MEETING REPORTS

Musculoskeletal Genetics and -Omics: Meeting Report from the 32nd Annual Meeting of the American Society for Bone and Mineral Research

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The 2010 Annual Meeting of the ASBMR was held in the gorgeous city of Toronto, capital of Ontario, from October 15-19. The steady number of active participants highlights the importance of this meeting for the field of calcified tissues and beyond. Indeed, this year's meeting was a success in general, and included many new and interesting studies concerning the genetics of musculoskeletal diseases. Recent advances in high-throughput "-omics" technologies have greatly increased the scale and scope of genomics research, and this was evident throughout the highly sophisticated research presented this year. Some highlights of the meeting regarding the molecular epidemiology of osteoporosis and related conditions are summarized here.

Genome-wide Association Studies (GWAS) for Osteoporosis

This year, as in previous years, GWAS have produced multiple interesting forays into the complex genetic regulation of osteoporosis susceptibility. GWAS have become better powered due to the availability of genome-wide genotyping in ever larger samples. Thus, Karol Estrada, a recipient of the ASBMR Young Investigator Award, presented on behalf of the international GEFOS consortium (1) a meta-analysis of 17 GWAS for lumbar spine and femoral neck bone mineral density (BMD), with ~ 2.7 million single nucleotide polymorphisms (SNPs) in individuals of mostly Caucasian (n = 32,000) ancestry. The meta-analysis detected 34 loci associated at stringent, genome-wide significant p-values $\leq 5 \times 10^{-8}$. Some of these 34 loci map within or near

genes involved in pathways relevant to known bone biology, *i.e.*, genes in the WNT pathway (*WNT16, AXIN1, WNT4, LRP5, CTNNB1, GPR177, FOXC2*), genes in the NF-κB pathway (*AKAP11, TNSFR11B, RANK, OPG*) and/or genes coding for some of the SOX family of transcription factors (*SOX4, SOX6, SOX9*). Interestingly, skeletal site specificity and sex specificity were observed for several loci, indicative of the complex genetic architecture of BMD variation and the multiple biological pathways involved in its regulation.

As a trade-off between a large sample size with normally distributed BMD and a sample with extreme BMD values, other investigators chose approaches such as extreme truncate selection. Thus, Duncan *et al.* (2) performed GWAS in 2,073 women (postmenopausal women aged 55-85 years) with either high (z = +1.5 to +4) or low (z = -1.5 to -4) total hip BMD. Following the GWAS, 127 SNPs were studied in replication cohorts from Australia and Europe (including > 10,000 women with normally distributed total hip BMD values). Three novel loci associated with BMD were identified in the discovery at p-values < 10^{-4} and confirmed in the replication set (p-values < 0.05); these pointed out biologically-relevant genes, *GALNT3, LTBP3*, and *CLCN7*, which were not discovered by previous GWAS with large sample sizes of normally distributed BMD. The authors thus make the case for the extreme ascertainment scheme, which corresponds to gains in power for gene discovery. However, three novel genes were discovered, and given that statistical power

depends on several factors, such as effect size, sample size, heterogeneity among studied samples, phenotype definition uncertainties etc., the potential power gain by the extreme ascertainment scheme needs to be further evaluated in numerical studies.

Other Osteoporosis-Related Traits and Conditions

Multiple quantitative traits serve as proxies for the risk of osteoporosis. There is still debate regarding the extent to which the genetic architecture is similar between the measured traits.

Quantitative Ultrasound (QUS)

Alireza Moayyeri, on behalf of the GEFOS Consortium (3), presented a meta-analysis of seven GWAS with heel QUS (12,286 participants) and two additional studies with heel pDXA (1,377 participants). Strong association signals were found for heel broadband ultrasound attenuation (BUA) on chromosome 7q31 (near/in *FAM3C, WNT16* and *C7orf58*) and on chromosome 6q25, as well as suggestive evidence of association on chromosomes 6q22, 16p13, 2p21 and 20q13. The top signal on chromosome 7q31 showed a partial overlap with BMD GWAS in the GEFOS study (see (1) above). This confirms the non-independent nature of DXA BMD and QUS at different skeletal sites; they seem to measure essentially the same properties of bones. The existing genetic correlation between BMD and QUS is not surprising, especially in light of another study, presented by Nguyen *et al*. (4). These investigators studied correlations between BMD and QUS measurements in adult individuals from 33 multigenerational families from the Dubbo Osteoporosis Genetics Study. BMD at the femoral neck (FN), lumbar spine (LS) and total body (TB) was measured by DXA, and speed of sound (SOS) at the distal radius (DR), midshaft of tibia and proximal phalanges was measured by Sunlight Omnisense QUS. Strong genetic correlations (ρ_G) were found between all three BMD measurements (ρ_G from 0.55 to 0.81), as well as between DR SOS and FN BMD (ρ_G =0.52) and LS BMD (ρ_G =0.40), but not with tibial or phalangeal SOS.

Bone Geometry

FN bone geometry is an important predictor of bone strength and risk of hip fractures. Two GWAS for FN bone geometry were also presented, one by a group from the University of Missouri, Kansas City Medical School (5), and another by the GEFOS Consortium (6).

Deng *et al*. (5) studied a sample of 2,500 unrelated Caucasians. A common genetic variant, rs6578987, located approximately 8kb downstream of the insulin-like growth factor 2 (*IGF2*) gene was suggestively associated with two indices of femoral bone geometry: cortical thickness ($p = 1 \times 10^{-7}$) and buckling ratio (BR, $p = 1 \times 10^{-6}$). Given the well-known function of IGF2 in bone metabolism, the study provided compelling evidence that IGF2 may affect FN bone geometry variation and thus the risk of hip fracture.

In a large meta-analysis (9,100 women and 4,100 men) of GWAS by the GEFOS Consortium, Hsu *et al*. (6) found that the most significant association with FN length (FNL) in both sexes was observed on chromosome 10q24, a locus where cytochrome P450 family member *CYP17A1* is mapped. Suggestive associations were found between genes for transmembrane proteins, *TPRA1/GPR175*, and neck-shaft angle (NSA) and between *TMEM38B* and FNL. Also, several female-specific associations were identified, such as an association between forkhead box Q1 (*FOXQ1*) with NSA, and between *OR4K14* and FN section modulus. Larger sample sizes are needed to formally test for sex-specific associations and to fine-map the above genes.

Paget's Disease of Bone

This year's Most Outstanding Clinical Abstract award went to Omar Albagha from the University of Edinburgh, who presented GWAS of Paget's disease of bone (PDB) (7). PDB is a late-onset metabolic bone disease characterized by focal areas of

increased bone remodeling affecting ~ 8% of older men and ~5% of women; its etiology is due primarily to increased activity of osteoclasts. The only mutated gene reported and replicated to date is *SQSTM1*, which encodes the protein sequestosome-1/p62. Therefore this GWAS excluded PDB cases with any *SQSTM1* mutations. In a sample of 750 PDB cases and 2,699 controls, GWAS discovered (and then replicated in an independent set), four top genes: *CSF1,* which encodes macrophage colony-stimulating factor (M-CSF) on chromosome 1p13; *OPTN*, which encodes optineurin on chromosome 10p13; *TNFRSF11A* (chromosome 18q21), which encodes RANK, a critical agent in osteoclast differentiation and function; and *TM7SF4* (which encodes DC-STAMP) on chromosome 8q22. The latter gene is a new addition to already-published PDB GWAS (8) thanks to a larger sample.

Osteoporosis and Related Conditions

The study of bone and mineral tissue biology can offer important insight into the pathophysiology of other processes and conditions; this was convincingly demonstrated by several groups.

Bone and Muscle

The genetics of sarcopenia and muscle dysfunction in relation to bone health was also included in the lectures presented either during the basic science or clinical sessions. Thus, Anna Rufo and Mattia Capulli of the University of L'Aquila, Italy (9) presented work on novel mechanisms underlying low BMD in Duchenne muscular dystrophy (DMD). DMD is an X-linked disease due to various mutations in the dystrophin gene. Furthermore, dystrophin-deficient (*mdx*) mice are a model of human DMD; compared to wild type animals, these mice showed reduced tibial trabecular and cortical bone, with decreased osteoblast and increased osteoclast activity. Besides mechanical failure, additional factors induce low bone density in DMD patients and *mdx* mice, including circulating IL-6 and other local cytokines. The investigators thus propose that IL-6 may link the muscular and the bone phenotype in muscular dystrophies

and therefore, an antibody for IL-6 might serve as a treatment option for DMD bones.

Bone and Cardiovascular Diseases

Both osteoporosis and cardiovascular disease are frequent in the aged population and seem to be associated with the aging process; this might be due to shared environmental or genetic determinants, or both. Kuipers and colleagues (10) calculated the genetic correlations between these two diseases in 377 adult individuals from seven large multi-generational families of African ancestry. They observed that the mean arterial diameter of the common carotid artery (measured by ultrasound) had a significant genetic correlation with total hip BMD measured by DXA (ρ_G = -0.47, P = 0.002), as well as with trabecular vBMD at the radius measured by pQCT (ρ_G = -0.48, P = 0.001).

Coronary artery calcification (CAC) is another measure of subclinical atherosclerosis. Yerges-Armstrong and colleagues (11) evaluated associations of the genotype scores (from LS BMD GWAS of (12)) with CAC measured by electron beam CT, in a modest sample of Old Order Amish. They found that an increasing number of high BMD alleles were associated with lower CAC quantity (p = 0.046), and especially that one BMD SNP on chromosome 13q14 seemed to be associated with CAC. These results indicate that a common genetic link may exist between bone and cardiovascular health.

A Multi-phenotype GWAS Approach to Identify Pleiotropic Effects

The majority of published linkage scans, candidate gene association studies and GWAS are focused on a univariate association analysis, which addresses one phenotype (or one outcome) at a time. Pleiotropic effects, defined as when a gene affects more than one phenotype or disease, have brought increasing attention recently. For example, a genetic variant in the glucokinase regulator gene (*GKCR*) has been reported to be associated with increased concentrations of plasma triglyceride and lower fasting glucose level in

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Europeans (13). In theory, studying correlated phenotypes/traits simultaneously increases statistical power and allows for a more efficient utilization of collected phenotypes. Yi-Hsiang Hsu (14) presented an approach for identifying novel candidate genes with potential pleiotropic effects on bone metabolism and glucose homeostasis. Significant genetic correlations of BMD with fasting glucose and insulin have been observed in the Framingham Study, which indicates that shared genetic determinants may regulate both bone and energy metabolism. Hsu *et al.* performed a multiple-phenotype GWAS analysis in 3,569 adult Caucasian men and women (mean age of 61 years) from the Framingham Study by modeling both BMD (LS and FN BMD) and glycemic phenotypes (fasting plasma insulin, proinsulin, and glucose levels) simultaneously using a newly developed approach, empirical-weighted linear-combined test statistics (eLC; (15)). They first performed univariate GWAS on each of those five phenotypes and then applied the eLC model to combine test statistics from each univariate analysis. Since this statistical approach does not require individual level genotype and phenotype information, the method is advantageous because: (1) GWAS meta-analysis results can be used and (2) it avoids the loss of statistical power due to missing data in one of the studied phenotypes. The most significant bivariate finding (p = 1.8 x 10^{-8}) was for a SNP located in the 3' UTR of the *LRRN1* gene and significantly associated with all of the pairs of traits such as BMD (either LS or FN) with either fasting plasma glucose or with fasting plasma insulin. Of note, the univariate associations were not genome-wide significant for either BMD or glycemic phenotypes (p ~ 1.8 x 10^{-5} for BMD and ~3.2 x 10^{-6} for glycemic traits). An additional three loci, including those located in or nearby *ANKRD44*, *IQCJ* and *TMEM16D* gene regions, also achieved a genome-wide significance threshold for bivariate analysis. From this study, it seems that the bivariate analysis of BMD and glycemic-related traits did identify additional loci/genes that would be missed in univariate GWAS analysis. The study demonstrated a useful approach for identifying pleiotropic effects underlying complex diseases and provided a path towards unraveling the joint genetic determinants involved in bone and other systems. However, more work in this area is surely needed: the function of those reported genes and how they contribute to the reciprocal physiology between bone metabolism and glucose homeostasis still need to be elucidated, and replication in independent samples is still required.

Prediction of Fractures Using Genetic Profiles

The contribution of genetic variation profiling to fracture risk was presented by Bich Tran, a recipient of an ASBMR Young Investigator Award (16). Using actual clinical data from the Dubbo Study, the investigators simulated 50 independent genes with allele frequencies ranging from 0.01 to 0.60 and relative risk from 1.01 to 3.0. Adding a simulated genetic risk score to the usual clinical risk factor model, the area under the receiver operating curve (AUC) increased from 0.77 to 0.88, with an improvement in the accuracy of fracture classification (specificity). In the presence of clinical risk factors, ~25 independent SNPs (a number similar to that identified in GWAS) were required to achieve an AUC of 0.85. These results suggest that profiling of genetic variations could enhance the predictive accuracy of fracture prognosis.

Mouse Quantitative Trait Loci (QTLs) for Osteoporosis

One alternative approach to understanding the genetic architecture of complex traits is to use a more tractable genetic system, such as mouse models. Moreover, a combination of both approaches, human GWAS and QTL analysis in mice, can be productive for novel discoveries and cross-validations. The guiding premise of this look-up approach is that analysis of homologous chromosomal regions in mice with QTLs for BMD may help focus follow-up studies on the genes most likely to affect variation of BMD in mammals across different species, which reduces potential false positive findings based on statistical signal alone. Nielson *et al.* (17) thus selected 3 QTLs strongly linked to BMD:

mouse proximal chromosome 12 (homologous to human chromosome 2p23-25), mid-chromosome 8 (human chromosome 16q12-23), and proximal chromosome 7 (human chromosome 19q12-13.3). They interrogated these regions in humans using the results of a published GWAS meta-analysis on FN and LS BMD in 19,195 individuals (12). For LS BMD, they found 3 novel genes (*GPATCH1*, *RHPN2*, and *PEPD* on chromosome 19q12-13.3) and 2 genes on chromosome 2p23-25 (*ASXL2* and *TPO*), but no genes on chromosome 16q12-23. The authors concluded that, by cross-examining selected human SNPs in regions homologous to previously identified mouse BMD QTLs, novel loci can be identified. This conclusion is similar to that of a mouse QTL meta-analysis (18) that found that 26 of the 28 reported GWAS loci examined were located within the confidence interval of a mouse QTL; 14 of the GWAS loci mapped to within 3 cM of a mouse QTL peak (18). These cross-species studies attest to a joint benefit of using information on syntenic linkages or associations in genetic epidemiological studies. However, of note, the number of mouse QTLs for BMD found thus far is larger than the number of loci found in GWAS of BMD. With the low resolution of the mouse QTL approach, each genomic region linked to a QTL is actually broader than the high resolution GWAS mapping.

Other Approaches to Identify Genes Underlying Skeletal Regulation

In addition to the work described above, several presentations utilized advanced technologies and expanded the landscape of the traditional concept of the SNP-phenotype association approach in the field of genetic epidemiological studies.

Integrative Genomics (Systems Genetics)

Charles Farber (19) presented a "systems genetics" approach to identify novel candidate genes for the regulation of BMD. The investigators' approach was to incorporate information on genotype, expression, and clinical traits together to construct regulatory networks that enable

one to prioritize candidate genes for diseases and improve understanding of disease etiologies. In this study, whole body BMD estimation, gene expression profiling and DNA genotyping were conducted in a group of F_2 mice derived from the C57BL6/J and C3H/HeJ strains. The detailed methods have been published previously (20;21). A network structure modeling, likelihood-based causality model selection (LCMS) was then applied. The authors found *bicaudal C homolog 1* (*Bicc1*) transcript levels to be regulated by a local expression QTL (eQTL), in which this eQTL was coincident with the BMD QTL. Based on the LCMS, *Bicc1* was predicted to be responsible for the effects of this locus. To validate this finding, several additional experiments were conducted, including the BMD measurement of *jcpk(+/-)* mice with heterozygous mutation in *Bicc1* and siRNA knockdown of *Bicc1*. The authors indeed found that *jcpk(+/-)* mice had a reduction of 4.9% in femoral areal BMD; *Bicc1* was also highly expressed in mature osteoblasts of mice. siRNA knockdown of *Bicc1* in primary calvarial osteoblasts resulted in reduced alkaline phosphatase activity and *in vitro* mineralization in a dose-dependent manner. Similar deficiencies were observed in differentiation of primary calvarial osteoblasts isolated from *jcpk(+/-)* mice. These findings suggest a role for the *BICC1* gene in osteoblast-mediated bone formation. In a human sample (adult men and women from the Framingham Study), SNPs located in the first intron of *BICC1* were found to be significantly associated with lower whole body BMD. One potential limitation of this study is that the initial gene expression profiling was performed for fat, liver and muscle tissues, but not bone cells/tissue. Tissue-specific expression has been observed in several studies and genes with specific functions in particular tissues may be differentially expressed across different tissue types. Genes that are only expressed in bone or that have very low expression in fat, liver and muscle tissue will not be identifiable in such a design. Yet, findings from this study highlight the advantage of using an integrative genomics approach to discover novel candidate genes and pathways for bone disorders and phenotypes.

A similar approach was also applied in a recently published GWAS study on BMD and hip geometry (22), which integrated gene expression profiling from human tissues (eQTL and expression SNPs (eSNPs)), animal and cellular models into GWAS analysis and prioritized candidate genes that were later successfully replicated in a larger scale meta-analysis. In addition to genetic variant genotypes, one can incorporate gene expression profiling from animal models, cellular models, and human tissues as well as from epigenetics, transcriptomics and proteomics in a network model, which may lead to improving association signal detection and better understanding of genetic association signals and provide a systems' view of the biological processes underlying disease susceptibility.

Epigenetic Regulation in Genetic Epidemiological Studies

Recently, the "missing heritability" phenomenon has been found in many of the GWAS of complex disorders/traits. Speculations in regard to the low proportion of heritability explained by SNPs have been offered; among them, other sequence variations and non-genetic variations (such as epigenetic regulation) are not considered, which is a serious limitation of the current GWAS approach.

Zhou *et al*. (23) presented a candidate gene approach to study the methylation patterns of CpG islands in the promoter regions of the *CYP2R1* gene and their association with serum 25(OH)D variation in response to vitamin D_3 supplementation. Healthy postmenopausal women were treated with calcium (1500 mg/day) and vitamin D_3 (1100 IU/day) for 12 months. Among the 446 treated participants, the authors selected 18 individuals with the highest dose-adjusted increased serum 25(OH)D levels as "responders" and 18 individuals with the lowest dose-adjusted increased serum 25(OH)D levels as "non-responders" to supplementation. Blood DNA was used for methylation profiling in the promoter of *CYP2R1*. The investigators found a hyper-methylation pattern (30%) in non-responders compared to hypo-methylation (8%) in responders, which suggested that

hyper-methylation in the promoter of *CYP2R1* may contribute to silencing of *CYP2R1* expression and then lead to a lower response to vitamin D_3 supplementation. Although some limitations should be recognized (the sample size is relatively small; the sites of CpG islands and their number were not reported; blood DNA may not be a suitable tissue for this particular experiment (methylation is usually tissue-specific); and there was no adjustment for potential confounders), this study certainly provides proof-of-concept that, in addition to genetic variants, an epigenetic profile may also affect the variation of phenotypes. Genetic variants (rs10766197 and rs12794714) in the promoter of *CYP2R1* were also found to be associated with serum 25(OH)D levels in 496 healthy Caucasian participants. Therefore, it would be interesting to know whether these two genetic variants are located in those estimated CpG methylation sites and whether the genetic variants modify the methylation status due to the substitution of DNA sequence or if both methylation and polymorphisms in the promoter regions have their own independent effect on the function of *CYP2R1*.

MicroRNAs (miRNAs) are non-protein-coding RNAs with a length of 22 (on average) nucleotides of short RNA molecules. The human genome may encode over 900 non-protein-coding RNAs that target about 60% of genes in many human cell types. miRNAs are genetic regulators at the post-transcriptional level by binding to complementary sequences of target mRNAs, which results in gene silencing. Dysregulation of miRNA has been found associated with disease (24). The 3'UTR of mRNA is the usual target site for miRNA. Reppe *et al*. (25) presented a study examining the association between miRNA expression levels (of bone biopsies) and total hip and LS BMD variation in 84 postmenopausal Caucasian women (50-86 yrs. of age). Those women were either healthy or of primary osteopenic or osteoporotic status with or without fractures (26). Bone biopsies were obtained at the same location (2 cm from the iliac crest and 2 cm from the iliac spine). Among 667

miRNAs tested, 24 miRNAs (false discovery rate (FDR) < 5%) were associated with total hip BMD adjusted for age. An additional analysis using a more sophisticated model, Lasso regression, identified 10 miRNAs that explained 32% of total hip BMD variation (9 of these 10 miRNAs were also among the 24 miRNAs at FDR < 5%). Based on prediction, the downstream targets of these identified miRNAs belong to adherens junction, TGF-β and Wnt signaling pathways. This study provides insight into the potential role of miRNAs in the regulation of bone metabolism and remodeling. It will also be interesting to learn whether the expression of predicted target genes is closely correlated with miRNA levels and whether the expression of target genes is also correlated with BMD variations. Further work should determine whether the sequence variants in the miRNAs or in the target genes do affect gene expression levels and, in turn, affect the variation of BMD, since SNPs identified from several GWAS may be located in miRNA coding or target regions. One of the potential limitations of this study is that the relatively small sample size may not have adequate statistical power to detect a moderate effect. The validation of miRNA levels by RT-PCR is also necessary.

A Genome-wide Search for Transcription Factor Binding and Histone Modifications

Transcription factors are essential for the regulation of gene expression. Transcription factors usually contain one or more DNA-binding domains, which attach to specific DNA sequences in the enhancer or promoter regions of adjacent genes that they regulate. Chromatin immunoprecipitation sequencing (ChIP-seq) is dedicated to localizing protein binding sites that may help identify functional elements in the genome. For example, in the case of transcription factors or histones as proteins of interest, one can determine their binding sites throughout the genome. Other proteins allow the identification of promoter regions, enhancers, repressors and silencing elements, insulators, boundary elements, and sequences that control DNA replication. Next-generation sequencing technology combined with ChIP-seq enables a genome-wide look at transcription factor binding.

Imai et al. (27) presented a study identifying androgen receptor (AR) binding sites, in the mouse genome, in MC3T3E1 cells, an osteoblastic cell line treated with 5α-dihydrotestosterone. ChiP-Seq identified 4,018 AR binding sites (p < 10^{-5}). About 7.7% and 5.8% of binding sites were mapped to within 10 kb upstream of the transcriptional start site and 10kb downstream of the transcriptional termination site, respectively. Additional studies are needed to validate the downstream genes that may potentially be regulated by AR. Meyer and Pike (28) reported a study identifying potential RUNX2-responsive genes by ChIP-seq analysis of RUNX2-binding sites on a genome-wide level in the same mouse MC3T3-E1 cells and correlated these sites with enhancer-specific histone marks. Approximately 4,822 RUNX2-binding sites were found. About 15% of these sites were within 5 kb of promoters, 36% were located within introns, and 41% were located more than 5 kb from transcriptional start sites of an adjacent gene. The majority of the identified sites were associated with increased levels of histone H4 acetylation (H4ac). The authors found RUNX2-binding to several transcription factor genes that are involved in osteoblast function (such as Sox9, Sp7, C/ebp-α, -β and -δ, c-Fos and c-Jun). RUNX2 also binds to its own promoters, which suggests that RUNX2 auto-regulates its own expression in osteoblasts. As a proof-of-principle, several previous known RUNX2-regulated genes, including Bglap2, Spp1, Alkp, Col1a1 and Fgf18 were also found to be RUNX2-binding genes in this experiment. A similar approach was used to identify VDR/RXR binding sites in LS180 cells, an intestinal/colon cell line (29). VDR-binding was detected at 728 sites under basal conditions. Interestingly, however, following activation by $1,25(OH)_2D_3$ treatment, the VDR became bound to over 8,150 sites; most of these binding sites are located within introns or distal intergenic regions. Similar to RUNX2-binding sites, many of these binding sites were associated with increased levels of

H4ac as well as RNA polymerase II density. Additional ChIP-seq found that CDX2-binding overlapped with 1,255 VDR-activated binding sites, which indicated a role for CDX2 in $1,25(OH)_2D_3$-related gene expression. Lee *et al*. (30) revealed tissue-specific VDR regulatory regions in bone cells: in MCF-7 cells (estrogen receptor) and in activated T cells (c-FOS). Using immunoprecipitation and co-immunoprecipitation experiments in progenitor cells, Gordon *et al*. (31) found that Pbx1 and Sirt2 co-localize on osteoblast-related gene promoters (osterix (*Sp7),* osteocalcin (*Bglap2*), and bone sialoprotein (*Ibsp*)) resulting in suppression of these genes while cells were actively proliferating. This evidence suggests that Sirt2 is required for Pbx1-mediated repression of osteoblast-related genes. Yasui *et al*. (32) also used a ChiP-seq approach and identified 903 Smad 2/3 target genes in osteoclast precursors (OCPs). Among 903 genes, 18 had histone modification, namely conversion from H3K4me3/H3K27me3 bivalent to H3K4me3 monovalent, with TGF-β treatment. With additional experiments, the authors concluded that the role of TGF-β in osteoclastogenesis is to maintain OCPs in an undifferentiated state and thus support RANKL-induced osteoclastogenesis.

Conclusion

Together with classical genetic epidemiological studies and novel sophisticated statistical techniques, such as multivariate GWAS, the molecular studies presented at the 2010 Annual Meeting of the ASBMR in Toronto offered some tantalizing glimpses of what next-generation research in the genetics of musculoskeletal disorders might look like. New experimental technologies are giving individual labs the opportunity to conduct large-scale genomic studies that were unimaginable just a few years ago. However, the magnitude and scope of information thus generated present a new conundrum in data management, analysis, and result interpretation. Given the quality of the science presented at this meeting, we are confident that the community will find creative and collaborative solutions for these and other challenges.

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