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REVIEW

Genetic regulation of bone strength: a review of animal model studies

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Population- and family-based studies have established that fragility fracture risk is heritable; yet, the genome-wide association studies published to date have only accounted for a small fraction of the known variation for fracture risk of either the femur or the lumbar spine. Much work has been carried out using animal models toward finding genetic loci that are associated with bone strength. Studies using animal models overcome some of the issues associated with using patient data, but caution is needed when interpreting the results. In this review, we examine the types of tests that have been used for forward genetics mapping in animal models to identify loci and/or genes that regulate bone strength and discuss the limitations of these test methods. In addition, we present a summary of the quantitative trait loci that have been mapped for bone strength in mice, rats and chickens. The majority of these loci co-map with loci for bone size and/or geometry and thus likely dictate strength via modulating bone size. Differences in bone matrix composition have been demonstrated when comparing inbred strains of mice, and these matrix differences may be associated with differences in bone strength. However, additional work is needed to identify loci that act on bone strength at the materials level.

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Introduction

It is now understood that hip, vertebral and wrist fracture risk are influenced by genetic factors.[1–4] Although fracture incidence can and has been used for heritability and forward genetic mapping studies,[3,5–9] data collection is restricted to retrospective measurements from clinical exams and medical records. Retrospective data often miss non-reported fractures and/or may miscategorize individuals who are phenotypically at risk but have not actually incurred a fracture.[8] Reduced skeletal strength has primarily been associated with loss of bone mass and concomitant changes in morphology. Extensive work has been carried out to establish the genetic underpinnings of bone mineral density (BMD) as a surrogate measure of strength, successfully identifying novel genes that have key roles in bone biology (reviewed in Hsu and Kiel).[6] Bone mass and geometry are phenotypes that can be easily, reliably and noninvasively measured in large populations of patients. This is a major reason for the success of genome-wide association studies (GWASs) for these phenotypes, as sufficient statistical power could be achieved and data were available for tens of thousands of genotyped individuals. However, the genetic correlations between BMD measurements in the spine and vertebral fracture incidence are modest. This suggests that studies in which genetic loci are mapped for surrogate phenotypes of strength[2] will not capture fracture incidence completely. As is true for any structure, bone strength is a function of the magnitude and architectural distribution of its mass, as well as the inherent compositional quality and resultant material strength of its constituent tissue matrix. Currently, measurement of these phenotypes requires invasive and typically destructive techniques, thus relegating their application to animal models, which allow collection of a more complete spectrum of genetic and phenotypic data. Given that osteoporotic fractures remain a substantial health burden in developed nations and are likely to increase in number with a proportionally aging population, there has been great interest in identifying the genes that regulate bone strength.

Animal Models Used for Genetic Studies of Bone Strength

Among model organisms, mice and rats are considered to be the most important for the study of human genetic skeletal diseases. The mouse genome, although 14% smaller than the

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human genome, is remarkably comparable at the nucleotide level. At the gene level, ~17 770 mouse genes have a known direct human ortholog (http://www.informatics.jax.org). Organizationally, the mouse and human genomes remain highly syntenic despite a quite long evolutionary distance between them.[10] Thus, genetic findings in mice are often concordant with genetic findings in humans[11] and have been used extensively in studies of the inheritance of bone mass and geometry.[11–17] Although larger than the mouse genome, the rat genome is still smaller than the human, but like the mouse the rat genome is highly orthologous to human. The majority of genes found in the human genome can be found in the rat without duplication or deletion.[18] Although many genetic mapping studies for bone phenotypes have been conducted using larger livestock species such as a sheep, cows and pigs, no direct measures of bone strength have been reported. Numerous studies have reported that, as in humans, fracture risk is highly heritable in horses,[19,20] but as for other large mammals no loci have been reported for direct measures of breaking strength.

Moving away from mammalian models, substantial work has been carried out examining the genetics of bone in chickens. Although this work was conducted in part for the benefit of the agricultural industry, there are sufficient similarities in basic bone physiology and anatomical patterning when comparing with mammals such that genetic findings from studies in poultry may be informative for interpreting mammalian genetics. At the genome level, there is homology between chickens and humans, and between chickens and rodents. Although only 2.5% of the chicken genome sequence could be directly aligned to the human genome, 75% of coding and between 30 and 40% of gene regulatory regions are conserved between species.[21] This degree of homology, although not perfect, may be sufficient to be informative for cross-species comparisons of genetic loci.

Types of Genetic Studies Conducted

Genetic studies can have two main starting points. In forward genetics studies one starts with a phenotype, with the goal of identifying the gene(s) and genetic polymorphisms that are responsible for that phenotype. In contrast, reverse genetics attempts to understand what phenotypes are affected by known polymorphisms in a gene already identified. In animal models, the historical workhorse of forward genetic screening has been the two-strain intercross. In short, two inbred strains are interbred once (resulting in F1 generation animals), and these F1 animals are either backcrossed to one of the original founder strains (N1F1 generation) or the F1 animals are bred together to make second-generation intercross animals (F2 generation). Then, either the N1F1 or the F2 animals (depending on study design) are phenotyped, genotyped at a reasonable marker density, and regions of the genome associated with the phenotype are identified. These so-called quantitative trait loci (QTL) harbor polymorphic differences when comparing the founder strains that are causative for some, or all, of the phenotypic differences between the two original strains.[22] There are a number of variations on this strategy, including the recombinant inbred lines (RI). Each RI population is a series of strains of mice descended from two or more progenitor strains but which has been bred to homozygousity.[23] Thus, unlike an F2 mouse, wherein each one is a genetic one in a million, the combination of alleles found in any one RI mouse is mirrored by all other RI mice of that same strain.

The drawback of these genetic mapping approaches has been that, although numerous genomic regions have been found that influence a phenotype, these regions are large and often contain hundreds of genes. Indeed, the mean QTL interval size for BMD, as mapped in mice, was found to be ~32cM.[11] One cM in genetic distance is roughly equivalent to 2 Mb in genomic distance for mice. At an average of 10 genes per Mb,[24] this equates to over 600 candidate genes, on average, per QTL.[25] As a result, the use of the two-strain, two-generation cross has started to fall out of favor for forward genetics studies. These two-strain populations are increasingly being replaced by studies using outbred populations of mice and rats. These include the use of commercially available outbred animals or 'designer' outbred populations, which are created by breeding two or more strains over the course of multiple generations in a manner that minimizes inbreeding.[26–29]

Additional study designs have been used to better define the genetic etiology of bone strength, including the use of congenic and consomic genetic reference populations.[23] A consomic, which is sometimes referred to as chromosome substitution, is a strain wherein all of the alleles for an entire chromosome from one inbred strain of mice or rats have been moved onto an otherwise pure background of a second strain. Similarly, a congenic is a strain with part of a chromosome moved from one genetic background to another. Both consomic and congenic strains are generated by selective breeding and thus take several years to generate.[30] However, the resulting animals allow confirmation or *de novo* establishment that genetic regions contain polymorphism(s) impacting phenotype and allow study of the biology associated with a QTL without necessarily knowing the causative gene.

Phenotypes of Bone Strength

The mechanical integrity of a whole bone, as for any force-bearing structure, is a product of geometrical size and shape, mechanical properties of its constituent material (that is, bone matrix) and the forces it must sustain. The most informative phenotypes describing bone strength are measurements of mechanical integrity derived from direct, destructive testing.[31] Surrogate measures obtained by imaging, such as dual X-ray absorptiometry or computed tomography, can provide only indirect measures of BMD, morphometry and size. Thus, the exercise of defining and measuring phenotypes describing the mechanical strength and integrity of whole bones (structural integrity) and constituent bone matrix (material integrity) inherently requires test methods of engineering mechanics. The regions of primary interest in skeletal phenotyping include anatomical sites comprising relatively large volumes of cancellous bone that are prone to osteoporotic fracture, such as the vertebral centrum, femoral neck and the distal radius. An engineering approach to measuring the mechanical integrity of these anatomical structures would include separate treatment of the geometry and constituent material integrity, the latter requiring careful preparation of uniform test specimens. As the bones from rodents are quite small, genetic studies have largely relied heavily on whole bone structural tests and predominantly on long bone cortical diaphyses subjected to flexural loading (bending).

Flexural tests of whole bones such as femur and tibia subject the central diaphysis to bending by resting a whole bone freely on end supports and subjecting the mid-span to force applied at one or two contact points (thus, a total of either 3 or 4 points of contact, providing the so-called 3-point or 4-point bending schemes).[32] This chosen loading scheme places the top span of bone length in compression and the bottom span in tension. Force is applied at a constant rate of deflection until failure, providing a force versus deflection relationship from which whole bone flexural stiffness is measured as the linear slope of the relationship (unit force per unit length of deflection). The maximum force attained is regarded as the flexural strength of the whole bone, and the integrated area under the force-displacement 'curve' is defined to be the whole bone fracture energy. Further subdivisions of mechanical behavior can be described as well, including the force and displacement at which whole bone behavior ceases to respond elastically and begins to 'yield' or deform plastically such that it will no longer retain its original shape. The force, displacement and energy might thus be parsed into that occurring during elastic versus 'post-yield' deformation. Importantly, the magnitudes of these mechanical test parameters depend on the size and shape of the diaphysis, the test configuration (for example, support span length and anatomical orientation of the specimen with respect to loading) and the inherent integrity of the constituent bone matrix as a material. As such, parameters derived from mechanical integrity tests of whole bones may reflect differences in size and shape or differences in constituent bone matrix integrity. Thus, a structural test performed in the absence of bone geometry measurement is insufficient to separate the effects of size and shape versus matrix integrity.

To estimate the material integrity of cortical bone matrix from the structural flexure test, accurate measurements of transverse (cross-sectional) geometry are required. Such geometrical measurements are most accurately and conveniently achieved with digital images acquired by X-ray computed tomography.[33] Notably, neither the cross-sectional area of the diaphysis nor the cortical thickness provides measures sufficient to estimate constituent bone matrix integrity from flexural tests. Mathematical descriptions from engineering mechanics beam theory provide all of the tools required for interpreting the test at the material scale, dictating that the cross-sectional second moment of area (colloquially referred to as moment of inertia) drives the mechanical behavior of flexure (or polar second moment of area for torsion). This measurement of cross-sectional size and shape provides a geometrical measure of diaphyseal stiffness and strength, which in combination with the structural test provides an approximation to a material test.

Many stipulations of beam flexure are violated by the cross-sectional asymmetry and non-constant size and shape throughout the length of the diaphysis, as well as its very small length:diameter ratio (which is $\sim$4:1 for rodent long bones but should be a minimum of 16:1 by ASTM D 790 standard.[34] As such, bone geometry is not captured by a single mid-diaphysis measurement, and whole bone stiffness measurement is compromised by shear forces imposed by the short length: diameter ratio. The mathematical reduction in the structural test loading configuration to mechanical stress (the concentration of force through a unit area) as an estimate of material strength is confounded by the lack of pure flexure due to the short length:diameter ratio. Moreover, calculation of mechanical strain and estimates of matrix material elastic modulus are greatly compromised by not considering the entire span of changing cross-sectional geometry.[35]

Summary of Genetic Mapping Studies

Studies in rats. In total, we identified four study populations in which bone strength was directly measured in a *de novo* cross between inbred strains for the purpose of conducting a forward genetic mapping study (**Table 1**), and these preliminary mapping studies have served as the foundation for more complex bioinformatics explorations.[36,37] As is highlighted in **Table 1**, 75 QTL for bone strength and geometry phenotypes were identified in these populations. It is immediately apparent that there is substantial co-mapping of loci for these various phenotypes, and, although true epistasis cannot be established from these data, for discussion they can be collapsed into $\sim$41 discrete loci. Of these 41, 23 represent loci to which only geometry and/or bone size phenotypes map (for example, total cross-sectional area, cortical area, width and polar moment of area), and 7 more co-map with one or more geometry phenotypes. On the basis of the known confounding issues for flexural bone breaking tests of long bones and femoral neck, it is reasonable to postulate that the majority of these 41 loci do not represent loci controlling bone matrix quality. Although they do represent a genetic region controlling whole bone strength, they do so by modulating the bone size, which is important for consideration when attempting to ascertain the biological processes modulated by these loci. This leaves 11 loci for which no geometry phenotype locus is reported as co-mapping. It is tempting to consider these loci as regulating bone matrix quality, but one must remain cognizant of the fact that this table lists only the reported QTL and makes no attempt toward identifying sub-significant loci for any phenotype. Likely, some of these 11 loci are impacting the bone matrix, but identifying such matrix loci may require refining test methods beyond those used in these studies.

At this stage, these studies represent loci, and loci represent statistical associations with a phenotype. Further, these loci are large and encompass too many genes at this point to name the causative gene for the most part. Toward this first point, Alam *et al.*[38] established that the locus on Chr 4 was indeed causative using congenic rats, and this has not been done for the other loci. In other species, using a series of nested congenic strains has been a successful method to narrow loci down to near single gene for other key bone phenotypes,[39] but this method is laborious and takes years to accomplish. For two of these loci, follow-up gene expression work was conducted. In these studies, expression of all genes within the loci was examined by microarray and genes demonstrating differential expression were examined for correlation with the phenotype(s) of interest.[36,37] A candidate gene must impact a phenotype by either altering the amount of a gene product or by altering its function. Thus, expression studies are an efficient tool for narrowing large loci to a testable number of candidate genes.

A plethora of additional bioinformatics techniques have been developed to narrow loci. The rat genome has now been sequenced for many of these strains (http://rgd.mcw.edu). These data can be used to narrow such large QTL to eliminate genes that are not polymorphic from the loci interval and may identify key genes harboring non-sense or mis-sense mutations

Table 1 Quantitative trait loci for size and strength mapped in rats

Chr	*Phenotype*[a]	*Bone*	*Peak or confidence interval (cM)*[b]	*Gender*[c]	*Strains*	*Reference*
1	Flexural strength	Tibia	17–42	Male and female	GKxF344	54
1	Cortical area	Tibia	10–59	Male and female	GKxF344	55
1	Polar 2nd moment of area	Tibia	10–59	Male and female	GKxF344	55
1	Flexural stiffness	Tibia	18–43	Male and female	GKxF344	54
1	Cortical area	Tibia	36–93	Male and female	GKxF344	55
1	Polar 2nd moment of area	Tibia	36–93	Male and female	GKxF344	55
1	Flexural strength	Femur	101.4	Female	F344xLEW	56
1	Cortical area	Tibia	95–124	Male and female	GKxF344	55
1	Polar 2nd moment area	Femur	110	Male and female	COPxDA	57
1	Total area	Femur	111	Male and female	COPxDA	57
1	Cortical area	Femur	112	Male and female	COPxDA	57
1	Neck width	Femur Neck	113	Male and female	COPxDA	57
1	Flexural strength	Femur	114	Male and female	COPxDA	57
1	Fracture energy	Femur	114	Male and female	COPxDA	57
1	Fracture force	Femur Neck	114	Male and female	COPxDA	58
1	Flexural stiffness	Femur	117	Male and female	COPxDA	57
2	Fracture	Femur	36	Male	COPxDA	58
	Force	Neck		Female		58
2	Fracture energy	Femur neck	88	Male and female	COPxDA	58
2	Flexural strength	Femur	102.2	Female	F344xLEW	56
2	Fracture energy	Femur	102.2	Female	F344xLEW	56
2	Polar 2nd moment of area	Femur	102.2	Female	F344xLEW	56
2	Cortical area	Femur	102.2	Female	F344xLEW	56
3	Cortical area	Tibia	9–42	Male and female	GKxF344	55
4	Polar 2nd moment of area	Femur neck	27.2–48.7	Female	F344xLEW	59
4	Fracture force	Femur neck	27.2–48.7	Female	F344xLEW	59
4	Fracture energy	Femur neck	27.2–48.7	Female	F344xLEW	59
4	Total area	Femur neck	27.2–48.7	Female	F344xLEW	59
4	Neck width	Femur neck	27.2–55.7	Female	F344xLEW	59
4	Polar 2nd moment of area	Femur	34–55	Male	PxNP	38
4	Flexural strength	Femur	34–55	Male	PxNP	38
4	Fracture force	Femur neck	34–55	Male	PxNP	38
4	Polar 2nd moment of area	Femur	57.7	Female	F344xLEW	56
4	Cortical area	Femur	57.7	Female	F344xLEW	58
4	Fracture force	Femur neck	87	Male and female	COPxDA	58
5	Cortical area	Femur neck	3	Male and female	COPxDA	58
5	Cortical area	Tibia	0–12	Male and Female	GKxF344	55
5	Flexural strength	Femur	68.3	Female	F344xLEW	56
5	Fracture energy	Femur	68.3	Female	F344xLEW	56
5	Flexural stiffness	Femur	68.3	Female	F344xLEW	56
5	Polar 2nd moment of area	Femur	68.3	Female	F344xLEW	56
5	Cortical area	Femur	68.3	Female	F344xLEW	56
5	Flexural strength	Femur	84	Male and female	COPxDA	57
6	Cortical area	Femur	16	Male and female	COPxDA	57
6	Polar 2nd moment of area	Femur	20	Male and female	COPxDA	57
6	Total area	Femur	20	Male and female	COPxDA	57
6	Bone area (longitudinal)	Tibia	30–60	Male	GKxF344	54
7	Polar 2nd moment of area	Femur	31	Female	F344xLEW	56
7	Total area	Femur	37	Male and female	COPxDA	57
7	Polar 2nd moment of area	Femur	40	Male and female	COPxDA	57
7	Total area	Femur neck	52	Male and female	COPxDA	58
7	Polar 2nd moment of area	Femur neck	53	Male and female	COPxDA	58
8	Bone area (longitudinal)	Tibia	43–59	Male and female	GKxF344	54
10	Fracture energy	Femur	8	Male and female	COPxDA	57
10	Cortical area	Tibia	5–18	Male and female	GKxF344	55
10	Polar 2nd moment of area	Tibia	5–18	Male and female	GKxF344	55
10	Total area	Femur neck	13	Male and female	COPxDA	58
10	Polar 2nd moment of area	Femur neck	43	Male and female	COPxDA	58
10	Compression strength	L5	58.5	Female	F344xLEW	56
10	Polar 2nd moment of area	Femur	73	Male and female	COPxDA	57
10	Total area	Femur	73	Male and female	COPxDA	57
12	Total area	Femur neck	43	Male and female	COPxDA	58
13	Polar 2nd moment of area	Femur	41	Male and female	COPxDA	57
13	Total area	Femur	41	Male and female	COPxDA	57
15	Flexural strength	Tibia	10–32	Male and female	GKxF344	54
15	Fracture energy	Tibia	25–37	Male and female	GKxF344	54
15	Cortical area	Femur	32	Male and female	COPxDA	57
15	Fracture force	Femur neck	36	Male and female	COPxDA	58
15	Fracture energy	Femur neck	36	Male and female	COPxDA	58
15	Cortical area	Tibia	55–69	Male and female	GKxF344	55
15	Polar 2nd moment of area	Femur	65.3	Female	F344xLEW	56
17	Cortical area	Femur neck	21	Male and female	COPxDA	58
18	Cortical area	Tibia	21–31	Male and female	GKxF344	55

Table 1 (Continued)

Chr	Phenotype[a]	Bone	Peak or confidence interval (cM)[b]	Gender[c]	Strains	Reference
18	Polar 2nd moment of area	Femur	29	Male and female	COPxDA	57
19	Compression strength	L5	5.2	Female	F344xLEW	56
X	Bone area (longitudinal)	Tibia	52–74	Male and female	GKxF344	54

[a]Mechanical integrity phenotypes were derived from long bone 3-point bending (flexure) tests or combined compression bending for the femoral neck. Mechanical phenotypes for flexure tests include whole bone flexural strength, stiffness and fracture energy. Phenotypes for femoral neck and vertebrae include fracture force and energy. Phenotypes describing long bone size and shape include bone width, cross-sectional total and cortical areas, and cross-sectional polar 2nd moment of area (the geometrical measure of bone strength and stiffness). No phenotypes are reported for bone matrix (material) mechanical integrity. [b]As provided in the literature. When more than one model was calculated, the peak for at the maximum LOD is provided. [c]Indicates gender of animals phenotyped. Not specificity of the locus.

that could be tested for causality (altered function). Using the assumption that a single gene would be responsible for a loci mapped by two different crosses to the same spot, one can combine the allele information for all strains to further eliminate genes from the interval.[40] Unfortunately, there is a paucity of co-mapping loci for strength wherein the same loci were found in multiple crosses and generating additional crosses could be beneficial. However, one needs to consider QTL from other species for the same or similar phenotypes that may have mapped to homologous regions. A wealth of information exists from mouse studies (see below). Combining species information has not been done for strength QTL but may be another successful method for narrowing these QTL. Covering the possible bioinformatics approaches that could be applied is beyond the scope of this review. The reviews by Peters *et al.*[23] and DiPetrillo *et al.*[40] provide details of these methods. Many of these techniques were originally developed for use with mouse QTL and could just as easily be applied to the QTL described in **Table 1**. Once one has found the gene(s) causative for a QTL, one needs to study the involvement of that gene in the phenotype(s) of interest. For mice, that means turning to transgenic models. This is now possible with rats[41,42] but is not a strategy that has been widely adopted.

Moving forward, populations such as the heterogeneous stock (HS) rat outbred population will likely replace the F2 model for forward genetics studies. The HS population is descended from eight genetically diverse founder strains: ACI/N, BN/SsN, BUF/N, F344/N, M520/N, MR/N, WKY/N and WN/N. Each HS rat represents a unique combination of alleles, analogous to each F2 intercross animal. As these rats have been bred for over for 50 generations, the density of visible genomic recombinations is higher than is possible with only two generations of interbreeding, leading to increased genetic mapping resolution, decreasing the number of candidate genes per loci and thereby increasing the likelihood of identifying the causative polymorphism(s).[43] Preliminary studies with this population show a high degree of heritability for key phenotypes such as flexural strength at the mid-diaphysis and femoral neck, yet a low degree of heritability for flexural fracture energy at mid-diaphysis.[44] Geometric phenotypes were not reported, precluding extrapolation of these findings toward making any inferences regarding bone matrix quality. Although loci for bone strength phenotypes have not yet been reported in the HS rat, QTL for bone mass have been, and the narrow confidence intervals of these QTL demonstrate the superior mapping resolution possible when using this population.[26] This highlights the superiority of the HS rat for any forward genetic mapping study.

Studies in mice. Genetic mapping studies in mice have followed similar strategies and approaches as for rats, in that whole bone strength was measured using similar methods (**Table 2**). Further, many more studies examining the genetic control of bone mass have been conducted in mice, but the review of these studies that lack a bone strength assessment as well is beyond the scope of this report. Thus, we are only reporting bone geometry phenotypes in **Table 2** for mouse populations in which whole bone strength QTL were mapped directly. This listing of 119 QTL can be binned conservatively into $\sim$50 QTL (based on peak locations provided by authors, assuming that QTL mapped 10 or more cM apart represent independent loci). As was observed for rats, in many instances, QTL for readouts of bone strength co-map with loci for bone size. Again, this demonstrates that many of these readouts for what are often attributed as capturing bone strength are in fact merely indirect measures of bone size. For example, the locus mapped to chromosome (Chr) 4 in mice, centered at $\sim$60 cM, is most likely a locus that affects bone size, leading to the mapping of whole bone strength to this genetic region. This hypothesis is supported by the phenotype of congenic mice carrying *c3h* alleles at this locus, on an otherwise C57BL/6J (B6) background. Female congenic mice have increased polar 2nd moment of inertia and concomitant increased flexural strength of the femur.[45]

Often body weight is used as a corrective factor in these studies, as is also done in the rat studies. Although generally there is a positive correlation between body weight and cortical cross-sectional area across growth within an inbred strain,[46] the relationship between cortical area and body weight breaks down when the alleles for body weight and cortical area begin to segregate independently (correlation coefficient = 0.521 (ref. 17)). It is unclear what bias this correction may introduce, as correlated traits are not necessarily caused by the same genes.[47] Indeed, this relationship between bone size and body size has been exploited in studies aimed at differentiating between bone robustness (total bone area/length), morphological compensation (cortical bone area/body weight) and the degree of bone mineralization (a surrogate for tissue quality). Using a combination of genetic mapping studies together with recombinant inbred lines and phenotyping of consomic mice, it was shown that these traits are independently inherited, demonstrating the complex genetic regulation of what is ultimately considered the strength of bone matrix.[13]

Given that bone strength is a function of size, shape and geometry, there is interest in determining the genetic control of bone quality and tissue level determinants of bone strength. The protein component of bone is the product of genes, and as such

Table 2 Quantitative trait loci for size and strength mapped in mice

Chr	*Phenotype*[a]	*Bone*	*Peak or confidence interval (cM)*[b]	*Gender*[c]	*Strains*	*References*
1	Flexural strength	Tibia	12–17	Female	B6xC3H	60
1	Flexural strength	Femur	20	Female	NZBxRF	17
1	Bone width	Femur	20	Female	NZBxRF	17
1	Yield stress	Femur	41	Male and female	HcB-8xHcB-23	16
1	Maximum stress	Femur	56	Male and female	HcB-8xHcB-23	16
1	2nd moment of area	Femur	66	Male and female	HcB-8xHcB-23	61
1	Post-yield strain	Femur	66	Male and female	HcB-8xHcB-23	16
1	Cortical area	Femur	67	Male and female	HcB-8xHcB-23	61
1	Flexural strength	Femur	103.8	Female	MRLxSJL	15
2	Maximum stress	Femur	36	Male and female	HcB-8xHcB-23	16
2	Cortical area	Femur	43	Male and female	HcB-8xHcB-23	61
2	Elastic modulus	Tibia	50–56	Female	B6xC3H	60
2	Flexural strength	Femur	54.6	Female	MRLxSJL	15
3	Fracture energy	Femur	5	Male and female	HcB-8xHcB-23	61
3	Flexural strength	Femur	11	Male and female	HcB-8xHcB-23	61
3	Flexural stiffness	Femur	11	Male and female	HcB-8xHcB-23	61
3	Cortical area	Femur	11	Male and female	HcB-8xHcB-23	61
3	Fracture energy	Femur	34	Male and female	HcB-8xHcB-23	61
3	Deflection at fracture	Femur	38	Male and female	HcB-8xHcB-23	61
3	Post-yield deflection	Femur	38	Male and female	HcB-8xHcB-23	61
3	Yield strain	Femur	38	Male and female	HcB-8xHcB-23	16
3	Maximum strain	Femur	38	Male and female	HcB-8xHcB-23	16
3	Yield force	Femur	47	Male and female	HcB-8xHcB-23	61
3	Yield stress	Femur	47	Male and female	HcB-8xHcB-23	16
3	Flexural strength	Femur	53	Male and female	HcB-8xHcB-23	61
3	Post-yield strain	Femur	73	Male and female	HcB-8xHcB-23	16
3	Flexural strength	Femur	85	Female	NZBxRF	17
4	Flexural strength	Tibia	17.9–79	Female	B6xC3H	60
4	Yield force	Tibia	48.5	Male and female	B6xDBA	14
4	Flexural strength	Tibia	48.5	Male and female	B6xDBA	14
4	Flexural strength	Femur	58	Female	B6xC3H	62
4	Polar 2nd moment of area	Femur	58	Female	B6xC3H	62
4	Fracture energy	Femur	58	Female	B6xC3H	62
4	Flexural stiffness	Femur	58	Female	B6xC3H	62
4	Flexural strength	Femur	61.9	Male and female	B6xDBA	14
4	Flexural strength	Femur	65	Female	NZBxRF	17
4	Cortical area	Femur	65	Female	NZBxRF	17
4	Yield force	Femur	66	Male and female	HcB-8xHcB-23	61
4	Flexural strength	Femur	66	Male and female	HcB-8xHcB-23	61
4	Flexural stiffness	Femur	66	Male and female	HcB-8xHcB-23	61
4	Cortical area	Femur	66	Male and female	HcB-8xHcB-23	61
4	2nd moment of area	Femur	66	Male and female	HcB-8xHcB-23	61
5	Flexural strength	Femur	30	Female	NZBxRF	17
5	Fracture energy	Femur	40	Female	NZBxRF	17
5	Bone width	Femur	45	Female	NZBxRF	17
5	Cortical area	Femur	50	Female	NZBxRF	17
5	Bone width	Femur	50	Female	NZBxRF	17
5	Flexural strength	Tibia	60–81	Female	B6xC3H	60
6	Flexural stiffness	Femur	7	Male and female	HcB-8xHcB-23	61
6	2nd moment of area	Femur	9	Male and female	HcB-8xHcB-23	61
6	Cortical area	Femur	10	Male and female	HcB-8xHcB-23	61
6	Maximum stress	Femur	10	Male and female	HcB-8xHcB-23	16
6	Fracture energy	Femur	11	Male and female	HcB-8xHcB-23	61
6	Flexural strength	Femur	12	Male and female	HcB-8xHcB-23	61
6	Bone width	Femur	30	Female	NZBxRF	17
6	Maximum stress	Femur	32	Male and female	HcB-8xHcB-23	16
6	Flexural strength	Tibia	51.5–74	Female	B6xC3H	60
7	Flexural stiffness	Femur	11	Male and female	B6xDBA	14
7	Yield force	Femur	25	Male and female	B6xDBA	14
7	Flexural strength	Femur	25	Male and female	B6xDBA	14
7	Flexural strength	Femur	30	Female	NZBxRF	17
7	Flexural strength	Tibia	50–65.4	Female	B6xC3H	60
7	Fracture energy	Femur	55	Female	NZBxRF	17
7	Cortical area	Femur	55	Female	NZBxRF	17
7	Bone width (ML dimension)	Femur	55	Female	NZBxRF	17
7	Bone width (AP dimension)	Femur	55	Female	NZBxRF	17
8	Flexural strength	Femur	15.7	Female	MRLxSJL	15
8	Flexural strength	Femur	64	Female	B6xC3H	62
8	Flexural stiffness	Femur	64	Female	B6xC3H	62
8	Polar 2nd moment of area	Femur	64	Female	B6xC3H	62
9	Flexural strength	Femur	40	Female	NZBxRF	17
9	Flexural strength	Femur	41.5	Female	MRLxSJL	15
9	Fracture energy	Femur	50	Female	NZBxRF	17

Table 2 (Continued)

Chr	*Phenotype*[a]	*Bone*	*Peak or confidence interval (cM)*[b]	*Gender*[c]	*Strains*	*References*
9	Bone width	Femur	50	Female	NZBxRF	17
9	Yield force	Tibia	53	Male and female	B6xDBA	14
9	Flexural strength	Tibia	53	Male and female	B6xDBA	14
9	Flexural strength	Tibia	50–71	Female	B6xC3H	60
10	Yield force	Femur	13	Male and female	HcB-8xHcB-23	61
10	Flexural strength	Femur	16	Female	B6xC3H	62
10	Fracture energy	Femur	16	Female	B6xC3H	62
10	Post-yield strain	Femur	17	Male and female	HcB-8xHcB-23	16
10	Flexural toughness	Femur	18	Male and female	HcB-8xHcB-23	16
10	Post-yield deflection	Femur	19	Male and female	HcB-8xHcB-23	61
10	Deflection at fracture	Femur	21	Male and female	HcB-8xHcB-23	61
10	Flexural strength	Femur	22	Male and female	HcB-8xHcB-23	61
10	Flexural stiffness	Femur	22	Male and female	HcB-8xHcB-23	61
10	Maximum strain	Femur	22	Male and female	HcB-8xHcB-23	16
10	Cortical area	Femur	24	Male and female	HcB-8xHcB-23	61
10	2nd moment of area	Femur	28	Male and female	HcB-8xHcB-23	61
10	Yield stress	Femur	28	Male and female	HcB-8xHcB-23	16
10	Maximum stress	Femur	37	Male and female	HcB-8xHcB-23	16
10	Elastic deflection	Femur	41	Male and female	HcB-8xHcB-23	61
10	Yield stress	Femur	42	Male and female	HcB-8xHcB-23	16
10	Flexural strength	Femur	50.3	Female	MRLxSJL	15
10	Bone width	Femur	60	Female	NZBxRF	17
11	Elastic modulus	Tibia	1.1–71	Female	B6xC3H	60
11	Fracture energy	Femur	60	Female	NZBxRF	17
11	Bone width (ML dimension)	Femur	60	Female	NZBxRF	17
11	Bone width (AP dimension)	Femur	65	Female	NZBxRF	17
11	Cortical area	Femur	75	Female	NZBxRF	17
12	Bone width	Femur	0	Female	NZBxRF	17
12	Flexural strength	Femur	5	Female	NZBxRF	17
12	Cortical area	Femur	5	Female	NZBxRF	17
12	Flexural stiffness	Femur	20	Female	NZBxRF	17
13	Flexural strength	Tibia	9–54	Female	B6xC3H	60
13	Fracture energy	Femur	56	Female	B6xC3H	62
13	Flexural strength	Femur	56	Female	B6xC3H	62
13	Max:Min 2nd moment of area	Femur	56	Female	B6xC3H	62
13	Flexural stiffness	Femur	56	Female	B6xC3H	62
14	Flexural stiffness	Femur	syntenic	Female	B6xC3H	62
14	Flexural strength	Femur	syntenic	Female	B6xC3H	62
15	Elastic modulus	Tibia	9.9–55.5	Female	B6xC3H	60
15	Flexural strength	Tibia	9.9–55.5	Female	B6xC3H	60
16	Flexural strength	Tibia	43–56	Female	B6xC3H	60
17	Flexural strength	Femur	6.6	Female	MRLxSJL	15
17	Flexural strength	Tibia	4.1–33.5	Female	B6xC3H	60
18	Flexural strength	Tibia	5–16	Female	B6xC3H	60
18	Cortical area	Femur	55	Female	NZBxRF	17
19	Elastic modulus	Tibia	26–55.7	Female	B6xC3H	60

[a]All mechanical integrity phenotypes were derived from long bone 3-point bending (flexure) tests. Mechanical phenotypes include whole bone flexural strength, stiffness and fracture energy. Phenotypes describing long bone size and shape include bone width, cross-sectional cortical area and cross-sectional 2nd moment of area (the geometrical measure of bone strength and stiffness). Phenotypes describing bone matrix (material) mechanical integrity combine the whole bone structural test with cross-sectional 2nd moment of area to estimate yield stress, maximum stress, post-yield strain and elastic modulus (matrix material stiffness). [b]As provided in the literature. When more than one model was calculated, the peak for at the maximum LOD is provided. [c]Indicates gender of animals phenotyped. Not specificity of the locus.

it stands to reason that there may be differences in bone matrix quality that are controlled at the level of genetic differences. This hypothesis is bolstered by the observation of interspecies differences in bone matrix composition. In a study by Courtland *et al*,[48] it was shown that the C57BL/6J, A/J and C3H/HeJ strains of mice have divergent bone matrix composition. To assess these differences from a strength point of view, this group milled a longitudinal section of cortical bone within the femur of a defined dimension and tested this section in tension to measure matrix strength directly. The authors concluded that A/J cortical bone matrix is more stiff and brittle than the other two strains. Similarly, Blank *et al.*[49] showed that there were differences in collagen cross-links and crystallinity when comparing two strains of recombinant congenic strains that also altered the biomechanical performance. Together, these studies highlight that the relationship between size, shape and material properties of bones is complex and that there is much work to be carried out to truly understand fracture susceptibility.

Studies in chickens. Although a number of QTL mapping studies have been conducted that report strength QTL using chicken bones, few of these studies actually conducted strength phenotyping. Rather, these studies examined bone mineral content and/or bone geometry as surrogate measures. Three studies reported direct measurements of bone strength in chickens. The first study crossed the domestic RJ and WL breeds of chickens and tested femurs in torsion. A single significant locus was reported for twist angle at fracture

(in degrees) on Chr 20. This locus is difficult to interpret given that no loci were found for torsional strength or stiffness.[50] In a second study, a QTL on chicken Chr 1 for long bone flexural strength was mapped in two white Leghorn lines.[51] However, bone size and shape were not measured in this study, clouding the interpretation of these results. The third study was substantially larger and involved Cobb–Cobb broilers bred to White Leghorn layers. QTL for tibial strength were reported on Chr 3, 11, 12, 15 and 26.[52] The QTL on Chr 3, 11 and 12 co-localized with loci for bone size, as was observed for the rodent studies. In sum, although promising findings have been made using chickens, additional work is required to further validate these results.

Summary and Conclusions

In summary, there has been extensive and varied work conducted in an attempt to identify genes that control the strength of long bone diaphyses. Less work has been conducted for more trabeculated sites such as the femoral neck and the vertebral body. A recurring theme from these studies is that QTL for whole bone structural strength co-map for bone size or/and bone shape QTL, a logically obvious and requisite outcome at such macroscopic length scale. As such, these QTL are not likely providing readouts of genetic loci governing bone matrix quality. Although there have been a few studies that have used body weight and/or bone length as putative correction for bone size, there have been few attempts made to account for actual cross-sectional size and geometry in order to refine structural phenotypes into matrix (material) quality phenotypes. Regardless, the limitations of any whole bone strength test must be carefully considered when interpreting these QTL results if the end goal is to determine the mechanism of action and biological processes the gene is acting onto manifest in the phenotype. At the matrix level, FTIR studies show that there are genetic differences in bone matrix composition, suggesting that variations may result in measurable differences in matrix strength. The whole bone mechanical strength tests commonly used in genetic studies may not adequately provide for reduction in structural properties to matrix (material) level properties that are necessary to elucidate differences in bone matrix quality (that is, matrix strength and stiffness). Mechanical test methods that can isolate and test matrix level properties with greater fidelity and precision will be beneficial toward identifying QTL and the underlying specific genes that regulate bone matrix quality. Because of the destructive nature of methods used to test bone material strength, this work will likely largely remain restricted to animal studies; however, if an alternative phenotype measurement technique was developed that could be used in human populations, GWAS in humans remains a possibility for the identification of bone strength genes. Our experience with bone mass phenotypes suggests that any such method would likely have to be applicable to large cohorts. GWAS for fracture risk (the closest clinical phenotype to bone strength) has been highly informative but does suffer from low statistical power issues. However, genes identified in animal studies can be tested in human populations as candidates for surrogate phenotypes such as fracture risk, thereby reducing the impact of statistical multiple testing penalties that must be applied[53] and increasing the information that can be extracted from GWAS data. In conclusion, although a great deal of work has been carried out to characterize the genetic control of bone strength, this work is only just the beginning.

Conflict of Interest

The authors declare no conflict of interest.

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