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MEETING REPORT

Genetics of osteoporosis and bone disease (ASBMR 2012)

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Meeting Report from the 34th Annual Meeting of the American Society for Bone and Mineral Research, Minneapolis, MN, USA, 12–15 October 2012

A broad swath of data, generated from a refreshing variety of study methodologies, was presented at the 2012 ASBMR meeting in Minneapolis MN that highlighted how far bone genetics has come. Several examples are presented herein.

New Directions for Single Phenotype Genome-Wide Association Study

Genome-wide association study (GWAS) no longer focusses just on spine and hip bone mineral density (BMD) in large adult cohorts, but rather it is being broadened to include previously under-examined populations, such as children, and for the identification of causative genes for specific skeletal conditions. Two examples are presented below. Dr Kemp presented data showing that skull BMD is an informative phenotype when measured in children,[1] as GWAS conducted using this phenotype yielded previously known BMD loci. However, a novel locus on *6q23.2* (*EYA4* gene) was also found. This was a relatively small sample for a GWAS, and it will be interesting to see what can be learned from larger cohorts. It is as yet unclear what the role of this *6q23.2* locus is in bone, and whether it is a locus whose primary function relates to growth and skeletal maturation. The Erasmus group presented an interesting late-breaking abstract that not only demonstrated that Scheuermann's disease, a form of osteochondrosis of the spine, has a prevalence of about 4% in the Dutch population, but that SNPs in the vicinity of *TLL1* are associated with this condition.[2]

Candidate Gene Identification

As illuminating as GWAS has been, association is still not causation and does not explain function. As resequencing becomes more affordable, the genetics field is moving, with mixed results, toward trying to find the causative polymorphisms for disease. The Framingham and Cardiovascular Health Study groups presented results from a targeted resequencing effort focused on four previously identified GWAS loci for BMD. They resequenced loci at *1p31.3 (WLS), 5q14.3 (MEF2C), 11p11.2 (LRP4)* and *20p12.2 (JAG2)* in 200 subjects who had extremely low BMD. They showed that rare variants in the vicinity of the *LRP4* gene have the potential to be causal.[3] The Oxford Endocrinology group presented work in which they identified the pathologic mutations causative for sub-types of familial hypocalciuric hypercalcemia (FHH) in genes other than the calcium-sensing receptor (*CASR*). They showed that FHH-2 is caused by mutations of *GNA11*,[4] a functional candidate that transduces the CASR signal, and found a three-nucleotide deletion that results in omission of the *Ile199* residue. Transfection studies in the cell line HEK293 (human embryonic kidney 293) cells confirmed its functional importance. This mutation was not found in non-FHH-2 patients, but the mutation cosegregated with the disorder in 21 members of an extended pedigree. In a second abstract, this group showed that FHH-3 arises from a mutation in *AP2S1*.[5] Whole-exome sequencing revealed a *Arg15Cys* missense mutation in three affected individuals from two unrelated FHH-3 kindreds. This residue is highly conserved evolutionarily, and functional assays in HEK293 confirmed its importance. The protein is a component of the fatty acid-binding protein-4 complex (also known as AP2) and the arginine residue appears to be necessary for recycling the CASR and other G-protein-coupled receptors to the cell membrane after ligand binding. This latter pair of abstracts illustrate how collaboration between skilled clinicians and bench scientists, recognizing and exploiting informative families, can advance the understanding of basic physiological mechanisms.

Use of Animal Models for Mapping and Gene Identification

Progress in identifying the genes underlying bone disorders is not restricted to human studies. A poster presented on Sunday by the Indiana University School of Medicine group highlighted one of the many rodent genetic reference populations that have been developed over time, but are only recently being exploited for bone genetics. These reference populations are vastly different than the two strain intercross populations that were the workhorse of rodent genetics for so many years.[6] In the poster

presented by Alam *et al.*,[7] genetic mapping using the heterogeneous stock rat (HS) was presented. This stock is actually a population of rats derived from eight inbred lines of rats that have been interbred for over 50 generations using a rotational breeding scheme. This population captures huge amounts of genetic variation and can facilitate genetic mapping with high resolution and precision.[8] Alam *et al.*[7] used the HS rats to map quantitative trait loci for both BMD and bone structure phenotypes that are comparatively difficult to measure in humans and discovered many sex-specific loci for these phenotypes. These loci are reasonably small in size, allowing for rapid candidate gene identification. Dr Farber presented his work conducting a modified GWAS study for BMD using data from 25 inbred strains of mice.[9] He presented loci mapping to mouse chromosomes (Chr) 2, 3 and 17, but focused much of his talk on the locus on Chr 3. Similar to human GWAS, loci mapped in this manner in mice are genetically narrow and the Chr 3 loci contained two genes. Using a combination of systems genetics and *in vitro* knockdown in osteoblasts, Dr Farber was able to show that *Lhfp* is a reasonable candidate for this locus and may serve to modulate osteoblast function. The Sanger group also presented some of their successes in finding bone genes. This group has been broadly phenotyping all mice going through the Sanger Institute Mouse Genetics Project and in doing so has identified nine genes previously not known to have a function in bone.[10] Hypothesis-free phenotyping projects such as this one have true potential to shift the study of bone biology in a major way, as they do not focus on a cell type or pathway, but rather attempt to catch all.

Pleiotropy

In a late-breaking poster, Dr Oei showed with the help of GWAS that genetic variation in the vicinity of the *SHFM1* (7q21.3) and *FAM201A* (18p11.21) genes is associated with osteoporotic fracture.[11] Using genome-wide complex trait analysis, additional fracture loci were identified. Collectively, these loci only showed a one-third overlap with known BMD loci, which serves as a reminder that understanding the genetics of fracture will require understanding more than just the bone. To further emphasize this point, it was shown that loci associated with BMD are putatively pleiotropic for other musculo–skeletal phenotypes/diseases such as osteoarthritis.[12] Several studies highlighted the genetic connection between muscle and bone. Specifically, two separate studies presented data suggesting that the *PPP6R3/LRP5* locus (*11q13*) is pleiotropic for muscle mass and bone traits, such as BMD,[13] and measures of hip geometry.[14] The Framingham group presented data suggesting loci for sarcopenic obesity (obesity coupled with low muscle mass) or dynapenic obesity (obesity coupled with low muscle strength) may also be associated with fracture.[15]

These pleiotropic loci are arguably the most informative, as they should provide more information about the etiology of disease and gene function than will loci mapped for a single phenotype, and demonstrate the interconnectedness of bone to other physiologic systems. Specifically, the existence of reciprocal signaling between bone and other tissues has been an area of growing interest over the past few years. In an oral presentation by Gorski *et al.*,[16] it was shown that targeted ablation of the proprotein convertase *Mbtbs1* (commonly known as *SKI-1* or *S1P*) in the osteocytes leads to maturity-onset weight gain of ~15% of body mass, but no compensatory increase in skeletal size or mineral content. Identifying the mechanisms by which the body mass increase occurs promises to advance understanding of bone's endocrine functions.

Signaling pathways in osteoblasts. The role of WNT signaling in mediating bone formation and mineralization continues to provide surprises. This year's meeting featured data showing that inhibition of canonical WNT signaling is necessary for normal bone mineralization.[17] These investigators found that mice in which β-catenin is constitutively overproduced display a hypophosphatemic and rachitic phenotype similar to that found in *Hyp* and *Dmp1*-knockout mice. To test the hypothesis that excessive canonical WNT signaling is important in the pathogenesis of hypophosphatemic rickets, they crossed *Dmp1*-knockout mice with mice harboring a *Col1a1* 2.3-kb promoter driving *Dkk1* expression. The mice harboring both engineered genes showed marked attenuation of the rickets and hypophosphatemia. These findings suggest that normal phosphate regulation and mineralization are inhibited by WNT signaling, and that its inhibition in the latter stages of osteoblast maturation is necessary for normal mineralization. These experiments highlight the need to consider that the impact of WNT signaling on the osteoblastic lineage differs according to the differentiation state of the target cells.

Interesting data were presented by a number of groups, demonstrating the importance in osteoblasts function of less-studied genes and pathways. In one of the last oral presentations of the meeting, Nozawa *et al.*[18] present data showing that heparan sulfate synthesis is needed for accumulation of normal skeletal mass, via their use of an osteoblast-specific *Ext1* knockout. *Ext1* encodes exostosin 1, an endoplasmic reticulum glycosyltransferase that catalyzes chain elongation of heparan sulfate. The *Ext1* gene is ubiquitously expressed, but loss of this gene in osteoblasts results in a marked decrease in vertebral BV/TV. Histomorphometry reveals an increase in osteoclast number and, concordantly, urinary deoxypyridinoline is increased in the knockout mice. The authors hypothesize that heparan sulfate functions as a substrate for binding osteoprotegerin in the extracellular space, as osteoprotegerin harbors a heparan sulfate-binding domain.

Conclusions

In summary, the genetics community is leading the bone field in new and interesting directions. It is clear the emphasis in genetics is no longer solely on location of genes and genetic variation, but rather is moving toward meaningful interrogation of function. Further, it has become clear that we cannot just examine bone phenotypes if we wish to understand bone disease. Last, although it is long been appreciated that we need to understand the impact of environment (that is, diet, exercise, and so on) on bone, the analysis methodologies and, more importantly, the comprehensive data sets needed to define the interactive relationships between environment and genes are now being developed. An example of this trend is provided by Dr Reeve's presentation of power calculations for finding gene x environment interactions for factors such as smoking, ethanol consumption, renal function and exposure to gonadal steroids

in the GEFOS and GENOMOS populations, concluding that recruiting an adequate sample is highly feasible.[19]

Conflict of Interest

The authors declare no conflict of interest.

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