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MEETING REPORT

Skeletal genomics: a report from the annual meeting of the American Society for Bone and Mineral Research (ASBMR 2014)

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Meeting Report from the ASBMR 2014 Annual Meeting, Houston, TX, USA, 12–15 September 2014

The 2014 Annual Meeting took place in Houston, Texas. The meeting had a fair representation of the genetic, genomic and other '-omic' research. **Figure 1** shows a percentage of abstracts dealing with some of the relevant topics, as returned by our keyword search in the total 1534 abstracts (we present relative amounts, as there was a fluctuation in the annual number of abstracts among the 4 consecutive years). One can see that the number of musculoskeletal (MSK) genetic topics remains stable.

One of the goals of genetic research in the skeletal field is by understanding key biochemical and physiological mechanisms to reveal molecular causes of rare and common diseases. This is a time of explosive growth in the field of human genetics, with whole-genome sequencing and bioinformatics driving a transformative paradigm shift; in parallel, powerful animal modeling approaches and advances in epigenetics are transforming our understanding of biological processes.[1] With the advent and proliferation of genome-wide association studies (GWAS), and, more recently, next-generation sequencing methodologies (NGS), allowing more detailed exploration of nucleic acid sequence and structure, we now have unprecedented opportunities to perform genetic studies not possible several years ago.[2] All these new developments had been reflected at the 2014 Annual Meeting.

New Resources for the –OMICS Studies

In an educational session, 'The Role of ENCODE in Advancing Musculoskeletal Research', Dr Elise Feingold, Director of the Encyclopedia of DNA Elements (ENCODE) project, introduced the project to the audience, and Dr Timothy Hubbard provided detailed guidelines for using ENCODE, specifically in MSK studies. ENCODE has 164 different assays including ChIP-seq, histone modification and RNA-seq on 128 cell types. The tutorials of using ENCODE were posted on the project's portal website https://www.encodeproject.org/tutorials. A noncoding DNA variant—for example, identified by GWAS or NGS—can be assigned an ENCODE-suggested functional role through a certain level of linkage disequilibrium. In addition, in the Meet-the-Professor Session on 'Epigenetic Regulators', Dr Jonathan Gordon talked about using epigenetic data including ENCODE to understand bone formation and regulation at the histone modification level. Dr Gordon pointed out that one of the factors to make epigenetic studies successful was a good collaboration with bioinformaticians because such studies require so called 'Big Data' expertise. One of the examples was a DNase-I hypersensitive site (DHS) profiling of the murine osteoblast genome performed by Phillip Tai from University of Vermont, who has identified highly dynamic regulatory modules at non-promoter regions.[3] They probed the presence of open chromatin using DNase hypersensitivity analysis during three stages of murine osteoblast (MC3T3) differentiation using high-throughput sequencing genome-wide and revealed novel transcriptionally active regions with conserved sequence patterns during osteoblastogenesis. Further, Chou *et al.*[4] used DHS data from ENCODE to identify which tissues or cells are most enriched with the DHS signals corresponding to bone mineral density (BMD)-associated loci. Their work pointed out that epigenetic peculiarities of not only osteoblast cells but also human muscle, skin and blood are reflective of BMD.

Matthew Warman and Ugur Ayturk, from Boston Children's Hospital, presented a very well organized and acclaimed Meet-the-Professor Session on RNA Sequencing. This hands-on knowledge contributed to a surge in applications to bone, such as measuring gene expression in mouse cortical and cancellous bone[5] and defining gene regulatory networks of osteogenesis by correlating differential gene expression and histone modification patterns.[6]

Genomic pipelines are thus rapidly producing large volumes of data ('Big Data'[7]); therefore, ingenious approaches and techniques are required to integrate the wealth of genetic data in a biological and medical context. Fortunately, new artificial-intelligence approaches are emerging, increasingly embracing the complex nature of biological systems. Knowledgeable bioinformatics specialists are needed to provide programing and computer science expertise to efficiently process, curate,

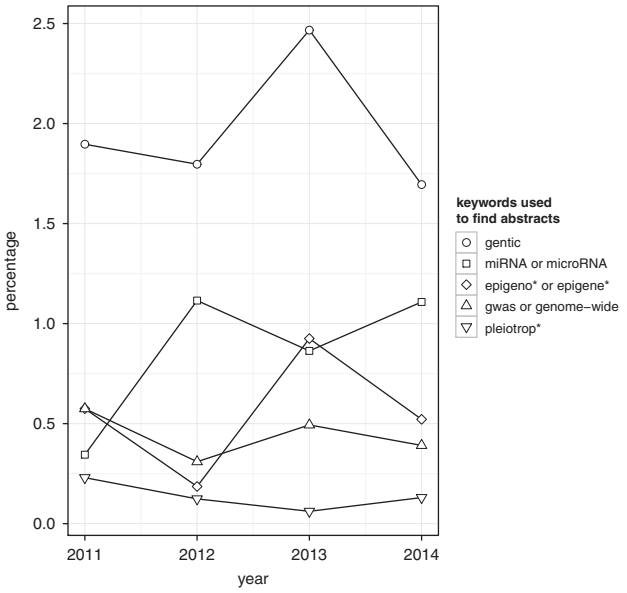

Figure 1 Abstracts dealing with genetic and genomic topics (relative to the total number of abstracts) for the years 2011–2014. The titles of online abstracts were queried for the keywords in the fol. domains: 1. Genetics; 2. Epigenetics; 3. GWAS-related; and 4. microRNA.

archive and analyze vast genomic data sets and to effectively utilize high-performance computing resources. ASBMR, as part of the International Federation of Musculoskeletal Research Societies, recently formed a Work Group on Big Data to promote this mission (see http://www.ifmrs.org/ifmrs-big-data-website-inventory).

Skeletal Genetics: Human Studies

MSK GWAS field has matured; it moves into the direction of confirmation and corroboration of results. Among interesting works worth to mention is a GWAS that HJ Choi [8] performed on a novel phenotype of DXA-derived 'trabecular bone score'. Generally, however, GWAS is gradually replaced with large-scale sequencing efforts in consortia of human cohorts. Hsu *et al.*[9] identified rare protein-coding variants associated with osteoporotic fracture by analyzing exome-chip data of 52 982 adult Caucasians from general population cohorts in CHARGE and GEFOS consortia. Three loci—*RPF1, SVIL* and *NLRC3*—were identified via single (common) variants. Six genes, *PPM1J, WAC, SMPDL3B, DAZL, MRPS23* and *NLRC3,* were identified as associated via gene-based analysis (which combined all-frequency variants). This work demonstrated the promising ability of exome-chip for identifying novel genes and protein-coding variants associated with complex disease. In addition, Zheng *et al.*[10] performed a large-scale whole-genome sequence-based analysis and discovered novel variants influencing BMD in population-based cohorts of Caucasians. The large-scale analysis used 31 508 individuals, including with 2868 whole-genome sequencing data, 2320 with whole-exome sequencing data and 26 320 with imputed genotype data. Several novel variants located near the *EN1* gene were identified to be associated with BMD, including a variant with minor allele frequency of 1.7%. The group then analyzed the bones of En1lacZ/+ knock-in mice and confirmed expression of this gene in bones. They then generated conditional En1Cre/flox muring mutants, who had higher trabecular number and higher tissue mineral density. This work demonstrated a promise sequencing-based study to identify low-frequency and rare variants associated with BMD at a genome-wide scale.[10] It also provided a good example of an intersection of bioinformatics and animal experimentation applied to the MSK genetics. Indeed, a validation is a standard requirement in genetic association studies, whereas the importance of genetic variations has to be confirmed by cellular experiments. Bioinformatics should effectively combine genomics, epigenetics and statistical modeling to inform us what animal experiments could be designed to evaluate and validate results.

'Getting the Best Out of Your Animal Models'

This was the topic of the Basic Evening, co-chaired by Dr Cheryl Ackert-Bicknell and Dr David Rowe. Notable was a presentation by Jorge Henao-Mejia of Children's Hospital of Philadelphia, titled 'One-Step Generation of Mice Carrying Multiple Mutations using Guided Nucleases', in which the presenter reviewed pros and cons of CRISPR/Cas9 and TALen genome editing systems to generate innovative genetically engineered mouse models in extremely short periods of time (months instead of a year).

Also, at this year's meeting, a new animal model was introduced into the MSK genetics field—a zebrafish (*Danio rerio*). Regenerating zebrafish caudal fin is a well-known model of post-amputation's *de novo* membranous bone formation (however, it was not widely studied by the bone experts). Ronald Kwon[11] of University of Washington used a transcriptomic analysis to reveal conserved osteogenic signatures during tail fin regeneration. Both he[12] and a group from Harvard[13] presented a technique of quantitative micro-computed tomography (μCT) analysis of zebrafish bone. This animal model was used as a validation of mechanism of action of a zinc finger protein 521 co-repressor complex in zebrafish osteogenesis.[14]

Pleiotropic Relationships Within and Beyond the Skeleton

Another important observation from this year's meeting is that complexity of biological systems, pleiotropic and epistatic relationships, and inherent redundancy of genetic regulation are well appreciated by the community. Moreover, although the bone is still in the center of the ASBMR's universe, there is more appreciation that it does not act in isolation. Thus, at the symposium on 'Muscles and Bones', in the first day of the meeting, Marco Brotto (University of Missouri—Kansas City) presented a talk on the *Mettl21c* gene, which was predicted to be associated with osteoporosis and sarcopenia by GWAS. Dr Brotto expanded on his efforts to validate this new gene's function in both bone and muscle cell lines. His findings are indicative of the inherent mechanisms of cross talk between muscles and bones. Further, Nuria Lara[15] from the same group reported on a deletion of a single allele of β-catenin in male mice, which results in changes in their muscle function. Notable, this allelic deletion was osteocyte-specific, which again emphasizes the role of this cell type as a master regulator of muscle–bone interactions.

Metabolic and endocrine system's interactions with the skeleton were in the focus of several studies. Thus, recently developed Phylogenetic Module Complexity Analysis (PMCA)[16] was applied to infer regulatory variants in the regions pointed out by multivariate GWAS of femoral neck BMD and metabolic risk factors.[17] PMCA tested variants by analyzing the flanking

region for cross-species conserved motif modules, exploiting evolutionary information while allowing for transcription factor binding site 'turnover'. Bivariate genetic association analysis produced 140 signals, among which the intronic variant at the *ADCY5* locus was suggested to be causal for the genetic correlation between BMD and glucose levels. By similarly applying a bivariate genome-wide association analysis to pediatric bone mineral content and bone area, Kemp *et al.*[18] identified two novel genetic variants, including one in the *KCNJ2* locus. This potassium channel regulator seems to possess pleiotropic functions: it was previously associated with primary tooth development and other development traits.

In a murine model, a pleiotropic quantitative trait locus (QTL) on mouse chromosome 4 (human 1p36), which affected bone size, shape and biomechanical performance, was dissected.[19] The QTL houses *Ece1*, the gene encoding endothelin converting enzyme 1, the gene linked to cardiac defects and autonomic dysfunction. These new data motivated further study of Ece1's biological role in bone.

Another bodily system with omnipresent reach is the intestine, which is a home for host-microbe interplay. Thus, J-Y Li[20] demonstrated that gut microbiota had a pivotal role in the bone loss induced by sex steroid deficiency. To determine the role of microbiota in the bone loss, they (similar to,[21] who reported on the gut microbiota regulation of bone mass in mice) used germ-free mice and control mice housed in standard conditions. This study fitted well into the recent paradigm shift, which focused on metagenomics—rather than intrinsic properties of the host—caused for the development of metabolic and autoimmune diseases.

Prediction of Disease Risk with Genetic Markers

SH Lee[22] performed targeted resequencing of 198 candidate genes in 982 post-menopausal Korean women. They then combined thus discovered functional variants into genetic risk scores (GRS): One with 19 common polymorphisms (SNPs) from 17 genes (GRS-common) and the other with additional 31 rare functional variants from five genes (GRS-total). Accuracy of fracture risk classification in the osteopenic patients was improved 6.8% by adding GRS-common to fracture risk assessment models and was further improved by adding GRS-total (9.6%, $P<0.001$). However, given that the base model (generally measured clinical risk factors such as age, sex and weight) explained about 50% of the risk, this performance did not seem overwhelming. It again has to be emphasized that the virtue of genomic discovery has a more fundamental value than application to diagnostics. Furthermore, the highly polygenic allelic architecture of osteoporosis and osteoporotic fracture, especially allele-by-environment interactions, makes the prediction task thankless. The work in this direction just started, which in the future may afford both prognostication of osteoporosis and personalized prevention feasible.[23]

Concluding Remarks

Continuous success in advancing the field of MSK genetics will depend on collaborations across large multi-disciplinary and multi-professional groups. There is a realization that growing intersection of bioinformatics, statistical modeling and experimentation is necessary as the strongest potential synergy to advance human genetics.[1] The ASBMR Annual Meeting is certainly one of the best avenues for this to happen.

Conflict of Interest

The authors declare no conflict of interest.

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