

Introduction to Skeletal Genetics

Michael J. Econs, MD

Departments of Medicine and Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, Indiana 46202

Corresponding author:

Michael J. Econs, MD, FACP, FACE

Glenn W. Irwin, Jr., Professor of Endocrinology and Metabolism

Director: Division of Endocrinology and Metabolism

Indiana University School of Medicine

541 North Clinical Dr.

CL 459

Indianapolis, IN 46202

Tel: 317-278-0682

FAX: 317-278-0658

E-mail: mecons@iupui.edu

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The skeletal genetics session highlighted some of the advances that have occurred over the past few years. While, the most progress has been made in Mendelian disorders, the field is beginning to have success in the study of the more complex trait, osteoporosis. Moreover, several discoveries in rare Mendelian conditions have profound implications for more common diseases and these discoveries are being exploited to develop new therapies for osteoporosis and other metabolic bone disorders.

Positional cloning studies have led to the identification of many genes that are responsible for Mendelian bone and mineral disorders. Many of these disorders are “human knock-outs” or “knock-ins” that have provided us with a wealth of information about normal bone and mineral physiology. One of the advantages of this approach is that it is not “hypothesis limited” and allows for identification of genes that would not have been thought of as good candidates for the disease. For example, until positional cloning studies determined that inactivating mutations in PHEX are responsible for X-linked hypophosphatemic rickets, no one hypothesized that inactivating mutations in an enzyme could be responsible for a renal phosphate wasting disorder [1]. Moreover, positional cloning studies of a very rare disorder, autosomal dominant hypophosphatemic rickets, resulted in identification of a previously unknown gene, FGF23 [2]. Further studies established FGF23 as a hormone that is important in day to day regulation of serum phosphorus and calcitriol concentrations. Moreover, this hormone plays a central role in several disorders of phosphate homeostasis [3]. A stunning example of success with the positional cloning approach is the identification of LRP5 as being responsible for high bone mass [4] and osteoporosis pseudoglioma [5]. This work led to discovery of an important pathway that regulates bone mass and has led to development programs within industry that target the Wnt signaling pathway to develop novel therapies for osteoporosis (see below).

Osteoporosis is a complex trait that is influenced by environmental and genetic factors. Current evidence indicates that there are many genes that influence the trait with no major gene that is responsible for a large proportion of the genetic influence. However, new approaches have been developed that may allow for successful identification of genes and that affect bone strength and fracture risk. Identification of genes that influence predisposition to osteoporosis has been a focus of several laboratories over the past several years. Identification of these genes will: 1) lead to molecular tests that predict osteoporosis risk and allow institution of early preventive measures; 2) provide insight into basic bone cell biology and other factors that affect BMD and predispose to osteoporosis; 3) allow for personalized medicine; and 4) provide molecular targets for therapeutic agents to influence BMD. Several different approaches have been used to identify these genes. These include candidate gene association studies, linkage studies (either large pedigree or sib pairs), and genome wide association studies. Any of these approaches can be combined with animal and/or in vitro studies to facilitate gene identification.

Examples of successful use of genetic studies in model organisms were presented by Dr. Robert Klein, who outlined linkage and follow up studies in mice. Mice are a commonly used model, whose last common ancestor with humans was approximately 80-100 million years ago, making them closer to humans, evolutionarily, than are dogs and cats [6]. Moreover, they are less expensive to keep than other mammals and there is a tremendous infrastructure for mouse genetics. F2 linkage studies have led to identification of several chromosomal regions that harbor genes that affect bone strength in mice. One such region, on mouse chromosome 11 has already yielded important data regarding bone strength [7]. After F2 studies demonstrated linkage between a region on mouse chromosome 11 and bone strength phenotypes, Klein and

colleagues made congenic mice that contained a small segment of chromosome 11 from C57BL/6 (B6) on a DGA/2 (D2) background. These congenic mice had increased peak BMD (whole body and femoral) compared to D2 mice.

They then performed microarray expression studies to find genes that are differentially expressed between tissues from the background strain (D2) and the congenics. They subsequently identified *Alox15* as a gene on mouse chromosome 11 that is differentially expressed and verified by RT-PCR that *Alox15* expression is 20 fold less in the congenics than the parent D2 strain. Examination of *Alox15* knock-out mice demonstrated that they have increased bone density and strength. Furthermore, pharmacologic inhibition of *Alox15* improves peak bone mass in mice and attenuates ovariectomy induced bone loss in rats [7]. Subsequent studies in humans [8, 9] demonstrate that polymorphisms in *ALOX12*, the functional homologue of mouse *Alox15*, are associated with variation in BMD in peak BMD. Thus, studies using a variety of techniques in mice led to the discovery of an important pathway that influences bone strength in humans and provide targets for pharmacologic intervention of osteoporosis.

As noted above, the identification of *LRP5* is an example of a stunning success, whereby studies in high bone mass [4] and osteoporosis pseudoglioma [5], both very rare conditions, led to discovery of a pathway that is critical to bone health. The history of high bone mass is an excellent example of a thoughtful clinician-scientist, Dr. Robert Recker, recognizing that a patient, who was referred for a routine consult, could have a genetic disorder that would be worthy of study. During his presentation, Dr. Recker summarized work from his laboratory characterizing the phenotype and discussed the implications of this phenotype for potential therapies for osteoporosis. Patients with high bone mass are biochemically normal and have increased BMD with an increase in BV/TV% resulting from increased trabecular thickness. However, the bone is morphologically normal. Developing a skeleton of normal size and shape requires the coordinated action of osteoclasts and osteoblasts. Positional cloning studies eventually demonstrated that this family's condition was caused by an activating mutation in *LRP5* that changed glycine 171 to valine [4]. Studies in other HBM kindreds identified additional activating mutations [10, 11]. An intriguing hypothesis is that these activating mutations alter the set point of bone to mechanical loads, in effect altering the "mechanostat" to favor positive bone balance. Inactivating mutations in *LRP5* result in osteoporosis pseudoglioma [5]. Collectively, these studies established that *LRP5* is a critical gene in normal bone development. Moreover, they identified a pathway, the Wnt signaling pathway that opened new avenues in bone biology and provided several targets for pharmaceutical development.

Dr. Bart Williams has used targeting approaches in mice to study the Wnt signaling pathway. During his presentation he summarized the Wnt signaling pathway and the roles of both *LRP5* and *LRP6* in this pathway. Dr Williams hypothesized that there is partial redundancy between *LRP5* and *LRP6* and tested this hypothesis in a variety of mouse models. In support of this hypothesis, the bone of *Lrp5*^{-/-}, *Lrp6*^{+/-} mice is significantly less dense than those that are only deficient in *Lrp5*. Furthermore, *Lrp5*-deficient mice carrying an osteoblast-specific deletion of *Lrp6* have extremely low bone mass (Williams JNMI, this issue). Moreover, humans with inactivating mutations in *LRP6* have osteoporosis as well premature cardiac disease [12].

The Wnt signaling pathway provides many targets for pharmaceutical intervention (Bodine JNMI this issue). These include *DKK1*, *SOST/Sclerostin*, and *SFRP1* in addition to *LRP5*. For example, treating rats with anti-*DKK1* antibody for 3 weeks results in increased bone formation rate with an increased midshaft cortical area and thickness and increased lumbar and whole leg BMD. Similarly, giving 2 months of treatment with humanized anti-sclerostin

antibody to cynomolgus monkeys increased BMD at the femoral neck, radius and lumbar spine and almost doubled vertebral load to failure [13]. In a phase one trial in 48 postmenopausal women anti-sclerostin antibody increased serum P1NP, osteocalcin and bone specific alkaline phosphatase [14]. Deletion of SFRP1 in the mouse increases femoral BV/TV by 79% [15]. Dr. Bodine reports that his group has screened over 400,000 compounds in a cell based reporter gene assay to find small molecule inhibitors of SFRP1. These studies yielded a number of small molecules, which were then optimized for pharmaceutically relevant characteristics. These studies produced a diphenylsulfone sulfonamide SFRP1 inhibitor that suppresses the ability of SFRP1 to induce apoptosis in preosteocytes *in vitro* and stimulates *ex vivo* bone formation. Further studies with similar molecules are currently underway.

In summary, skeletal genetic studies have had remarkable successes over the past several years. Several pharmacologic therapies are under development as a direct result of these studies. Studies currently underway hold the promise of identifying additional genes and pathways that will be of direct relevance to patients.

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