of **expression quantitative trait loci** (eQTLs) [32]. Just like a clinical trait, GWASs can be used to identify associations for the expression of a gene [33]. These analyses identify sets of genetics variants, or eQTLs, that influence transcript levels of any gene expressed in a given cell type or tissue. There are two types of eQTL, local and distant [34]. Local eQTLs influence the transcript levels of genes in close proximity; whereas distant eQTLs influence gene expression over a long genomic distance. The identification of eQTLs is a logical follow-up to a GWAS, given that the vast majority of GWAS loci are due to noncoding variants that presumably have a role in gene regulation. A typical analysis consists of identifying local eQTLs for genes located within a GWAS locus and then determining if the two signals are due to the same sets of variants (referred to as colocalizing eQTLs) [35,36].

One of the major considerations for eQTL studies (and for that matter the generation of any other -omics dataset) is the cell type or tissue used for the generation of gene expression profiles. Recently, the Genotype-Tissue Expression (GTEx) project demonstrated that many eQTLs are tissue specific, thus, ideally the transcriptomics data would be from a disease relevant source [37]. In the context of bone GWASs, this would be either bone tissue or bone cells, for example, **osteoblasts** and **osteoclasts** [37,38]. However, to date only three small studies have generated bone-relevant eQTL data. One such study generated microarray profiles on transiliac bone biopsies from 84 postmenopausal women [39]. These data were used to identify colocalizing eQTLs for a GWAS of vertebral volumetric BMD [21]. Microarray profiles of undifferentiated osteoblasts from 95 individuals have also been used to identify eQTLs and inform several bone GWASs [16,17,40]. More recently, eQTLs were identified in cultured primary osteoclasts using RNA-seq profiles in 158 individuals. These data were used to identify colocalizing osteoclast eQTL for genes in eight loci [41]. Integrating transcriptomics data from non-disease-relevant sources has also been shown to provide insight given the expectation that some eQTLs will be active across many different tissues [37]. For example, peripheral blood eQTLs were recently used to identify *ASB16-AS1* and *SYN2* as potentially causal BMD GWAS genes [42]. Furthermore, GTEx expression data from thyroid tissue were used in a recent study to link the expression of *MARK3* to BMD-associated variants on chromosome 14q32.32 [43].
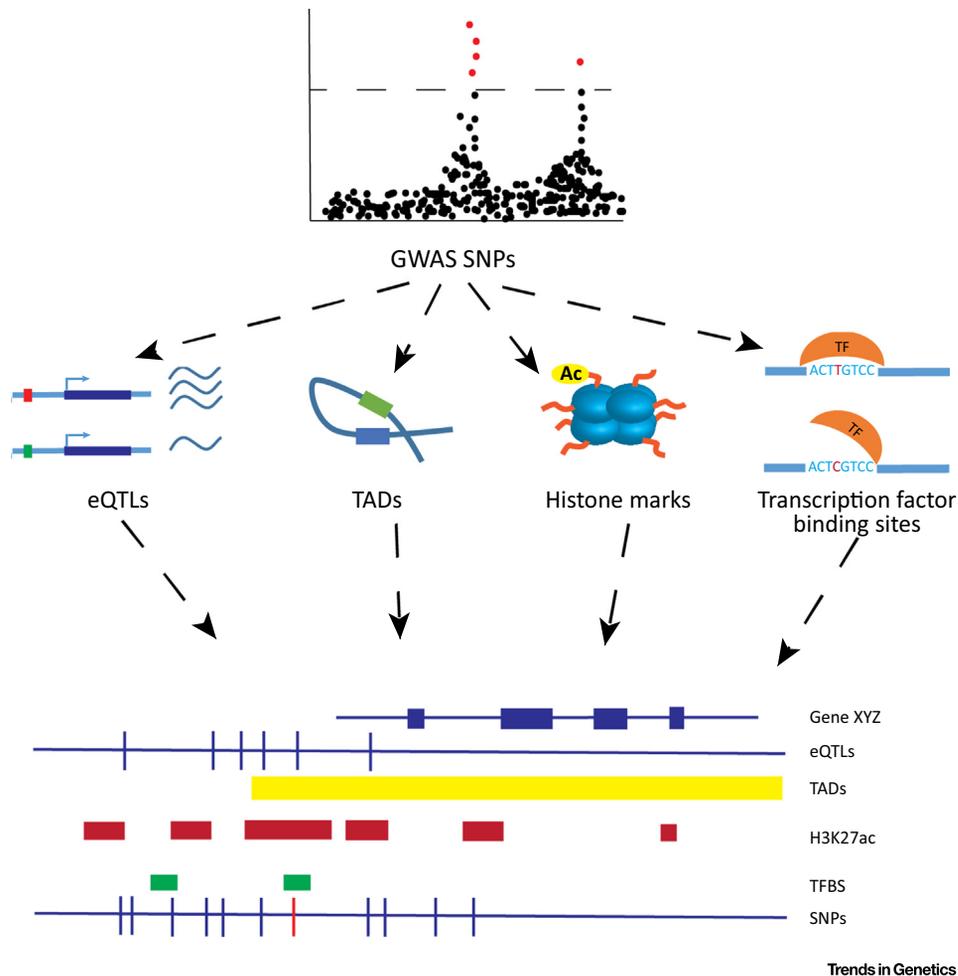
Figure 2. Systems Genetics Approaches for Prioritizing GWAS Data. By integrating biological data with data from GWAS, SNPs affecting traits can be prioritized for functional follow-up. For example, transcriptomic, biophysical and epigenetic data pertaining to lists of GWAS SNPs can be leveraged in order to prioritize the most likely causal SNPs (red bar, bottom row). Abbreviations: eQTL, expression quantitative trait locus; GWAS, genome-wide association study; H3K27ac = histone H3 lysine 27 acetylation; TAD, topologically associating domain; TFBS, transcription factor binding site.

Another systems genetics approach that has proven useful for informing GWASs is the integration of epigenetics data [44]. As mentioned above, the vast majority of BMD GWAS loci implicate only noncoding variation that presumably impacts gene regulation. Thus, it is likely that most causal GWAS variants reside in regulatory elements, such as promoters and enhancers, which can be identified as regions of open chromatin marked by histone modifications, including H3K4me1, H3K4me2, H3K4me3, and H3K27ac. In support of this hypothesis, studies have demonstrated an enrichment for GWAS variants overlapping enhancers in disease-relevant tissues [45–48].

Epigenetic data have recently been used to inform BMD GWASs [49]. Using publicly available data, an eQTL in blood cells was identified for *LINC00339* that colocalized with a BMD GWAS association on chromosome 1p36.12. It was then found, using chromosome conformation capture (HI-C) data, that one of the eQTL SNPs (rs6426749) was located in a genomic region

interacting with the promoter of *LINC00339*. Using epigenetics data from the ENCODE project, this SNP was found to overlap and influence the activity of an enhancer element in osteoblasts by altering a binding site for *TFAP2A* [50]. Furthermore, alteration of *LINC00339* expression influenced the transcript levels of a nearby gene, *CDC42*, which plays a key role in bone modeling and remodeling [51]. Using a similar approach, another recent study determined that the BMD GWAS SNP rs9533090 affects the expression of *RANKL*, which plays a central role in osteoclastogenesis, by disrupting an *NFIC* binding site and enhancer activity [52,53]. These studies demonstrate the power of systems genetics approaches that combine multiple data types to unravel the molecular consequences of BMD-associated variants.

There are limitations to using eQTL data to inform GWASs. As described above, the most powerful sets of eQTL data (e.g. GTEx) are from non-bone tissue. While such data have been informative for identifying colocalizing eQTLs, it is likely that well-powered eQTL studies in bone tissue and cells will provide more insight. It has also become evident that tissue and cell-type specificity is a critical factor when trying to dissect how GWAS loci influence BMD. As a result, not only do we need efforts focused on generating data in bone tissue and cells, but also specific bone cell populations at different stages of their life cycle exposed to varying stimuli. It should also be noted that differences in the genetic backgrounds (with differences in linkage-disequilibrium structure) of GWASs and eQTL studies affect the interpretability of results. This can be resolved by efforts to generate both types of data from racially diverse populations.

Network-based Approaches

As described above, systems genetics approaches are critical for identifying individual genes contributing to BMD. However, gene discovery is just the beginning. The next step is to understand how variants, genes, and their products work together to enable proper bone function. GWASs for BMD have identified many genetic loci implicating disparate biological processes and mechanisms, suggesting a complex web of networks operating within and between various bone cell types. Identifying these interactions is important as they can inform our understanding of emergent properties of bone that are not evident from the function of individual genes in isolation. This is analogous to identifying a car battery and alternator as elements involved in starting an engine. However, it would be impossible to understand their true function without knowing that they worked together in the electrical system of the car. It is also likely that genetic variation is a major perturbation that shapes underlying biological networks. As a result, systems, rather than reductionist, approaches to bone genetics are critical to understand the role of genetics in systems-level function. Understanding bone molecular networks and how they are influenced by genetic variation is also important in the context of discovering and evaluating potential antiosteoporotic therapeutic targets [54–56].

Biological Networks

Networks are prevalent in all aspects of our lives. The internet, social media, and economic markets are all examples of networks that impact us daily. In biology, many types of networks exist including protein–protein interaction, transcriptions factor binding, metabolic, and gene regulatory networks. Mathematically, a network (or graph) is a set of **nodes** (elements) connected by **edges**, which represent relationships between nodes [57]. Edges can be directed or undirected and either weighted or unweighted. An undirected gene coexpression network represents the relationships in coexpression between genes without an indication of which node is upstream of the other, while a directed network models the information flow between nodes (e.g., increased expression of gene A causes increased expression of gene B). Weights can represent the strength of evidence for the edge or the strength of the relationship

between nodes. Methods used to generate and analyze networks are indispensable to systems genetics, as they allow for a shift of focus from reductionist methods, like GWASs, to more holistic, systems-level approaches. Mostly due to the scarcity of bone-relevant data, and the relative paucity of investigators applying such approaches, the use of network biology in the bone field has lagged behind others. However, there are emerging use cases. For example, by combining BMD GWAS data with functional genomic analysis, a PU.1-dependent transcription factor network essential for osteoclast differentiation has been identified [58].

Coexpression Networks

The most popular types of biological networks used in systems genetics applications are based on coexpression. There are many methods for generating coexpression networks and one of the most widely used is weighted gene coexpression network analysis (WGCNA) [59]. WGCNA organizes transcriptomics data into modules, or clusters, of coexpressed genes. It does this by analyzing coexpression (i.e., correlation in expression) across a set of perturbations, such as genetic background in mice or environmental exposures in a human population. Modules have been found to have a number of important features, such as containing functionally related genes that may be subject to coregulation by similar factors [59,60]. As a result, one can think of coexpression network analysis as a way to organize biology in an unbiased way, similar to the way that file folders are used to organize documents by topic.

There are two aspects of coexpression networks that make them particularly useful for systems genetics studies. First, unlike many other popular biological networks they retain tissue- or cell-type-specific information. While recent advances in proteomic technology have facilitated the study of protein–protein interactions *in vivo*, the majority of extant data is generated through *in vitro* methods, which may not accurately reflect physiological interactions [61]. Second, unlike other biological networks, coexpression modules can be related to phenotypes from the individuals used to generate the transcriptomic profiles. For example, a WGCNA network was recently generated from blood cells in individuals with BMD measurements [62]. These data were then used to identify a module whose behavior (as summarized by its first principal component) was correlated with BMD. Once trait-correlated modules are identified, they can be further analyzed to identify key genes and relationships. For example, highly connected hub genes have been shown to drive modular associations with a trait [63]. A recent study generated a WGCNA network using bone transcriptomic data on 96 strains from the Hybrid Mouse Diversity Panel (HMDP) [64,65]. An osteoblast-lineage specific module was identified (module 9) and shown to be highly correlated with femoral BMD in the same HMDP strains. The study showed that knockdown of the top two module 9 hub genes (*Maged1* and *Pard6g*) altered osteoblast proliferation, differentiation, and mineralization *in vitro* and knockout of *Maged1* decreased BMD in mice [63,65]. The authors mapped the first principal component of module 9 and demonstrated that the overall expression levels of module 9 genes were influenced by a local eQTL for *Sfrp1*, a key regulator of osteoblastogenesis [66]. This demonstrates how coexpression network analysis in a genetics population can be used to understand the systems-level organization of genes. Similarly, another study generated a WGCNA network using gene expression data from female transiliac bone biopsies in humans. Through the integration of BMD GWAS data, this study identified a gene module and several candidate genes (*HOMER1* and *SPTBN1*), with putatively important roles in bone mass regulation [67].

Another use of coexpression networks is to inform GWASs. A number of studies have demonstrated that network information is a useful prioritization strategy for predicting causal genes for sets of GWAS associations [68]. As an illustration, a recent study mapped genes located in 64 BMD GWAS associations onto the HMDP bone network described above [43,68].

This led to the identification of two modules that were enriched for genes implicated by GWASs. Using information on module genes with known roles in bone, it was predicted that novel module genes located in GWAS loci were causal and likely altered BMD via a role in osteoblasts. Two of the module genes, *MARK3* and *SPTBN1*, were experimentally confirmed to influence BMD when perturbed in mice. This study indicates that viewing GWAS data through the lens of a disease-relevant coexpression network can begin to highlight how key GWAS genes function together to regulate BMD.

Bayesian Networks (BNs)

Although initially described in the mid 1980s, Bayesian networks (BNs) have only recently begun to gain traction in biological research [69]. BNs are directed, **acyclic graph** representations of conditional dependencies between random variables [57]. The directed, acyclic nature of the graphs is informative for reconstructing systems-level relationships between genes. For example, in a systems genetics context it is possible to apply a BN **structure learning** algorithm to a WGCNA module, as the dependence of gene expression on other genes can be observed in a hierarchical manner, which allows for an elucidation of the direction of the flow of molecular information. One scenario is where BN analysis methods are applied to trait-relevant WGCNA modules, in order to direct relationships between genes and identify key regulatory elements (Figure 3). This strategy was used in a recent study, where an undirected coexpression network was constructed. Directional relationships between nodes were then established using BN analysis. This led to the identification of causal network structures relevant to late-onset Alzheimer's disease pathology, as well as the identification of TYRO Protein Tyrosine Kinase Binding Protein as a key regulator [70]. In another study, a BN generated from coexpression modules was used to reveal regulatory driver genes affecting coronary artery disease [71]. To our knowledge, BNs have not yet been applied in a systems genetics context in the bone field, and therefore provide an exciting avenue for future research.

A great advantage of BNs is that they allow for the incorporation of prior knowledge, which allows for more informative modeling of gene relationships within modules. For example, network structure learning can be biased by 'whitelisting' high-confidence edges (such as well-known gene–gene relationships or protein–protein interactions) *a priori*, or 'blacklisting' improbable edges. Disparate data sources can be easily incorporated into BNs as well. For example, a BN from a WGCNA module can also include SNP nodes and trait nodes, in order to model information transfer from genetic element to gene expression and phenotypic outcomes [72].

Network-based approaches are not without limitations. One limitation involves the quality and type of the investigated bone phenotype. For example, BMD can be assayed in different anatomical locations by several different methods, which can lead to heterogeneity in the data that can obfuscate meaningful network relationships, or lead to network connections that are artificial and not mechanistically viable. Furthermore, a phenotype such as BMD is actually a composite of many different aspects of bone, which can also exacerbate the problems of interpretability. Therefore, careful selection of phenotypes should be performed *a priori*. Furthermore, biological networks often encompass multiple cell types, tissues, and physiological microenvironments. *In silico* analyses based on data from *in vitro* sources, such as cultured osteoblasts, will not uncover many physiological relationships that exist *in vivo*. This drawback is not unique to network analyses and pervades biological science, but should be carefully considered when designing experiments and drawing conclusions. Methodological drawbacks exist as well. A significant drawback of using BNs to model biological relationships is in their acyclic nature. In biological processes, structures like feedback and feed-forward loops are

(A)

Gene expression profiles

(B)

WGCNA network

(C)

Bayesian network
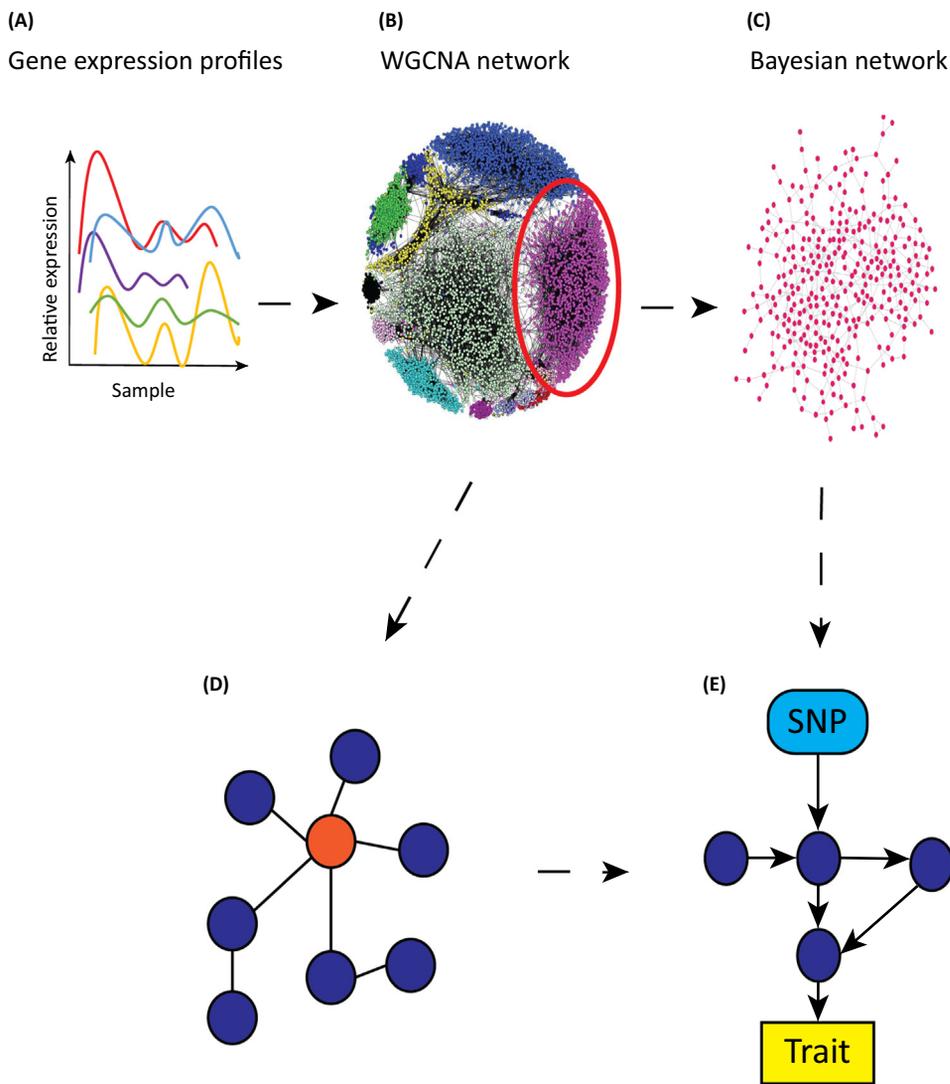
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Figure 3. Network Analysis Can Reveal Systems-Level Information. In a typical network analysis workflow, gene expression profiles (A) (typically RNA-seq) can be analyzed from a network perspective. In this example, a coexpression network (B) is generated using WGCNA. In a WGCNA network, modules (colored clusters, B) consist of gene expression profiles connected by undirected edges, which signify the strength of the connection between two genes. A trait-correlated WGCNA module (within red circle, B) can be further dissected through BN analysis (C). Highly connected hub genes (orange node, D) can signify functionally important genes. BNs differ from WGCNA networks in that they contain directed edges, and are acyclic (E). Furthermore, diverse biological information, such as SNP and trait data (blue and yellow boxes respectively, E) can be incorporated into BNs as prior information to improve network reconstruction. An advantage of BN analysis is the generation of more mechanistic hypotheses. Abbreviations: BN, Bayesian network; WGCNA, weighted gene coexpression network analysis.

prevalent. As BNs are acyclic, these network structures will be missed. Furthermore, depending on the algorithm used, it can be computationally impractical to learn the network structure of large sets of genes. These shortcomings make the aforementioned strategy of using BNs to dissect WGCNA modules an attractive one [73].

Concluding Remarks and Future Perspectives

In the past decade or so, advances in sequencing technology have completely revolutionized biological science. In practically every biological field, a wealth of -omics data is being generated. However, our understanding of the underpinnings of biological processes and diseases is still far from complete. This is evident in the bone field, as many genetic associations with BMD have been described, yet we still know few of the responsible genes. These limitations reinforce the need for complementary strategies, such as systems genetics, to further advance our understanding of bone genetics.

One of the major limitations of genetic studies of bone is the primary focus on BMD. Although BMD is the single strongest predictor of osteoporotic fracture, there are many individuals with normal BMD that fracture [74,75]. The use of BMD has been necessitated by the difficulty, or impossibility, of measuring other aspects of bone fragility in humans. For example, biomechanical properties of bone strength, the single most important fracture-related trait, can only be measured in cadavers. Due to these limitations, a possible alternative is to use GWAS and systems genetics in mice and rats as a way of developing a more complete understanding of osteoporosis [76–78].

The adaptive nature of bone creates an additional layer of complexity in understanding osteoporosis. Studies have demonstrated that recombinant inbred mice with different genetic backgrounds build functional bones in different ways. For example, mice with genetically slender bones will compensate for this deficiency by increasing cortical thickness and mineralization, whereas mice with mineralization defects will increase bone size [79,80]. This genetically based covariation in traits serves as an example of a system adapting to perturbations. It also illustrates the importance of understanding not only how genetic variation impacts individual

Outstanding Questions

To date, the genetic analysis of osteoporosis has focused on connecting variants to genes to disease. Equally important will be understanding how genetic variation impacts gene networks and how network perturbations lead to disease.

The bone field has generated several large GWASs of BMD. Should we now focus on the generation of other -omics data to facilitate systems genetics approaches? If so, what data types are lacking, which tissue/cell types should be the focus, and what are the most pressing questions to examine?

Does a best approach or algorithm for network-based analyses exist? What are the best practices for validation and interpretation of the methodology and results of network analyses?

How can systems genetics approaches be utilized to understand genetically influenced relationships between traits, which ultimately underlie bone strength?

Box 2. Nascent Approaches in Systems Genetics

In order to capitalize on advances in biological knowledge, such as the recent abundance of -omics datasets, continuous development of analytical approaches that can utilize large, varied data is necessary. **Machine learning** is an example of such approaches, and has generated a high level of interest in recent years. Machine learning is a broad term that encompasses many widely used algorithms and techniques; for example, hierarchical clustering, a main component of WGCNA network construction, and linear regression are considered machine learning algorithms. In the bone field, machine learning approaches have been utilized to predict bone loss rates, osteoporosis and bone fracture risk [84–87].

In the context of systems genetics, machine learning approaches are of interest due to their utility in analyzing large, highly dimensional data, such as multiomic data sets [88]. Within machine learning, deep learning approaches (such as artificial neural networks) represent the state of the art, and refer to a class of approaches that learn patterns in data through structuring algorithms in hierarchical layers. Deep learning approaches can be utilized to learn patterns in data for the prediction of traits and for the identification of important data features. For example, patterns that classify samples as healthy or diseased can be discerned in multiomic datasets, facilitating the prediction of diseased individuals from input data, as well as identifying causal determinants of disease, leading to a deeper understanding of the biological networks underlying phenotypes [89]. Similarly, machine learning approaches can also be used to learn patterns in data that lead to the identification of more accurate phenotypes and biomarkers [90]. Deep learning approaches can even be used to predict genetic elements, such as enhancers, from genomic data [91]. To our knowledge, such approaches have not yet been implemented in in the context of the systems genetics of bone, but have begun to be utilized in other biological fields [92–95].

In addition to being limited by their relatively recent genesis, the use of such approaches has been limited in the bone field due to the same limitation that affects network analyses; the sparsity of large, high-quality data sets encompassing multiple omics sources. In order for the bone field to ascend to the forefront of systems genetics, it is imperative to generate such data sets from relevant samples.

traits, but also the relationships between traits. A more encompassing approach to systems genetics has the potential to begin to understand how genetic variation contributes to these relationships and overall system function.

In the field of systems genetics it is of importance to develop approaches for the effective understanding and utilization of available data (Box 2). As biology is inherently complex, it is unreasonable to believe that a single or a few types of genetic analyses will be sufficient to gain a thorough understanding of the genetics of complex bone traits. We argue that more realistic models of biological processes can be generated and analyzed by synthesizing and incorporating seemingly disparate data sources. While barely scratching the surface, the systems genetics approaches described herein provide an avenue for such an endeavor. Of course, our understanding of many systems-level principles is still evolving (see Outstanding Questions). With increasingly accessible computational resources and by training researchers adept in the computational sciences, the transition to understanding bone biology and the impacts of genetic variation from a holistic, systems perspective will be within our reach.

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