

## MEETING REPORTS

### Genetics of Osteoporosis: Meeting Report from the 31st Annual Meeting of the American Society for Bone and Mineral Research

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The genetic study of osteoporosis is one of the most important areas in bone and mineral research. At this year's ASBMR Annual Meeting, great efforts were made in genome-wide association (GWA) studies of osteoporosis. Complementary to GWA studies, gene-gene interaction analysis, functional analysis, and epigenetics have become efficient tools to reveal genetic mechanisms of osteoporosis.

#### GWA Studies

At this year's meeting, the GWA study approach used to identify genes contributing to bone mineral density (BMD) and fracture risk attracted attention. BMD has been identified as the major risk factor for susceptibility to osteoporotic fractures and is currently the predominant study phenotype for osteoporosis. For example, using the dense Affymetrix 500K SNP gene chip method, two genes, parathyroid hormone (*PTH*) and interleukin 21 receptor (*IL21R*) were found to be associated with femoral neck BMD in two independent Caucasian samples (1). In another GWA study, SNPs in the cation channel, sperm-associated, beta (*CATSPERB*) and ADAM metallopeptidase with thrombospondin type 1 motif, 18 (*ADAMTS18*) genes provided evidence of association with femoral neck BMD (2). Interestingly, *ADAMTS18* was previously found to be associated with hip BMD in an independent GWA study (3). Associations of several other polymorphisms with BMD in

previous GWA studies, e.g., SNPs from catenin beta like 1 (*CTNBL1*), low density lipoprotein receptor-related protein 5 (*LRP5*), *osterix* and intergenic regions on chromosomes 2 and 4, were replicated in younger American men and women (4). Based on an Efficient Mixed-Model Association algorithm, one study (5) performed a GWA analysis for BMD and gene expression values using expression SNPs (eSNPs) in the Hybrid Mouse Diversity Panel (HMDP), and identified a novel candidate gene named additional sex combs like 2 (*ASXL2*) for BMD. Besides SNPs, longer sequences of DNA insertions and deletions collectively called copy number variants (CNVs) are also rich and important markers for human diseases. At this year's meeting, one genome-wide CNV association study on osteoporosis found a CNV in one novel gene, vacuolar protein sorting 13 homolog B (*VPS13B*), associated with spine, hip and femoral neck BMD and cross-sectional area, cortical thickness and buckling ratio (6).

Despite some candidate genes associated with BMD that were discovered by GWA studies, few of those genes were associated with osteoporotic fractures. Genetic factors associated with variations in BMD and risk of osteoporotic fractures overlap, to some extent, but are not all identical (7). Recent genetic studies of osteoporosis have focused primarily on BMD, whereas genetic studies using osteoporotic fractures as the

direct study phenotype have been rather limited. However, at this year's meeting, genetic studies of osteoporotic fractures received more attention. For instance, one oral presentation (8) reported a GWA study using Affymetrix 550K SNP chips to localize susceptible genes for incident osteoporosis fractures during an average of 20-years' follow-up. Several associations achieved a genome-wide significance level ( $p < 5 \times 10^{-7}$ ), i.e., SNPs on the fibrillin 1 (*FBN1*) gene and chromosome 1p34. However, in this study several types of fractures, such as hip fractures and non-vertebral fractures, were analyzed together, which may lead to false positive results since fractures at different sites may have different genetic mechanisms (9;10).

Osteoporosis and obesity are closely-related diseases (11). Osteoblasts and adipocytes share the same progenitor, bone marrow mesenchymal stem cells (MSCs), and can transdifferentiate into each other (12). Since there is significant genetic correlation between the two diseases (13), results from a bivariate GWA study to detect pleiotropic genes that may exist for obesity and osteoporosis were revealed at this year's meeting. Four candidate genes, cadherin-associated protein, alpha 3 (*CTNNA3*), phosphodiesterase 3A (*PDE3A*), inositol polyphosphate-4-phosphatase, type II (*INPP4B*) and protein kinase C, theta (*PRKCC*), were bivariately associated with both femoral neck geometry and obesity phenotypes (14). Interestingly, one study (15) indicated that a Monte Carlo-based algorithm that was developed on the basis of a Bayesian block clustering model was suitable to examine pleiotropic SNPs associated with multiple osteoporosis-related phenotypes.

### Gene-Gene Interaction

Recent results from GWA studies have suggested that the risk of fragility fracture is determined by multiple genes. However, these genes collectively account for a small proportion of fracture cases. It has been hypothesized that there exists a network of genes that interact with each other in the determination of fracture risk. An interesting study (16) was designed to test this

hypothesis. The results suggest that several genes, particularly estrogen receptor 1 (*ESR1*), low density lipoprotein receptor-related protein 4 (*LRP4*), tumor necrosis factor receptor superfamily, member 11b (*OPG*), and *SOST* are involved in the determination of fragility fractures, most likely via complex gene-gene interactions. Incorporation of gene-gene (and gene-environment) interactions may be important for future strategies of individualized prognosis and management of osteoporosis.

### Functional Analysis

Further functional studies are needed to confirm the role of SNPs identified by a genetic epidemiology approach. An interesting study (17) used a luciferase reporter gene assay to assess the functionality of SNP rs312009 in the *LRP5* gene that had been found to be associated with lumbar spine BMD in a cohort of postmenopausal Spanish women. Although no significant differences were observed between the two alleles of the SNP in the context of the full wild-type promoter, mutation of the runt-related transcription factor 2 (*Runx2*) binding site produced a decrease in transcription levels only in the context of the C-allele of the polymorphism. Similar efforts to perform functional analysis for statistically significant SNPs have been made in other studies (3) using an electrophoretic mobility shift assay (EMSA) to confirm potential changes in transcription factor binding to the target gene *ADAMTS18* caused by the relevant SNP. Such studies provide the potential next step for GWA studies.

Currently, gene microarray expression analysis is used mainly to search relationships between diseases and certain genes at the mRNA level, which shed light on gene function. When trans-iliac bone biopsies from 84 postmenopausal females from the age of 50 to 85 years were submitted to global gene expression analysis, eight transcripts were found to be significant at a 5% false discovery rate (FDR) level for total hip BMD, explaining 53% of the BMD variation when adjusting for the influence of age (18). Among the 8 genes, only dickkopf homolog 1 (*DKK1*) and *SOST*

showed associations with bone phenotypes previously observed, e.g., *DKK1* levels inversely proportional to bone loss (19), and *SOST* polymorphisms contributing to BMD variation (20;21), while the other 6 genes are novel for bone biology, especially for low BMD. In another gene expression study (22), global gene expression profiles of human trabecular bone, from lumbar spine laminae and iliac crest, upon mechanical loading were compared. Genes serving as markers of bone cells were upregulated in the spine vs. iliac crest although the numbers of bone cells at these two different sites are similar, indicating anatomical and micro-architectural site-related changes in bone cell function.

There is increasing evidence that osteogenic cells are present not only in bone marrow but also in peripheral blood (23) and that these cells can be identified using staining for alkaline phosphatase (AP). Although the underlying biology of circulating osteogenic cells remains to be fully defined, these cells are easily accessible and may provide novel tools to understand the pathogenesis of osteoporosis. One study (24) presented at this year's meeting established the utility of circulating hematopoietic lineage negative (lin-)/AP+ osteoprogenitor cells as novel tools to study gene expression differences between postmenopausal women undergoing rapid vs. slow bone loss, and identified several key pathways (Wnt, interferon, TGF $\beta$ , and prostaglandin) that may mediate bone loss in postmenopausal women.

### Epigenetics

Besides the traditional central dogma involving DNA, mRNA and protein in molecular genetics, recently epigenetic mechanisms have been found to regulate gene expression. Epigenetics refers to reversible, heritable changes in gene regulation that occur without a change in DNA sequence, including microRNA (miRNA) expression, DNA methylation and histone modification (25). Epigenetic regulation has been implicated as a key regulatory mechanism in the etiology of human complex diseases (26). At this year's meeting, results were presented from

several epigenetic studies performed in the bone field.

miRNAs are short non-coding RNA molecules that regulate post-transcriptional gene expression by translational repression and target mRNA degradation. An oral presentation (27) suggested that human miR-151 may be involved in monocyte-related osteoclastogenesis and thus the etiology of osteoporosis. Also, miR-204/211 were found to act as important endogenous negative regulators of Runx2 that promote adipogenesis and inhibit osteogenesis of MSCs (28). In addition, a set of miRNAs, such as miR-140, miR-206, miR-1, miR-133a and miR-133b, were identified to be involved in BMP2-mediated osteoblast differentiation (29). In chondrocyte biology, miRNAs are also essential for normal chondrocyte proliferation and differentiation processes (30).

DNA methylation is a type of chemical modification of DNA that involves mainly the reversible methylation of cytosine. It is one of the most important epigenetic regulatory mechanisms for disease etiology, possibly by changing the promoter activities of the genes that have a fundamental role in disease. In general, high density CpG site regions are hypomethylated. However, one study (31) noticed that a high density CpG-site region (-1112/-888) of the stromal-derived factor 1 (*SDF1*) promoter/enhancer is hypermethylated in human MSCs before induction of chondrogenesis. In addition, the study reported that hypermethylated CpG-sites in this region tended to be demethylated after induction of chondrogenesis. These results suggest that the expression of *SDF1* is tightly regulated in undifferentiated MSCs, possibly, in part, through DNA methylation.

Histone modifications are covalent modifications that occur on the N-terminal tails of histone proteins that protrude from nucleosomes. The altered chromatin configuration can allow or prevent access to the transcription machinery (32). An oral presentation (33) suggested that the homeobox A10-pre-B-cell leukemia homeobox 1 (HOXA10-PBX1) complex acts as an important regulator of chromatin

remodeling to initiate and control osteoblast-related gene expression and commitment of mesenchymal progenitors to the osteoblast lineage. Specifically, HOXA10 was found to be a positive regulator of chromatin remodeling as mesenchymal progenitors isolated from HOXA10-null mice had altered levels of CBP/p300, BRG1 and histone methylation and acetylation reflecting increased chromatin activation. Knockdown of PBX1 by short hairpin RNA (shRNA) disrupted the recruitment of histone deacetylases (HDACs) to osteoblast gene promoters, leading to increased H4K16 acetylation and subsequent gene expression. One study undertaken in genetic hypercalciuric stone-forming (GHS) rats showed that the vitamin D receptor (VDR) regulated calcium transport by transient receptor potential cation channel, subfamily V, member 6 (TrpV6) and calcium-sensing receptor (CaSR) by modifying histone and chromatin structures (34). Together, the newly discovered epigenetic mechanisms for the expression of genes important for bone metabolism await further study.

### Summary

GWA studies are powerful methods to identify novel genes underlying bone metabolism. It is necessary to perform further statistical genetic and functional studies to replicate and confirm these results. In addition, epigenetics is providing new knowledge for understanding the regulation of bone-related gene expression.

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