IBMS BoneKEy. 2011 October;8(10):444-456
http://www.bonekey-ibms.org/cgi/content/full/ibmske;8/10/444
doi: 10.1138/20110537

MEETING REPORTS

Update on the Genetic Basis of Disorders of the Musculoskeletal System: Meeting Report from the 3rd Joint Meeting of the European Calcified Tissue Society and the International Bone and Mineral Society

May 7-11, 2011 in Athens, Greece

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The 3rd Joint Meeting of the European Calcified Tissue Society and the International Bone and Mineral Society took place in the enchanting capital of Greece, the birthplace of democracy and of many wise men of ancient times. Athens has been a wonderful host of magnificent events in modern times, and now it was time to bring together scientists working in the field of musculoskeletal research from Europe and other countries throughout the world. The success of the 3rd Joint Meeting of the ECTS and IBMS was reflected in the attendance of 3,000 delegates and an exciting array of high-quality scientific presentations and discussions. In addition to the invited program, which included 7 symposia, 6 workshops and the annual joint ECTS/ASBMR debate, oral communication and poster sessions were selected from the 600 submitted abstracts. These activities were complemented by new investigator and meet-the-professor sessions, a dedicated training course and an allied health professionals session, thereby maintaining the ECTS and IBMS commitment to ensuring a balanced and successful program of interest to investigators in all disciplines.

The field of genetics was one of those disciplines well-represented at the meeting through a wide range of abstracts that provided an integrated view extending from the cellular and tissue levels up to the organism and population levels. This outcome was the result of applying several state-of-the-art high-throughput techniques for genetic research extending from next-generation whole-exome sequencing to genome-wide microarray genotyping and gene expression profiling, without fully leaving behind traditional applications for single nucleotide polymorphism (SNP) genotyping of candidate gene polymorphisms. Novel findings were reported for monogenetic syndromes of the musculoskeletal system, common disorders of the skeleton (*i.e.* Paget's disease) as well as for the common form of osteoporosis. Another interesting highlight was the overlap of the genetic determinants of osteoporosis with other chronic conditions like osteoarthritis and diabetes mellitus.

From the molecular perspective, it was interesting that several bone pathways arose as prominent across several research efforts. Undoubtedly the Wnt signaling pathway was corroborated as a critical pathway related to bone formation and shown to influence the risk of several musculoskeletal diseases. Several abstract presentations complemented the symposium on "LRP5 and Wnt Signaling in Bone" that included invited speakers Matthew Warman, Bart Williams and Michaela Kneissel. All three speakers provided a comprehensive view of the intricate properties of Wnt signaling regulation, some of which merit further highlighting here.

Wnt Signaling and Bone

Matthew Warman's talk (1) focused on explaining how LRP5 functions to produce high bone mass. Under normal conditions

LRP5 is inhibited by DKK1 or sclerostin (*SOST*). Dr. Warman's murine models elegantly show how the high bone mass phenotype can be the result of missense mutations in *LRP5* affecting the canonical Wnt signaling pathway, mainly through reduced DKK1 inhibition of the receptor. On the other hand, loss-of-function mutations in *LRP5* can cause low bone mass by producing variations in the amino-terminal of the receptor that lead to increased ligand affinity or reduced repression by DKK1 or *SOST*.

There are several potential therapeutic applications targeting DKK1 and *SOST*. This is of special interest considering the limited number of anabolic compounds available for the treatment of osteoporosis. Recently, it has been proposed that DKK1 and/or *SOST* could be influenced by PTH (2), which is one of the few currently approved anabolic treatments for osteoporosis, *i.e.*, teriparatide (TPD). While at the beginning of TPD treatment a strong turnover effect is observed, after 12 months of use the effect diminishes. Idolazzi and colleagues (3) tested if this phenomenon is correlated with changes in either DKK1 or *SOST* levels. They studied 55 women in a randomized controlled trial for 18 months, randomizing subjects to either TPD (20 mcg/daily) or placebo. While no differences were observed in *SOST* levels, the results indicated that long-term (> 12 months) treatment with TPD was associated with an increase in serum levels of DKK1, which in turn might be associated with TPD's declining pharmacological efficacy. These results suggest again that inhibition of DKK1 is a potential anabolic strategy for the treatment of osteoporosis, considering that DKK1 is a negative regulator of osteoblast differentiation and hence of bone formation. This potential has been illustrated by results from murine models. While complete deletion of Dkk1 leads to early embryonic lethality, McDonald *et al*. (4) showed that > 50% of *Dkk1(-/-);Wnt3(+/-)* mice (HOM/HET) are viable. Whole body BMD was increased by 14% in female HOM/HET mice vs. wild-type (WT/WT) mice ($P < 0.01$). MicroCT measurements were even more remarkable in the HOM/HET group, showing up to a 416% increase in trabecular bone mass compared to the WT/WT group. Cortical bone volume was also increased by 25%, with overall bone formation rates increasing by 58%.

Dr. Warman (1) also emphasized additional aspects of the biology underlying *SOST* activation, specifically highlighting the influence of mechanosensing. His experiments showed how sclerostin levels decline with loading, while *Lrp5*(-/-) mice showed no response to mechanical loading. Such effects were also evident in heterozygous mice. These findings with regard to mechanosensing are nicely corroborated at the cellular level by the work of Galea *et al*. (5) showing by quantitative real-time PCR gene expression methods that the regulation of *SOST* by mechanical strain involves activation of the EP4 receptor and the ERK signaling pathway in Saos2 osteoblast-like osteosarcoma cells.

At the human population level, the relationship between high bone mass and sclerostin levels was studied by Gregson *et al*. (6) within the setting of the DINAG consortium. They collected a case set of individuals with high bone mass not carrying *SOST* mutations and compared their sclerostin levels with controls. Sclerostin levels increased with age in both cases and controls but were more prominently elevated in cases. Under the assumption that a reduction in the expression of sclerostin is related to an increase in BMD, these results could indicate that the elevated sclerostin levels in high bone mass cases reflect a regulatory response as part of a feedback loop rather than a primary disturbance. The authors concluded that sclerostin levels are elevated in high bone mass cases, possibly as a compensatory response to reduced strain associated with having increased bone mass. Another study examining 19 patients with sclerosteosis from van Lierop *et al*. (7) showed an absence of circulating sclerostin and increased amino-terminal extension propeptides (PINP) levels in these patients. In addition, the authors showed a clear gene-dose effect on circulating sclerostin levels. In this way, lower circulating sclerostin levels in carriers could represent decreased synthesis of sclerostin

by osteocytes, leading to less inhibition of bone formation.

In general, these studies on sclerostin production may lead to the development of alternative therapeutic strategies for enhancing bone mass. In fact, some progress has already been made in that direction, as presented by Xiaodong Li (8), who gave an overview of how Amgen has applied this knowledge to create a sclerostin antibody that showed a 25% increase in lumbar spine (LS) BMD in ovariectomized rats. Recent results from a phase I trial in humans showed a dose-dependent effect with increased bone formation and, importantly, also decreased bone resorption. Current results from phase II trials will be presented at a future meeting. Arasu *et al*. reported their latest results from a case-control study of 230 cases with hip fracture and 270 randomly selected controls in the Study of Osteoporosis Fracture (SOF) (9) comprising 9,704 women. Women in the highest quartile of sclerostin levels were older and had higher BMD. In addition, they had 1.9-increased odds for fracture (95% CI, 1.2-3.5) per SD increment in sclerostin levels. Contrary to expectations, a positive correlation between sclerostin levels and BMD was observed, where the odds ratio (OR) increased to 3.4 after correction for BMD. The authors concluded that the effects on incident fracture are independent from those on BMD, while an effect through bone turnover remains to be investigated. Another potential therapeutic application for sclerostin was suggested by the work from McDonald *et al*. (10) examining bone repair in the *SOST* knockout (KO) mouse. Their findings show that an absence of sclerostin resulted in enhanced endochondral ossification and a denser hard callus, implying potential for more rapid fracture repair.

Bart Williams described experiments evaluating the roles of the Wnt ligands, Lrp5 and Lrp6, in regulating bone development (11). Induced β-catenin (*CTNNB1*) mutations result in severe low bone mass likely inducing the death of newly-born mice (seemingly by bone failing to keep pressure on vital organs). A phenotype similar to the β-catenin KO can be obtained by simultaneous mutation of both *Lrp5* and *Lrp6*. While *Lrp5* mutations seem to produce a bone phenotype exhibiting reduced cortical thickness, increased trabecular space and reduced trabecular volume, effects of *Lrp6* mutations seem to be related to an earlier onset of low bone mass phenotypes. On the other hand, the authors' efforts assessing the 19 Wnt molecules described thus far for the signaling pathway were based on mutating one of the recently identified proteins responsible for Wnt secretion, i.e. Wntless (Wls), formerly known as *Gpr177*. The consequences of mutating *Wls* were as strong as those observed by producing β-catenin mutations whose critical effects on human bone biology are highlighted by a recent GWAS meta-analysis that has identified common variants in *WLS*/*GPR177*, *CTNNB1* and *LRP5* influencing BMD variation (12).

Michaela Kneissel spoke about the "conundrum" of Wnt/β-catenin signaling in bone, focusing particularly on *SOST*/Lrp5 interactions and the ongoing dilemma surrounding the proposal that gut-derived serotonin depresses bone formation and the plausibility that the positive effect of Lrp5 on bone formation could be exerted via inhibition of serotonin synthesis in the gut (13). Data from different KO mouse models presented by Brommage *et al*. during the meeting (14) showed no evidence to support an interaction of circulating gut-derived serotonin and bone mass. They first evaluated blood and intestinal serotonin levels in *Lrp5* KO mice with low bone mass. Second, they evaluated bone mass in *Tph1* KO mice with low intestinal serotonin levels, and finally they tested bone mass in normal and ovariectomized mice treated with LP-923941, an inhibitor of Tph1. Results of the first experiment showed that *Lrp5* KO mice had normal serotonin levels in the circulation and the gut, as well as normal *Tph1* expression levels in the duodenum and colon. Their subsequent studies showed that, compared to WT animals, *Tph1* KO adult mice exhibited no essential differences in bone mass, the bone volume to trabecular volume ratio or cortical thickness measured at the fifth lumbar vertebral body, distal femur metaphysis or mid-shaft femur. In contrast to the positive control group treated

with TPD that had increased serum levels of amino-terminal extension propeptides (PINP) and bone mass at all three skeletal sites, Tph1 inhibition reduced serotonin levels in the blood and gut, but no changes in bone mass in the sham ovariectomized osteoporosis model were observed. Controversial increases in bone mass after pharmacological Tph1 inhibition (observed in C57BL/6 mice), together with increased bone mass (in growing but not in older adult *Tph1* KO mice), point to potential different roles at the local and endocrine levels.

Additional work from Dr. Kneissel illustrated how osteocyte Wnt/β-catenin signaling is required for normal bone homeostasis, exerting effects on both bone formation and resorption. Under normal conditions, *SOST* blocks β-catenin signaling upon Lrp5/6 activation. The low bone mass phenotypes induced by loss-of-function *SOST* mutations require intact β-catenin to have an effect on bone resorption as shown in homozygous *Dmp-1*-Cre (osteocyte-specific, β-catenin-deficient) mutant mice (15). These *Dmp-1*-Cre mice display no obvious abnormalities at birth, but their bone mass accrual is strongly impaired by early-onset bone loss affecting both the axial and appendicular skeleton. Such a low bone mass phenotype is not the result of impaired bone formation (having normal osteoblast function and osteocyte density), but is instead the consequence of increased osteoclast number and activity.

Genome-wide and Candidate Gene Association Studies of Osteoporosis Traits

The most recent results of the Genetic Factors of OSteoporosis (GEFOS) Consortium were presented by Estrada and colleagues (16) and represented thus far the largest study of its kind, involving a meta-analysis of 2.5 million HapMap-imputed SNPs from 17 GWAS of LS and femoral neck (FN) BMD pursued in more than 32,000 individuals. The meta-analysis yielded 82 loci associated with either FN or LS BMD, of which 34 reached genome-wide significant (GWS) levels ($P < 5 \times 10^{-8}$) and the remaining 48 reached suggestive levels ($P < 5 \times 10^{-6}$) and which together explain up to 6% of the variance in BMD. These 82 top-associated SNPs were tested for association with the risk of any type of osteoporotic fracture in 6,900 cases and 21,500 controls drawn from the 17 BMD studies. In a literature-based pathway analysis, the authors concluded that a large proportion of the identified BMD loci contain genes clustered within the Wnt signaling, OPG-RANK-RANKL, and the mesenchymal cell differentiation biological pathways. Once again the Wnt pathway appeared prominent in these results, with several loci mapping within or near genes involved in Wnt signaling (*WLS*, *WNT4*, *WNT16*, *AXIN1*, *LRP5*, *CTNNB1*, *LRP4*, *SP7*, and *RUNX2*). Six of the 82 BMD loci tested for association with osteoporotic fracture risk remained significant after Bonferroni correction ($P < 6 \times 10^{-4}$) and included variants mapping to 2p16, 7q21.3, 10q21.1, 14q32.12, 4p16.3 and 18p11.21. The most significantly associated variant mapped to a protein of unknown function in the 18p11.21 locus and had the G-allele associated with increased osteoporotic fracture risk at the GWS level (OR = 1.13 [95% CI, 1.09-1.17], $P = 4 \times 10^{-13}$). All 82 loci are undergoing additional replication in a sample from more than 80,000 independent individuals. These results show for the first time how these pathways have relevance for skeletal biology at the human population level and confirm BMD as a surrogate phenotype for genetic studies of fracture risk.

In addition to the several Wnt factors identified by the GWAS approach, other independent candidate gene studies postulated additional Wnt factors and other elements from other biologic pathways to be involved with osteoporosis-related traits. One of these investigations evaluated genetic variation in the *LRP4* gene within the Odense Androgen Study (17), including four common non-synonymous coding polymorphisms located in the extracellular region of the LRP4 protein. The study included 1,404 Danish men and found the most significant associations with total body BMD ($P = 4.7 \times 10^{-5}$) and two SNPs associated with different bone geometry parameters, confirming previous results from GWAS studies (12;18;19). Researchers from the same group prioritized variants in

the secreted frizzled-related protein 4 (*sFRP4*) gene to be studied in younger men from the Odense Androgen Study (20). The selection of *SFRP4* as a candidate gene was based on the low BMD phenotype of the senescence-accelerated mouse P6 (SAMP6), which was previously shown by Nakanishi *et al*. to result from overexpression of *Sfrp4*, a modulator of the Wnt signaling pathway (21). By binding to Wnt ligands, sFRP4 is able to suppress osteoblast proliferation *in vitro*. Boudin *et al*. found that common genetic variation mapping to *sFRP4* is associated with FN BMD and several hip geometry parameters (20).

Additional loci and candidate gene studies were presented by Annie Kung and colleagues focusing on Chinese populations. A linkage and gene-based association study replicating four previously-identified significant quantitative trait loci (QTL) for BMD variation on chromosome 2q24, 5q21, 7p21 and 13q21 was performed on 1,459 individuals from 306 pedigrees of Southern Chinese origin. These investigators reported successful replication of the linkage signal on chromosome 5q21 for FN BMD with a LOD score of 1.38 (nominal p value = 0.006) (22). Subsequent QTL-wide gene-based association analysis in 800 subjects with extreme BMD identified *CAST* and *ERAP1* as novel BMD candidate genes in the identified locus. *In-silico* mRNA expression data analysis determined that the expression of *CAST* was significantly decreased upon *BMP2* stimulation in a MC3T3-1b osteoblast cell line. These findings provide further evidence that 5q21 is a BMD QTL, and that variants in *CAST* and most likely *ERAP1* may be associated with FN BMD variation. In a second study, Xiao *et al*. postulated periostin (*POSTN*) as a novel candidate gene for BMD variation and vertebral fracture risk. Periostin is an extracellular matrix protein secreted by osteoblasts that is also involved in the regulation of osteoblast differentiation and bone formation. Variants in *POSTN* were investigated in an extreme truncate design of BMD levels involving 1,572 subjects with replication assessed in 2,509 additional subjects. The minor allele of a SNP located 2.3 Kb upstream of *POSTN* was significantly associated with lower BMD (P = 0.0007) and 1.3-times higher risk of vertebral fracture (P = 0.007). Another study by Swanberg *et al*. assessed the influence of genetic variation in the major histocompatibility complex (MHC) locus *MHC2TA* in relation to BMD levels, bone loss and fracture risk in young and elderly Swedish women (23). This is an interesting new study after the initial reports from early GWAS (18) claiming association of variants in the MHC region as influencing BMD variation. This candidate study showing *MHC2TA* SNPs affecting MHC expression and showing association with BMD and fracture risk merits further investigation, in a more powered setting, to determine an effect of the MHC region on BMD and fracture that is not resulting from the proneness of the associations in the locus to be driven by population stratification.

Loci containing genes coding for proteins that are part of the OPG-RANK-RANKL pathway have also been identified as being prominently associated with LS BMD in the current and previously reported GWAS meta-analyses (16;18;19;24). A series of candidate gene and functional studies highlighted the critical importance of this pathway in bone biology. In a very elegant study, Formosa *et al*. provided robust evidence of functionality for SNPs in the osteoprotegerin (OPG) gene (*TNFRSF11B*) using RNA splicing analysis (25). Three functional SNPs in the OPG gene were previously reported as associated with LS BMD in Maltese women (26;27). In the current study, the authors showed how a haplotype assembled from such functional SNPs results in expression of lower quantities of the full OPG transcript, which in turn is associated with higher bone resorption. Another study by Niti and colleagues (28) evaluated the functional consequences of *RANKL* over-expression in a novel transgenic model in so-called transgenic-human (Tghu) RANKL mice. Quantitative bone analysis revealed a mild phenotype in a low copy number Tghu-RANKL line, which presented with trabecular bone loss as well as reduced biomechanical properties. In the high copy number Tghu-RANK line, trabecular bone loss developed together with severe cortical bone porosity

and decreased bone strength. In addition, the Tghu-RANKL mice were able to rescue the osteopetrotic phenotype of mutant mice expressing an inactive form of endogenous RANKL. The results of this transgenic model show that the human RANKL protein is fully active in the mouse.

The Genetics of Paget's Disease of Bone

After the successful discovery of genetic variants in *CSF1*, *OPTN* and *TNFRSF11A* associated with Paget's disease of bone in a GWAS on 750 cases and 1,000 controls (29), this year Albagha and colleagues reported findings from a subsequent effort. In their current study they increased the sample size of their original GWAS by including 2,700 additional controls and tested for replication in an independent set comprising 500 cases and 500 controls (30). Applying this strategy they found another intronic variant near the *TM7SF4* gene associated with a 1.4-times increased risk for Paget's disease ($P = 7.4 \times 10^{-17}$). To identify the functional variant driving this association, they sequenced the promoter and coding regions of this gene in 50 cases and 10 controls with standard DNA sequencing methods. After genotyping the additional 500 cases and 500 controls, a variant in the promoter region showed consistent association (OR = 1.32; combined $P = 3.1 \times 10^{-8}$). Then, using a luciferase reporter assay, they showed that the risk allele increased protein expression by 20% compared to the non-risk allele. These results suggest this is the functional variant driving the association in the GWAS report that has been published recently (31).

While juvenile Paget's disease is a rare disorder caused by inactivating mutations in *TNFRSF11B*, mutations in the OPG gene have never been found in patients with the common adult form of Paget's disease. A previous study in Belgian and British samples suggested that common genetic variants in *TNFRSF11B* were nominally associated with Paget's disease with evidence for sex-specificity in males (32). In the current study presented by Alonso and colleagues (33), 32 variants in *TNFRSF11B* were tested in a gender-stratified analysis within a larger dataset including 750 cases and 2,700 controls. While a nominally significant association was observed in males, this association was not significant after adjusting for multiple testing. Hence, the authors concluded that common variants at this locus do not seem to play a major role in regulating the risk of Paget's disease.

Another study conducted by Obaid and colleagues examined a SNP in the *OPTN* gene in relation to disease severity and complications of Paget's disease in a case collection including 635 patients without *SQSTM1* mutations from the PRISM trial (34). *OPTN* encodes optineurin, a ubiquitous cytoplasmic protein with a possible role in the NF-κB signaling pathway, but its role in bone metabolism is yet to be defined. A disease severity score (range 0-46) was devised based on several clinical features including the number of affected bones, clinical evidence of bone deformity, the presence of bone pain, bone fractures, a need for orthopedic surgery, and the use of a hearing aid for deafness. In addition, quality of life was assessed by the SF36 physical summary. The risk allele of the common variant in *OPTN* was significantly associated with both Paget's disease risk ($P = 6.9 \times 10^{-13}$; OR = 1.5) and higher severity score (6.02 ± 0.11) compared to non-carriers (5.39 ± 0.11; P = 0.03). There was also a trend (P = 0.09) for reduced quality of life scores in patients who carried the risk allele (36.8 ± 1.41) compared to those who did not (39.3 ± 0.5). The authors say that confirmation in larger studies is needed, yet conclude that these findings could be of clinical value in identifying patients who are *SQSTM1*-negative and are at risk of developing severe disease.

Developmental Genetics

During an invited speaker session, Stefan Mundlos showed how the process of bone formation in the perichondrium is controlled by Hox genes (35). These genes encode homeodomain-containing transcription factors and are arranged in four gene clusters (Hoxa, Hoxb, Hoxc, and Hoxd) that comprise to date a total of 39 genes in humans. Their expression patterns are differentially regulated during development, particularly for the Hoxd cluster, whose

repression depends on a chromosomal region upstream of Hoxd13 (36). Mundlos showed that the process of bone formation in the perichondrium is controlled by *Hoxd13* together with *Hoxa13* via the control of BMP and *Runx2* expression; his results also show how Hoxd13 is essential but not sufficient to induce bone formation. In another study, Eelen *et al*. showed results highlighting how the cluster of FoxO transcription factor genes plays an important role in skeletal homeostasis; their goal was to determine the role of FoxOs in growth plate chondrocytes during endochondral ossification. They showed that mice lacking *FoxO1* displayed a significant increase in the length of the hypertrophic zone of the growth plate compared to control littermates. This feature was most obvious in the distal growth plates of the tibia and radius. Serum levels of osteocalcin, TRAP5b and CTX were also found to be significantly increased as compared to WT. Overall, these data show that the expression of FoxO transcription factors in chondrocytes is indispensable for normal growth plate organization and control of skeletal growth and bone volume. Future work is needed to elucidate FoxO target-gene populations, as well as the role of indirect FoxO action on other transcription factors in the control of bone cell biology, particularly with regard to molecular pathways like Wnt/β-catenin that are controlled by FoxOs (37).

The Genetics of Other Bone Diseases

Emma Duncan and colleagues presented late-breaking results on mapping genes for skeletal dysplasias using state-of-the-art whole-exome sequencing technology (38). There are more than 380 different skeletal dysplasias described in humans, usually of very rare occurrence with a prevalence ~2.5 per every 10,000 births. Nevertheless, their investigations have proven relevant for the understanding and potential treatment of common diseases; studying rare conditions such as sclerosteosis and van Buchem disease has played a pivotal role in the development of the promising anti-sclerostin treatment for osteoporosis (8). In this way, ascertaining genes for skeletal dysplasias has been quite successful. Genetic determinants for more than 200 of them have been identified using traditional linkage mapping. Nevertheless, collecting pedigrees with a sufficient number of meioses to run linkage is not always feasible, particularly considering such severe phenotypes are likely to affect survival and reproductive potential. Given the relatively rare minor allele frequencies of the genetic determinants underlying the skeletal dysplasias, GWAS are unlikely to be successful for their identification, and practically impossible to map when rare *de-novo* mutations are involved. Duncan *et al*. showed quite elegantly the potential of using whole-exome sequencing for the identification of genetic candidates underlying the occurrence of skeletal dysplasias of unknown origin. They studied a family including three affected siblings negative for mutations in the *RMRP* gene responsible for the anauxetic dysplasia syndrome (OMIM#607095). After exclusion filtering of known non-deleterious variants, they narrowed the search to ~100 variants with deleterious potential. Using a Mendelian analysis approach, they concluded that a compound heterozygous model was the only one fitting the disease transmission mechanisms. Four genes fitted this mechanism, of which only *POP1* was found to be invariably predicted to result in altered protein function by diverse bioinformatics algorithms. Two damaging mutations in a highly evolutionary conserved region were shown to affect *POP1*, which is the largest constituent of the RNAse MRP complex that interacts with *RMRP* (39). A similar success story was shown for yet another unknown dysplasia, highlighting how whole-exome sequencing is a promising technology for the identification of rare Mendelian disorders.

A series of other studies also focused on the underlying genetics of rare bone diseases. Kühnisch *et al*. showed how a novel skeletal disorder found in a large Turkish family, characterized by collateral ligament calcifications of small joints, progressive spondyloarthropathy, osteopenia, mixed hearing loss, mental retardation, and absence of obvious articular cartilage mineralization, was caused by a missense mutation in *ANKH* (40). ANKH is a membrane protein involved in the regulation

of pyrophosphate levels, and so these findings nicely corroborate findings from Ank KO mice and demonstrate a loss-of-function despite normal protein expression. In another study, Sarrion *et al*. performed a mutation analysis of the exons and flanking regions of the *EXT1* and *EXT2* genes in 18 Spanish patients with multiple osteochondromas (41). Osteochondromas are the result of excessive chondrocyte proliferation and bone growth at the juxtaepiphyseal regions of long bones; though benign they can result in short stature and in some cases malignant transformations. Even though *EXT1* on 8q24 and *EXT2* on 11p11-p13 are known loci containing mutations causing multiple osteochondromas, the authors reported 17 null mutations of which eight are novel. Finally, Coudert *et al*. analyzed the transcriptome of differentiated osteoclasts from 16 autosomal dominant osteopetrosis type II (ADO II) patients and 31 controls using an Illumina BeadChip array (42). ADO II is a rare disorder characterized by increased bone mass. This disease is explained primarily by mutations in the *CLCN7* gene but the mechanism by which these mutations act is unknown. Coudert and colleagues found that the integrin B5 gene was 1.5-times up-regulated and the perforin gene was 2-times down-regulated in the ADO II case expression profiles as compared to those of controls. The authors conclude that these two genes are potential novel drug targets for the treatment of this rare disease, though replication is required.

The Genetics of Osteoarthritis

The ECTS/IBMS meeting offered an unprecedented number of studies on osteoarthritis (OA), with a broad range of basic molecular and genetic studies demonstrating the importance of known bone pathways in the pathophysiology of OA. An expression study by Zupan *et al*. using qPCR showed that in 31 patients with OA and 23 with osteoporosis, *TNFRSF11A* (RANK) was three-fold and *TNFSF11* (RANKL) was six-fold over-expressed in osteoporotic than in osteoarthritic tissue. This study suggests that differential expression of genes coding for RANK and RANKL may play a role in the two conditions.

A series of other studies postulated how Wnt signaling pathway factors may also play a role in the pathophysiology of OA. In an expression study by Delgado-Calle and colleagues, the authors compared β-catenin levels in osteoblast cultures from patients with fractures to those from patients with OA (43). They also explored whether differences in sclerostin expression could explain differences in Wnt activity. They found less nuclear β-catenin in osteoblast cultures from patients with fractures than in those with OA. Yet, they found no differences in β-catenin gene transcription, consistent with possible decreased Wnt pathway activity. No differences were found in *SOST* and/or alkaline phosphatase expression. The authors concluded that compared to the profiles observed in osteoblasts derived from patients with OA, Wnt activity is reduced in patients with hip fractures, but likely not through increased sclerostin expression. In contrast, another study by Abed and colleagues examining primary human subchondral osteoblasts derived from the tibias of patients with OA undergoing knee arthroplasty did find differences in *SOST* expression and reduced Wnt/β-catenin signaling (44). As compared to controls, elevated *SOST* expression was present in osteoblasts derived from OA patients that also exhibited abnormal mineralization. The authors propose such elevated *SOST* expression can be responsible for the observed reduced Wnt/β-catenin signaling and subchondral bone sclerosis due to abnormal osteoblast functions seen in the pathogenesis of OA.

Two studies using murine OA models also studied gene expression. Chen *et al*. showed how mice with postnatal β-catenin conditional activation display severe defects in intervertebral disc tissue with significant loss of growth plate cartilage and extensive osteophyte formation (45). They also showed that *Mmp13* and *Runx2* were over-expressed in chondrocytes. These findings indicate that activation of β-catenin induces Mmp13 expression in a Runx2-dependent manner. The other study used a mouse KO model to evaluate the influence of estrogen

on the development of OA (46). Here Azuma and colleagues examined the joints of estrogen receptor alpha KO (ERαKO) mice. They found that the cartilage of ERαKO mice was significantly thinner than that of WT female mice. Gene expression analyses using microarrays in chondrocytes of ERαKO mice were performed to further examine estrogen signaling in chondrocytes. These analyses showed that cholecystokinin and *Sfrp1* gene expression profiles were decreased in ERαKO mice compared to those of WT mice. These results suggest that estrogen interactions with cholecystokinin and the Wnt signaling pathway potentially modulate chondrocyte differentiation.

Finally, Castano-Betancourt *et al*. examined a SNP in the *GDF5* gene previously shown to be associated with height (47), OA (48) and fracture risk (49) in relation to hip morphology (50). The authors propose that *GDF5* variants are associated not only with bone length but also with other hip geometry parameters, such as bone width and variation in femoral head shape, which have been related to hip OA and can explain the observed association with fracture risk.

Conclusion

The molecular and genetic studies presented at the 3rd Joint Meeting of the European Calcified Tissue Society and the International Bone and Mineral Society provided valuable insight into diverse areas of musculoskeletal research at the basic, translational and clinical levels. The most striking feature of this research is the confluence of genetic discoveries across different phenotypes (*e.g.*, Wnt factors) arising from molecular studies at the cellular, tissue and organismal levels and genetic studies at the patient and population levels. This validates the call to unite approaches across fields and to extend the current scope of genetic research to include different aspects of systems biology. Undoubtedly this more holistic approach will advance knowledge and most importantly allow for translation of exciting new discoveries into the clinical realm.

Online links

Abstracts: http://www.ects-ibms2011.org/prog/planner.htm (ECTS login required)

Webcasts: http://web28.streamhoster.com/ects/athens2011/webcast.html

Acknowledgements

The authors wish to thank Martha Castano-Betancourt for her help in compiling the abstracts from the field of osteoarthritis and Amanda Sherwood, Executive Director of the European Calcified Tissue Society (ECTS) for providing reference material for this manuscript.

Conflict of Interest: None reported.

Peer Review: This article has been peer-reviewed.

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