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Systems Analysis of Bone

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Abstract

The genetic variants contributing to variability in skeletal traits has been well studied, and several hundred QTLs have been mapped and several genes contributing to trait variation have been identified. However, many questions remain unanswered. In particular, it is unclear whether variation in a single gene leads to alterations in function. Bone is a highly adaptive system and genetic variants affecting one trait are often accompanied by compensatory changes in other traits. The functional interactions among traits, which is known as phenotypic integration, has been observed in many biological systems, including bone. Phenotypic integration is a property of bone that is critically important for establishing a mechanically functional structure that is capable of supporting the forces imparted during daily activities. In this paper, bone is reviewed as a system and primarily in the context of functionality. A better understanding of the system properties of bone will lead to novel targets for future genetic analyses and the identification of genes that are directly responsible for regulating bone strength. This systems analysis has the added benefit of leaving a trail of valuable information about how the skeletal system works. This information will provide novel approaches to assessing skeletal health during growth and aging and for developing novel treatment strategies to reduce the morbidity and mortality associated with fragility fractures.

Fracture risk is a heritable condition. Because fractures are associated with substantial morbidity, mortality, and cost [92], there is currently a large effort to identify genes leading to increased fracture susceptibility. The vast majority of these studies aim to map genes that influence skeletal traits and that contribute to overall skeletal strength. Except for rare genetic mutations, the range of genetic variants in the general population do not alter the functionality of bones under normal daily activities. That is, individuals with small, slender bones relative to their body size tend to function as effectively as individuals with wide, robust bones. It is only under extreme load conditions that it becomes apparent which heritable characteristics may be problematic. A primary challenge for genetic analyses is that the biological basis of fracture risk is extremely heterogeneous (Figure 1). There are many different biological pathways that can lead to a “fragile” bone, where fragility refers to the propensity to fracture. The various pathways altering bone function leads to a funnel-effect, such that multiple biological, genetic, and/or environmental variants can lead to a similar outcome. This biological heterogeneity complicates the search for fracture susceptibility genes, because the genes leading to fracture risk will be different for each person. To reduce fracture risk requires diagnostic measures that can differentiate between these pathways. This is necessary to develop novel treatment strategies that are based on the biological needs of the individual rather than that of the general population.

Despite the biological heterogeneity, the factor that is common to all biological pathways is that mechanical functionality is altered. To understand how functionality is altered will require a broader understanding of bone as a system. Thus, the genes that regulate the biological processes involved in establishing function may have greater value than the genes regulating individual traits. This review is written from the perspective of a bio-engineer and focuses on how bone as a system is able to buffer genetic variants to establish function and how this process may contribute to fracture susceptibility. The guiding principle is that

fracture susceptibility genes will be identified by understanding how genetic variants compromise function. This requires a better understanding of how the skeletal system adjusts its structure and tissue-quality to match overall stiffness and strength with loading demands. The goal of this review is to focus on what is currently known about the relationship between genetic variation and phenotypic variation as it pertains to bone functionality.

Bone Structure

There are approximately 206 bones in the adult skeleton, and each varies widely in size and shape. The overall size and shape of a bone and the distribution of these tissue types are critically important for providing mechanical function. Most bones fall into discrete morphological categories such as the long bones (e.g., femur, tibia), short bones (e.g., the carpal bones of the hand), flat bones (e.g., skull), and irregular bones (e.g., vertebrae). Bones are comprised of two types of skeletal tissues and these are called cortical bone and trabecular bone (Figure 2). Cortical bone, also known as compact bone, is the hard, dense tissue that makes up the shaft (diaphysis) of all long bones and the outer layer of nearly all short, flat, and irregular bones. Trabecular bone, also known as cancellous bone, exhibits a spongy appearance and is found near the ends of all long bones, as well as the central region of bones like the vertebral body. The cells responsible for constructing and maintaining bone include the osteoblast (“builder” cells), the osteoclast (“killer” cells), and the osteocyte (“sensor” cells). The physiological roles of each cell type and the manner in which they communicate among each other and with other systems have been reviewed extensively [30,38,131].

Heritability

Genetic background plays a critical role in the susceptibility to osteoporotic and fragility fractures. Prior work has shown that fracture incidence [45] and peak adult bone mass [32,64,106,118,124,139] are heritable traits. Estimates of the narrow sense heritability, h^2, for bone mineral density (BMD) generally exceed 50%, depending on the bone site examined and the method of assessment. Heritability varies with age for most skeletal traits [128], and BMD shows a maximum heritability value of 84% at approximately 26 years of age [55]. Heritability values range from 25–48% for diaphyseal width [123], to 54% for the width of the iliac crest [19,58], to 50–68% for calcaneal ultrasound attenuation [29,46], to 60–90% for anthropometric measures such as body height, standing/sitting height, arm span, chest circumference [19]. Even after adjusting for age, height, and weight, measures of the cross-sectional size of the metacarpal, spine, and hip show heritabilities of 58%–62% [87,88]. This indicates that genetic factors affect the transverse growth of a bone differently than longitudinal growth. Serum markers that are representative of the biological activity of the two primary bone cells (osteoblasts and osteoclasts) also show large heritabilities [67], indicating that the biological controls of bone formation and resorption are under genetic control. The effects of genetic factors are present during infancy [20] and prior to puberty [43], indicating that genetic susceptibility to a condition like osteoporosis may be determined early in life. Genetic factors also regulate the relationship among traits. Seeman et al [119] showed that genetic factors account for 50% of the covariance between lean mass and femoral neck BMD.

Skeletal Functionality

Bones serve many physiological functions. In addition to functioning as a reservoir for minerals and hematopoietic cells, bones also function to support the body, to protect vital organs, and to facilitate movement via attachments with tendons, ligaments, and muscles [27]. These latter functions depend critically on the mechanical stiffness and strength of

bone. Bones must be sufficiently stiff (i.e., resistant to deformation) and strong (i.e., resistant to failure) to support the loads associated with weight bearing and joint movement. For a review of basic biomechanical terms, please refer to the paper by Turner and Burr [135]. To be functional under daily load conditions, bones must be capable of withstanding enormous forces, sometimes on the order of 2–3 times body weight [31]. Bones must also be fatigue resistant, given that the average individual takes approximately 1–3 million steps per year [133]. Finally, bone is expected to support these forces using minimum mass [28], because bone tissue is metabolically expensive to maintain and excessive tissue is energetically demanding during movement.

In basic engineering terms, bone stiffness and strength are determined from the morphological traits and the tissue-quality traits (Figure 3). The morphological traits include measures of the amount of bone (size) and the distribution (shape) of bone tissue in space. Both measures are necessary because torsional and bending loads result in a non-uniform load pattern, such that the load is supported primarily by the tissue located furthest from the geometric centroid of a region of interest. Small increases in bone diameter translate to large increases in bone stiffness and strength. Tissue-quality traits refer to the tissue-level mechanical properties. Bone microstructure exhibits a field of cylindrical structures called osteons (Figure 4), whereas trabecular bone shows packets of lamellar-structured tissue. Both are comprised of similar constituents such as collagen, mineral, proteoglycans, and water. Tissue-level mechanical properties vary greatly across bones having very different functions [26], indicating that bone cells can modulate material properties to achieve a particular global function [104]. The biological processes that regulate the degree of mineralization relative to function are not entirely understood.

Reductionism: Genetic regulation of single traits

The majority of genetic analyses in bone have been conducted with the goal of mapping QTLs to identify genes that can be used to reduce fracture susceptibility. There are many approaches available to identify genetic variants, and the details of these approaches have been extensively reviewed in the literature [53,56,100,111,149]. The motivation for most studies and the selection of traits for analysis have largely been based on identifying genetic variants that affect bone mass and size. Over 350 QTLs have been mapped for bone traits, and several genes have been identified regulating bone mass such as LRP5 [86], Alox15 [72], and Darc [40].

Bone Mineral Density

The vast majority of studies have used bone mineral density (BMD) as the phenotypic trait in genetic studies. BMD is typically measured using dual-energy x-ray absorptiometry (DXA) and has been associated with fracture risk in many clinical studies [95]. Further, many organizations (e.g., World Health Organization, National Osteoporosis Foundation) promote the use of BMD as a clinical measure of bone health. BMD, like fracture strength, is a composite index that depends on the size, shape, and composition of the bone, as these later determinants define the attenuation of radiation used to measure bone mineral content.

Beamer and colleagues [5] reported that BMD varied significantly among inbred mouse strains. Although BMD was already known to be a heritable trait [3], this study made it possible to use a mouse model to rapidly map trait-causing genes. Since then, a large number of genetic analyses have been conducted to identify QTLs regulating BMD [7,8,11,13,34,61,73,74,78,85,90,97,103,137,153]. To date, a QTL regulating BMD has been identified on all 19 autosomes and the X-chromosome in the mouse genome (Figure 5a). In most studies, the QTLs account for ~35% of the population variance for femoral BMD and ~24% of the population variance for vertebral BMD [8]. QTLs regulating BMD vary with

skeletal site [8] and with gender [39,103]. QTLs show positive and negative effects on BMD values, and are generally additive in nature [8]. BMD QTLs often co-localize with QTLs regulating body weight, lean weight, and fat mass [61], whereas in some studies [97] BMD was found to be independently regulated. Beamer et al [6] used nested congenic sub-lines and revealed that a major BMD QTL consisted of 3 nested QTLs, each with distinct effects on BMD.

Morphology

Reducing the complexity of bone traits like BMD into a series of constitutive traits is important, because variation in complex traits like BMD and fracture strength can arise in very different ways [137]. Like BMD, morphological traits are also quite complex and cumulative results of studies mapping QTLs regulating bone morphology have identified QTLs on nearly all mouse chromosomes [13,14,33,34,68,74,78,80,110,122,137,148,152,153] (Figure 5b). Klein et al [74] measured total cross-sectional bone area and corrected this for body weight in a cohort of F_2 and Recombinant Inbred mouse strains derived from C57BL/6J (B6) and DBA/2J (DBA). This normalization provided a measure of bone size relative to body weight, which could be considered roughly equivalent to a measure of bone slenderness. They identified 4 chromosomes present in both genders and 1 exclusive for males and 2 exclusive for females. Drake et al [33] conducted a genetic analysis using an F_2 intercross between B6 and DBA, and showed that QTLs regulating bone length are different from those regulating bone width, suggesting that the genetic controls of longitudinal growth (an endochrondral process) are distinct from the genetic regulation of lateral growth (an appositional process). A few studies mapped QTLs regulating trabecular bone traits. Bower et al [14] identified 4 QTLs regulating various aspects of trabecular architecture of the proximal tibia.

Mechanical Properties

Mechanical properties of bone are determined most accurately from *ex vivo* tests. These tests are destructive, and they typically quantify the mechanical behavior of a whole bone under axial, torsional, or bending loads [17]. Whole bone mechanical properties like stiffness, strength, ductility, and toughness represent the summed effects of all underlying traits. Because mechanical function depends on morphological traits and tissue-level mechanical properties, it is not surprising to find that the QTLs for bone mechanical behavior tend to co-localize with the QTLs for bone morphology and tissue-composition [78,80,85,147,152] (Figure 5c). Yershov et al conducted a genetic analysis of humeri from HcB/Dem Recombinant Congenic (RC) strains and found that QTLs regulating whole bone mechanical properties co-localized with a QTL on chromosome 1 regulating bone morphology and a QTL on chromosome 10 regulating matrix mineralization. Although this co-localization may be evidence of pleitropy or closely linked loci, this co-localization may also be explained by structure-function relationships. Li et al [85] mapped QTLs associated with whole bone failure load in young F_2 mice derived from MRL/MpJ x SJL/J mice. They identified 6 chromosomes associated with failure load, and these QTLs explained 23% of the trait variance among the F_2 population, three of these were concordant with BMD. Kollar et al [78] examined female F_2 mice from B6 and C3H and used principal components analysis (PCA) to test for pleitropic effects regarding whole bone mechanical properties and bone morphology. QTLs regulating tissue-level stiffness have been identified on mouse chromosomes 3, 8, 12, and 13, indicating that there are genes that directly regulate the composition and organization of the extracellular matrix [63].

Responsiveness to Mechanical Loading

Bone is known to respond to changes in its loading environment by increasing or decreasing the amount of tissue [140]. Analysis of inbred mouse strains showed that this responsiveness

varies with genetic background [1,65], suggesting that the adaptive process of bone is genetically controlled. Robling et al [110] tested the hypothesis that genes controlling bone size exert their effect by influencing this adaptive response. They compared the response of a congenic mouse strain derived from B6 mice carrying a portion of the C3H genome shown previously to be a QTL regulating volumetric BMD [8] and subsequently shown to involve an increase in external diameter of the femoral diaphysis [137]. Kesavan et al [69] applied bending loads to the tibiae of 10-week old female F_2 mice derived from an intercross between C3H (poor adaptor) and B6 (good adaptor). RNA expression levels of bone sialoprotein (BSP) and alkaline phosphatase (AP), two markers representing an anabolic response, showed heritabilities of 87% and 91% respectively. They mapped QTLs regulating the fold change in the gene expression levels of BSP and AP. The basal level of BSP and AP in the non-loaded control tibiae mapped to mouse chromosomes 4, 18 and 4, 10, 16, 18, respectively, showing overlap in QTLs regulating the adaptive response of bone under loaded and non-loaded conditions.

Integration of Morphological Traits: The genetic basis of bone size and shape

QTLs regulating variability in individual traits has been well studied, and it is clear from these studies that each trait is influenced by multiple genes. However, because bone is incredibly adaptive, not all genetically varying traits result in altered skeletal function [60]. Thus, it will be difficult to identify fracture susceptibility genes by searching for QTLs regulating individual bone traits. One approach to resolve this issue is to better understand how a complex system like bone deals with genetic variants affecting size and mass. This will lead to the identification of genetic variants that alter the ability of the system to establish function.

Studies following the guiding principles of morphological integration [102] have provided insight into the genetic architecture that establishes function in the skeletal system. These studies have largely been conducted in the context of evolutionary changes in the morphology of the cranium, and in particular the mouse mandible. The anatomical relationship among landmark positions that determine the shape of the mandible is critical for mastication. Morphological integration refers to the interdependence among morphological elements that give rise to a functional organism. The interdependence among traits can arise within an individual because morphological elements share a common function during development (functional integration) or are subject to a common external stimulus (developmental integration). This interdependence results in significant correlations among many adult skeletal traits. The stimulus integrating multiple traits during development may be a common muscle force or a hormonal signal (e.g., growth hormone) that regulates cellular activity [22]. Thus, the degree to which traits are co-inherited reflects the degree to which those traits function and develop together [41].

Examination of the functionality of a complex structure like the mandible provided important insight into the genetic architecture contributing to variation in a complex skeletal structure. The systematic examination of the mouse mandible showed that genetic regulation of this complex structure may be in the form of modules. The mandible was decomposed into the alveolar region, which supports the tooth roots, and the ascending ramus, which supports the muscles involved in mastication. These modules correspond to functional or developmental sub-units within the structure. Bailey [4] quantified the relative distances between specific anatomical landmarks on the mandible and examined the variation in these distances among a panel of Recombinant Inbred mouse strains derived from C57BL/6By and BALB/cBy inbred mouse strains. Variation in the correlation coefficients among the landmark distances were consistent with developmental events, suggesting that landmark

distances which tend to correlate after segregation of genomic regions may have a developmental origin. A regression analysis showed that correlations were higher for landmarks that tended to be in close proximity to each other, indicating that genes tend to affect small localized regions rather than expansive regions. This suggested that localized genetic control may be necessary to obtain a functionally adapted structure.

Cheverud [21] examined craniofacial traits of the rhesus macaque. The results showed that morphological integration (correlation) was greater among functionally and developmentally related traits compared to unrelated traits. Traits that tended to develop together, or were subjected to similar functional stimuli, were found to correlate significantly in the adult structure. In a later study, Cheverud et al [22] found that of the QTLs exhibiting pleitropic effects, 50% were associated with the ascending ramus only, 27% were associated with the alveolus only, and 23% affected the entire mandible. These studies suggested that morphological integration may arise from pleitropy within each phenotypic module. An analysis of the fluctuating asymmetry within the mouse mandible also showed that the mandible can be viewed as a modular structure, and that this modularity results from direct developmental interactions [76]. Cheverud et al [23] identified 23 QTLs that regulate the relationship between specific mandibular traits relative to overall mandible length. Approximately 30–40% of the relationship QTLs overlapped with QTLs identified for individual traits or mandible length, indicating that 60–70% of the identified relationship QTLs may involve differential epistasis. The non-random distribution of QTL effects was also observe by Mezey et al [93]. Pleitropy in the mouse mandible appeared to be localized to sites having developmental and/or functional relationships, indicating that modularity of gene effects is important for establishing functionality in a complex structure.

Modularity was confirmed in a similar study that used Procrustes analysis to assess genetic variation in the spatial location of anatomical landmarks for the mouse mandible [75]. This analysis provided a way to identify QTLs on a landmark-by-landmark basis. They found that shape variation tended to be small in scale and that shifts in landmarks were often accompanied by opposite shifts in neighboring landmarks. The overall effect of these subtle, compensatory shifts was that there was no major change in overall shape of the mandible. This analysis showed that shape variation results from the effect of loci having distinct effects in localized regions, and not from QTLs having a more homogeneous effect. Further, these studies indicated that compensatory changes occurred within the structure, suggesting that compensation may be critical for establishing function in a complex system like the mouse mandible.

Genetic Regulation of the Relationship Among Traits

Because complex structures are a conglomeration of multiple traits, it is important to understand how the relationship among traits is regulated. Relationship QTLs have been identified for the mouse mandible by studying allometry, which is a measure of the relationship between local measures of structure relative to overall bone length. An analysis of F_2 mice revealed 23 relationship QTLs on 13 chromosomes for the mouse mandible [23]. Only 30% of these mapped to the same location as a specific trait QTL, and 40% mapped to mandibular length QTLs. This analysis showed how local length measures varied as a function of overall mandible length as well as genotype.

Li et al [84] used structural equation modeling (SEM) to understand the interactions among QTLs and how genetic variants contributed to the relationship between body weight and bone geometry. They used a genetically randomized experimental approach by taking advantage of meiosis to create a cohort of mice with natural, non-pathological genetic variation. They used an iterative procedure to identify a Path Model that best fit the data and

found that the relationship between bone length and transverse bone size is mediated primarily through accompanying changes in body weight. They identified QTLs influencing bone size directly, as well as QTLs influencing bone size indirectly through body size. This indicated that longitudinal growth and transverse bone expansion are regulated by different sets of genes. This study provided a valuable systems approach to understand the nature of pleitropic effects affecting the relationship among body weight and bone size. Thus, allelic variation affects multiple bone traits and this impact is mediated through multiple physiological pathways.

Drake et al [34] examined the genetic and metabolic determinants of bone mass in older female mice. They analyzed the co-localization of QTLs for various traits to test whether the presence of pleitropic loci accounts for phenotypic correlations among traits. A hierarchical cluster analysis of trait variation showed that bone mass, bone density, and cortical thickness formed a common cluster, whereas body weight clustered with adipose tissue measures and bone strength clustered, surprisingly, with several metabolic traits. They showed that increasing the number of traits analyzed simultaneously increased the power to detect coincident QTLs for metabolic and bone density traits, suggesting that these traits may be regulated by a common QTL (pleitropy) or a set of closely linked QTLs. Further studies showed that these pleitropic effects may be attributed in part to the gene encoding 5-lipoxygenase (5-LO; Alox5) [91].

Functional Adaptation During Development

The studies examining genetic variation in the context of morphological integration demonstrated that functionally related traits arise during development. Mechanical functionality is established during growth through a critical biological process known as functional adaptation. Functional adaptation, simply put, means that “input determines output.” Functional adaptation matches structure to function through a strain-based biological feedback system [47,113,136]. The physical forces engendered during daily activities induce tissue-level strains, which are the physical cues directing skeletal growth, the development of trabecular architecture, peak bone mass, cross-sectional shape, matrix architecture, and the anatomical relationships within and among skeletal elements [22,44,49,99,102,116,127,142]. Functional adaptation thus ensures that structures like long bones are sufficiently stiff to support loading demands and sufficiently strong to resistant fracturing [116]. Alterations in functional adaptation would be expected to lead to an under-designed (or over-designed) structure. The variation in bone size and shape observed across species [116], between populations [117,126], between bones[15,115], and between sexes [51,89] has largely been explained by the functional adaptation of various structures to their particular loading regimens. However, the inter-individual variation in bone size and shape within a single population has garnered little attention [59,154], despite these traits being significant determinants of fracture risk [9,10,25,36,50,52,129].

Studies of diaphyseal growth provided insight into the biological processes that are responsible for covarying traits to establish function. Because transverse growth of diaphyseal structures involves expansion plus cortical drift [42,105], the periosteal and endosteal surface expansions required to match cortical area with body weight [99,127] involves regulating the relative activity levels of osteoblastic and osteoclastic cell populations working on two surfaces [35,109]. Thus, the mechanism for biological regulation of phenotypic covariation resides at the cell-population level (Figure 6). The biological factor(s) controlling the relative activities of these cell populations during growth is not fully understood, but likely involves the interaction between growth factors and local physical forces [82]. Hormonal [71,138] and mechanical [15,16,51,57,99,144] factors are known to influence expansion of the periosteal and endosteal surfaces during growth

[12,62,83,127,141,142,145]. The biological mechanism that matches structure with function must therefore be capable of precisely coordinating the expansion of the periosteal and endosteal surfaces in order to match structure with body mass. Osteoblastic and osteoclastic activities have been shown to vary with genetic background [109,121], but there has been no study showing how genetic variation in the coordination of the relative activities of these cell populations affects function.

Functionally related traits are thought to result from a common biological control affecting growth [150]. IGF-I has been hypothesized to be involved in the functional adaptation of bone, but the mechanism by which this occurs is unclear [134]. IGF-I, which is produced primarily by the liver under growth hormone control, acts as a systemic hormone as well as an autocrine/paracrine growth factor, and may mediate the response of bone to mechanical loading [54]. Strong correlations between IGF-I levels and BMD [81F] and fracture risk [48] suggest that IGF-I plays an important role in bone strength. Prior work showed that circulating IGF-I levels correlated with bone mass and bone length [112] and are critical for periosteal expansion [151]. IGF-I increases both osteoblast [125] and osteoclast [96] differentiation, and appears to be a coupling agent for bone formation and resorption through regulation of the OPG/RANKL ratio [24,114]. This is important because transverse expansion of the femoral mid-diaphysis involves the activities of both osteoblasts and osteoclasts owing to cortical drift. Circulating IGF-I levels correlate with femoral cross-sectional area independent of bone length in mice [112] and in humans [98], suggesting that circulating IGF-I levels regulate the amount of bone relative to body weight.

Because mechanical forces are a critical stimulus for the development of bone size and shape, it is possible that skeletal variation may arise indirectly through genetic variants affecting muscle mass and activity levels, which are key determinants of the loads applied to bone. These relationships were examined by Sharkey and Lang [120] using a panel of F_2 mice derived from an intercross between C57BL/6 and DBA/2. They used structural equation modeling, and identified several QTLs having direct and indirect effects on bone traits. Because bone is mechanically responsive during growth, the QTLs having direct effects on bone may represent genes that alter the responsiveness of bone to its external loading demands. In contrast, QTLs that influence bone indirectly (with no direct effects on bone) may represent genes that do not alter the responsiveness of bone but alter the stimulus imposed on the system during ontogeny. These studies show that variation in bone size can arise from multiple genetic pathways.

Phenotypic Integration: The genetic basis of mechanical functionality and fragility

Prior work examining the interaction among morphological traits has generally been conducted in the context of the evolution of changes in skeletal shape. These studies did not consider integration in the context of skeletal fragility, nor did these studies consider the interaction between tissue-quality and bone morphology. Phenotypic integration refers to the interactions among traits that contribute to overall functionality of an organ or organism [107]. Bone is an example of a robust system in which phenotypic integration provides multiple ways for bone to be sufficiently stiff and strong to support the physical forces associated with daily activities [108]. Recent studies examining how the skeletal system dealt with genetic variations affecting bone slenderness revealed new insight into phenotypic integration operating in mouse [59] and human long bones [132]. Slenderness (Figure 7), which is a measure of bone width relative to length, is a particularly important trait variation that threatens functionality, because under physiological bending and torsional loads, small reductions in bone width lead to large reductions in stiffness and strength [143]. Consequently, genetic or environmental variants that impair transverse bone expansion

during growth and that lead to a slender adult phenotype significantly threaten the ability of biological processes to match structure with function. This is an important variation to study because bone slenderness is a highly heritable trait [66,77,130] and is a critical determinant of fracture risk throughout life [2,9,18,25,35–37,50,52,70,79,94,129,146].

Functional interactions among traits were examined using a panel of AXB/BXA Recombinant Inbred (RI) Mouse Strains [59]. RI mouse strains have a unique pattern of genetic randomization between their parental strains (A/J, C57BL/6J) which creates subtle, non-pathological trait variation that can be used to measure the tendency of different traits to co-segregate or correlate [101]. Depending on the set of A/J and B6 genes that were inherited, the RI strains are expected to build mechanically functional bones in slightly differently ways (Figure 8). The expectation is that as one bone trait varies in each RI strain, other traits will covary in a particular way to achieve organ-level functionality. Functionality was described in terms of how well bone stiffness matched loading demands, which was assumed to be proportional to body weight. A Path Analysis revealed that genetic variation in transverse bone growth relative to longitudinal growth was functionally related to cortical thickness and matrix mineralization. Thus, slender bones were compensated by increases in cortical thickness and matrix mineralization. However, this occurred at the expense of increased brittleness, as the increased mineralization correlated with reduced tissue-ductility. At the other extreme, wide (robust) long bones were compensated by thin cortices and lower mineralization. This compensation avoided excessive bulk by keeping mass to a minimum. This analysis indicated that the amount of tissue used to establish mechanical functionality was highly regulated or constrained [28,47]. Thus, compensatory interactions among external size, cortical thickness, and matrix mineralization were critical for establishing mechanical function while using minimum mass. Thus, the increased fragility of slender femora may not be a result of reduced bone size and mass as originally thought [9,94], but a result of accompanying changes in tissue-quality. This analysis revealed that phenotypic integration was critical for establishing function in the skeletal system, and that phenotypic integration may also contribute to fracture susceptibility by giving rise to genotype-specific trait sets that are more damageable and brittle (Figure 9).

Summary

This review shows that variation in skeletal traits can arise from multiple QTLs. Given the complexity of the biological processes in bone, gene variants affecting one trait do not necessarily mean that function will also be altered. This is because bone is highly adaptive and is able to compensate for many genetic variants. Learning how bone establishes and maintains function when faced with a wide range of genetic variants will lead to a better understanding of how bone functions as a system and how genetic variants compromise this functionality. This area of research will lead to a new understanding of the biological processes that are responsible for matching skeletal stiffness and strength with loading demands. Thus, genes involved in this process will likely play a direct role in establishing skeletal strength. This systems biology approach will provide new targets for genetic analyses and the identification of genes that are directly involved in regulating skeletal strength and mechanical fitness. Further, these studies provide a way to properly evaluate the effects of engineered mutations on skeletal function, as the outcome provides a way to evaluate how genetic variants affect multiple traits simultaneously. This area of research will benefit efforts to better understand skeletal health. Understanding how the system co-adapts traits to maintain function provides a novel way to evaluate whether a skeletal growth pattern will lead to a structure with stiffness that is properly matched to loading demands. This also has the benefit of providing a way to simultaneously evaluate whether there is an underlying biological defect and to be able to specify this defect accurately. Importantly, this systems approach makes no assumption about the underlying biology and thus may provide

a way to improve diagnosis by identifying at-risk individuals earlier in life, to identify novel biological pathways that can be used to treat individuals earlier and more effectively, and to individualize medicine by treating the individual based on their biological needs rather than that of the general population.

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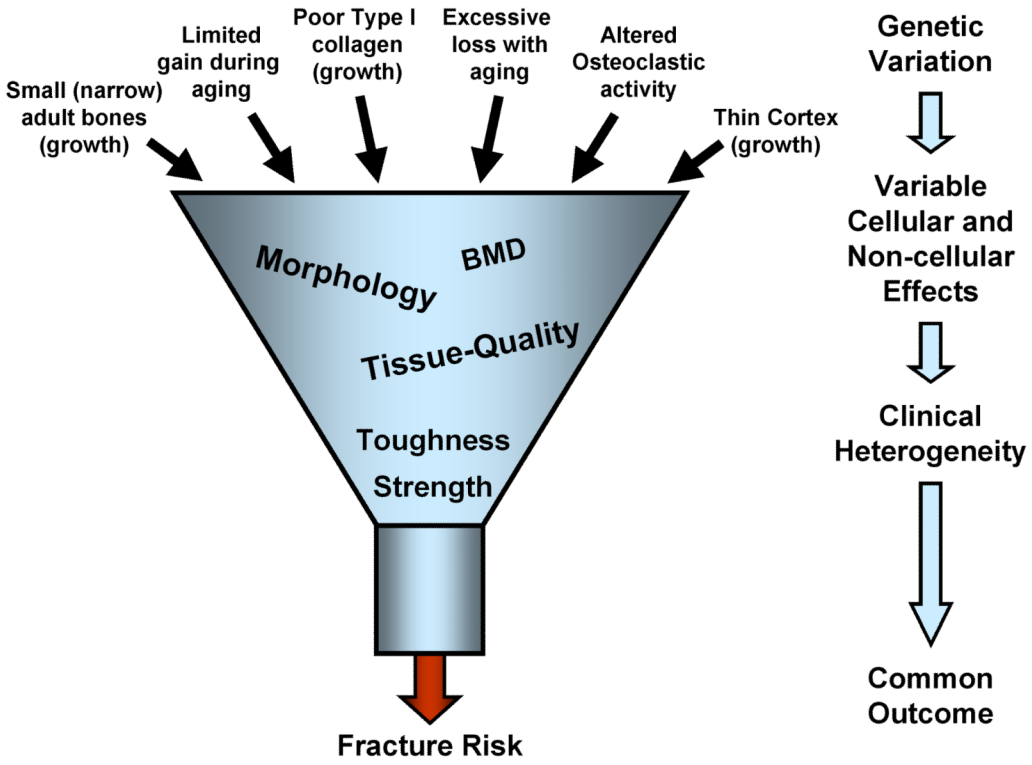

Figure 1.
The "Funnel Effect" represents the large number of biological and genetic pathways that lead to a skeletal structure that is more susceptible to fracturing. These and other biological pathways present tremendous heterogeneity in the underlying genetic basis of fragility.

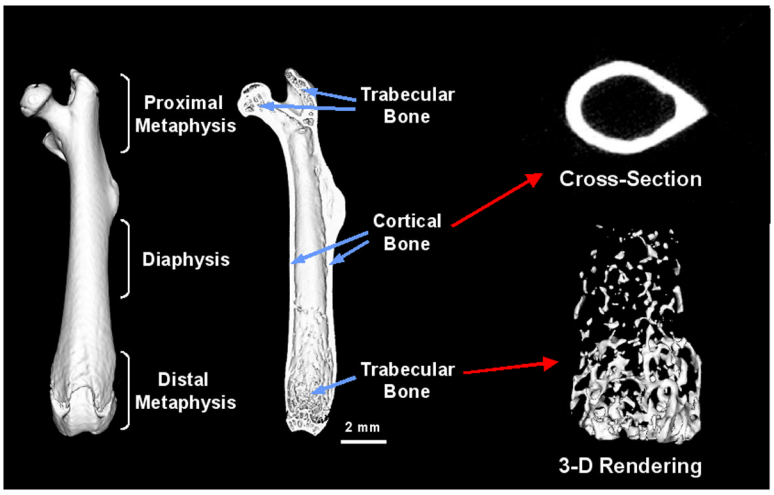

Figure 2.
A 3-dimensional tomographic rendering of a mouse femur reveals the complexity of bone structure. Each bone is comprised of a hard dense outer shell called cortical bone and a spongy-like material called trabecular bone.

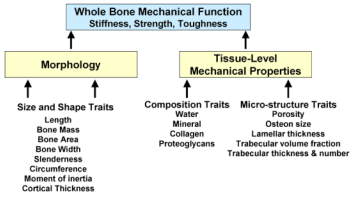

Figure 3.

Whole bone mechanical function is defined by morphological traits and tissue-level mechanical properties. Each trait category can be further reduced to a large number of traits that can be readily measured for genetic analyses. The traits listed are representative of a larger list of possible traits.

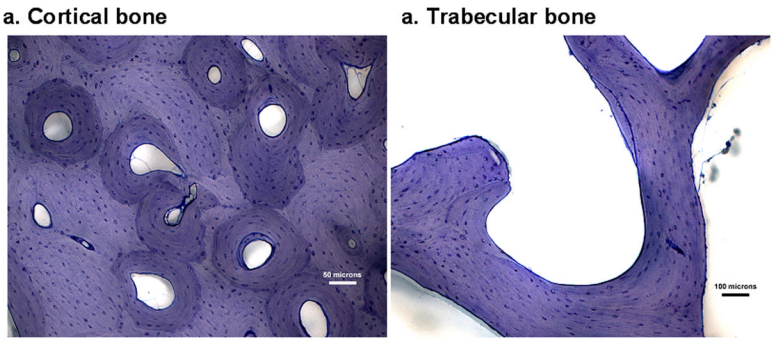

Figure 4.

Bone tissue exhibits a highly organized microstructure. a) Cortical bone is comprised of a field of osteons interspersed amidst interstitial tissue. b) Trabecular bone is comprised of packets of lamellar tissue.

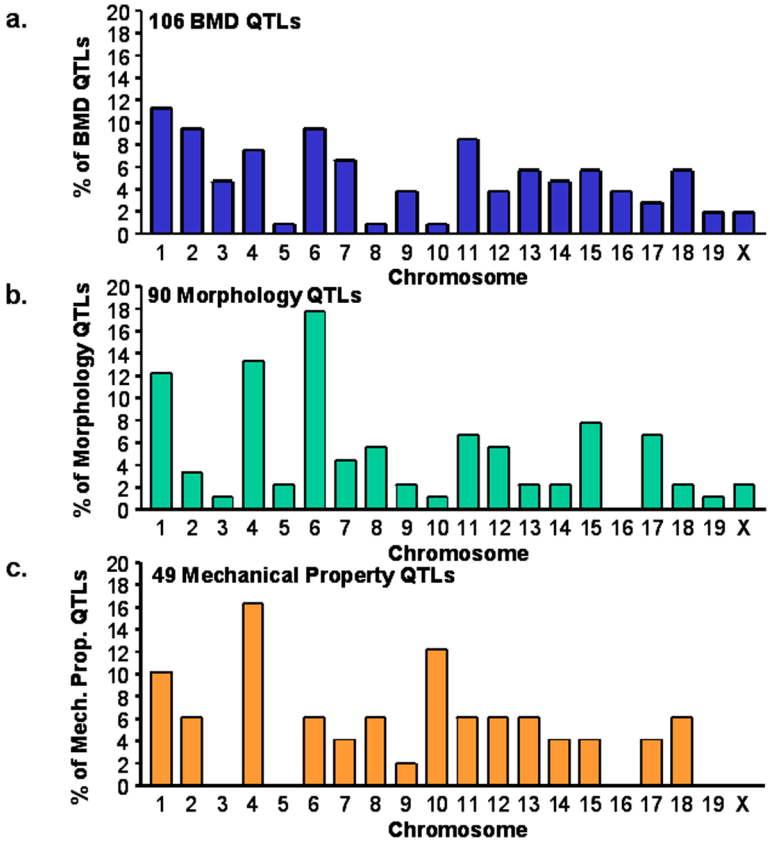

Figure 5.

A review of the literature revealed that over 350 QTLs have been identified for mouse bone. QTLs have been identified for many traits, including a) bone mineral density, b) bone morphology, and c) bone mechanical properties. The graph shows the fraction of identified QTL as a function of chromosomal location.

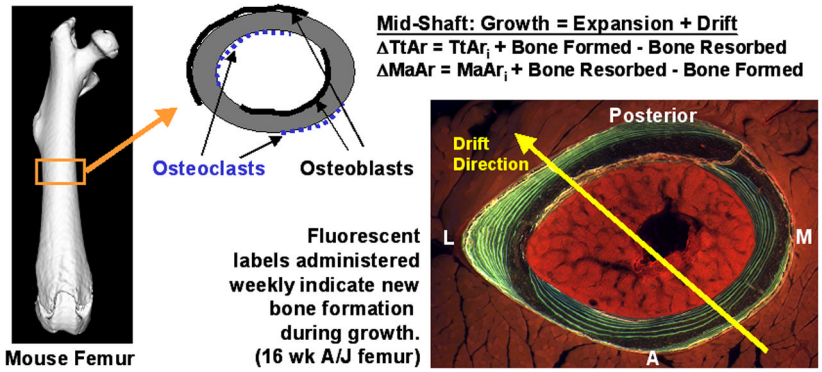

Figure 6.

Femoral mid-shafts grow by expanding and drifting simultaneously, as shown by the fluorescent labels in the histological cross-section. Expansion of each surface involves a mathematical relationship between bone formed and bone resorbed on the outer (sub-periosteal) and inner (endosteal) surfaces. Regulating periosteal expansion (ΔTtAr) and marrow expansion (ΔMaAr) so cortical area matches loading demands involves the coordination of osteoblastic and osteoclastic cell populations working on two surfaces.

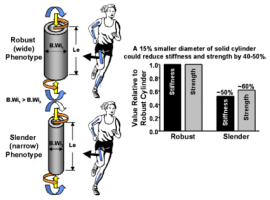

Figure 7.
Bone slenderness varies widely among individuals, independent of height, and could lead to significant reductions in bone stiffness and strength if not compensated.

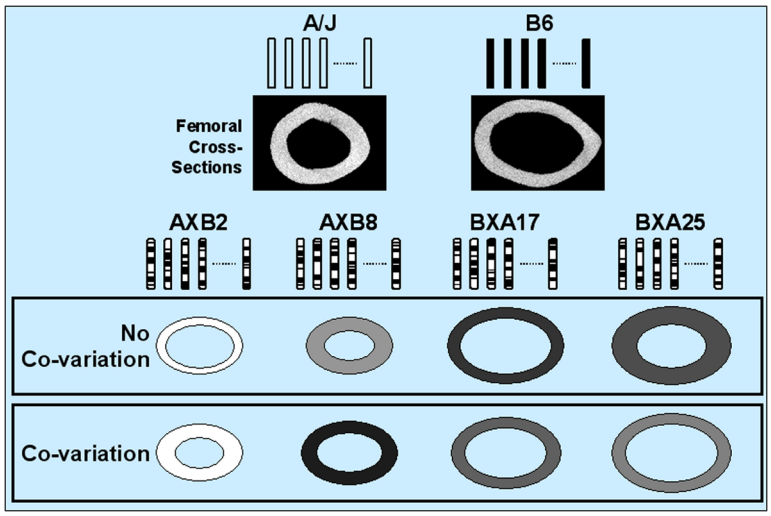

Figure 8.

Recombinant Inbred (RI) mouse strains derived from A/J and B6 inbred mouse strains can be used to study functional interactions among bone traits. If bone does not possess biological processes that covary traits then each member of the RI panel will exhibit a random set of traits. However, if bone possesses biological processes that covary traits in a highly ordered way then each member of the RI panel will exhibit a set of traits that is well adapted to weight-bearing loading demands. An examination of the traits across the panel will thus reveal the nature of this covariation.

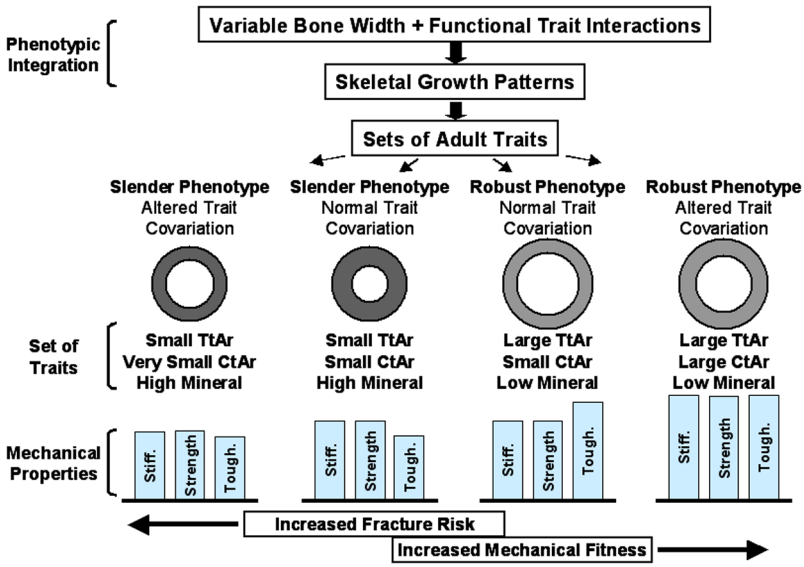

Figure 9.

Variation in phenotypic integration during growth may lead to different sets of adult traits that contribute to fracture risk in different ways: via reduced strength and/or reduced toughness. Risk will depend on bone size and the degree to which covariation was altered.