Genetics of osteoporosis – Utility of mouse models

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Although recent clinical reports show promise, unraveling the very complex genetic basis of skeletal development will be difficult because of the genetic and cultural heterogeneity of the patient populations. Workers investigating the determinants of bone mass in humans have limited ability to intervene in the genetics, personal environment, or skeletal biology of their subjects. One approach to this problem is the use of appropriate animal models to pinpoint candidate genes for more focused human investigation.

An ideal model that can be used for all studies in bone research does not exist. Whether or not an animal model is useful depends largely on the specific objectives of the study and frequently involves tradeoffs between such factors as realism, reproducibility of results and feasibility. Birds, mice, rats, rabbits, dogs, sheep, pigs, and non-human primates have all been the subject of experimental osteoporosis research. Each of these animal systems has its own advantages and disadvantages, but the obvious requirement for a reasonably detailed knowledge of basic genomic structure currently limits the choice for genetic animal models of osteoporosis to mice, rats and non-human primates. Of the three currently available options, the mouse is arguably the model of choice because: (1) mice are much cheaper to house and easier to handle, (2) mouse genetic resources are quite extensive, and (3) once candidate genes are identified, the ability to manipulate them in mice and to deduce unambiguously their role in disease is unparalleled.

Moreover, gene targeting has reached new heights in mice, but is barely on the horizon in other animals. With gene targeting perhaps as the ultimate arbiter for establishing cause-and-effect relationships between candidate genes and osteoporosis susceptibility, the mouse is apt to remain the primary experimental model system for the foreseeable future.

Current murine research in this field is heavily dependent upon inbred mice of different strains that exhibit marked differences in parameters of skeletal integrity. A strain of a species is inbred when virtually every genetic locus is homozygous. What this means is that all individuals within an inbred strain share a set of characteristics that uniquely define them compared to other strains. Typically, inbred strains are derived from 20 or more consecutive generations that have been brother x sister mated; the strain can then be maintained with this same pattern of propagation. Individual animals within an inbred strain are as identical as monozygotic twins. There are several qualities of inbred strains that make them especially valuable for research. The first is their long-term relative genetic stability. This is important because it allows researchers to build on previous investigations. Genetic change can occur only as a result of mutation within an inbred strain and share a set of characteristics that uniquely define them compared to other strains. Typically, inbred strains are derived from 20 or more consecutive generations that have been brother x sister mated; the strain can then be maintained with this same pattern of propagation. Individual animals within an inbred strain are as identical as monozygotic twins. There are several qualities of inbred strains that make them especially valuable for research. The first is their long-term relative genetic stability. This is important because it allows researchers to build on previous investigations. Genetic change can occur only as a result of mutation within an inbred strain. A second important quality of inbred animals is their homozygosity because inbred strains will breed true. Once the characteristics of a strain are known they can be reproduced repeatedly allowing for replicate experimentation as well as for studies by other investigators. The influence of genotype upon a particular characteristic can be investigated by placing mice from several inbred strains in a common environment. Observed differences must then be, within limits, the consequence of genetic factors. By reversing this strategy, and placing mice from a single inbred strain in a variety of environments, it is possible to estimate the importance of environmental influences upon a parameter of interest. Thus, inbred animals can be used to determine whether genetic variation in the expression of a characteristic exists and the environmental malleability of the characteristic. Experiments with inbred strains also have some limitations. While strain differences are easily demonstrated, it is often very difficult to attach much meaning to these differences, because the genes and gene products involved are usually unknown. Because comparisons of mice from two or more strains do not usually provide any information about the nature of the genetic differences, crosses between genotypes must be used to analyze patterns of genetic influence.
Additionally, when using an inbred strain to investigate any type of phenomenon, it is important to be aware that the observations may be relevant only to that strain. Because an inbred strain differs from all others, there will be characteristics unique to it. It is therefore important to use more than one strain to confirm that any observation obtained pertains to the species and not just to the strain studied.

Osteoporosis researchers have performed genotype-phenotype linkage (or quantitative trait or QTL) analyses in large populations of genetically heterogeneous mice derived from various combinations of inbred strains in the hopes of obtaining a more complete picture of the polygenic control of bone mass and an improved understanding of the complex interactions and physiological mechanisms involved. Results from these complementary studies are beginning to define the landscape of the genetic regulation of bone fragility and partition this quantitative trait into separate genetic components amenable for more detailed evaluation. However, the ultimate goals of complex trait analysis are to identify coding sequences and to understand their biological roles at a molecular level remain the major challenge. Initial QTL analyses on an adequately-sized experimental population rarely succeeds in narrowing map positions to less than 10-20 centiMorgan (cM) or about 1/4 of the length of the average mouse chromosome. This broad QTL region includes about 250 to 500 genes (25 genes per cM). This is because the phenotypes of individual animals are easily swayed by the influence of unlinked or environmental noise. Positional cloning of human disease genes has demonstrated that even when the position of a gene has been defined within one or two million base pairs and all the DNA sequences within that region have been isolated, identification of the relevant gene can still be a formidable task. Fortunately, new experimental strategies for fine QTL mapping, development of transgenic technologies, and more traditional approaches employing congenic strains, promise to eventually bridge the gap between cloning and disease.

QTL fine mapping involves careful analysis of recombinants within an interval previously found to contain the gene. For a compilation of the various experimental designs currently available, the reader is referred to an excellent recent review by Darvasi. Once the QTL has been resolved to such a narrow region, an examination of candidate genes within that region can take place and transform a conventional positional cloning strategy into a positional candidate approach.

Transgenic technology creates a very effective tool for analyzing the physiological roles of specific genes. A transgenic animal contains a segment of exogenous genetic material stably incorporated into its genome, resulting in a new trait that can be transmitted to further generations. Two widely used methods introduce exogenous genetic material into the genome: 1) microinjection of one-cell fertilized embryos and 2) genetic manipulation of embryonic stem (ES) cells. In contrast to traditional "gain-of-function" mutations, typically created by microinjection of the gene of interest into the one-celled zygote, gene-targeting via homologous recombination in pluripotent ES cells allows one to precisely modify the gene of interest. Employing ES methodology, investigators have generated site-specific deletions ("knock-outs"), insertions ("knock-ins"), gene duplications, gene rearrangements, and point mutations. In addition to facilitating the study of known candidate genes, molecular complementation (transfer of specific genes) of selected phenotypes is a potentially important tool for gene identification.

Classical transmission genetics can also be used to transfer a gene of interest from a donor strain or mutant onto the genetic background of an inbred strain. Using this approach, one is able to transfer regions containing risk or protective QTLs, or even multiple QTLs, onto appropriate background strains. Such congenic strains are produced by repeated backcrossing to the background inbred strain and genotypic selection of the desired allele at a marker or markers at each backcross generation. After 7 backcross generations, the congenic and background strains can be expected to be about 98% genetically identical except for the transferred (introgressed) chromosomal region. The primary advantage of the congenics is that the influence of an individual QTL on any trait can be tested using the congenic vs. background strain comparison at any level from the molecular to the physiological. Any differences found would strongly implicate a QTL in the introgressed chromosomal region as the cause of the differences. When there are several congenic strains for a given QTL, their differing sites of recombination can aid in attaining higher resolution mapping of the QTL with respect to neighboring markers. The near elimination of "genetic noise" due to unlinked loci greatly aids the search for candidate genes associated with each QTL, and for studies of differential gene expression. Ultimately, congenic strains can greatly facilitate positional cloning of a QTL. Knowing the genetic markers defining the boundaries of a QTL region automatically indicates the candidate genes residing within the region. In addition, congenic strains provide an invaluable resource for further defining specific genes of interest and for in-depth studies of the mechanisms by which they affect skeletal phenotype. A number of groups have now reported the generation and initial characterization of congenic strains bearing skeletal-relevant QTLs. Finally, it may be possible to combine the mapping data present in congenic strains with expression analysis (e.g., complementary DNA microarray analysis) to identify, without bias about potential roles, putative target genes underlying a given QTL.

In a complex disorder such as osteoporosis, experimental approaches that can either manipulate or hold constant biological variables that determine a given skeletal trait provide a crucial opportunity to systematically examine the pathophysiologic processes that contribute to osteoporosis vulnerability. Murine studies per se provide an attractive interface between forward and reverse genetics. As candidate genes are nominated as having important skeletal functions, the tools of molecular biology will allow the genetic and epigenetic diversity underlying their expression and function to be more fully examined.
References

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