J Musculoskel Neuron Interact 2002; 2(3):225-231

Review Article

Hylonome

Genetics and bone
Using the mouse to understand man

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Abstract

The rationale for use of inbred strains of mice in bone research is well recognized and includes: a) practical factors (economics of scale, rapid development of adult status, pre-existing knowledge, down-sized technologies) and b) proven methodologies for genetic studies (polygenic trait analyses, mapping tools, genomic sequencing, methods for gene manipulation). Initial investigations of inbred strains of mice showed that femoral and lumbar vertebral volumetric bone mineral density (BMD, mg/mm^3) by pQCT varied in excess of 50% for femurs and 9% in vertebral BMD. Two strains - low BMD C57BL/6J (B6) mice and high BMD C3H/HeJ (C3H) – were investigated for insights to their BMD diversity. B6C3F2 females derived from intercrossing B6C3F1s were raised to adult skeletal status at 4 months, then necropsied for phenotyping of bone and genotyping of genomic DNA. 1000 F2 females were genotyped for PCR product polymorphisms on all 19 autosomes at ~15 cM. Genome wide analyses for genotype-phenotype correlations showed 10 chromosomes (Chrs) carried genes for femoral and 7 Chrs for vertebral BMD. LOD scores ranged from 2.90 to 24.4, and percent of F2 variance accounted for ranged from 1 to 10%. Analyses of main effects revealed both dominant-recessive and additive inheritance patterns. Both progenitor strains carried alleles with positive and negative effects on BMD of each bone sites. A remarkable array of additonal skeletal phenotypes (femur and vertebral geometry, strength measures, serum markers) also proved polygenic in nature, with complex segregation patterns. Verification of BMD quantitative trait loci (QTLs) was undertaken by creating congenic B6 strains carrying individual QTL regions from C3H. Following 6 cycles of backcrossing a QTL-containing region from C3H to the B6 strain, N6F2 congenic strain mice were aged to 4 months, then genotyped for the QTL region and phenotyped for skeletal traits. Comparison of mice homozygous for C$_3$H alleles versus homozygous for B6 alleles in the QTL regions showed that femoral BMD increased or decreased significantly in congenic strains, as was predicted from F2 data. Gender differences specific to BMD QTLs have been revealed, as have more than 30 additional phenotypes associated with cortical and trabecular structural parameters and biomechanical properties.

Keywords: Genetics, Bone, Man, Inbred Strains of Mice

Introduction - historical perspective

A low bone mineral density (BMD) has become the most established and identifiable risk factor for osteoporotic fractures. The proliferation of newer tools to measure bone mass has resulted in widespread testing and has also led to the realization that BMD is a complex trait normally distributed across various populations. In addition, the data produced by these tools also provided the first clues that a syndrome once characterized as an age-related traumatic disorder with back pain and fractures, is, in fact, a heritable disease. More recently, BMD studies of mother-daughter pairs, twins and large sib cohorts estimated the heritability of this trait to be between 50 and 70%[1]. This finding led most investigators to hastily conclude that the genetic influences on BMD were "oligogenic"; i.e. the phenotypic variation in BMD was caused by the actions of a limited number of genes with discrete effects. Fueled by this concept, the last ten years have been characterized by a flood of candidate-gene association studies in both small and large unrelated cohorts[1]. Although data from these papers were conflicted, and have yet to yield major genes that define osteoporotic risk, such studies, combined with genome wide scanning of multi-generational families, served to reinforce the complex and polygenic nature of the genetic influence on bone acquisition. Hence,

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Accepted 15 August 2001

we still have the daunting task of defining the role of multiple genes that individually, or in concert, moderate the acquisition and maintenance of peak bone mass.

In addition to the complex multifactorial nature of genetic influences, three other factors have emerged that further complicate our search for osteoporosis genes. First, it is now clear that individual genetic determinants of BMD are strongly influenced by other genes that do not have direct effect themselves on BMD; i.e. epistasis[1,2]. Second, there are numerous environmental factors that may modulate expression of one or more genes[1]. Third, most investigators have come to recognize that the BMD phenotype is only a surrogate for fracture. Defining "bone density genes" provides limited information in respect to prediction of future fractures, in part because there are other contributing factors that affect the eventual health of any given bone. The emergence of measurement tools with high resolution of bone microstructure has further heightened this awareness. Finally, and probably most importantly, there is now a growing realization that BMD represents the sum of several temporally related processes beginning with skeletal development and including modeling, remodeling and consolidation. Decomposition of bone mass into intermediate phenotypes, such as BMD, cross-sectional area, shape or a biochemical marker such as IGF-I, is likely to yield more mechanistic insights not only into the overall processes of peak acquisition, but also into the determinants of skeletal strength.

Rodent models for testing hypotheses related to skeletal disorders are not new. The ovariectomized rat is a well-established tool for testing new therapies for osteoporosis, as well as for understanding how estrogen deprivation affects the bone remodeling unit. Adding to data from the rat are new models of laboratory mice that carry specific gene deletions (knockouts), gene additions (transgenic), or spontaneous mutation. These mice are currently at the forefront of basic research, specifically to test how a known gene may regulate diverse skeletal actions. For example, targeted over-expression of IGF-I in transgenic mice using the osteocalcin promoter is characterized by a marked increased in both cortical and trabecular bone density at 6 weeks of age[3]. Similarly, mice globally lacking expression of the Cbfa1 gene (i.e., null mutation) are characterized by the absence of osteoblast differentiation, failure to mineralize bone, and lethality at birth[4]. Finally, spontaneous mutants such as the osteopetrosis mouse (gene symbol, op) which lack a functional mcsf gene and its product, fail to exhibit differentiated osteoclasts which are required for normal bone resorption[5]. Such gene mutations, induced or spontaneous, are extremely informative in respect to understanding single gene action in bone biology.

An entirely different approach that has caught the attention of the bone field utilizes the power of the mouse as a genetic tool to uncover genes whose normal allelic variation regulates BMD. And for good reason. Over the last thirty years, inbred strains of mice have helped identify genetic determinants of various disease states with both single and polygenic bases[6]. Although investigators in the bone field have been a little late in recognizing these models, several factors have hastened their utilization. First, technology was developed to measure BMD accurately, easily, and relatively inexpensively, in mice. Use of pQCT, peripheral DXA, and full body DXA, by both *ex-vivo* and *in vivo* methods, now allow investigators to measure BMD and appreciate large differences among knockouts, transgenics, mutants and healthy inbred strains[7]. MicroCT has, for the first time, provided an opportunity to define three dimensional microstructural aspects of bone, and in conjunction with newer methods to measure bone strength, have opened the door for elucidating determinants of bone quality[8]. Finally, the power of breeding strategies to isolate quantitative trait loci (QTL), and to test their effects either singly or in combination with other genetic determinants, has permitted hypothesis testing for individual or clusters of genetic loci[9].

There are several confounding factors that have plagued human genetics studies in the last two decades, making gene identification exceedingly difficult. These have included the complex nature of the phenotype regulated by numerous genes, significant gene x gene and environment x gene interaction, and the multifactorial nature of bone quality[1,10]. Mouse studies have made these much more amenable to resolution. Furthermore, the homology between human and mouse genomes, as well as the intense efforts to map every gene in both species, provides more impetus to use this animal as a tool for defining the heritable determinants of osteoporotic risk. In this paper, we will elucidate the role of several mouse model systems for determining the polygenic basis of osteoporosis. We will not examine mutant, transgenic or knockout models, in part because we want to de-emphasize the role of single genes in producing extreme pathology. Rather this review will focus on normal allelic variation in inbred strains of mice, an animal model more directly applicable to understanding the BMD trait in humans.

Inbred strains of mice

There are many types of mice available for genetic and biologic studies. In general, mice have become the workhorses of biomedical research because of their ease of breeding and reproductive capacity, their short life span, and the availability of large numbers of genetic markers in the mouse genome. Probably more importantly though, and unlike the rat, dozens of different inbred mouse strains have been available since the early decades of the twentieth century. These inbred strains of mice were developed by repeated matings between siblings for at least 20 consecutive generations[6]. This resulted in nearly 100% homozygosity at all alleles across the mouse genome (except for any spontaneous mutations which arise), thereby providing researchers with a plethora of genomically identical mice. Hundreds of pure inbred strains are available at The Jackson Laboratory alone.

The second feature of inbred strains that makes them powerful genetic and physiologic tools is that an individual

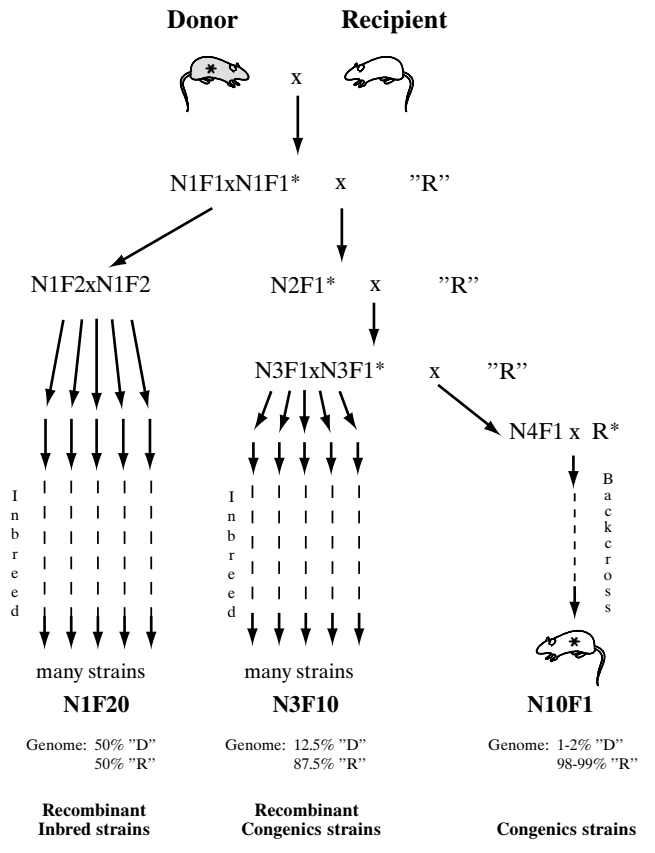

Figure 1. Mating systems for genetic analyses
The composite figure illustrates uses of two inbred mouse strains for analyses of a genetically regulated phenotypic trait. The C57BL/6J and C3H/HeJ strains characterized by low and high volumetric bone mineral density (BMD) are depicted.
First, intercrossing of N1F1 progeny yield classic F2 progeny that are equally effective for mapping single gene traits and polygenic traits that are either quantitative or qualitative in nature.
Second, mating pairs of N1F2 mice together and then breeding their decendents yields a set of Recombinant Inbred (RI) strains. If the B6 and C3H inbred strains are utilized as progenitors, at the N1F20 generation, the mice within a 'line' will be inbred, with better than 99% of all loci homozygous for either B6 or C3H alleles. Each RI strain is designated, BXH-1 (consecutive numbers for each strain), and will have inherited on average one half its genes for each of the progenitor strains.
Third, backcrossing twice to one progenitor strain (N1F1 x "R"; N2F1 x "R"), followed by decendent mating withing a line, yields a set of Recombinant Congenic strains, each of which carries 12.5% of it genes from the donor strain and 87.5% of the genes from the recipient strain. The desire is to capture subsets of genes that regulate a complex trait in a series of related but distinct inbred strains. This method is particularly useful for analyses of phenotypes that depend on modifier genes for expression.
Finally, continued backcrossing to one of the progenitor strains (N3F1 x "R"; etc.) yields a Congenic Strain that carries a segment of a chromosome transferred from a donor strain to a recipient strain. The transfer is accomplished by at least 10 cycles of backcrossing. The region containing the gene or QTL of interest is found by genotyping each generation of progeny and mating the identified carrier of the donor segment to the recipient strain.

inbred strain differs from all other strains. Each strain has its own set of phenotypic characteristics that make it unique and allow innumerable differences in physiologic behavior. One such difference is in the wide variation in BMD among inbred strains. Thus, by choosing two inbred strains that differ in a trait of interest, a cross can be made to enumerate, locate, and define heritability of the genes that contribute to that trait.

Crossing two inbred strains of mice, i.e., C57BL/6J and C3H/HeJ, results in hybrid F1 mice that are genetically identical with each other and heterozygous at all loci. As illustrated in Fig. 1, intercrossing F1 mice results in F2 progeny in which genetic alleles for BMD have randomly reassorted into new combinations, such that at any given locus an F2 mouse will be homozygous for either progenitor strain alleles (i.e., b6/b6 or c3/c3) or heterozygous (i.e., b6/c3). BMD regulatory loci that are not genetically linked to each other will independently segregate in these F2. The net effect of alleles at all BMD regulatory loci yields the BMD for each mouse.

Since there are now more than 8000 genotypic markers that are polymorphic across inbred strains, investigators can identify quantitative trait loci (QTLs) by genotyping and phenotyping the F2 progeny[11]. Analyses of the F2 progeny in the extremes of the phenotypic distribution allows rapid identification of major effect loci, whereas analyses of all F2 progeny yields major and minor effect loci, as well as the opportunity to assess trait variance accounted for by each locus and gene x gene interactions. Genetic linkage is established by testing for association of progenitor alleles with high or low expression of a particular phenotype using various computer software programs[11,12]. The initial QTLs typically reside in chromosomal regions of 10 to 40 centiMorgans (cM: recombination distances between specific markers) - regions of the chromosome with hundreds of potential candidate genes. Fine mapping and congenic construction (see below) define narrower regions of the chromosome and allow for positional cloning and gene sequencing to be undertaken. This type of QTL strategy has been successful in identifying genes associated with obesity, atherosclerosis, epilepsy and cancer susceptibility in the mouse.

Beamer et al. in 1996 first described large differences in femoral and vertebral BMD, as measured by pQCT, among various inbred strains of mice[7]. Subsequently, other investigators reported similar findings using planar radiography, whole body BMD by DXA and site specific regions of interest (i.e. vertebrae, femur and tibia) by peripheral DXA technology[13-15]. More recently, microCT has been utilized to detect differences in trabecular bone structural parameters in vertebrae, femorae and tibiae among inbred strains[8]. For each pair of strains differing in a bone phenotype, the strategy has been to map QTLs by crossing the progenitor strains, then intercrossing their F1 hybrids to produce large numbers of F2 progeny (range 250-1000 males and females). Data from several sets of such F2 progeny

have now been reported for the BMD phenotype and include: C3H/HeJ (C3H:) vs. C57BL/6J (B6: low BMD), Castaneus/EiJ (CAST: high BMD) vs. B6 (low BMD), SAMR1(high BMD) vs. SAMP6 (low BMD), AKR/J (high) vs. SAMP6 (low BMD), B6 (high BMD) vs. DBA/2J (D2 low BMD) [13, 14-17].

Bone mass is a complex phenotype that includes mineral content, size (length, width, cross-sectional area), trabecular connectivity and shape. Bone strength is determined by these parameters as well as other variables affecting overall bone quality. Depending on measurement, and the instrument used to define it, a bone density phenotype varies within a given strain as well as between inbred strains. For example, B6 is a low bone density strain compared to C3H, when defined by volumetric measurements of the femur such as pQCT, but B6 is a high density strain in comparison to DBA/2 when whole body areal BMD using DXA technology is the density phenotype of choice[14,17]. In part, this can be related to the shape as well as the size of bone and its individual components. The B6 femurs have thinner cortices, a more elliptical shape and lower volumetric BMD than C3H animals. However, in comparison to DBA/2 mice, the periosteal circumference of B6 is greater, hence areal measurement of the femur by DXA can actually show relatively greater apparent BMD for this strain. Since size, shape and mineral are all critical components of strength, the phenotype under study becomes critical not only for assigning QTLs, but also for attempting to understand the biomechanical mechanisms that ultimately define both bone morphology and strength.

Mapping "bone density genes" can be extremely productive in F2 mice because there is independent segregation of unlinked genes for this polygenic phenotype. Surprisingly, progenitor differences are not mandatory before performing genetic analyses of polygenic traits, as the F2 population, with its independent assortment of loci, will reveal genetic determinants whose actions might not be evident in the progenitor inbred strains or may be hidden by actions of epistatic loci[18]. Moreover, QTLs in the F2s can display alleles with actions that appear contrary to expectations based on the progenitor strains' phenotype. For example, the high BMD C3H inbred strain has been found in one QTL analysis to be carrying two genes yielding a 'low density' in F2 progeny[16]. Similarly, Benes et al. found that two AKR QTLs (i.e. Chr 7, 11) were associated with low areal BMD when these alleles were homozygous in F2 mice from an AKR (high density) x SAMP6 (low density) cross[13]. Thus, F2 mice provide an invaluable tool for locating and enumerating QTLs, as well as delineating allelic effects. Moreover, the phenomena of transgenesis (gene recombinantions in the F2s resulting in phenotypic values that are greater or lesser than the progenitor strain phenotype) can offer critical insight into the complex genetic influence on a specific phenotype[13].

Another exciting aspect of gene mapping in mice is the evolving story surrounding trabecular structure and strength. Newer technology with microCT has provided investigators with the opportunity to study trabecular spacing, number, connectivity, and three dimensional structure, in the vertebrae and femorae of mice. These determinants are likely to produce even more phenotypes for QTL analyses, especially in respect to understanding "bone quality". Recent work by Turner et al. suggests that data on vertebral and femoral neck breaking strength in one inbred strain can differ considerably from that at the mid-diaphysis of the femur in the same strain[8]. For example, C3H femurs have a high BMD and thick cortex and therefore are stronger than B6 femurs by three point bending in the mid-diaphysis. However, vertebral strength by compression testing is reduced in C3H compared to B6. MicroCT analyses have revealed a markedly reduced number of trabeculae in C3H vertebrae[8]. These data are consistent with the fact that although C3H has higher apparent BMD (even in the vertebrae!!) by projectional methodology, trabecular BMD, when quantitated properly, is actually reduced. This would suggest there are distinct genetic determinants within a given strain that define cortical vs. trabecular BMD. Moreover, these findings support long standing clinical observations that different bone compartments acquire peak bone mass at different times, and that there can be very disparate BMD values between the spine and hip in an individual. Furthermore, response to both anti-resorptive and anabolic treatment clearly differs from one skeletal site to another. Hence, there has to be a multitude of osteoporosis genes, some of which regulate both trabecular and cortical components, some which must determine distinct skeletal compartments, and some which must be temporally activated or suppressed during the acquisition and consolidation phase of acquiring bone mass.

Finally, it should be noted that the search for mouse BMD genes permits a more in-depth analysis of the biology related to acquisition and maintenance of BMD. For example, C3H mice have high cortical bone density, in part because their bone marrow stromal cells proliferate more rapidly, their osteoblasts are programmed to synthesize more IGF-I and alkaline phosphatase than B6 bone cells, and their rate of bone formation is much higher than B6 mice. By histomorphometry, C3H bone shows high rates of bone formation and modest bone resorption rates compared to B6 mice[19,20]. These data combined with biochemical markers of bone turnover reflecting similar alterations in bone remodeling, demonstrate that the genetic determinants of bone density affect the basic cellular processes of bone. Intermediate skeletal phenotypes, such as IGF-I, N-telopeptides, or alkaline phosphatase, can also be examined in F2 mice for QTL analysis, thereby providing additional approaches to understanding the cellular mechanisms responsible for acquisition of peak bone mass.

Recombinant inbred strains

Recombinant inbred (RI) strains are generated by outcrossing two progenitor strains and then intercrossing

these F1 hybrids to produce F2 progeny, as illustrated in Fig. 1. Then, pairs of sister-brother F2 mice are selected at random to serve as founders for each RI strain. These F2 mice are mated to produce F3 generation mice and this process sibling mating within each RI line is repeated 20 generations. The result is new inbred strains nearly 100% homozygous at all loci, yet which have a different combination of genes from the same original progenitor strains[6]. This constitution can be maintained indefinitely by continual brother-sister matings. Since the original founders were selected at random, many distinct RI strains can be generated from the original progenitors (more than 12 such sets of RI strains exist at The Jackson Laboratory). These RI strain sets are named by the capital letter of each strain separated by 'X' (e.g. B6 and DBA/2 gave rise to BXD-) plus a number for the particular strain (e.g. BXD-16)[6]. Unlike classic inbred strains, the genotype of a RI strain is somewhat limited by the fact that there are only two possible alleles (e.g., B6 or DBA/2) at each locus. More importantly, there is limited recombination because homozygosity sets in relatively quickly during the intercrosses. In addition, because both DBA/2 and B6 inbred strains were originally derived from the same founder animals more than 70 years ago, a number of loci are not polymorphic. However, the strength of the RI strategy is that hundreds of genomic markers for each strain are identified, and publicly available databases can be utilized for rapid linkage studies and for determining map distances, with as few as 1-2 mice phenotyped from each RI strain. To establish genetic linkage, the investigator simply phenotypes each of the strains in a RI set (e.g. 12 BXD strains) to obtain a strain distribution pattern for the phenotype. The strain distribution pattern for the new phenotype is compared with strain distribution patterns of known polymorphic loci previously established in that RI set. When congruence between strain distribution patterns is found, linkage of the phenotype to a specific chromosomal region is established. By using known recombination sites in the mouse RI strains, one can mate RIs to parental strains (e.g. BXD-8 to DBA/2 and B6) to obtain new F1 progeny. Then by linkage between the genotype and phenotype, the QTL can be placed either above or below the recombination break point. This approach is called the recombinant inbred segregation test strategy (RIST) and has been successfully used by one group to help refine QTLs locations for bone density genes[21].

Klein et al. was the first group to employ RI strains to define BMD QTLs. Their group utilized 19 BXD RI strains to show: 1) whole body areal BMD is heritable (h2=0.50); 2) gender and strain specific effects are present, and 3) BMD differed by 30% among individual RI strains, suggesting that combinations of genes played a big part in overall peak bone acquisition[14] (Klein et al., 1998). Using published databases containing more than 1500 genetic markers. identified QTLs on Chrs 2, 7 and 11 with major effects[22]. Interestingly, some QTLs were only present in males, while others only in females, and three QTLs (Chrs 1, 18, 19) appeared gender independent[23]. Moreover, several of these QTLs were found to be similar to those identified independently by other groups using different strategies (i.e. F2 matings). Finally, from this same group, Klein et al. recently reported that RIST allowed them to narrow the QTLs on Chrs 2 and 11 by more than 10 cM[22]. Hence, RI strains have provided a tool for rapidly establishing linkage of whole body areal BMD QTLs, as well as for further resolution of large QTL regions into smaller segments.

Congenic strains

Congenic strains are generated to test the effect of individual or multiple linked loci from a donor strain placed on the genetic background of a recipient strain[24]. As illustrated in Fig. 1, the strategy is based on repetitive backcrossing to the recipient strain while genotyping for the donor strain's alleles in the subsequent backcross generations. With each generation, the homozygosity of the recipient background increases from 50% at N1F1 to 99.9% at N10F1. The residual heterozygosity resides at the region of interest carrying the donor alleles. Hence, the genetic background of the chromosomal region of interest has been switched from donor to recipient. Congenic strains are particularly useful for confirming the QTL existence, fine mapping of the QTL, and for testing the quantitative effect of individual QTLs. For example, if there is a strong BMD QTL on Chr 1 from C3H mice, as found by F2 analysis, congenic strain mice in which the Chr 1 QTL is now placed on a B6 background, allows the investigator to test the effect of this single genetic locus on a low bone density background. The congenic strain would be named B6.C3H-1 (recipient.donor-chromosome). Multiple QTLs can be combined to test for gene x gene interactions. Congenic strain construction takes about 18 months and may be labor intensive, but it provides an essential means of evaluating the biology regulated by the QTL, as well as refining the map position of individual loci. However, there are two caveats to this approach: 1) "passenger" loci adjacent to the QTL of interest travel with the donor QTL may affect the phenotype and affect mapping precision; 2) the QTL phenotype may be the net result of linked genes with different effects within the QTL region. In each case, fine mapping and generation of nested congenics can overcome these problems[6].

Congenics have recently become extremely valuable tools for bone biologists, not only for studying the quantitative effect of individual QTLs, but also for more completely understanding the phenotype and its underlying physiology. Thus, one moves from breeding strategies and QTL analysis to individual congenic construction in order to define the locus of interest, as well as to test precisely how that locus could affect the phenotype. Two groups have reported generation of congenics using their most promising QTLs for either whole body BMD or femoral BMD. These loci include Chrs 1, 2, 4, 5, 6, 11, 13, 14 and 18[16, 22]. In addition, one group has developed congenic strains for the serum

IGF-I phenotype on Chr 6 and 15. The power of this strategy is illustrated by some very recent studies[20].

Klein and colleagues constructed congenic mice for the Chr 2 areal BMD QTL and reported that after the fourth generation, D2.B6-2 mice (i.e. mice carrying a B6 QTL) on a nearly 93% homozygous D2 background had a difference of nearly one standard deviation in whole body BMD compared to inbred D2 mice[22]. Moreover, this effect occurred only in female mice. Beamer et al. reported that the Chr 1 QTL from C3H mice had an approximately 8% increase in femoral BMD when placed on a B6 background in N6 congenics[16]. Similar findings were also noted for the Chr 4 QTL. On the other hand, a "low bone density QTL" from C3H in Chr 6 was associated with a 3.5% reduction in BMD, when donated to a B6 background. Not unlike the studies from Orwoll et al., this effect was only noted in female mice[23]. Hence, congenic strains can provide insight into the effect of individual QTLs in respect to bone and to the mechanism of such action.

The power of the congenics extends beyond simple tests for QTL effects. For example, Beamer et al. reported that although the congenic on Chr 1 had a statistically significant effect on femur BMD, microCT analysis revealed a much greater effect on vertebral trabecular bone[25]. In fact, vertebral bone density (measured as BV/TV) was nearly 35% greater in the Chr 1 congenic compared to progenitor B6 mice at 16 weeks. These findings, also noted for the Chr 4 QTL congenic, support the thesis that bone micro-structure may be altered dramatically while BMD may change only modestly. If confirmed, these data provide more impetus for defining aspects of bone quality and their relationship to skeletal fragility, an issue very relevant when considering the skeletal response to long term anti-resorptive therapy. Finally, Rosen et al. have noted that one of the strongest QTLs for serum IGF-I in B6C3F2 mice is likely to be the Chr 6 QTL noted for BMD[20]. Moreover the congenic B6.C3H-6 mice at N10 generated from this QTL, show not only reduced femoral and vertebral BMD, but also markedly lower serum IGF-I concentrations. This model provides further proof that the congenics can offer insight into the mechanisms whereby peak bone mass is acquired.

Recombinant congenic strains

A fourth system available for genetic and biological studies of polygenic traits, such as BMD, is illustrated in Fig. 1. Recombinant Congenic (RC) Strains represent a combination of the attributes found in RI strains and congenic strains of mice. As can be discerned from Fig. 1, two backcrosses are made to a recipient strain to achieve progeny that carry 12.5% of genes from the donor strain. Sibling progeny from the N3F1 cross are then incrossed to inbred status as shown. The intent of this system is to isolate small subsets of genes that regulate a complex trait within distinct inbred strains. Demant and colleagues have successfully used RC strains to genetically analyze both colon and lung cancer in mice[26,27]. In addition, Blank et al. have reported that genetic linkage to regions on 11 different chromosomes could be demonstrated for bone strength, ash percentage, and morphological parameters using the set of 27 HcB/Dem RC strains[28]. These strains were derived from donor C57BL/10ScSnA and recipient C3H/DiSnA strains. Thus, RC strains are very suitable for analyses of complex traits and may be most valuable for assessment of genes that interact in subtle ways not easily identified by standard statistical means.

Mouse to man

This review has pointed out some of the models investigators have used to define the genetic determinants of bone density in mice. But, it is quite obvious that despite major efforts by several groups, no mouse "bone density" gene has yet been cloned. Still, inbred, recombinant inbred and congenic strains, as model systems, offer a wealth of information related to acquisition and maintenance of peak bone mass. With the advent of more rapid genotyping and congenic generation techniques, it seems certain that these putative QTLs will yield numerous genes that contribute to the variance in bone density within a mouse strain. Moving from mouse genes to human genes using published maps, as well as with the ongoing genome sequencing projects, may actually turn out to be easier than once thought[29]. More of a challenge, however, will be to understand the full effects of a particular gene on bone cell function, the interactions with environmental factors, and perhaps even more importantly, the regulators of such genes. Nevertheless, it has become clear that the power of the mouse for bone biologists lies in several relevant factors: a) the strong homology (60-70%) between human and mouse genomes; b) ongoing efforts to completely map and sequence mouse and human genomes, thereby permitting more rapid identification of putative bone density genes; c) the ease and rapidity of conducting crosses among various strains of mice; d) the relative control that investigators have over environmental factors that modulate genetic determinants of bone density; and, e) the rapid acceleration in knockout and transgenic technology permitting functional testing of putative bone density "genes".

Clearly, the last two factors are the most appealing and compelling. In contrast to human studies, strict regulation of dietary factors, physical activity, life style, and environment, is relatively straightforward in the mouse. Moreover, except for the sex chromosomes, each of the mice in an inbred is an identical twin to the next, carrying the same genome as all others within that strain. This makes it considerably easier to refine the search for various bone density genes and to test their responsiveness to various perturbations, all within a defined life cycle. Finding "osteoporosis" genes in mice provides an unmatched opportunity to test their role in all aspects of bone biology, and indeed, such findings can then be used to further our understanding of the pathophysiology of this debilitating disease.

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