ASBMR Symposium

Bone-omics: Translating Genomic Discoveries into Clinical Applications

September 15, 2016
Georgia World Congress Center
Atlanta, Georgia

ASBMR® 2016
Annual Meeting

GENERAL MEETING INFORMATION

Registration
Registration desks will be open for new registrants in the Georgia World Congress Center in the Registration Hall, Main Entrance on **Thursday, September 15 from 7:00 am – 6:00 pm.**

Speaker Ready Room
Speakers must check into the Speaker Ready Room in advance of their presentation. At that time, speakers may review their slides. The Speaker Ready Room is located in Room A406 in the Georgia World Congress Center. The Speaker Ready Room will be open on **Thursday, September 15 from 7:00 am – 5:00 pm**

Organizing Committee
Douglas Kiel, MD, MPH
Fernando Rivadeneira, MD, PhD
Cheryl Ackert-Bicknell, PhD
Eric Orwoll, MD

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The American Society for Bone and Mineral Research (ASBMR) recognizes the generous support provided by:

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This activity has been planned and implemented by Creighton University Health Sciences Continuing Education (HSCE) and The American Society for Bone and Mineral Research (ASBMR) for the advancement of patient care. Creighton University Health Sciences Continuing Education is accredited by the American Nurses Credentialing Center (ANCC), the Accreditation Council for Pharmacy Education (ACPE), and the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing education for the healthcare team.

AMA PRA Statement
Creighton University Health Sciences Continuing Education designates this live activity for a maximum of 6 *AMA PRA Category 1 Credit(s)* ™. Physicians should claim only the credit commensurate with the extent of their participation in the activity

AAPA accepts AMA category 1 credit for the PRA from organizations accredited by ACCME.

Online Evaluation to Receive CME
The online evaluation to receive CME will be available beginning Thursday, September 22. You will receive an email from ASBMR with instructions on how to claim credit.

Target Audience
This meeting will bring together national and international investigators currently working in the field of genomics as well as young and established investigators, industry scientists, NIH intramural scientists and program staff, clinicians, endocrinologists and basic and translational researchers.

Learning Objectives

Upon returning home from the meeting, participants should be able to:

1. Appraise the latest contributions of the field of genomics to the understanding of skeletal disease mechanisms, ranging from basic molecular biology to animal models and bidirectional translation to humans, particularly within the scope of precision medicine and targeted treatments.
2. Provide participants with an updated view of how new technological innovations and novel methodological approaches in the field of genomics offer the possibility to translate the new knowledge into palpable clinical applications.
3. Promote how medicine can be revolutionized by these novel approaches and is already evident in the area of therapeutics.

ASBMR Expectations of Authors and Presenters

Through ASBMR meetings, the Society promotes excellence in bone and mineral research. Toward that end, ASBMR expects that all authors and presenters affiliated with the ASBMR Symposium on Bone-omics: Translating Genomic Discoveries into Clinical Applications will provide informative and fully accurate content that reflects the highest level of scientific rigor and integrity.

ASBMR depends upon the honesty of the authors and presenters and relies on their assertions that they have had sufficient full access to the data and are convinced of its reliability.

Furthermore, ASBMR expects that:

- Authors and presenters will disclose any conflicts of interest, real or perceived.
- Authors of an abstract describing a study funded by an organization with a proprietary or financial interest must affirm that they had full access to all the data in the study. By so doing, they accept complete responsibility for the integrity of the data and the accuracy of the data analysis.
- The content of abstracts, presentations, slides and reference materials must remain the ultimate responsibility of the author(s) or faculty.
- The planning, content and execution of abstracts, speaker presentations, slides, abstracts and reference materials should be free from corporate influence, bias or control.
- All authors and presenters (invited and abstracts-based oral and poster presenters) should give a balanced view of therapeutic options by providing several treatment options, whenever possible, and by always citing the best available evidence.

Disclosure Policy

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Meeting Evaluation

An online evaluation form for the ASBMR Symposium on Bone-omics: Translating Genomic Discoveries into Clinical Applications will be available on the ASBMR Website at www.asbmr2016.org after the meeting and sent to you via email. Your participation in this evaluation is extremely important to us. Please take a moment to complete the evaluation of this meeting to aid in planning future meetings. Thank you in advance for your feedback.

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No abstract presented at the ASBMR Symposium may be released to the press before its official presentation date and time. Press releases must be embargoed until one hour after the presentation.

Future ASBMR Annual Meeting Dates

ASBMR 2017 Annual Meeting
Colorado Convention Center, Denver, Colorado, USA
September 8 – 11, 2017

ASBMR 2018 Annual Meeting
Palais des Congrès de Montréal, Montreal, Quebec, Canada
September 28 – October 1, 2018

THURSDAY, SEPTEMBER 15, 2016

CONTINENTAL BREAKFAST

8:00 am – 9:00 am   **Room A411**

THE –OMICS REVOLUTION IN MOLECULAR MEDICINE

9:00 am – 10:30 am   **Room A411**

Co-Chairs:
Eric Orwoll, M.D., Ph.D., Oregon Health & Science University
Joan McGowan, Ph.D., National Institute of Arthritis, Musculoskeletal & Skin Disease

9:00 am   **25 years Since the Human Genome Project: New Tools and Technologies to Lessen the Burden of Disease**
Eric Boerwinkle, Ph.D., University of Texas Health Science Center
Disclosures: None

9:30 am   **Integrating –omics Platforms: A Glimpse into the Future of Clinical Practice**
Nicholas J. Schork , Ph.D., J. Craig Venter Institute
Disclosures: None

10:00 am   **Integrating –omics and Phenomics: Elucidating Molecular Mechanisms Influencing Therapeutic Decision Making**
Greg Gibson, Ph.D., Georgia Institute of Technology
Disclosures: None

BREAK AND POSTER VIEWING

10:45 am – 11:30 am   **Room A412**

MODEL CELLS AND ORGANISMS TO UNDERSTAND GENOME BIOLOGY

11:30 am – 1:00 pm   **Room A411**

Co-Chairs:
Cheryl Ackert-Bicknell, Ph.D., University of Rochester
Michael Econs, M.D., Indiana University School of Medicine

11:30 am   **Induced Pluripotent Stem Cells (iPSCs) for the Understanding of Human Musculoskeletal Disease**
Pamela G. Robey, Ph.D., National Institute of Dental and Craniofacial Research
Disclosures: None

12:00 pm   **International Mouse Phenotyping Consortium: Past, Present and Future… Making Mouse Models for All Protein Coding Genes**
Steve Brown, Ph.D., Medical Research Council Harwell, United Kingdom
Disclosures: None

12:30 pm   **Systems Biology: Gene and Networks Underlying the Molecular Basis of Musculoskeletal Disease**
Charles Farber, Ph.D., University of Virginia
Disclosures: None

LUNCH AND POSTER VIEWING

1:00 pm – 2:00 pm   **Room A412**

BIG DATA AND NETWORKS

2:00 pm – 3:30 pm   **Room A411**

Co-Chairs:
Fernando Rivadeneira, M.D., Ph.D., Erasmus MC Rotterdam, The Netherlands
Lynda Bonewald, Ph.D., Indiana University School of Medicine

2:00 pm   **Model Cells, Organisms and Human Populations: BIG DATA in Musculoskeletal Research**
André G. Uitterlinden, Ph.D., Erasmus Medical Center Rotterdam, The Netherlands
Disclosures: None

2:30 pm   **Integration of Large-scale Functional and Comparative Genomics Datasets**
Manolis Kellis, Ph.D., Massachusetts Institute of Technology
Disclosures: None

3:00 pm   **Methods for Integrating Data to Uncover Genotype-phenotype Interactions**
Marylyn D. Ritchie, Ph.D., Pennsylvania State University
Disclosures: Geisinger Health System and Regeneron Genetics Center, 1

BREAK

3:30 pm – 3:45 pm   **Room A411**

GENOMICS APPLIED IN THERAPEUTICS

3:45pm – 5:15 pm   **Room A411**

Co-Chairs:
Emma Duncan, Ph.D., Royal Brisbane and Women's Hospital, Australia
Douglas Kiel, M.D., M.P.H., Hebrew SeniorLife, Harvard University

3:45 pm   **Promoters and Enhancers: DNA Regulatory Elements as Drug Targets**
Matthew Maurano, Ph.D., New York University
Disclosures: None

4:15 pm   **Genomic Discoveries for the Development of New Medications**
Matthew Nelson, M.D., Ph.D., Glaxo SmithKline
Disclosures: GlaxoSmithKline, 4, GlaxoSmithKline, 5

4:45 pm   **The Translational Landscape of Genomic Research: From Cells and Model Organisms to Human Treatments**
Robert Plenge, M.D., Ph.D., Merck Sharp & Dohme
Disclosures: Merck, 5

CLOSING REMARKS

5:15 pm - 5:30 pm   **Room A411**

POSTERS

All posters presented at the ASBMR Symposium on Bone-omics: Translating Genomic Discoveries into Clinical Applications will also be presented at the ASBMR 2016 Annual Meeting, September 16 - 19, 2016, in the Georgia World Congress Center.

*Denotes Presenting Author

P01

The histone methyltransferase EZH2 is a novel therapeutic target in multiple myeloma bone disease. Juraj Adamik*[1], Peng Zhang[1], Quanhong Sun[1], Rebecca Silbermann[2], G. David Roodman[3], Deborah L. Galson[1] [1]University of Pittsburgh, USA, [2]Indiana University, USA, [3]University of Indiana, Veterans Administration Medical Center, USA

Multiple myeloma (MM) is the most frequent cancer to involve the skeleton, with over 80% of patients developing osteolytic bone lesions due to hyperactivation of osteoclasts (OCL) that can result in severe bone pain and frequent pathological fractures and enhanced mortality. These bone lesions rarely heal even after therapeutic remission due to MM-induced suppression of bone marrow stromal cells (BMSC) differentiation into functional osteoblasts (OB), enhancing their support of MM growth, survival, and drug-resistance. We reported that MM cells induce increased recruitment of EZH2, the histone methyltransferase component of the Polycomb repressive complex 2 (PRC2), to the *Runx2* gene in the murine pre-OB cell line MC4 resulting in H3K27me3-mediated repression of *Runx2*, thereby causing repression of OB differentiation. In the present study, we show that H3K27me3 levels are elevated on the *Runx2* promoter in MM patient derived BMSC. Importantly, we found that the selective EZH2 inhibitor GSK126 enhances the active architecture at the *Runx2* promoter in both BMSC from MM patients and MM-treated MC4 cells, and rescues the osteogenic potential and mineralization capability. Therefore, we investigated the effects of GSK126 on MM cells. GSK126 inhibition of EZH2 induced dose-dependent, caspase 3-mediated, apoptosis of 5 human MM cell lines (U266, RPMI, MM1.S, H929 and JJN3) and the murine 5TGM1 MM cell line. In addition, GSK126 showed synergistic MM cell cytotoxic effects when used in combination with a suboptimal dose of the proteasome inhibitor bortezomib. Lastly, we analyzed the effects of GSK126 on pro-inflammatory, myeloma-amplified osteoclastogenesis. We reported that expansion of bone marrow monocytes (BMM) in the presence of MM1.S conditioned media significantly enhanced formation of TRAP-positive multinucleated OCL. We found that GSK126 significantly blocked the upregulation of OCL marker genes NFATc1, RANK, Cathepsin K, OSCAR, and DC-STAMP resulting in suppression of myeloma-enhanced, RANKL-induced osteoclastogenesis. Our study demonstrates that selective inhibition of the epigenetic modifier EZH2 using GSK126 in vitro decreases MM cell survival and positively regulates the bone microenvironment by suppressing OCL formation and improving the osteogenic potential of BMSC. These data suggest that targeting EZH2 activity may prove a valuable therapeutic strategy to improve bone health and limit disease progression in MM patients.

P02

Transcriptomic Analysis of Whole Bone Marrow Cultures Treated with Osteoinductive Agents: BMP-2 (bone morphogenetic protein 2) or TPO (thrombopoietin). Marta B. Alvarez*[1], Paul J. Childress[1], Nabarun Chakraborty[2], Duncan E. Donohue[2], Rasha Hammamieh[2], Todd O. McKinley[3], Melissa A. Kacena[3]. [1]Indiana University School of Medicine, USA, [2]US Army Center for Environmental Health Research, USA, [3]Indiana University School of Medicine, Department of Orthopaedic Surgery, USA

Despite advances in surgical techniques and implants, healing segmental bone defects (SBDs) and non-unions present ongoing challenges for orthopaedic surgeons. BMP-2 is effective in healing SBDs, but has side effects, including an increased risk for cancer. We have shown that TPO has the ability to heal SBDs as well as stimulating muscle healing, angiogenesis, and modulating immune function. The purpose of this study was to explore the differences in healing signatures between BMP-2 and TPO by quantifying transcriptomic changes in cultured whole bone marrow (BM) cells. We hypothesized that expression patterns of these two agents would be unique and that TPO signatures would result in an expanded set of wound healing genes. Femoral BM cells were collected from C57BL/6 mice and cultured in the presence of BMP-2 (200ng/ml), TPO (10 ng/ml), or saline for 3 days (n=4/group). RNA was isolated and converted to cDNA. Gene expression analysis was done using high throughput dual-dye cDNA microarrays (Agilent Inc.). Pairwise t-test with p<0.005 found 756, 1033, and 2488 transcripts differentially expressed by BMP-2 vs. saline, TPO vs. saline, and BMP-2 vs. TPO treatment groups, respectively. The genes were annotated using the Systems Biology tools: Ingenuity Pathway Analysis® (IPA) and the Database for Annotation, Visualization, and Integrated Discovery (DAVID). Expression of select genes relevant to this study were validated using qPCR (data not shown). Using principle component analysis (PCA) of the raw data, we plotted the first principle component (PCI) vs. the second (PC2), which cumulatively accounted for 41% of total variance. Using DAVID and IPA analysis, we found that TPO treatment activates genes involved in wound healing, fluid balance, coagulation, and other profiles. TPO differentially activated integrins and actin (A2 and B), proteins known to be important for osteoblast function and are integral to the mechanical responsiveness of bone. TPO up-regulated angiopoietin 1 and platelet derived growth factor, which regulate blood vessel growth and mesenchymal differentiation into osteoblasts, respectively. In contrast BMP-2 treated cells primarily stimulated T lymphocyte activation. This work distinguishes the modes of actions of TPO and BMP-2, which will inform development of TPO as a novel bone healing agent.

P03

A High-Fat Diet Induces Changes in the Bone Composition of Murine Humeri Independent of Total Body Bone Mineral Density. Michael-John G. Beltejar*[1], Jun Zhang[2], Dana A. Godfrey[1], Michael J. Zuscik[1], Douglas Adams[3], Cheryl L. Ackert-Bicknell[1]. [1]Center for Musculoskeletal Research, University of Rochester Medical Center, USA, [2]Department of Orthopedics, Zhejiang Provincial People's Hospital, China, [3]Department of Orthopaedic Surgery, University of Connecticut Health, USA

Previous studies have implicated a high-fat diet in changes to bone mineral density (BMD). However, BMD alone is an imperfect predictor of bone strength, as contributions from bone composition are not captured. For example, in diabetes, patients can have fractures despite normal BMD but have alterations in mineral:matrix and carbonate:phosphate ratios. Studies of inbred mice suggest that bone composition is controlled as a genetic trait, but alterations in these parameters of bone composition in response to dietary fat remains understudied. The aim of this study was to determine if dietary and genetic interactions affect bone composition. Male mice from 3 strains (A/J, C57BL/6J (B6), and NOD/H1LtJ), were placed on either a high-fat (60% kcal from fats) or lean diet (10% kcal from fats) from 6 to 21 weeks of age. Both diets were iso-caloric for protein and the fat and carbohydrate sources were identical between the two diets. Area BMD and percent fat of the whole body (sans the head) were measured using DXA. Attenuated Total Reflectance Fourier Transformed Infrared Spectroscopy (ATR-FTIR) was used to determine the carbonate to phosphate ratio (which reflects bone age) and mineral to matrix ratio (which correlates with bone strength) of humeri. Differences in the parameters among strains and diets were determined using a two-way ANOVA with interactions and Tukey's HSD post hoc test. All mice on a high-fat diet had elevated body fat. Otherwise, the effects of a high-fat diet were unique in each of the three strains. A/J mice had an increase in BMD, B6 had a decrease and NOD mice remained unchanged (Fig. 1). However, A/J mice experienced a decrease in the mineral:matrix and increase in the carbonate:phosphate ($p < 0.0001$, Fig. 1). B6 experienced the opposite changes in these compositional parameters ($p < 0.0001$, Fig. 1). The www.differences between the A/J and the B6 mice on the lean diet mirrors previously published differences between these 2 strains. Interestingly, there were no changes in these compositional parameters between diets in NOD mice. These results demonstrate that there is indeed an interaction between genetics and diet that impacts bone at the level of composition. The effects of this interaction are beyond what is captured by DXA measures of bone mass. Compositional changes in bone are understood to result in changes in bone mechanical properties and thus dietary fat mediated changes in composition have the potential to impact bone strength.
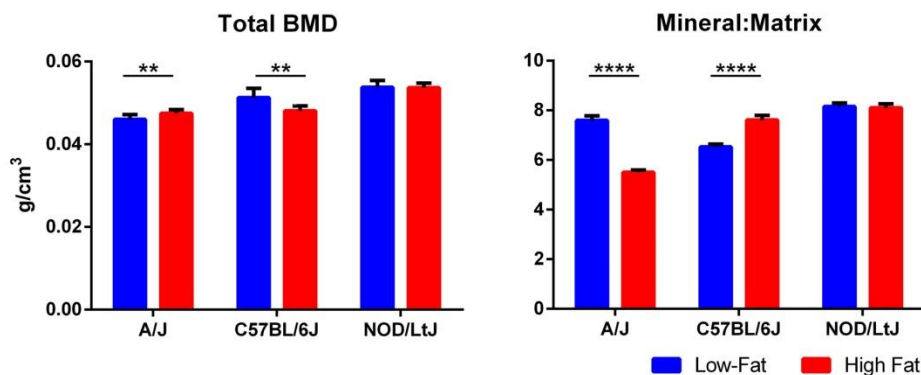
Figure 1. High-Fat Diet Changes to Total BMD and Bone Composition

P04

Generation and Phenotypic Characterization of a Lrp4 R1170Q Knock-In Mouse Model. Eveline Boudin*[1], Igor Fijalkowski[1], Stephan Sonntag[2], Gretl Hendrickx[1], Timur A Yorgan[3], Thorsten Schinke[3], Geert Mortier[1], Wim Van Hul[1]. [1]Centre of Medical Genetics, University & University Hospital of Antwerp, Belgium, [2]PolyGene AG, Rümlang, Switzerland, [3]Department of Osteology & Biomechanics, University Medical Center Hamburg, Germany

Throughout the years, it became clear that proteins deficient in rare monogenic sclerosing bone disorders such as sclerostin or cathepsin K are important regulators of bone remodeling and promising targets for the development of therapeutic agents for osteoporosis. We were recently able to show that mutations in the *low density lipoprotein receptor related protein 4* (*LRP4*) gene are the cause of some cases of sclerosteosis, a rare monogenic bone disorder marked by syndactyly and hyperostosis of the skull and tubular bones among other symptoms. LRP4 is shown to regulate bone formation by facilitating the inhibitory actions of sclerostin on the Wnt/β-catenin signaling pathway. So far, three patients with sclerosteosis causing mutations in LRP4 have been described. Furthermore, we recently demonstrated that the mutation W1186S causing mutation in LRP4 results in highly increased sclerostin levels in the sclerosteosis patient (z-score +7), indicating that LRP4 has a role in sequestering sclerostin at the bone surface. As LRP4
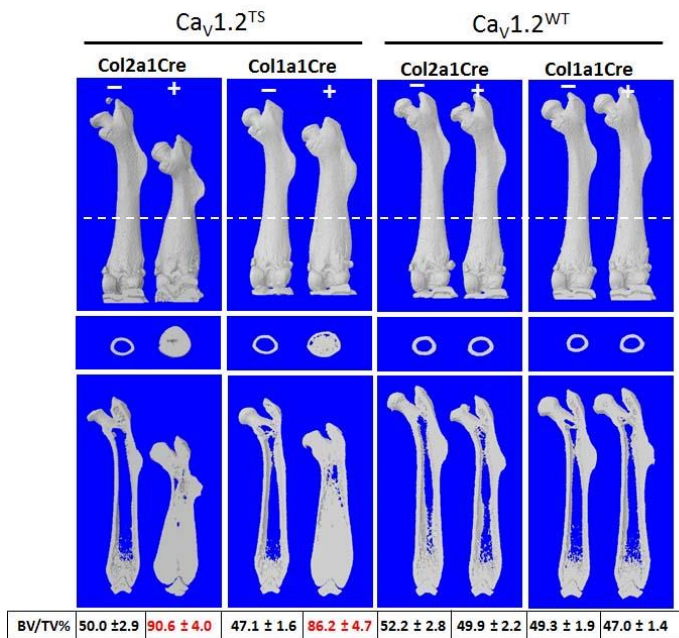
clearly is an important regulator of bone formation and an interesting therapeutic target for treatment of osteoporosis, increased knowledge on the mechanism of action of LRP4 is required. In order to further elucidate the mechanism whereby LRP4 facilitates the inhibitory actions of sclerostin, we generated in collaboration with Polygene (Rümlang, Switzerland), a *Lrp4* R1170Q knock-in (KI) mouse model. R1170Q is the most recently identified sclerosteosis causing *LRP4* mutation and the arginine at position 1170 seems to have an important role in the binding of sclerostin since this amino acid is mutated in two different sclerosteosis patients. The mutation is located at the inside of the third β-propeller domain which is shown to be important for the binding of sclerostin. Previous studies demonstrated that complete loss of *lrp4* in mice results in perinatal lethality. Here, we show that homozygous R1170Q KI mice are born at the expected frequencies and that they are viable and fertile. Furthermore, preliminary µCT analysis results of the skull and femur of 4 month old mice clearly demonstrate an increased bone mass at the tubular bones and at the skull in the presence of either one or two copies of the mutant allele compared to wild type mice. More detailed phenotyping is ongoing and results will be presented. However our preliminary data indicate that the Lrp4 R1170Q knock-in mouse is a good model to study the role of Lrp4 in the regulation on bone remodeling.

P05

Ca2+ signaling through the Ca$_V$1.2L-type Ca2+ channel regulates bone formation. Chike Cao*[1], Anthony Mirando[2], Amy McNulty[2], Farshid Guilak[2], Matthew Hilton[2], Geoffery Pitt[1]. [1]IonChannel Research Unit; Department of Medicine, Duke University Medical Center, USA, [2]Department of Orthopaedic Surgery, Duke University Medical Center, USA

Timothy Syndrome (TS) is a multi-organ disorder characterized by cardiac arrhythmias and developmental abnormalities. TS patients carry a de novo gain-of-function mutation in the Ca$_V$1.2 L-type calcium channel, which has been best characterized in excitable cells such heart, brain, and hormone-secreting cells. The TS mutation reduces channel inactivation and increases Ca^{2+} influx into the cell. The myriad phenotypes in TS patients reveals critical but not yet known roles of Ca$_V$1.2 in multiple non-excitable tissues. We previously reported that Ca^{2+} influx through Ca$_V$1.2 regulates chondrocyte hypertrophy and hyperplasia during mandible development. Here, we investigate the potential role of Ca$_V$1.2 in bone formation. We first found that during skeletogenesis, Ca$_V$1.2 was highly expressed in perichondrium and/or periosteum by using a Ca$_V$1.2 *lacZ* reporter mouse. Striking expression of Ca$_V$1.2 was also observed in the proliferating chondrocytes within the growth plate at P10. Targeted expression of the TS-causing Ca$_V$1.2 mutant channel (Ca$_V$1.2^{TS}) in the osteoblast lineage cells with *2.3Col1a1-Cre*, in the chondrocyte lineage cells with *Col2a1-Cre*, or in both cell types with *Prx1-Cre*, dramatically enhanced bone mass. Histological analysis confirmed that both primary and secondary ossification centers were occupied with excessive bone. In primary bone marrow stromal cell (BMSC) cultures, expression of Ca$_V$1.2^{TS} promoted osteoblast differentiation. RNASeq analysis revealed that Ca$_V$1.2^{TS} in BMSCs enhanced the expression of extracellular matrix proteins. Gene ablation of Ca$_V$1.2 in BSMCs inhibited osteogenesis. The L-type calcium channel blocker verapamil (5 µM) significantly decreased MC3T3-E1 mineralization, suggesting the role of Ca$_V$1.2 in bone formation was mediated by Ca^{2+} signaling. We conclude that Ca^{2+} influx through Ca$_V$1.2 is required for bone formation and increased Ca^{2+} signaling through the TS-causing mutant channel promotes osteoblast differentiation. Our data suggest that increasing Ca^{2+} signaling through Ca$_V$1.2 could be an attractive therapeutic strategy for osteoporosis.

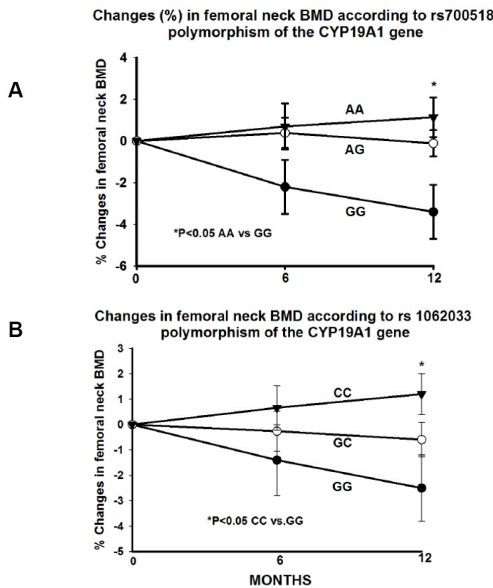|	Ca$_V$1.2^{TS}				Ca$_V$1.2^{WT}			
	Col2a1Cre		Col1a1Cre		Col2a1Cre		Col1a1Cre	
	−	+	−	+	−	+	−	+
BV/TV%	50.0 ±2.9	90.6 ± 4.0	47.1 ± 1.6	86.2 ± 4.7	52.2 ± 2.8	49.9 ± 2.2	49.3 ± 1.9	47.0 ± 1.4

P06

A haplotype in the CYP19A1 gene is associated with variable musculoskeletal response to testosterone therapy in men with hypogonadism. Georgia Colleluori*[1], Lina Aguirre[2], Richard Dorins[2], David Robbins[2], Clifford Qualls[3], Dean Blevins[1], Dean Blevins[1], Dennis Villareal[4], Reina Villareal[4]. [1]Baylor College of Medicine, USA, [2]New Mexico VA Health Care System, USA, [3]University of New Mexico School of Medicine, USA, [4]Michael E. DeBakey VA Medical Center, USA

Background: A haplotype in the CYP19A1 gene (which encodes aromatase) is associated with estrogen levels, bone mineral density (BMD) and fracture prevalence and this is mostly due to the single nucleotide polymorphisms (SNPs) rs700518 and rs1062033. These SNPs are in linkage disequilibrium (LD) and show in-vitro differences in allelic expression and activity. The effect of these linked SNPs on the response and susceptibility to side effects from testosterone (T) therapy in men with hypogonadism is unknown. Our objective is to evaluate the effect of the rs1062033 and rs700518 SNPs on 1)the musculoskeletal response and 2)changes in hematocrit (HCT) and prostate specific antigen (PSA) with T therapy in men with hypogonadism. Methods: Single arm clinical trial involving 105 men, 40-74 yo, average morning T (2 samples)<300 ng/dL, given T cypionate 200 mg every 2 weeks IM. Areal BMD and body composition by DXA; C-telopeptide (CTX) by ELISA; genotyping by Taqman SNP allelic discrimination assay. Results: Figure 1 shows that those with GG genotype for the rs700518 (Fig.1A) and GG genotype for the rs1062033 (Fig. 1B) experienced bone loss at the femoral neck despite T therapy at 6 mo which became significant at 12 mo compared to AA for rs700518 and CC for rs1062033 who had increases in BMD, while GA and GC had intermediate changes. CTX increased among patients with the GG compared to those with GA+AA genotypes at 6 mo (24.7±18.9vs.-20.5±10.2%, p0.04) and 12 mo (171.9±61.1vs.9.2±28.4%, p0.01) for the rs700518. There were no differences in HCT and PSA changes at 6 and 12 mo. All decreases total and trunk fat mass at 12 mo but it was greater in AA than GA+GG for total and trunk (-13.2±2.50vs. -6.9±1.4%, p0.04) fat in rs700518 and in CC than the GC+GG for total and trunk (-10.7±6.7vs.-6.3±6.8%, p0.02) fat in rs1062033. All had increases in lean mass at 12 mo but the AA genotype had greater increase than the GA+GG (6.46±1.04vs.3.96±0.60%, p0.04) in rs700518 and CC than GC+GG (5.9±4.9vs3.5±3,3%, p0.03) in rs1062033. The 2 SNPs are in partial LD (r²=0.22) in our sample. Conclusion: Subjects with the GG genotype in both SNPs respond the least to T treatment (decrease BMD; attenuated decrease in body fat and increase in lean mass). AA genotype of rs700518 and CC genotype of rs1062033 are the best responders: stable BMD and marked improvement in body composition. GA and GC genotypes showed intermediate responses. So far no genotype is predisposed to increased side effects

Figure 1

A — Changes (%) in femoral neck BMD according to rs700518 polymorphism of the CYP19A1 gene

B — Changes in femoral neck BMD according to rs 1062033 polymorphism of the CYP19A1 gene

P07

TGFb Regulates the Stability of Sox9. George Coricor*, Rosa Serra. University of Alabama at Birmingham, USA

One out of two people in the U.S. will experience some form of Osteoarthritis (OA) in their lifetime. Members of the Transforming Growth Factor-β (TGFβ) superfamily are important factors that stimulate chondrocyte matrix biosynthesis. Mice with a dominant-negative mutation of the TGFβIIR develop a degenerative joint disease resembling OA. Another key chondrogenic factor, SOX9, is important for maintaining chondrocyte function. Patients with OA show decreased levels of SOX9 and TGFβ receptors. Our overall hypothesis is that TGFβ regulates SOX9 to maintain articular cartilage. We utilized two cell culture models for these experiments, ATDC5 cells, and primary bovine articular chondrocytes. We showed that TGFβ stabilizes SOX9 protein and increases phosphorylation of SOX9 in both ATDC5 and bovine articular chondrocytes. SOX9 can be phosphorylated on three Serine residues S64, S181, and S211. We mutated these serine sites to alanine then over expressed the mutants in ATDC5 cells to determine which

phosphorylation site is important for TGFβ-mediated stability of Sox9. We found that mutation of Serine 211 is required to maintain normal SOX9 protein levels and that TGFβ was not able to increase the stability of SOX9-S211A mutant. The results suggest that TGFβ may phosphorylate SOX9 on S211 to stabilize it. The S211 site is in a motif that is targeted by p38 kinase. TGFβ can signal through a canonical/Smad-mediated pathway or non-conical pathways, including p38. We tested the hypothesis that Smad2/3 and/or p38 regulate the phosphorylation and stability of SOX9. First, we determined that knock down of Smad2/3 using siRNA, blocked TGFβ-mediated stability of Sox9. Then we showed that stabilization of SOX9 by TGFβ was blocked in cells treated with p38 inhibitors suggesting that TGFβ requires both Smad2/3 and p38 activity to regulate SOX9 protein levels. Previously, we showed that Papss2 is a downstream target of TGFβ. We utilized siRNA for SOX9 to show that TGFβ requires SOX9 to regulate Papss2. We also showed that p38 is required for TGFβ-mediated regulation of PAPSS2 suggesting a new mechanism for TGFβ-mediated gene regulation in chondrocytes. Understanding how TGFβ stabilizes SOX9 may lead to identification of preventative targets of both age-related and post-traumatic OA.

P08
Individual Variants and Genetic Risk Score for Puberty Timing Associate with Pediatric Bone Mineral Density. Diana Cousminer*[1], Jonathan Mitchell[1], Alessandra Chesi[1], Sani Roy[1], Heidi Kalkwarf[2], Joan Lappe[3], Vincente Gilsanz[4], Benjamin Voight[5], Sharon Oberfield[6], John Shepherd[7], Andrea Kelly[1], Shana McCormack[1], Struan Grant[1], Babette Zemel[1]. [1]Children's Hospital of Philadelphia, USA, [2]Cincinnati Children's Hospital Medical Center, USA, [3]Creighton University, USA, [4]Children's Hospital of Los Angeles, USA, [5]University of Pennsylvania, USA, [6]Columbia University Medical Center, USA, [7]University of California San Francisco, USA

Background: Later puberty is associated with lower areal bone mineral density (aBMD) in adulthood, especially in females. Genome-wide association studies (GWAS) have reported 130 puberty-associated variants, but the impact of this variation on pediatric aBMD during skeletal development is unknown. Methods: In the European-ancestry subset of the multicenter, longitudinal Bone Mineral Density in Childhood Study (BMDCS) of healthy children ages 5 to 20 years, aBMD of the spine, total hip, femoral neck, and distal radius, and bone mineral content (BMC) of the total body less head (TBLH) were measured (n=1450). Using sex-stratified linear mixed models in STATA, we tested 126 of the 130 known puberty-associated alleles for association with age- and sex-specific aBMD (or BMC) Z-scores (aBMDz), adjusted for height z-score, study center, age, physical activity, dietary calcium, and body mass index z-score. The Bonferroni-adjusted significance threshold was $P=3.9 \times 10^{-4}$. Additionally, we constructed combined-allele genetic risk scores (GRS) weighted on menarche-delaying (female GRS) or age at voice break-delaying (male GRS) effect estimates and tested these scores for associations as described above. We also tested whether the GRS had consistent associations with bone outcomes across age and pubertal (Tanner) stage (ie statistical interactions). Results: Three puberty-delaying alleles were negatively associated with aBMDz in girls (rs2600959 at the radius, and rs9475752 and rs1400974 at TBLH). The female GRS was associated with 0.2 yr later menarche ($P=8.2 \times 10^{-5}$), but associations with aBMDz scores were inconsistent in girls. The male GRS was strongly negatively associated with aBMDz scores at all skeletal sites (all sites, $P<0.04$). In boys, the puberty-delaying allele at rs113388806 associated with lower total hip and spine aBMD, and at rs2063730 with lower femoral neck aBMD, in older, but not younger boys (GRS x age interactions, $P=9.7 \times 10^{-6}$, 3×10^{-4}, and 4.9×10^{-4}, respectively). We also observed significant negative GRS x age and GRS x Tanner stage interactions with aBMD at many of the skeletal sites in both sexes. Conclusions: Concordant with epidemiological observations, puberty-delaying loci associate with lower aBMD during skeletal growth, although individual associations are sex-specific and vary across age and Tanner stage. These studies highlight the utility of genetic studies to investigate the complex relationship between pubertal development and bone health.

P09
Heritability of Bone Mineral Density and Content in Childhood and Adolescence. Diana Cousminer*[1], Alessandra Chesi[1], Jonathan Mitchell[1], Sani Roy[1], Heidi Kalkwarf[2], Joan Lappe[3], Vincente Gilsanz[4], Sharon Oberfield[5], John Shepherd[6], Andrea Kelly[1], Shana McCormack[1], Benjamin Voight[7], Babette Zemel[1], Struan Grant[1]. [1]Children's Hospital of Philadelphia, USA, [2]Cincinnati Children's Hospital Medical Center, USA, [3]Creighton University, USA, [4]Children's Hospital of Los Angeles, USA, [5]Columbia University Medical Center, USA, [6]University of California San Francisco, USA, [7]University of Pennsylvania, USA

Background: Heritability is the amount of variance in a trait that is attributed to genetic factors. To our knowledge, only one previous study used genotype data to assess heritability of bone mineral density (BMD) in children aged 9 yr (Kemp et al., 2014). To date, no study has assessed the heritability of BMD or bone mineral content (BMC) at specific skeletal sites across childhood and adolescence. Aim: To determine heritability estimates for BMD and BMC across clinically important skeletal sites in children and adolescents. Methods: In the multicenter, multiethnic, longitudinal Bone Mineral Density in Childhood Study (BMDCS) of healthy children ages 5 to 20 yr, BMD and BMC of the spine, total hip, femoral neck, distal radius, skull, total body, and total body less head (TBLH) were measured in up to 1876 boys and girls. We used GCTA-Restricted Maximum Likelihood (GREML) to estimate the proportion of genetic variance (Vg), using age- and sex-specific areal BMD (or BMC) Z-scores at each site, adjusting for study center, cohort, age, sex, physical activity, dietary calcium, and body mass index z-score (except for skull). GREML accounts for relatedness and ancestry with a genetic relationship matrix. Additionally, we performed bivariate GREML to compare Vg pairwise between skeletal sites. Results: BMD had larger Vg estimates than BMC at all skeletal sites. Each skeletal site showed varied contributions from genetic and residual factors. Heritability estimates ranged from Vg (SE)=0.38 (0.1), $P=2.6 \times 10^{-5}$ at the skull to Vg (SE)=0.11 (0.08), P=0.08 at the radius for BMD and from Vg (SE)=0.23 (0.07), $P=3.8 \times 10^{-4}$ for the total body to Vg (SE)=0.08(0.07), P=0.1 at the radius for BMC.

Additionally, height z-score-adjusted (HAZ) Vg estimates were higher than unadjusted phenotypes at all skeletal sites except the radius. For the bivariate correlations, both phenotypic and genotypic correlations were similar across most skeletal sites. All skeletal sites showed a patchwork of shared and unique genetic contributions. Conclusions: In comparison to Kemp et al., our estimates of Vg were slightly lower, possibly due to the broad age range assessed, which may mask variations in heritability at different ages. However, our results represent pediatric heritability estimates at the most detailed skeletal resolution to date for BMD and BMC, and will serve as a resource for genetic studies aiming to distill the specific genetic variants impacting these traits

P10
Secreted microRNAs from Prostate Cancer Cells: Novel Therapeutic Targets. Nicholas Farina*, Cody Callahan, Coralee Tye, Joseph Boyd, Gary Stein, Janet Stein, Jane Lian. University of Vermont, USA.

Introduction: More than 80% of men with aggressive prostate cancer (PCa) develop metastatic disease and skeletal complications. Secreted microRNAs (miRNA) are emerging as biomarkers for monitoring disease progression and as therapeutic targets for PCa. Here we tested the hypothesis that miRNAs secreted from prostate cancer cells in the primary tumor function to alter the normal tissue environment and promote PCa. *Methods:* Use global miRNA (Affymetrix miRNA v4.0 arrays, miRBase v20) and gene expression (by stranded RNA-seq analysis) to 1) define cohorts of miRNAs secreted from prostate cancer cells representing different disease stages, 2) identify expression levels of their target mRNAs. RNA was isolated from normal prostate epithelial, early stage AR+ LNCaP, and metastatic PC-3 cells and from their conditioned media. Global expression of both secreted and extracellular miRNAs were interrogated with RNA-Seq profiles to identify differential expression (DE) of miRNA targets predicted from reciprocal miRNA – mRNA expression changes. *Results:* Secreted miRNAs candidates for promoting PCa progression were identified by comparing DE miRNAs between the 3 prostatic cell lines and their conditioned media. Numerous miRNAs are found to be both secreted and expressed robustly in the cell layer, while unique subsets of miRNAs in each cell line are either preferentially secreted or retained within the cell. Putative mRNA targets of these miRNAs were identified in our gene expression data from the reciprocal mRNA-miRNA relationships. The miRNAs were found to be associated with pathways known to play a role in PCa and metastatic bone disease including ErbB, Wnt, TGF-beta, and MAPK signaling, as well as biological processes unassociated with PCa. Functional *in vitro* studies were performed for miRNAs that were overexpressed or inhibited in highly metastatic PC-3 cell lines, by assaying, invasion, migration, and proliferation. For example, inhibition of miR-30a-5p, a known tumor suppressor, increases migration rate of RWPE-1 and PC-3 cells to heal a cell layer scratch, while overexpression prevents wound closure in RWPE-1 cells.*Conclusion:*Our combined data identify, for the first time, miRNAs secreted from prostate tumor cells that promote metastatic events of prostate cancer. Several of these miRNAs have been reported to be elevated in PCa patient serum and can provide diagnostic/prognostic biomarkers of tumor development, response to treatment, and recurrence.

P11
The Association Of Circulating miRNAs With Bone Mineral Density, Microarchitecture And Prevalent Fracture In The OFELY Cohort. Elodie Feurer*, Casina Kan, Martine Croset, Elisabeth Sornay-Rendu, Roland Chapurlat. INSERM UMR 1033, France

Objective Current biomarkers of osteoporosis lack sensitivity. The objective of this work was to study whether selected circulating microRNAs are associated with bone mineral density (BMD) and microarchitecture, along with prevalent fracture in a cohort of French women. Material and methods In this cross-sectional analysis from the 14th annual follow-up of the OFELY study, microRNAs were measured from 200 μL of serum by RT qPCR (Exiqon, Denmark). We chose 32 microRNAs that seemed relevant in their association with bone metabolism, based on prior literature. BMD was measured by DXA and the microarchitecture was assessed with high resolution peripheral quantitative computed tomography. Prevalent low energy fractures were ascertained using radiographs and medical records. Results MicroRNAs measurements were obtained from 682 subjects: 99 pre-menopausal women (mean (SD) age 49 (2.5)) and 583 post-menopausal women (mean (SD) age 68 (8.7)). One pre-menopausal woman and 122 post-menopausal women had at least one prevalent fracture. All the 32 analyzed microRNAs were significantly correlated with age (correlation coefficient between 0.09 and 0.49). The miR-16-5p was the most correlated with age (r -0.49, p<0,000 1). In univariate analysis, most of the microRNAs were significantly associated with femoral neck bone mineral density and with radius and tibia total volumic bone mineral density. In multivariate analysis, none of these results remained significant after adjustment for age. Similarly, the prevalent fractures were not associated with the serum levels of microRNAs after adjustment for age. Conclusion We showed a constant association between the level of circulating microRNA with age but we were not able to reveal an association with bone parameters, and especially with prevalent osteoporotic fracture.

P12

Micro-RNA Expression Profiling in Paget's Disease of Bone and Osteoporosis and their Modulation by Intravenous Bisphosphonates. Simone Bianciardi[1], DANIELA MERLOTTI[2], Guido Sebastiani[1], Marco Valentini[1], Stefano Gonnelli[1], Carla Caffarelli[1], Isabella Anna Evangelista[1], Simone Cenci[3], Ranuccio Nuti[1], Francesco Dotta[1], Luigi Gennari*[1]. Department of Medicine, Surgery & Neurosciences, University of Siena, Italy, [21]Department of Medicine, Surgery & Neurosciences, University of Siena, Italy; [2]Division of Genetics & Cell Biology, San Raffaele Scientific Institute, Milan, Italy, Italy, [3]Division of Genetics & Cell Biology, San Raffaele Scientific Institute, Milan, Italy

Since their discovery, microRNAs (miRs) have emerged as critical post-transcriptional regulators of stem cell lineage commitment and bone development. Nonetheless, despite the massive increase in bone turnover, whether dysregulation of miRs is involved in Paget's disease of bone (PDB) remains unknown. Here, we performed a serum miRNA expression profile (Taqman Human MicroRNA Array Card Set v3.0) in peripheral blood mononuclear cells (PBMCs) from 25 PDB pts (13 with and 12 without *SQSTM1* mutation) and 25 age-matched subjects with or without osteoporosis (OP). Moreover, miRs from 10 of these PDB cases and the 10 OP pts before and 6 months after therapy with intravenous bisphosphonates (BP) were transcribed from plasma samples and pooled into pre- and post-treatment groups and compared to controls in a subsequent miR expression profile analysis. Data from Array Cards was exported using ViiA7 RUO software and then analyzed using Expression Suite software v1.0.1 (Applied Biosystem). All values were normalized using different housekeeping miRs. Overall, 24 miRs were significantly upregulated (n=21) or downregulated (n=3) in PBMCs from PDB pts than in non-pagetic controls. Conversely, 14 miRs were significantly dysregulated in OP than non-OP pts. The most consistent associations were observed with miR-15b, -19a, -19b, -23b, -30b, -424, -501, and -502.3p for PDB and miR-1, -23b, -124a, -148b, -182, -190, -451, and -487.a in OP. For most of the PDB-related miRs the association was significantly higher in carriers of *SQSTM1* mutation; moreover in these*SQSTM1*-mutated pts 20 additional miRs were specifically upregulated. The analysis of serum miRs identified partly different associations between OP or PDB cases and controls at baseline. Of interest 3 miRs were specifically upregulated in serum of OP patients than in controls (miR-1, -124a, -520e, -542.3p) and their levels were significantly decreased by BP treatment, while miR-125a.3p, -362.3p,-502.3p, and -627 were reduced by BP therapy in PDB. In addition 4 serum miRs (miR-493, -512.5p, -525, and -548c.5p) were underexpressed or undetected in serum of PDB and OP patients at baseline than in controls and their levels increased after BP treatment reaching similar values than in controls. Validation studies in larger samples are ongoing to confirm the above associations and assess the role of the identified miRs as potential biomarkers or therapeutic targets in PDB as well as in other disorders of bone metabolism.

P13

Identification of Epigenomic Regulators of Osteoblast Function. Carole LE HENAFF[1], Nicola PARTRIDGE[2], Frederic JEHAN[1], Valerie GEOFFROY*[1]. [1]Inserm U1132, France, [2]New York University, USA

Molecularly characterized epigenetic networks that control bone formation and are altered during aging are necessary to uncover new potential targets for osteoanabolic therapy. We aimed to identify osteoanabolic epigenomic regulators which are involved in osteoblast phenotype and differentiation. This study consists of siRNA screening for epigenomic regulators of osteoblastic differentiation and validation. To do this, we have developed a colorimetric alkaline phosphatase (ALP) assay for the initial high-throughput screening in 96 well-plates and we chose to use the FhSO6 human cell line (fetal osteoblast progenitors from fetal bone marrow stroma) that has high ALP activity and reduced type 1 collagen expression after dexamethasone stimulation. We have employed a custom siRNA library targeting 347 epigenetic regulators which contain siRNAs mainly against histone and chromatin modifiers, DNA methylation partners and histone chaperones. We identified 36 siRNAs that significantly increased and 13 siRNAs that strongly decreased ALP activity. These siRNA knockdown studies confirmed that the Polycomb group proteins (EZH2, SUZ12) down-regulate ALP activity and, conversely, the BAF complex (ARID1A, ARID2, SMARCC1) increases ALP activity. Based on the GUItars software statistical analysis, the epigenetic regulators that exhibit the strongest effect on ALP activity were KDM5D that specifically demethylates Lys-4 of histone H3, PRDM11 that contains a *PR domain similar to* the SET domain of histone methyl transferases and RING1, a transcriptional repressor that is associated with the multimeric Polycomb group protein complex. To further validate our best candidates, we will determine their effects on gene expression and on Runx2 transcriptional activity or activity of the canonical Wnt/β-catenin pathway using specific integrated reporter plasmids to quantitate osteoanabolic activity. For this we will use the STRO1A cell line (human immortalized osteoprogenitors) that is less differentiated than the FhSO6 cell line. From this work, we expect to obtain a better understanding of how epigenetic mechanisms participate in bone formation and bone loss during ageing.

P14

Succinate and its G-Protein-Coupled Receptor Stimulates Osteoclastogenesis and Bone Loss. Yuqi Guo*, Tao Yu, Jian Yang, Xin Li. New York University, USA

Bone fragility is a complication of diabetes mellitus, a metabolic disorder characterized by chronic hyperglycaemia with dysregulation in metabolites. The pathophysiological link between diabetes and compromised bone metabolism is not well understood. Recent findings in humans and rodents revealed that impaired osteoblast-mediated bone formation, accelerated osteoclastic-mediated bone resorption, microstructural defect, and poor bone quality are associated with diabetes mellitus. However, regarding the cellular effect, it remains elusive whether there is a direct link between abnormal metabolites resulted from hyperglycemia and the impaired bone cell
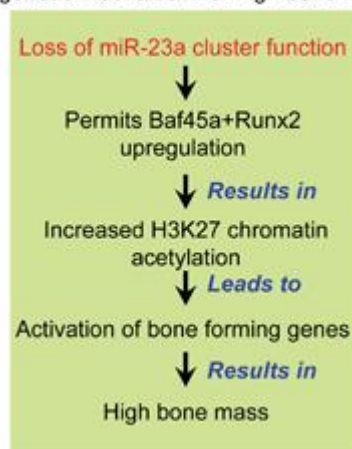
functions. We investigate the metabolomics of bone marrow stromal cells (BMSC) derived from hyperglycemic (type-2 diabetes, T2D) and normal (Wildtype, WT) mice using liquid chromatography-mass spectrometry. In comparison to WT BMSCs, 142 metabolites were significantly regulated in T2D BMSCs, in which 126 were upregulated and 16 were downregulated. Succinate, an intermediate metabolite that is catalyzed to fumarate by succinate dehydrogenase (SDH) in Krebs cycle, increased by 30-fold in diabetic BMSCs. It is known that deficient SDH activity leads to succinate accumulation. Indeed, both human and mouse BMSCs cultured with high glucose medium exhibit significantly reduced SDH activity and increased succinate levels. Excessive succinate can act as an extracellular ligand through binding to its specific receptor (SUCNR1), G-protein coupled receptor. Interestingly, SUCNR1 expresses exclusively in osteoclastic but not osteoblastic lineage cells indicating BMSC-derived succinate regulates osteoclasts in a paracrine mode under hyperglycemic condition. Results from succinate colorimetric assay, osteoclast enumeration, bone histomorphometry and micro CT analysis support that an abnormal accumulation of succinate is associated with the enhanced bone loss in T2D mice. Excessive succinate derived from BMSCs or administration of exogenous succinate stimulates osteoclastogenesis in vitro and bone loss in vivo. Furthermore, we synthesize a SUCNR1 antagonist to block SUCNR1 activation which successfully impedes succinate-enhanced osteoclastogenesis in vitro and in vivo. We further prove that succinate activates osteoclastogenesis via the canonic NF-κB pathway while administration of metformin, which suppresses succinate accumulation in bone marrow, attenuates succinate-enhanced osteoclastogenesis and bone resorption in vitro and in vivo. Our study reveals a novel osteoclastogenesis modulation mechanism mediated by an intermediate metabolite succinate accumulated in bone microenvironment. Thus, targeting succinate signaling could provide a specific and effective treatment for improving bone health in diabetes.

P15
MiR-23a-cluster Regulates BAF45a to Control a Tissue-specific Epigenetic Mechanism for Bone Formation. Mohammad Hassan*, Tanner Godfrey, Harunur Rashid, Amjad Javed. School of Dentistry, University of Alabama, USA

As over 53 million adults in the United States have low bone mass due to arthritis and osteoporosis, novel therapeutic strategies that protect from progressive and chronic bone loss are clearly warranted. The purpose of the proposed research is to refine our central hypothesis that the miR-23a cluster controls bone synthesis and homeostasis by regulating osteoblast-specific transcription factor Runx2 and chromatin remodeling factor BAF45a/PHF10 expression. Current studies do not have any insight on how epigenetic activation by histone acetylation of bone-specific chromatin is maintaining bone mass in vivo. Understanding of this gap is pivotal for further studies to identify specific targets for future therapies, which protect against bone loss. Gene regulation by microRNAs (miRNAs or miRs) is a key regulatory event in many biologic processes including skeletogenesis. Recently, we found that miR-23a cluster represses Runx2 expression, inhibits osteoblast-specific genes, and finally blocks osteoblast differentiation in vitro. However, the inhibitory role of miR-23a cluster during the development and remodeling of bone and the chromatin repression mechanism, leading to this inhibition of essential genes required for bone formation, is unrevealed. To study the role of miR-23a cluster in bone synthesis, we created an inducible miR-23a cluster knockdown mouse model (miR-23aCl^{ZIP}). Interestingly, our knockdown mice developed high cortical and trabecular bone. RNA sequencing from these mice significantly increased the expression of Runx2, and BAF45a/PHF10. Furthermore, we observed that BAF45a interacted with RUNX2, and together promote osteoblastogenesis. Osteoblast-specific BAF45a knockdown mice (BAF45a$^{f/f}$, osteocalcin ^{cre}) significantly reduced bone development. Knockdown of BAF45a in miR-23a cluster-depleted osteoblasts re-established miR-23a cluster-mediated inhibition, established a direct regulatory link to govern osteogenesis. ChIP assays with bone essential promoters using differentiated miR-23aCl^{ZIP} calvarial cells revealed higher BAF45a/PHF10 binding and H3K27 acetylation when compared with H3K27 methylation. Together, our findings strongly suggest that H3K27 acetylation of the bone-specific open chromatin is a key chromatin modification event for enhanced osteogenesis.

Epigenetic mechanism for high bone mass

Loss of miR-23a cluster function
↓
Permits Baf45a+Runx2 upregulation
↓ *Results in*
Increased H3K27 chromatin acetylation
↓ *Leads to*
Activation of bone forming genes
↓ *Results in*
High bone mass

P16
Regulation of FGF23 Production by Extra-Long Gsa Variant XLas in Acute Kidney Injury. Qing He*[1], Marc Wein[1], Jordan Spatz[1], Antonius Plagge[2], Paola Divieti Pajevic[3], Harald Jüppner[1], Murat Bastepe[1]. [1]Endocrine Unit, Department of Medicine, Massachusetts General Hospital & Harvard Medical School, Boston, MA, USA, [2]Department of Cellular & Molecular Physiology, Institute of Translational Medicine University of Liverpool, Liverpool, United Kingdom, [3]Department of Molecular & Cell Biology, Goldman School of Dental Medicine, Boston University, Boston, MA, USA

FGF23 is a bone-derived endocrine hormone regulating the renal excretion of phosphate and the synthesis of 1,25-dihydroxyvitamin D (1,25(OH)$_2$D). FGF23 levels are significantly elevated in chronic kidney disease and directly related to mortality in this condition. FGF23 levels are also elevated in acute kidney injury and correlate with increased risk of death or need for dialysis. Mechanisms underlying the kidney injury-induced FGF23 production are poorly defined. The *GNAS* locus encodes the α-subunit of the stimulatory G protein (Gsα) and its extra-long variant XLαs. Gsα stimulates cAMP generation and plays important roles in skeletal development and mineral ion homeostasis. XLαs is expressed in bone and can also stimulate cAMP generation when overexpressed. In addition, evidence from certain patients with *GNAS* mutations and mouse models indicates that XLαs is involved in the actions of calciotropic hormones and bone metabolism. We have now found that at postnatal day 10 (P10) XLαs knockout (XLKO) mice exhibit hyperphosphatemia (11.4±0.43 vs. 10.2±0.21 mg/dl in WT), increased serum 1,25(OH)$_2$D (224.8±3.49 vs. 142.5±4.02 pmol/L in WT), and reduced serum FGF23 (204.4±10.50 vs. 398.7±10.14 pg/mL in WT). FGF23 mRNA levels in XLKO femurs were also significantly reduced (57.7±3.26% of WT levels). CRISPR/Cas9-mediated knockout of XLαs in a murine osteocytic cell line (Ocy454) significantly decreased FGF23 mRNA (14.8±2.11% of control levels), indicating that XLαs regulates FGF23 expression in a cell-autonomous manner. FGF23 levels in adult XLKO mice were also decreased (389.0±10.09 vs. 501.5±12.90 pg/mL in WT); however, the difference was not statistically significant (p=0.079). We then tested whether XLαs is required for increased FGF23 production in response to acute kidney injury by injecting high dose folic acid into XLKO and WT littermates. Folic acid led to the expected deleterious effects in renal tubules of both XLKO and WT mice, as determined by histological analyses, and resulted in comparable increases in serum phosphate and blood urea nitrogen (BUN) levels. In contrast, the elevation of serum FGF23 was significantly lower in XLKO mice than WT littermates (1389.4±158.11 vs. 3474.8±199.32 pg/mL in WT at 24 hours after folic acid injection, p<0.01). These results suggest that FGF23 expression is regulated directly by the action of XLαs in bone cells, and that XLαs plays an important role in kidney-injury induced FGF23 production.

P17
Sc65-null mice provide evidence for a novel endoplasmic reticulum complex regulating collagen lysyl hydroxylation.
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Sc65 (Synaptoneal Complex 65) is an endoplasmic reticulum protein that belongs to the Leprecan family which include the prolyl 3-hydroxylases (P3H1, P3H2, P3H3) and cartilage associated protein (CRTAP). We and others have shown that mutations in both *CRTAP* and *LEPRE1* (encoding P3H1) cause recessive forms of Osteogenesis Imperfecta. Sc65 and CRTAP are both non-enzymatic proteins and share high homology suggesting Sc65 may also function in bone homeostasis. Utilizing a gene trap allele, we recently demonstrated that loss of Sc65 results in low bone mass. Here, a new global knockout mouse (Sc65-KO) derived by deleting exons 7 and 8 using homologous recombination is described. Mice null for Sc65 exhibit a similar bone loss phenotype as the previous model with decreased BV/TV and the loss of cortical and trabecular bone. To ascertain Sc65 function in bone, co-immunoprecipitation (co-IP) of Sc65 candidate interactors in mouse fibroblasts followed by mass spectrometry was performed. These experiments identified several fibrillar procollagen α-chains as likely substrates of Sc65 supporting the idea that Sc65 plays a role in collagen modification, similar to other Leprecans. At the biochemical level, mass spectrometry of type I collagen peptides showed severe under-hydroxylation at helical cross-linking sites K87 and K930/933 in collagen α1(I) and α2(I) chains both from bone and skin which are known LH1 preferred substrate residues but with no effect on sites of prolyl 3-hydroxylation. Direct co-IP assays showed Sc65 interaction with lysyl-hydroxylase 1 (LH1, Plod1), prolyl 3-hydroxylase 3 and cyclophilin B. Western blot revealed dramatic reduction of LH1 and P3H3 in primary osteoblasts and skin fibroblasts from *Sc65*-KO mice. Size exclusion chromatography confirmed that Sc65 and P3H3 form a stable complex in the ER that affects the activity of lysyl-hydroxylase 1 potentially through interactions with the enzyme and/or cyclophilin B. Further testing showed that *Sc65*-KO mice also have fragile skin with less tensile strength than control mice, consistent with a collagen cross-linking abnormality. Collectively, these results indicate that Sc65 is a novel adapter molecule that stabilizes a unique ER-resident complex that is essential for proper collagen lysyl-hydroxylation. Loss of Sc65 leads to complex instability and defective fibrillar collagen modifications which negatively impacts bone, skin and likely other connective tissues.

P18

A Missense Mutation in the ZIP14 Gene Results in Abnormal Skull Growth in Patients with Hyperostosis Cranialis Interna.

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Hyperostosis cranialis interna (HCI) is a rare autosomal dominant skeletal disorder characterized by intracranial hyperostosis and osteosclerosis of the calvaria and skull base, whereas the remainder of the skeleton is not affected. Symptoms are caused by entrapment of cranial nerves due to progressive bone overgrowth. We previously localized the disease causing gene on chromosome 8p21 in a family with HCI. With exome sequencing we now identified a mutation (p.L441R) in the *SLC39A14* (*ZIP14*) gene, which encodes a transmembrane protein that mainly functions as a zinc (Zn^{2+}) transporter. Subcellular localization studies indicated that mutant ZIP14 is no longer present at the membrane and mainly localizes in the cytoplasm. Subsequent Zn^{2+} uptake and transport experiments with respectively ^{65}Zn and Fluozin-3AM were performed. Here, the p.L441R mutation impairs the ability of ZIP14 to transport Zn^{2+} into the cell, but also causes entrapment of Zn^{2+} in the cell. Moreover, by performing a cAMP-responsive luciferase reporter assay, we observed a 5-fold increase in activation of cAMP-CREB signaling by mutant ZIP14, whereas overexpressing wildtype ZIP14 slightly decreased cAMP levels. Skull tissue from a HCI patient was obtained and immunohistochemistry experiments were performed with an anti-ZIP14 antibody. Here, expression of ZIP14 was detected in skull osteoblasts and not in osteocytes, whereas no osteoclasts could be detected. Moreover, we performed µCT analysis of *Zip14*$^{-/-}$ (KO) mice. Compared to wildtype littermates, tibia length of KO mice was significantly reduced, however no difference in the skull phenotype was observed. *In vivo* analysis of the p.L441R mutation was initiated by the development of a Zip14^{L438R}floxed mouse model and crossing these with different Cre mouse lines. Ubiquitous expression of Zip14^{L438R} after crossing with Sox2-Cre mice resulted in perinatal lethality. Conditional knock-in of Zip14^{L438R} in osteoblasts or osteoclasts was achieved by crossing Zip14$^{flox/flox}$ mice with either Runx2-Cre or Cathepsin K-Cre mice, respectively. Currently, skeletal phenotyping of osteoblast and osteoclast specific knock-in mice at the age of 6 months is being performed by µCT analysis, dynamic bone histomorphometry and histological stainings of calvaria and long bones.

P19

Identifying Genetic Mediators of Periodontitis in Mice. Sarah Hiyari*[1], Azadi Naghibi[2], Sotirios Tetradis[2], Flavia Pirih[2]. [1]University of California, Los Angles School of Dentistry, USA, [2]UCLA School of Dentistry, USA
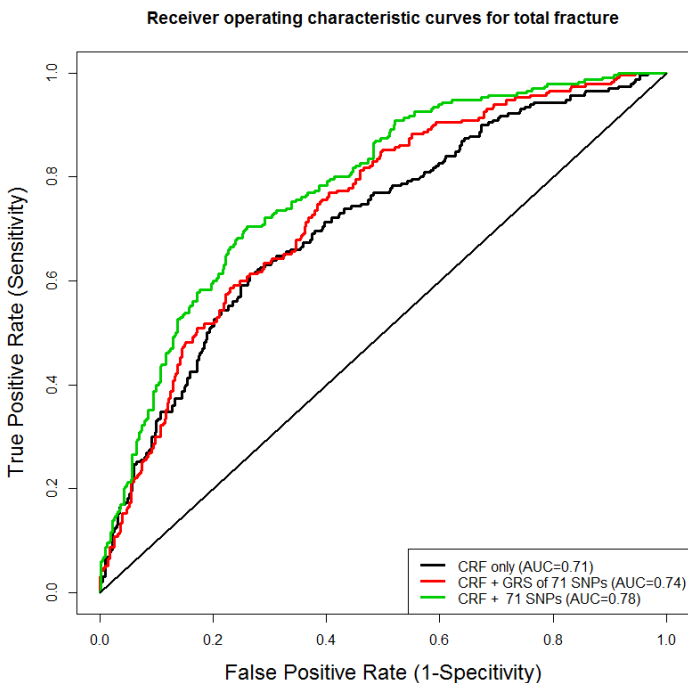
Periodontitis (PD) is characterized by bacterial infection and inflammation of the gingiva and alveolar bone surrounding the teeth. If left untreated, PD causes bone resorption that can ultimately lead to tooth loss. Additionally, PD affects ~47% of the U.S. population over age 30. Interestingly, twin studies have shown PD to be 50% heritable. Objective: Identify genetic mediators and cellular populations associated with PD susceptibility and severity. Methods: *P. gingivalis* (*P.g.*)-Lipopolysaccharide (LPS) was injected in between 1st and 2nd maxillary molars in 78 strains of the Hybrid Mouse Diversity Panel (HMDP) twice a week for 6 weeks. Following sacrifice, maxillae were scanned using microCT and linear bone loss was quantitated. Factored Spectrally Transformed Linear Mixed Modeling (FaST-LMM) was used to identify genetic loci associated to bone loss. A/J and C57BL/6J were further analyzed through: a) microarray b) *in vitro* osteoclastic potential, and c) *in vivo* histology including H&E, anti-p65 (NF-κB), anti-COX2, anti-TNFα, anti-TGFβ, anti-MMP13, anti-CD3 (T-cells), anti-neutrophil NIMP-R14, and anti-Cxcl10 after LPS injections. Results: We observed a strain-dependent 6-fold difference in LPS-induced bone loss across the HMDP. Focusing on the 5 parental strains, A/J was the most resistant and C57BL/6J was the most susceptible to *P.g.*-LPS-induced bone loss. Additionally, heritability was 50% for our trait. Microarray of A/J and C57BL/6J showed statistically differentially expressed genes induced by LPS including members of the Cxcl chemokine family, which correlated with our FaST-LMM data. *In vitro* data confirmed that C57BL/6J exhibit more osteoclastic potential compared to A/J (p=0.0195). *In vivo*analysis revealed that C57BL/6J had increased osteoclasts, NF-κB, COX-2, TNF-α, TGF-β, MMP-13, T-cells, neutrophils, and Cxcl10 after LPS injections. Conclusions: Heritability of bone loss in mice is similar to PD in humans. We identified known genes/loci, as well as, novel gene candidates that may be important for LPS-induced bone loss. Future work will validate candidate genes that contribute to PD. The ultimate goal of our study is to understand PD pathophysiology in order to identify individuals at high risk for PD.

P20

Prediction of Fragility Fracture Risk by Genetic Profiling. Thao P. Ho-Le*[1], Jacqueline R. Center[2], John A. Eisman[3], Hung T. Nguyen[4], Tuan V. Nguyen[5]. [1]Centre for Health Technologies, University of Technology, Sydney, Australia, [2]Bone Biology Division, Garvan Institute of Medical Research; St Vincent Clinical School, UNSW Australia, [3]Bone Biology Division, Garvan Institute of Medical Research; St Vincent Clinical School, UNSW Australia; Notre Dame University School of Medicine, Australia, [4]Centre for Health Technologies, University of Technology, Australia, [5]Centre for Health Technologies, University of Technology, Sydney; Bone Biology Division, Garvan Institute of Medical Research; School of Public Health & Community Medicine, UNSW, Australia

Aim: Several common genetic variants associated with bone mineral density (BMD) have been identified by genomewide association studies. However, the contribution of these variants to fracture prediction remains largely unknown. This study sought to define the

predictive value of a genetic profiling in fracture prediction. Methods: Seventy-one BMD-associated genetic variants were genotyped in 556 men and 902 women aged from 60 years who were participants of the Dubbo Osteoporosis Epidemiology Study. A genetic risk score (GRS) was constructed for each individual by summing the weighted number of risk alleles for each SNP, with the weight being log odds ratio. The incidence of fragility fracture was ascertained from X-ray reports between 1990 and 2009. Femoral neck BMD was measured by dual-energy X-ray absorptiometry. The magnitude of association between GRS and fracture risk was assessed by the logistic regression model with and without clinical risk factors. The utility of GRS was assessed in terms of area under the ROC curve (AUC) and integrated reclassification index (NRI). Results: Individuals with a fracture had greater GRS than those without a fracture (P<0.001). The odds of fracture was increased by 47% (odds ratio [OR] 1.47; 95%CI, 1.28-1.69) for each score increase in GRS. After adjusting for age, gender, prior fracture, fall and femoral neck BMD, the association between GRS and fracture risk remained statistically significant (OR 1.44; 95%CI, 1.24-1.67). For hip fracture, increase in GRS was also associated with an elevated risk of fracture (OR 1.66; 95%CI, 1.38-2.0). The AUC for the model with clinical risk factors was 0.71 (95%CI, 0.67 to 0.75); when GRS was added to the model, the AUC value was increased to 0.74 (95%CI, 0.70 to 0.78), and the NRI was improved by 23% at cut-off value of (0, 0.1, 0.2, 1). For hip fracture, the addition of GRS into the clinical risk factors based model resulted in an improvement of AUC from 0.80 (95%CI, 0.74 to 0.86) to 0.87 (95%CI, 0.82 to 0.91), and yielded an NRI gain of 19% in correct reclassification of fracture vs non-fracture. Conclusion: A genetic profiling of BMD-associated genetic variants could improve the accuracy of fracture prediction over and above that of clinical risk factors alone. Given the high prevalence of fracture in the general population, the clinical utility of cumulative genetic risk score can help stratify individuals by fracture status.

Receiver operating characteristic curves for total fracture

- CRF only (AUC=0.71)
- CRF + GRS of 71 SNPs (AUC=0.74)
- CRF + 71 SNPs (AUC=0.78)

P21

Genetic Determinant of Trabecular Bone Score (TBS). Lan T Ho-Pham*[1], Didier Hans[2], Linh D Mai[3], Minh C Doan[3], Tuan V Nguyen[4]. [1]Bone & Muscle Research Division, Ton Duc Thang University, Vietnam, [2]Center of Bone disease, Lausanne University Hospital, Switzerland, [3]Department of Internal Medicine, Pham Ngoc Thach University of Medicine, Vietnam, [4]Osteoporosis & Bone Biology Program, Garvan Institute of Medical Research; School of Public Health & Community Medicine, University of New South Wales; Centre for Health Technologies, University of Technology Sydney, Australia

Introduction. Trabecular bone score has emerged as a predictor of fragility fracture, but its etiological determinants have not been well documented. In this study, we sought to determine the genetic contribution to the variation of TBS in the general population. Methods. This study was designed as a population based family study, which involved 556 women and 189 men from 265 families. The participants were recruited from Ho Chi Minh City (Vietnam). The individuals aged 53 years (SD 11). We measured lumbar spine bone mineral density (BMD; Hologic Horizon) and then derived the TBS from the BMD scan. A biometric model was applied to the data to partition the variance of TBS into two components: one due to additive genetic factors, and one due to environmental factors. The index of heritability was estimated as the ratio of genetic variance to total variance of a trait. Bivariate genetic analysis was conducted to estimate the genetic correlation between lumbar spine BMD and TBS. Results. TBS was strongly correlated with lumbar spine BMD (r = 0.73; P < 0.001). TBS was also significantly associated with age and height, and the two factors accounted for 33% variance of TBS. After adjusting for age and height, average TBS in men was higher than women (1.37 [SD 0.09] vs 1.31 [SD 0.11]). Results of genetic analysis showed that the index of heritability of TBS was 0.51 (95% CI, 0.44 - 0.58), which was not much different from that of lumbar spine BMD (0.53; 95% CI, 0.42 - 0.60). Moreover, the genetic correlation between TBS and LSBMD was 0.34

(95% CI, 0.19 - 0.45). Conclusions. These data, for the first time, suggest that half of the variance in TBS between individuals is under genetic influence, and this effect magnitude is similar to that of lumbar spine BMD. This finding provides a scientific justification for the search for specific genetic variants that may be associated with TBS and fracture risk.

P22
Impaired Fracture Healing in Fgf2 Heterozygous and Homozygous Knockout Mice. Marja Hurley*, Kimberly Pontoja, Liping Xiao. UConn Health, USA

FGF2 enhances fracture (FX) repair in young animals and a clinical trial of FGF2 in Japan demonstrated its efficacy in enhancing FX healing in young human subjects. However there are no studies on its efficacy in FX repair in old animals or humans. We published that there was a significant decrease in FGF2 in old human mesenchymal progenitor cells (HMDPCs) compared with young, a significant reduction in FGF2 in bones of aged WT mice, and accelerated fracture healing in young 18kDaFGF2Tg mice. We posit that in aged and Fgf2Het mice with low endogenous FGF2 or Fgf2KO mice, FX healing would be impaired. Femur FX healing was determined in 4 months-old WT, Fgf2Het and Fgf2KO male mice. At 21 days post FX (PFD21), x-ray showed calluses were less mineralized in Fgf2het and Fgf2KO compared with WT. MicroCT at PFD35 still showed less mineralization in Fgf2Het and Fgf2KO (Fig. 1A). At PFD35 while callus remodeling was almost complete in WT, there was still ongoing callus remodeling in Fgf2Het and Fgf2KO and the ratio of bony/total callus area was decreased in Fgf2Het compared with WT and further decreased in Fgf2KO (Fig. 1B). Femur FX healing in young (2-months) and aged (24-months) WT and Fgf2KO mice was determined. At PFD14 x-rays showed less mineralized calluses in aged WT and in young and aged Fgf2KO mice. Bony/total callus area was decreased in aged WT compared with young WT mice but was similar to that observed in young and aged Fgf2KO (Fig.1C). To assess whether impaired healing in Fgf2KO was associated with decreased periosteal cell proliferation, femur FX was performed on 6-month-old female WT and Fgf2KO mice. Immediately after FX, mice were sc injected with 100ug/kg FGF2 or vehicle and sacrificed on PFD2. Twenty-four hours prior to sacrifice mice were injected intraperitoneal with EdU (5-ethynyl-2'-deoxyuridine) to assess proliferation. Femurs were harvested and frozen sections stained for EdU, then counterstained with 4'6-diamidino-2-pheynlindole(DAPI) to identify nuclei. Compared with WT decreased EdU labeled periosteal cells were noted in vehicle treated Fgf2KO femurs that were partially rescued by FGF2 (Fig.1D). In summary, FX healing is impaired in young Fgf2Het, young Fgf2KO mice, aged WT and aged Fgf2KO mice. FGF2 treatment increased periosteal cell proliferation in the early post fracture period in femurs of WT and Fgf2KO mice. Impaired periosteal cell proliferation due to reduced FGF2 expression could contribute to the impaired FX healing.

C=cortical bone, P= periosteum
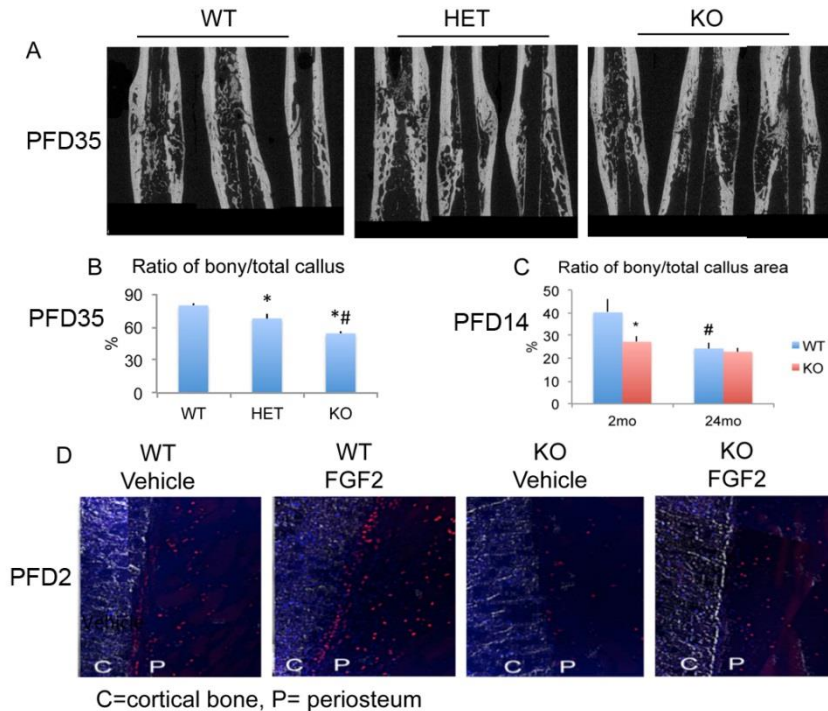
P23

Osteoblasts Inhibit Osteoclast Formation by Targeting Prdm1 via theMechanism Underlying Matrix Vesicle-Mediated Transfer of miR-125b.

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Matrix Vesicles (MVs) secreted from osteoblasts and chondrocytes serve as initial mineralization sites in the osteoid. Recent evidence indicates that MVs share common hallmarks with exosomes, suggesting MVs as vesicular lipid transporters involved in cell-cell communications. Current trends with the roles of exosomal miRNAs raise the question whether MVs include miRNAs that play a fundamental role in bone. Our global analysis of miRNAs identified 172 miRNAs in MVs isolated from mouse osteoblast cultures. We reported that not only MVs but also miR-125b inhibits osteoclast formation in mouse bone marrow macrophage (BMM) cultures. A positive correlation between miR-125b levels in MVs and antiosteoclast activity *in vitro* and miR125b accumulation in the bone matrix led us to uncover the role of MV-miR-125b in bone *in vivo*. We generated transgenic (Tg) mice overexpressing miR-125b under the control of the human osteocalcin promoter. Tg mice were grossly normal and born in Mendelian ratios. Micro-CT analysis revealed that Tg mice had increased bone volume systemically. This structural change mostly arose in trabecular bones that occupied marrow cavities. Without significant effects on the number of osteoblasts, decreased number of osteoclasts was observed in Tg mice. Calvaria cells and BMMs from Tg mice differentiate into osteoblasts and osteoclasts, respectively, in a similar manner to those from the wild type (WT). To clarify the molecular mechanism underlying impaired osteoclastic bone resorption in Tg mice, we searched miR-125b target genes in BMMs. Of predicted targets of miR-125b, *Prdm1*, a transcription repressor, was downregulated in BMMs treated with MVs. miR-125b also decreased*Prdm1* levels in the mouse RAW264.7 macrophage cell line, with resultant increases in the *Irf-8* and *Mafb* antiosteoclast factor genes. miR-125b decreased 3' UTR luciferase activity of the *Prdm1* gene. Inhibition of the binding between the 3'UTR of *Prdm1* and miR-125b rescued impairment of osteoclast formation in RAW264.7 cells treated with either MVs or MiR-125b. Thus, our findings reveal that osteoblasts inhibit osteoclast formation by targeting *Prdm1* via the mechanism underlying MV-mediated transfer of miR-125b. This may provide a novel therapeutic target for osteolytic bone diseases.

P24

Legumain is a Novel Regulator of Bone Formation and Deregulated in Postmenopausal Osteoporosis. Abbas Jafari*[1], Diyako Qanie[2], Thomas L. Andersen[3], Li Chen[2], Nicholas Ditzel[4], Sundeep Khosla[5], Harald T. Johansen[6], Per Kjærsgaard-Andersen[7], Jean-Marie Delaisse[8], Basem M. Abdallah[9], Daniel Hesselson[10], Rigmor Solberg[6], Moustapha Kassem[2]. [1]Department of Cellular & Molecular Medicine, University of Copenhagen, Denmark, [2]Endocrine Research Laboratory (KMEB), Odense University Hospital & University of Southern Denmark, Denmark, [3]Department of Clinical Cell Biology (KCB) Institute of Regional Health Science University of Southern Denmark, Denmark, [4]Endocrine Research Laboratory (KMEB), Odense University Hospital & University of Southern Denmark., Denmark, [5]Endocrine Research Unit, Mayo Clinic College of Medicine., USA, [6]Department of Pharmaceutical Biosciences, School of Pharmacy, University of Oslo, Norway, [7]Department of Orthopaedic Surgery, Vejle/Lillebaelt Hospital, Denmark, [8]Department of Clinical Cell Biology, Vejle/ Lillebaelt Hospital, Institute of Regional Health Research, University of Southern Denmark, Denmark, [9]Department of Endocrinology & Metabolism, Endocrine Research Laboratory (KMEB), Odense University Hospital & University of Southern Denmark, Denmark, [10]St Vincent's Clinical School, UNSW, Australia

It is increasingly recognized that secreted factors have an important role in mediating the functions of human skeletal (stromal, mesenchymal) stem cells (hMSC). We have determined the secretome of human MSC at the baseline and during osteoblast (OB) differentiation using stable isotope labeling by amino acids in cell culture (SILAC) and identified several novel regulators of hMSC differentiation. In the present study, we show that legumain (also known as asparaginyl endopeptidase) is a novel regulator of bone formation and its expression level and cellular localization are abnormal in the bone microenvironment of postmenopausal osteoporotic patients. Legumain expression and activity were down-regulated during *in vitro* and *in vivo* OB differentiation of hMSC. Silencing of legumain expression or pharmacological inhibition of its activity in *ex vivo* hMSC cultures, enhanced OB differentiation, and *in vivo* heterotopic bone formation, and reduced adipocyte (AD) differentiation. Ectopic expression of legumain in hMSC reduced *ex vivo* OB differentiation and enhanced AD differentiation. We found that the inhibitory effects of legumain on OB differentiation are mediated through degradation of the bone matrix protein fibronectin. At the organismal level, legumain-deficient zebrafish show precocious OB differentiation accompanied by increased vertebral mineralization. A similar phenotype was obtained by post-embryonic pharmacological inhibition of legumain activity. Legumain serum levels in women decrease by ageing, but these levels do not depend on estrogen or osteoporosis status. However, histological analysis of legumain in the bone microenvironment patients with postmenopausal osteoporosis revealed localized ectopic expression of legumain in bone marrow adipocytes and reduced adjacent trabecular bone volume. Our data demonstrate a novel role of legumain in regulation of hMSC lineage fate and bone formation and suggest that altered proteolytic activity in the bone microenvironment may contribute to development of osteoporosis.

P25

Epigenomic Signature of Bisphosphonate use. Roby Joehanes*, Yi-Hsiang Hsu, David Karasik, Douglas Kiel. Institutes for Aging Research; Hebrew SeniorLife; Harvard Medical School, USA

Bisphosphonates (BP) have been the first line drug for treating osteoporosis. However, their use has been reported to be associated with several rare side effects, such as osteonecrosis of the jaw and atypical femur fracture. The incidence of such side effects has been reported to correlate with the duration of BP use, raising the possibility that exposure to BP could affect DNA methylation, which could play a role in the risk for these side effects. We hypothesized that BP use induces alteration of DNA methylation signatures that eventually contribute toward such side effects. We investigated the DNA methylation in whole blood of 2,648 Framingham Osteoporosis Study participants (1,210M/1,348F) using the Illumina HumanMethylation BeadChip 450K over 485,512 cytosine-phosphate-guanine (CpG) sites. Of these samples, 293 (27M/266F) were BP users (210 alendronate, 72 risedronate, and 11 ibandronate). We used linear mixed effects models with methylation proportion (beta) as the outcome, BP use as the variable of interest, adjusting for sex, age, smoking status, menopause status, blood cell count, family correlation structure, and applicable technical covariates, such as batch and lab effects. We adjusted for multiple testing using Benjamini and Hochberg's False Discovery Rate (FDR). At a significance level of FDR<0.05, 15 CpG sites were significantly associated with BP use (TABLE). Gene-set enrichment pathway analyses revealed genes *ACVR1*, *ABCG1*, *LRRC1*, *SLC9A1*, *CASK*, and *CLIC1*, which are involved in estradiol downregulation response. In conclusion, our results suggest that BP may have induced DNA methylation patterns that lower estradiol response, which is consistent with recent findings (PMID: 26358930) that estradiol levels differ between BP responders and non-responders.

Probe ID	Chr.	Location	Gene Symbol	Coef.	SE	P	FDR
cg17501210	6	166,970,252	RPS6KA2	0.0187	0.0030	3.0E-10	0.0001
cg14622782	X	41,782,111	CASK	0.0161	0.0028	1.4E-08	0.0033
cg08863422	2	232,290,901		0.0171	0.0031	4.9E-08	0.0079
cg18909389	6	31,701,110	CLIC1	-0.0112	0.0021	2.0E-07	0.0210
cg00712762	1	36,351,470	EIF2C1	-0.0073	0.0014	2.2E-07	0.0210
cg15614653	2	129,104,464		0.0119	0.0023	2.8E-07	0.0223
cg06500161	21	43,656,587	ABCG1	-0.0101	0.0020	3.6E-07	0.0251
cg01298945	14	73,259,435	DPF3	0.0063	0.0013	4.5E-07	0.0272
cg25130381	1	27,440,721	SLC9A1	-0.0085	0.0017	7.8E-07	0.0368
cg22321559	X	16,738,200	SYAP1	0.0035	0.0007	8.1E-07	0.0368
cg23663298	11	61,912,356	INCENP	-0.0070	0.0014	9.2E-07	0.0368
cg26403843	5	158,634,085	RNF145	-0.0158	0.0032	9.5E-07	0.0368
cg00495036	2	158,733,490	ACVR1	-0.0035	0.0007	1.1E-06	0.0368
cg23370548	1	6,471,486		0.0111	0.0023	1.1E-06	0.0368
cg11475305	6	53,663,860	LRRC1	0.0039	0.0008	1.1E-06	0.0368

P26

PLS3 Sequencing in Childhood-onset Primary Osteoporosis Identifies Two Novel Mutations. Anders J Kämpe*[1], Alice Costantini[1], Riikka E Mäkitie[2], Nina Jäntti[1], Helena Valta[3], Minna Pekkinen[2], Mervi Mäyränpää [4], Fulya Taylan[1], Hong Jiao[5], Outi Mäkitie[6]. [1] Department of Molecular Medicine & Surgery & Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden, [2]Folkhälsan Institute of Genetics & University of Helsinki, Helsinki, Finland, [3]Children's Hospital, University of Helsinki & Helsinki University Hospital, Helsinki, Finland, [4]Children's Hospital, University of Helsinki & Helsinki University Hospital, Helsinki, Finland, Sweden, [5]Department of Biosciences & Nutrition, & Science for Life Laboratory, Karolinska Institutet, Stockholm, Sweden, [6]Department of Molecular Medicine & Surgery, Karolinska Institutet, Sweden; Folkhälsan Institute of Genetics & University of Helsinki, Helsinki, Finland; Children's Hospital, University of Helsinki & Helsinki University Hospital, Helsinki, Finland, Sweden

Early-onset osteoporosis caused by mutations in the X-chromosomal gene *PLS3* was first described in 2013 as a rare condition mainly affecting males. To investigate this further, we screened two separate patient cohorts for *PLS3* mutations. Cohort I consisted of 31 patients referred to the Metabolic Bone Clinic, Children's Hospital, Helsinki for suspected early-onset osteoporosis of unknown genetic etiology; several had a positive family history. Cohort II consisted of 64 otherwise healthy children (67% boys) aged 4-16 years who during a 12-month period had been treated for a fracture at the Children's Hospital, Helsinki, and had a significant fracture history not caused by secondary osteoporosis or osteogenesis imperfecta. We Sanger sequenced all coding exons and exon-intron boundaries of *PLS3*(NM_005032) to identify disease-causing variants. In Cohort I, 2 patients (6.5%) were positive for novel *PLS3* mutations that were deemed causative of their phenotype. The first patient was a male who was diagnosed with osteoporosis at the age of 12 years based on multiple vertebral and bilateral femoral fractures. He had a nonsense mutation (p.R256*), inherited from his mother who had osteopenia. The second patient was a girl with multiple long bone and vertebral fractures and low BMD (Z-score -6.6) at the age of six years. She had a *de novo* missense mutation (p.N446S); other genetic causes were excluded by exome-sequencing of the family. In Cohort II, no pathogenetic variants were identified. In total, we found 4 different single nucleotide variants (SNVs) within coding regions, 3 synonymous and 1 missense variant; all were predicted benign. The synonymous variant *rs140121121* has been associated with osteoporosis, but was not enriched in our cohorts. In conclusion, based on our results, screening for disease-causing *PLS3* mutations should be included in the investigation of both male and female patients with early-onset osteoporosis but is not indicated in patients only fulfilling the criteria for a clinically significant fracture history.

P27
Bone Strength Estimated by Failure Load Shares Genetic Composition with Areal BMD: The Framingham Study Families.

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Genetic factors contribute to the risk of long bone fractures, mostly due to effects on bone strength. High-resolution peripheral quantitative computed tomography (HR-pQCT) estimates bone strength in compression using micro finite element analysis (μFEA). The goal of this study was to investigate if the HR-pQCT-based μFEA-estimated bone failure load is heritable and to what extent it shares genetic regulation with long bone's aBMD measured by DXA. Bone microarchitecture was measured by HR-pQCT (Scanco Medical, AG) at the distal tibia (n=1,059) and distal radius (n=1,022) in adults of European ancestry from the Offspring Cohort of the Framingham Heart Study (average age 72.2 years, range 48-95; 57% women). Radial and tibial failure load (FL) in compression was estimated by μFEA (in Newton). Femoral neck (FN) and ultradistal forearm (UD) aBMD were measured by DXA (Lunar DPX-L, g/cm^2). Heritability estimates (h^2) and bivariate correlations were calculated (adjusting for age, sex, and height) using a variance components model in SOLAR. FL values at the non-weight-bearing distal radius and at the weight-bearing distal tibia were highly correlated: r=0.906. Estimates of h^2 adjusted for covariates ranged from 0.518-0.522 (radius) and 0.497-0.505 (tibia). Additional adjustment for local aBMD dramatically decreased both radius and tibia FL h^2 estimates: 0.222 and 0.380, respectively. In bivariate analysis, there were high phenotypic and genetic correlations between multivariable-adjusted FL at radius and UD aBMD (ρ_P =0.826, ρ_G =0.954), while environmental correlations (ρ_E) were lower (0.696; Table 1). Similar but lower correlations were observed between tibial FL and FN aBMD (ρ_P = 0.577, ρ_G = 0.703, ρ_E= 0.432 respectively; Table 1). These data from extended families of a population-based cohort of adult men and women suggest for first time that, in adults of European ancestry, bone strength estimated by failure load is heritable and shares genetic composition with areal BMD, regardless of the specific skeletal site (slightly lower between tibial FL and aBMD of the hip since these bones are not the same). Further work is necessary to identify specific variants underlying genetic differences in long bone structure and strength.

Table 1: Descriptive statistics, heritability of failure load and its phenotypic (ρ_P), genetic (ρ_G) and environmental (ρ_E) correlations with proximal aBMD

		Radius			Tibia	
FL, Newtons (mean±SD)		2518.2± 788.8			6437.5±1751.1	
h^2 (mean±SE)		0.522±0.127 P=0.000026			0.497±0.120 P=0.000027	
		correlations with UD aBMD			correlations with FN aBMD	
model of adjustment	ρ_P	ρ_G±SE (p0, p1)	ρ_E±SE (p0)	ρ_P	ρ_G±SE (p0, p1)	ρ_E ±SE (p0)
Age and sex	0.819	0.936±0.050 (0.00004; 0.092)	0.704±0.079 (0.00064)	0.594	0.713±0.085 (0.00006; 0.00048)	0.453±0.141 (0.035)
Age, sex, and height	0.826	0.954±0.046 (0.00002; 0.155)	0.696±0.080 (0.0009)	0.577	0.703±0.086 (0.00012; 0.0009)	0.432±0.142 (0.039)

p0: p value for difference from 0; p1: p value for difference from 1.0

P28
GATA4 Regulates RUNX2 expression in osteoblasts.
Susan Miranda, Aysha Khalid*, Gustavo Miranda-Carboni. University of Tennessee, USA

GATA4 is a zinc-finger transcription factor that is essential in various tissues, such as heart, kidneys and intestine, and more recent studies showed that it also has a role in bone mineralization. Previously we have shown that *in vivo* deletion of *Gata4*, driven by Cre-recombinase under the control of the Col1a1 2.3 kb promoter, results in incomplete perinatal lethality, decreased trabecular bone properties, and abnormal bone development in P0 mice. Analysis of the trabecular bone of 14 week old males and females by Micro-CT showed significantly reduced values for BV/TV and trabecular (Tb) number, while Tb separation was greater in the femur and tibia of knockout mice. However, deleting *Gata4* did not have any effect on cortical bone properties. Histological analysis of the tibia and femur showed that knockouts not only had reduced Tb number and increased Tb separation, but also had reduced osteoblast surface/bone surface (ObS/BS), osteoblast perimeter and osteoclast perimeter. Analysis of the cDNA of bone marrow mesenchymal stem cells from wild type control (WT) and cKO mice differentiated for 14 days in osteogenic media demonstrated a significant reduction in *Runx2* gene expression and alizarin red staining in osteoblasts from cKO mice as compared to controls. Gene expression levels determined by qPCR for *Runx2* were also reduced in primary calvarial cells infected with lentivirus expressing shRNA directed to *Gata4* (shGATA4), as compared to GFP (shGFP). To determine if *Runx2* is a direct target of GATA4, ChIP was performed, and indeed GATA4 is recruited to the *Runx2* promoter. Furthermore, when *Gata4* is knocked down, the chromatin at the *Runx2* promoter is not open, as detected by ChIP with antibodies to the open chromatin mark H3K4me2 (histone 3 lysine 4 dimethylation). Together

the data suggest that GATA4 binds near *Runx2* promoter, helps open the chromatin to regulate *Runx2* expression and subsequent bone mineralization.

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Intestinal microbiome present in Crohn disease impairs the skeletal health and linear growth. Anu Maharjan*, Young Huh, Maureen Bower, Hong Yuan, Young Truong, Ian Carroll, Francisco Sylvester. University of North Carolina, USA

Background: Crohn disease (CD) is a chronic inflammatory disorder of the gastrointestinal tract. In children CD is frequently associated with significant bone mass deficits and sarcopenia present from diagnosis and persisting even when CD is in clinical remission. Aim: We hypothesized that a dysbiotic enteric microbiota in pediatric CD inhibits skeletal and muscle growth. Methods: We performed fecal microbiota transplant (FMT) from 2 children with newly diagnosed, untreated CD and 2 unaffected age-matched healthy controls (HC) into 15 germ-free (GF) wild type 129S6/SvFv (7 male and 8 female, 6 week old) mice. We measured bone mineral density (BMD), fat mass, and fat-free mass using dual X-ray absorptiometry (DXA) 8 days post-FMT (Lunar Piximus). We tracked daily weight and obtained colon length and histology post-sacrifice. Results: Male mice that received FMT from CD developed colitis and lost significant percentage of weight from baseline compared to mice that received HC FMT (HC 5.23 ± 2.62 %; CD -29.41 ± 3.54 %; p = 0.0007 on day 7. However, BMD in male mice did not decrease irrespective of whether they received CD or HC FMT. On the other hand, female mice that received CD FMT had lower BMD (g/cm^2) than HC in the presence of colitis relative to baseline (% change HC 0.41 ± 1.45; CD -5.57 ± 1.26; p = 0.036). Male mice that received CD FMT with colitis had significant loss in fat-free mass (g) (HC 3.4 ± 0.8; CD -24.2 ± 5.7; p = 0.009) and in fat mass (g) (HC 35.4 ± 13.7; CD -32.4 ± 4.8; p = 0.003), but not in female mice. Conclusion: In this model, a CD dysbiotic intestinal microbiome impairs both bone and muscle health but effects depend on sex. This suggests gender influence on colitis susceptibility and the effects of colitis on BMD and body composition.

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Transcriptomic Analysis of Osteoclasts from Autosomal Dominant Osteopetrosis type2 (ADO2) Mice harboring the G213R Mutation of the Chloride-Proton Antiporter type 7 (ClC7). Antonio Maurizi*[1], Nadia Rucci[1], Tina Schleicher[2], Yadhu Kumar[2], Anna Teti[1], Mattia Capulli[1]. [1]Dept. of Biotechnological & Applied Clinical Sciences, University of L'Aquila, Italy, [2]GATC Biotech AG, Germany

Heterozygous mutations of ClC7 induce ADO2, impairing osteoclast resorption lacuna acidification. We observed that this mutation also increases lysosomal pH (+1.64 pH units, p<0.001) and induces Golgi enlargement (+1.8fold, p=0.008), thus suggesting that the osteoclast function can be impaired at various levels. To verify this hypothesis, primary osteoclasts were obtained from WT and ADO2 mice carrying the ClC7 G213R mutant and subjected to RNA deep sequencing by Illumina Hi Seq 2500 technology. We found 387 over- and 63 under-expressed transcripts in ADO2 vs WT osteoclasts (p<0.05). The highest expressed genes were grouped by the Gene Ontology terms. Statistically significant enrichment was observed for genes involved in the biological processes of cell adhesion (p<0.0001), integrin signaling (p<0.001) and regulation of apoptosis (p=0.05). Grouping these genes for their molecular functions, revealed an enrichment of the metal and ion binding function (p<0.05), peptidase activity (p=0.008) and integrin binding function (p=0.01). The analysis of the cellular components revealed an enrichment of transcripts for proteins localized in endoplasmic reticulum (p<0.001) and basement membrane (p<0.001). Conversely, biological processes such as immune response (p=0.04), chemotaxis (p<0.0001), cell proliferation (p<0.001) and regulation of endocytosis (p=0.05) were enriched in the group of under-expressed transcripts of ADO2 vs WT osteoclasts. Interestingly, further investigations using the KEGG pathway analysis revealed higher expression in ADO2 of genes involved in osteoclast differentiation (p<0.05). Accordingly, in vitro osteoclastogenesis was faster and more prominent in ADO2 bone marrow cultures vs WT (1.7fold,p<0.01), probably triggered by increased circulating PTH levels (1.98fold,p=0.045) and consequent increased number of osteoclast precursors in the ADO2 bone marrow (1.4fold,p=0.05). Of note, other pathways more active in ADO2 osteoclasts were cytokines-cytokine receptors (p<0.001), Jak/Stat signal (p=0.001), extracellular matrix-receptor (p<0.001) and focal adhesion (p<0.001). All together, these results suggest that the heterozygous impairment of ClC7 function in ADO2 osteoclasts does not cause only a defective bone resorption, but also an impairment of pathways essential for multiple osteoclast functions. We expect this research to pave the way for the identification of druggable targets that could improve the osteoclast function in ADO2.

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Functional validation of a key bone mineral density locus determined by genome-wide association studies. Robert D Maynard*[1], Fernando Rivadeneira[2], Carolina Medina-Gomez[2], Kwangbom Choi[3], Cheryl L Ackert-Bicknell[1]. [1]Center for Musculoskeletal Research, University of Rochester, USA, [2]Department of Internal Medicine, Erasmus University Medical Center, Netherlands, [3]The Jackson Laboratory, USA

Genome-wide association studies (GWAS) for bone phenotypes have aided in the discovery of novel loci, but functional validation is lacking for many of these novel loci. GWAS has repeatedly identified a locus for BMD and fracture risk at 7q31.31. *WNT16* is adjacent to this locus and has a defined role in bone biology, but a contribution to this locus of the neighboring gene, *CPED1*, cannot be excluded. *CPED1* is an uncharacterized gene with no verified function. Our data has shown that in mice, the open reading frame

for this gene is composed of at least 24 exons. Similarly, we have found that this gene is widely expressed. Relevant to bone, we have determined that *Cped1* is expressed in mouse cortical bone, calvarial osteoblasts, and in multipotent C3H10T1/2 cells differentiated with osteogenic media. Conversely, no expression was observed in the macrophage line, RAW264.7, which can be stimulated down the osteoclast lineage. The translated protein putatively contains an N-terminal cell surface signal peptide, a cadherin-like domain, and a PC esterase domain with predicted acyltransferase and acylesterase activity for the modification of glycoproteins. In maturing calvarial osteoblasts, the pattern of expression of *Cped1* is highly correlated with other extracellular matrix proteins such as the glycoprotein Osteonectin (*Sparc*, $R^2 = 0.99$) and Type I collagen (*Col1a1*, $R^2 = 0.83$). We have established that the 3rd exon is alternately spliced out resulting in at least 2 transcripts and thus 2 putative protein products. The expression of both isoforms increases during osteoblastogenesis. To understand the role of these isoforms in osteoblast function, siRNAs targeting either exon 1 (present in both transcripts) or exon 3 (isoform specific) were delivered to C3H10T1/2 cells concurrent with osteogenic media, and cells were differentiated for up to 10 days. By day 5, cells treated with siRNA against exon 1 had reduced alkaline phosphatase (ALP) staining (Fig 1A), and this coincided with a 99% reduction in message (Fig 1B). Interestingly, *Cped1* exon 3 siRNA led to increased ALP staining and an 85% increase in expression (Fig 1B), suggesting that these *Cped1* isoforms are not redundant in function. A similar observation was made on day 10 post knockdown (Fig 1A). In summary, these data strongly suggest that the two known isoforms of this gene play opposing roles in osteoblast maturation and that this gene may have a key role in bone biology.
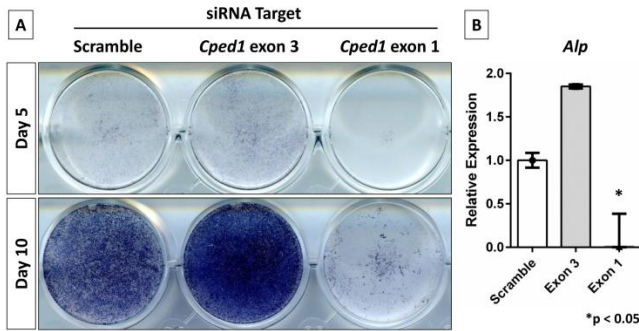
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Genome-Wide Association Study of DNA Methylation Identifies a Novel Locus Associated with Bone Mineral Density.

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Purpose: Genetic and environmental determinants of osteoporosis risk may converge through the epigenome, providing a tool to better understand the pathophysiology of osteoporosis. Since the epigenetics of bone mineral density (BMD) has been largely unexplored in humans, we undertook a comprehensive genome-wide assessment of the influence of epigenetic changes on BMD. Methods: We undertook an international, large-scale epigenome-wide association study of BMD using the Illumina Infinium HumanMethylation450 array to measure site-specific DNA methylation across four cohorts in up to 4,559 samples of European descent. Using these data, we aimed to identify differentially methylated probes (DMPs) associated with femoral neck and lumbar spine BMD. We used whole-blood since epigenetic changes are partially stable across tissues, immune cells may influence BMD, and some bone cells arise from monocyte-macrophage precursors. We performed sex-pooled and stratified analyses, controlling for age, weight, smoking status, sex (for pooled analyses), estimated white blood cell proportions, and random effects for relatedness and batch effects. We defined statistical significance of DMPs as being associated with BMD at a 1% false-discovery rate (FDR). Results: We identified one DMP significantly associated ($P = 2.92 x 10^{-10}$) with femoral neck BMD in females (N = 3,312). The DMP maps to the liver carboxylesterase 1 (*CES1*) gene 5' UTR. The DMP is within a novel locus for bone biology as there are no previously identified genetic associations with BMD and *CES1*. Ongoing analyses aim to explore the tissue specificity of the signal across blood and additional tissues, and assess whether corresponding gene expression changes are observed. Further follow-up experiments to assess genetic interaction with the DMP, understand the sex-specificity of the association, and the function of DNA methylation and *CES1* with respect to BMD are underway. Results for sex-pooled and male-only analyses were not statistically significant. Conclusion: Undertaking the most comprehensive genome-wide analysis to-date for the role of whole-blood methylation in BMD, we identified a novel epigenetic locus for bone biology mapping to *CES1*. Interrogation of the function of this region will help to understand how environmental and genetic effects converge upon the epigenome to regulate BMD.

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Measles Virus Nucleocapsid Protein Expressing Osteoclasts Increase Expression of SPHK1/S1P/S1PR3 to Enhance Osteoblast Differentiation in Paget's Disease. Yuki Nagata*[1], Yasuhisa Ohata[1], Jolene Windle[2], David Roodman[3], Noriyoshi Kurihara[1]. [1]Medicine / Hematology- Oncology, Indiana University, USA, [2]Human & Molecular Genetics, Virginia Commonwealth University, USA, [3]Medicine / Hematology-Oncology, Indiana University; Roudebush VA Medical Center, USA

Paget's Disease (PD) is the most exaggerated example of coupled bone remodeling with increased osteoclast (OCL) formation and increased osteoblast (OB) differentiation. Blocking OCL formation in PD decreases bone formation, demonstrating that the enhanced OB differentiation in PD is driven by OCLs. However, the OCL-derived factors increasing OB differentiation in PD are just being identified. We reported that OCLs from PD patients express measles virus nucleocapsid protein (MVNP), which increases IL-6, IGF1 and ephrinB2 expression in OCLs and ephB4 on OBs to increase OB differentiation. However, these factors do not totally account for the increased OB differentiation in PD. Since we previously showed that MVNP-expressing OCLs (MVNP-OCLs) express high levels of sphingosine-1-phosphate (S1P), and S1P was reported to increase OB differentiation via S1P receptor 3 (S1PR3) (JCI 2013), we examined the roles of S1P and S1PR3 in OB differentiation in PD. We found that mature MVNP-OCLs produce high levels of Sphk1 and S1P compared to WT OCL. Further, S1P production was dependent on IL-6 since Shpk1 expression was decreased in OCLs from MVNP/IL-6 KO mice. We then assessed S1PR1 and R2 expression on MVNP-OCLs, since we found that S1P binding S1PR1 inhibits immature OCL precursor (pre-OCL) proliferation, while S1P binding S1PR2 inhibits pre-OCL motility and fusion. MVNP expressing pre-OCL expressed high levels of S1PR1 during proliferation, while S1PR2 increased with pre-OCL differentiation. We then tested if S1P (10 μM) blocks OCL formation by highly purified MVNP and WT pre-OCL. S1P blocked OCL formation by both MVNP and WT pre-OCL, suppressing both pre-OCL proliferation and fusion, and decreasing levels of DC-STAMP, NFATc-1 and ADAM8. We then determined if MVNP-OCLs induced S1PR3 on OBs in co-cultures of MVNP-OCLs and OBs. MVNP-OCLs increased S1PR3 expression on OBs and enhanced OB differentiation to a greater extend than WT-OCL. To confirm that S1PR3 mediated the enhanced OB differentiation, we treated OCL/OB co-cultures from MVNP and WT mice with an S1PR3 antagonist (VPC23019) or S1PR3 agonist (VPC24191). VPC23019 blocked OB differentiation in MVNP-OCL/OB co-cultures while VPC24191 enhanced SP7 and Col-1 levels in OBs to greater extent in MVNP-OCL/MVNP-OB co-cultures compared to WT-OCL/WT-OB. These results suggest that S1P produced by PD-OCL acts as a coupling factor to block OCL formation, enhance S1PR3 expression in OB, to increase bone formation in PD.

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Novel Genetic Variants are Associated with Increased Vertebral Volumetric BMD, Reduced Vertebral Fracture Risk, and Increased Expression of SCL1A3 and EPHB2. Carrie Nielson*[1], Ching-Ti Liu[2], Albert Smith[3], Cheryl Ackert-Bicknell[4], Sjur Reppe[5], Johanna Jakobsdottir[3], Christina Wassel[6], Thomas Register[7], Ling Oei[8], Nerea Alonso Lopez[9], Edwin Oei[8], Neeta Parimi[10], Elizabeth Samelson[11], Mike Nalls[12], Joseph Zmuda[13], Thomas Lang[14], Mary Bouxsein[11], Jeanne Latourelle[15],Melina Claussnitzer[11],Kristin Siggeirsdottir[3], Priya Srikanth[1], Erik Lorentzen[16], Liesbeth Vandenput[16], Carl Langefeld[7],Laura Raffield[7], Greg Terry[7], Amanda Cox[7], Matthew Allison[17], Michael Criqui[17], Donald Bowden[7], M. Arfan Ikram[8], Dan Mellström[16], Magnus Karlsson[18], JeffreyCarr[19],Matthew Budoff[20],Caroline Phillips[21], L.Adrienne Cupples[2], Wen-Chi Chou[22], Richard Myers[23], Stuart Ralston[9], Kaare Gautvik[5], Peggy Cawthon[10], Steve Cummings[10], David Karasik[11], Fernando Rivadeneira[8], Vilmundur Gudnason[3], Eric Orwoll[1], Tamara Harris[21], Claes Ohlsson[16], Douglas Kiel[11], Yi-Hsiang Hsu[11]. [1]Oregon Health & Science University, USA, [2]Boston University School of Public Health, USA, [3]IcelandicHeart Association, Iceland, [4]University of Rochester Medical Center, USA, [5]Lovisenberg Diakonale Hospital, Norway, [6]University of Vermont College of Medicine, USA, [7]Wake Forest School of Medicine, USA, [8]Erasmus MC, University Medical Center, Netherlands, [9]University of Edinburgh, United Kingdom, [10]California Pacific Medical Center Research Institute, USA, [11]Harvard Medical School, USA, [12]National Institutes of Health, USA, [13]University of Pittsburgh, USA, [14]University of California, San Francisco, USA, [15] Boston University School of Medicine, USA, [16]University of Gothenburg, Sweden, [17]University of California, San Diego, USA, [18]Lund University, Sweden, [19]Vanderbilt, USA, [20]Los Angeles Biomedical Research Institute, USA, [21]National Institute on Aging, USA, [22]BROAD Institute of MIT & Harvard, USA, [23]Boston University School of Medicine, USA

Genome-wide association studies (GWAS) have revealed numerous loci associated with DXA-measured hip and spine areal bone mineral density (aBMD). We completed the first GWAS meta-analysis (N=15,275) of lumbar spine volumetric BMD (vBMD) measured by quantitative computed tomography (QCT), allowing for the unique examination of the trabecular bone compartment. SNPs that were significantly associated with vBMD were also examined in two GWAS meta-analyses of cohorts of older adults to determine associations with morphometric vertebral fracture (N=21,701) and clinical vertebral fracture (N=5,893). Expression QTL analyses of human iliac crest biopsies were performed in a sample of 84 postmenopausal women, and the murine osteoblast expression of genes implicated by eQTL or by proximity to vBMD-associated SNPs was examined. We identified significant vBMD associations with five loci, including variants near 3 loci reported in hip and lumbar spine aBMD GWAS (1p36.12, containing *WNT4* and*ZBTB40;* 8q24, containing *TNFRSF11B*; and 13q14, containing *AKAP11* and *TNFSF11*). Two loci (5p13 and 1p36.12) also contained associations with radiographic and clinical vertebral fracture, respectively. The minor allele of the most significant variant in 5p13 (rs2468531; minor allele frequency [MAF]=3%), which has not previously been linked to BMD or fracture, was associated with higher vBMD ($\beta = 0.22$, $p = 1.9 \times 10^{-8}$) and decreased risk of radiographic vertebral fracture (OR = 0.76; false discovery rate [FDR] p = 0.01). The minor allele of the most significant variant in 1p36.12 (rs12742784; MAF=21%) was associated

with higher vBMD (β = 0.09, p = 1.2 x 10^{-10}) and decreased risk of clinical vertebral fracture (OR =0.79; FDR p = 8.6 x 10^{-4}). Both SNPs are located in the non-coding part of the genome and were associated with increased mRNA expression levels in human bone biopsies: rs2468531 with *SLC1A3* (β = 0.28, FDR p = 0.01, involved in glutamate signaling and osteogenic response to mechanical loading) and rs12742784 with *EPHB2* (β = 0.12, FDR p = 1.7 x 10^{-3}, functions in bone-related ephrin signaling). Both genes are expressed in murine osteoblasts. This is the first study to link *SLC1A3* and *EPHB2* to clinically relevant vertebral osteoporosis phenotypes in older adults. These results may aid in elucidating vertebral bone biology, leading to novel approaches to reducing vertebral fracture incidence.

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Vitamin D Deficiency Due to a Recurrent Gain-of-Function Mutation in CYP3A4 Causes a Novel Form of Vitamin D Dependent Rickets. Jeffrey Roizen*[1], Dong Li[2], Lauren O'Lear[3], Muhammad K Javaid[4], Nicholas Shaw[5], Peter Ebeling[6], Hanh Nguyen[7], Christine Rodda[8], Kenneth Thummel[9], Hakon Hakonarson[2], Michael Levine[3]. [1]Division of Endocrinology & Diabetes The Children's Hospital of Philadelphia, University of Pennsylvania Perelman School of Medicine, USA, [2]Center for Applied Genomics, The Children's Hospital of Philadelphia, University of Pennsylvania Perelman School of Medicine, USA, [3]Division of Endocrinology & Diabetes, The Children's Hospital of Philadelphia, University of Pennsylvania Perelman School of Medicine, USA [4]National Institute for Health Research (NIHR) Musculoskeletal Biomedical Research Unit, University of Oxford, United Kingdom, [5]Birmingham Children's Hospital & University of Birmingham, United Kingdom, [6]Monash University, Monash Medical Centre, Clayton, Victoria, Australia, [7]Monash University, Monash Medical Centre, Clayton, Victoria, Australia, [8]NorthWest Academic Centre, University of Melbourne, Australia, [9]Department of Pharmaceutics, University of Washington, Seattle

We evaluated two unrelated and non-consanguineous adolescent females, one originally from Jordan and one from Australia, who had presented with early-onset rickets, reduced serum levels of 25(OH)D and 1,25(OH)$_2$D, and deficient responsiveness to vitamin D$_2$/D$_3$, as well as to activated forms of vitamin D. Targeted analyses of the *CYP2R1, CYP27B1* and *CYP24A1* genes revealed normal sequences, thus we analyzed genomic DNA from both patients by whole exome sequencing (WES). We identified an identical heterozygous single nucleotide change in both subjects in exon 10 of the *CYP3A4* gene (c.902T>C) that results in replacement of isoleucine by threonine at codon 301 (p.I301T). This change was not present in other first-degree relatives of the two probands, nor in public databases (1,000 Genomes Project, 6503 exomes from the Exome Sequencing Project (ESP6500SI), and Exome Aggregation Consortium dataset (ExAC v0.3)) and existing data from more than 2,000 WES samples in the CHOP database. CYP3A4 (EC 1.14.13.97) is a P450 enzyme that metabolizes xenobiotics and many steroid hormones. Because induction of CYP3A4 by anticonvulsants leads to vitamin D deficiency via generation of the inactive vitamin D metabolites principally 4b,25(OH)$_2$D and 1,23R,25 (OH)$_3$D, we compared the activity of the p.I301T mutant to that of wild type CYP3A4 and CYP24A1 (the principle vitamin D degrading enzyme) in HepG2 cells also expressing a 2-hybrid reporter consisting of the ligand-binding domain of the VDR fused to the yeast GAL4 DNA binding domain plus a firefly luciferase reporter gene under the control of nine copies of the yeast Gal4 UAS. The p.I301T mutant showed increased inactivation of calcitriol (Figure 1) relative to wild type CYP3A4 at multiple calcitriol concentrations (p<0.05 by 2-way ANOVA) and post-hoc multiple-comparison-adjusted analyses confirmed significant differences at 30 ng/mL (p < 0.05), and relative to CYP24A1 at 30 ng/mL (p<0.01). As measurements are done in whole cells, the kinetic data are apparent values. The apparent catalytic efficiency [Vmax(app)/Km(app)] of the wild type was 0.015, and of the mutant was 0.14. Thus, CYP3A4 (p.I301T) is nearly 10-fold more active than the wild type enzyme. In summary, we have identified a recurrent missense mutation in *CYP3A4* that results in increased CYP3A4 activity and enhanced inactivation of vitamin D metabolites. We propose that this gain-of-function mutation of *CYP3A4* causes a novel form of vitamin D dependent rickets.

Figure 1: CYP3A4 (p.I301T) degrades 1,25(OH)$_2$D$_3$ significantly more than wild type CYP3A4
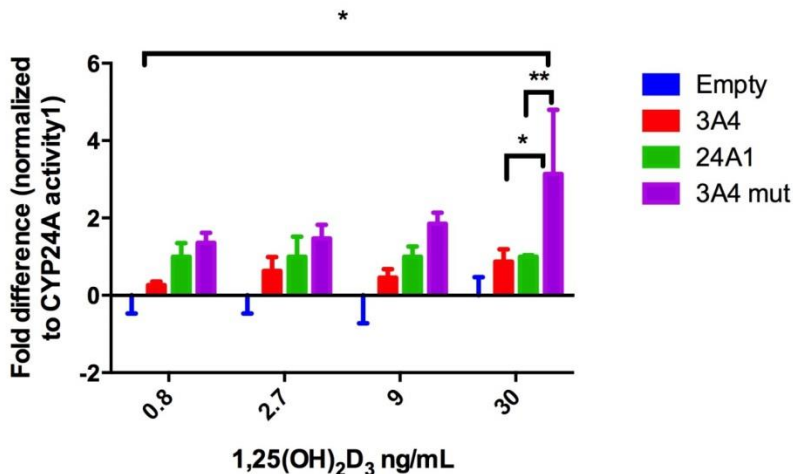
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Description of a novel variant in SMAD3 presenting in a patient with osteoporotic fractures and arterial dissection. Laura Ryan*, Dawn Allain. The Ohio State University Wexner Medical Center, USA

Introduction: Osteoporotic fracture and bone fragility has long been associated with disorders of connective tissue development, such as Marfan syndrome, Ehlers-Danlos syndrome and osteogenesis imperfecta. We present a case of a woman with arterial deficiency in combination with osteoporotic fragility fractures in whom a novel *SMAD3* variant was noted, and review the experience of this grouping of findings within the research data repository at our institution. Case: A 43yo Caucasian female presented initially to our endocrinology clinic an unusual presentation of multiple, low-trauma fractures, including a recent fracture of the proximal humerus after a fall from standing height. She continues to have regular menstrual cycles. Her family history revealed aortic dissection in her father. She did not exhibit blue sclerae, hypertelorism, bifid uvula or hyperflexibility. Her bone mineral density testing revealed osteoporosis. She then developed pupillary asymmetry and facial droop and was diagnosed with bilateral vertebral artery dissections. Subsequent MRI revealed worsening dissection. She was referred to the adult genetics department at our institution. Findings: Genetic testing included a 16 gene next generation sequencing panel along with concurrent targeted array CGH analysis. This evaluation revealed a c.154G>A mutation in *SMAD3*, a previously unreported mutation, and the presumptive cause of the phenotypic presentation of both arterial dissection and osteoporotic fracture in our patient. Using our research data repository, a query revealed that only 4 out of 821,318 individuals treated at our institution over the previous 5 years had a similar combination of findings. Discussion: SMAD proteins were initially described and identified as mediators of transcriptional activity in the transforming growth factor-beta (TGF-β) family. It has been noted that Smad3 is instrumental in osteoblastic bone mineralization, and that disruption of Smad3 in mice causes osteopenia and decreased bone formation. Blockade of Smad3 signaling by overexpression of Smad7 resulted in markedly suppressed RANKL-induced osteoclastogenesis in murine cell lines. Through TGF-β signaling, *SMAD3* may also play a role in the activation of sclerostin gene expression. The potential presence of an abnormality in connective tissue development should be considered in a patient who presents with pre-menopausal fragility fractures.

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Loss of the Longevity Gene SirT1 Dysregulates Chondrocytes and Leads to an Arthritic Phenotype In Vivo, Via Impaired Autophagy. Pradeep Kumar Sacitharan*[1], Tonia Vincent[1], James R Edwards[2]. [1]Kennedy Institute of Rheumatology, University of Oxford, United Kingdom, [2]The Botnar Research Centre, University of Oxford, United Kingdom

Ageing is universally linked to skeletal deterioration. Common mechanisms may control both processes, where dysregulation may predispose to bone loss and osteoarthritis (OA). The epigenetic deacetylase SirT1 controls lifespan and decreases with age. However, the role of SirT1 in joint disease is unclear. Human samples, novel genetically modified mice, a surgical disease model and a new therapeutic approach were employed alongside advanced cellular and molecular studies to explore the hypothesis that SirT1 is dysregulated in OA human cartilage, disrupting normal lifespan-protecting mechanisms such as autophagy, which can be targeted to reduce OA. Autophagy degrades unwanted proteins, and is defective in ageing. Inhibition of SirT1 in HTB94 chondrocytes decreased chondrogenic (COL2A1, ACAN, SOX-9, p<0.01) and autophagy markers (BECN1, ULK-1, LC3, p<0.01). LC3 protein conversion, essential for autophagosome formation, was positively regulated with SirT1 activity in chondrocytes (western blot, FACS (p<0.001)) and cartilage explants from LC3-GFP reporter mice (p<0.0001), demonstrating changes in SirT1 alters autophagic flux. Immunoprecipitation analysis showed direct SirT1 binding with ATG5,-7, beclin1, and LC3 and dysregulated acetylation in line with pharmacological activation (SRT1720;500nM) or inhibition of SirT1 (EX-527;100nM). Human OA cartilage showed decreased SirT1 compared to healthy cartilage (p<0.05). A previously undescribed inducible, articular cartilage-specific SirT1 deletion murine model (SirT1fl/flx Aggrecan CreERT2 mice (SirT1Agg)), showed greater cartilage degradation, increased OA disease score and epiphyseal volume (vs WT, p<0.001) on histomorphometric and uCT analysis at 2, 6, 12 mths. SirT1Agg mice showed decreased ACAN, COL2A1 (p<0.001) and autophagy markers BECN1, ULK-1, LC3, ATG5, ATG7, ATG13 (p<0.01), decreased LC3 protein in hip explants (p<0.01), and decreased autophagosome number (SEM, p<0.05). Induction of experimental OA (destabilisation of the medial meniscus (DMM) surgery) exacerbated cartilage degradation in SirT1Agg mice vs WT and decreased LC3 staining. However, activation of autophagy using Spermidine (5mM) protected against DMM-induced joint destruction in WT (p<0.001) but not in SirT1Agg mice. These studies suggest that declining SirT1 in ageing human cartilage, or SirT1 deletion in murine chondrocytes, results in OA due to dysregulated autophagy, and which may be rescued by increasing autophagic flux.

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Transgenic Mouse Model for Reducing Oxidative Damage in Bone. Ann-Sofie Schreurs*[1], Eric Moyer[1], Akilesh Kumar[1], Samantha Torres[1], Tiffany Truong[1], Candice Tahimic[1], Joshua Alwood[1], Charlie Limoli[2], Ruth Globus[1]. [1]NASA ARC, USA, [2]UC Irvine, USA

Bone loss can occur due to factors such as age, radiation, microgravity, all of which increase Reactive Oxygen Species (ROS); and is characterized by alterations in the balance between bone formation and resorption. Hence, we hypothesized that suppression of excess ROS in both osteoblasts and osteoclasts will improve overall bone microarchitecture and mass. To test our hypothesis, we used transgenic mCAT mice which overexpress the human anti-oxidant catalase gene targeted to the mitochondria, the main site for endogenous ROS production. mCAT mice have a longer life-span than wildtype controls and have been used to study various age-

related disorders. To stimulate skeletal remodeling, 16-week-old mCAT mice or wildtype mice were exposed to treatment (hindlimb-unloading and total body-irradiation) or sham treatment (control). Tissues were harvested two weeks later for skeletal analysis (microcomputed tomography), biochemical analysis (gene expression and oxidative damage measurements), and ex vivo bone marrow derived cell culture (osteoblastogenesis and osteoclastogenesis). mCAT mice expressed the transgene and displayed elevated catalase activity in skeletal tissue and ex vivo osteoblast and osteoclast cultures derived from marrow. In addition, when challenged with treatment, bone from wildtype mice showed elevated levels of malondialdehyde (MDA, indicating oxidative damage) whereas mCAT mice did not. Correlation analysis revealed that increased catalase activity correlated with decreased MDA levels and that increased oxidative damage correlated with decreased percent bone volume (BV/TV). Furthermore, ex-vivo osteoblast colony growth positively correlated with osteoblast catalase activity. mCAT mice displayed reduced BV/TV and trabecular number (Tb.N) relative to wildtype, as well as increased structural model index (SMI) in cancellous axial bone (tibia). No genotype effects on microarchitecture or compressive mechanical properties were observed in the 4th lumbar vertebra. Treatment caused bone loss in wildtype mice, as expected. Treatment also caused deficits in microarchitecture of mCAT mice, although less severe than wildtype mice in some parameters (percent bone volume, structural model index and cortical area). In conclusion, our results indicate endogenous ROS signaling in both osteoblast and osteoclast lineage cells contribute to skeletal development and remodeling and quenching oxidative damage could play a role in bone loss prevention.

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Differential Regulation of Post-traumatic Osteoarthritis Associated Genes in Str/ort, MRL/MpJ and C57BL/6 Mice. Aimy Sebastian*[1], Jiun C. Chang[1], Deepa K. Murugesh[2], Sarah Hatsell[3], Aris N. Economides[3], Blaine A. Christiansen[4], Gabriela G. Loots[2]. [1]UC Merced, School of Natural Sciences, USA, [2]Lawrence Livermore National Laboratories, Physical & Life Sciences Directorate, USA, [3]Regeneron Pharmaceuticals, USA, [4]UC Davis Medical Center, Department of Orthopedic Surgery, USA

Joint injury, particularly tears of the anterior cruciate ligament (ACL), often result in post-traumatic osteoarthritis (PTOA), yet the mechanisms contributing to PTOA development after joint injury are still poorly understood. To better understand the molecular events that trigger PTOA development and progression we profiled injury-induced gene expression changes in knee joints of three mouse strains with varying susceptibility to PTOA: STR/ort (highly susceptible), C57BL/6 (moderately susceptible) and MRL/MpJ (resistant). Right knee joints of 10 weeks old male mice were injured using a non-invasive tibial compression overload mouse model of PTOA that mimics ACL rupture in humans. Histological evaluation of injured joints at 12 weeks post injury revealed severe cartilage erosion in STR/ort, moderate cartilage erosion in C57BL/6, and minimal cartilage damage in MRL/MpJ. Next, we examined transcriptional changes in injured joints compared to uninjured contralateral joints using RNA-seq at 1 day and 1 week post injury. At 1 day post injury, we identified 637, 810, and 380 differentially regulated genes (>1.5 fold) in the injured joints of STR/ort, C57BL/6 and MRL/MpJ, respectively, including 172 genes commonly changed in all three strains. By comparing age-matched uninjured controls from all three strains we found that several immune/inflammation responses related genes including *Ccl2*, *Ccl7*, *Cxcl1*, *Cxcl5* and *Il6* were highly expressed in STR/ort compared to C57BL/6 and MRL/MpJ, and their expression was increased immediately post injury in all three strains. However, the expression of many of these genes returned back to normal by 1 week post injury in C57BL/6 and MRL/MpJ but remained elevated in STR/ort. At 1 week post injury, 818 and 874 genes were found to be differentially regulated in injured joints of STR/ort and C57BL/6, respectively. Only 574 genes were found to be differentially regulated in injured joints of MRL/MpJ, and 269 of these genes overlapped with genes identified in STR/ort and C57BL/6. Among the transcripts differentially regulated at 1 week post injury, we found *Adamts4*, *Aldh1a2*, *Cyp26b1* and *Il1r1* to be significantly up-regulated, and muscle related proteins *Myl2*, *Myh7*, *Tnnc1* and *Tnni1* to be significantly down-regulated exclusively in STR/ort and C57BL/6. The transcriptome level data generated in this study can be further explored experimentally to identify candidate genes that could be targeted to prevent PTOA development.

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Deletion of Axin1 in Osteoblast Progenitor Cells Leads to Delayed Endochondral Bone Formation through Inhibition of Osteoclast Formation. Bing Shu*[1], Yongjian Zhao[1], Chunchun Xue[1], Rong Xie[2], Yongjun Wang[3], Di Chen[2]. [1]Longhua Hospital, Shanghai University of Traditional Chinese Medicine; Spine Research Institute, Shanghai University of Traditional Chinese Medicine, China, [2]Rush University Medical Center, USA, [3]School of Rehabilitation Science, Shanghai University of Traditional Chinese Medicine; Spine Research Institute, Shanghai University of Traditional Chinese Medicine, China

Axin1 is a key regulator in canonical Wnt/β-catenin signaling pathway and its role in endochondral bone formation is currently unknown. We have recently generated *Axin1* conditional knockout (cKO) mice (*Axin1^{Oxs}*) by breeding *Axin1^{flox/flox}* mice with *Osx-Cre* mice. The phenotypes of bone development and postnatal bone formation were analyzed in new born and 1-, 2-, and 4-week-old *Axin1^{Oxs}* mice. The result of immunohistochemistry (IHC) showed that expression of β-catenin was increased on the surface of trabecular bone and on the endosteal surface of the cortical bone in *Axin1^{Oxs}* mice. Alizarin red/Alcian blue staining of whole skeletal preparation showed no significant difference in the overall skeletal pattern formation in new born *Axin1^{Oxs}* mice. The hypertrophic zone of tibia growth plate of new born and 1-week-old *Axin1^{Oxs}* mice was significantly expanded in *Axin1^{Oxs}* mice. Large numbers of chondrocytes and unresorbed cartilage matrix were found in the middle of the bone marrow cavity in new born and 1-week-old *Axin1^{Oxs}* mice and in the secondary ossification center of 2- and 4-week-old *Axin1^{Oxs}* mice. Chondrocyte apoptosis in the bone marrow cavity of tibia was reduced and osteoclast formation in metaphyseal and subchondral bone areas of tibia was significantly decreased in *Axin1^{Oxs}* mice, demonstrated by decreases in tartrate-resistant acid phosphatase (TRAP), matrix metalloproteinase 9

(MMP9) and Cathpesin K staining. IHC results also showed increased osteoprotegerin (OPG) expression in tibiae of *Axin1^Osx* mice. The results of real-time PCR showed that ratio of *Opg/receptor activator for nuclear factor-κB ligand* (*Rankl*) was much higher in primary calvarial cells of *Axin1^Osx* mice. Osteoclast formation in bone marrow cells (BMCs) cultured with the conditioned media (CM) collected from primary calvarial cells derived from *Axin1^Osx* mice was significantly decreased compare to the BMCs cultured with the CM derived from Cre-negative calvarial cells. The results of IHC staining also detected decreased expression of nuclear factor of activated T-cells cytoplasmic 1 (NFATc1) and c-fos in *Axin1^Osx*mice. In conclusion, deletion of *Axin1* in osteoblast progenitor cells caused reduction of osteoclast formation, which in turns leads to the inhibition of chondrocyte apoptosis, cartilage matrix resorption and endochondral bone formation in*Axin1^Osx* mice.

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Novel BAT precursor-derived cell lines with normal Gnas methylation maintain predominantly maternal Gsa expression: new tools for studying paternal Gsa regulation. Olta Tafaj*[1], Harald Jüppner[1], Steven Hann[2], Matthew Warman[2], Lee S. Weinstein[3]. [1]MGH, USA, [2]Boston Children's Hospital, USA, [3]National Institutes of Health, USA

Pseudohypoparathyroidism type Ia (PHP1A) is caused by heterozygous, inactivating mutations affecting exons 1-13 of the maternal GNAS allele that encode the α-subunit of the stimulatory G protein (Gsα). These mutations cause resistance to parathyroid hormone (PTH) and frequently other hormones mediating their actions through Gsα-coupled receptors. No hormonal resistance is observed when the same or similar *GNAS* mutations occur on the paternal allele. Observations similar to those in PHP1A patients had been observed in mice lacking Gnas exon 1 or 2, thus leading to the hypothesis that the paternal exon 1 promoter undergoes tissue-specific silencing through as-of-yet undefined mechanisms. Cells with predominantly maternal Gsα expression are present in proximal renal tubules (PRT) and in subpopulations within thyroid, pituitary and central nervous system. Taking advantage of a SNP in Gnas exon 11 (rs13460569; C in 129Sv; G in C57/BL6), we recently showed that predominantly maternal Gαs expression is maintained throughout life in brown adipose tissue (BAT). To further understand the mechanism underlying silencing of the paternal Gnas allele in BAT, we isolated primary mature brown adipocytes from newborn wild-type (WT) mice and showed that predominantly maternal Gsα expression is maintained in these cells. Because mature adipocytes are post-mitotic, we isolated adipocyte precursor cells (APC) from neonatal mice derived from matings between floxed *Gnas*exon 1 (E1fl/fl) females and WT males that were for homozygous SNP rs13460569 (C or G, respectively). After immortalization, clonal APC lines (n=19) were generated and parent-specific Gsα expression was investigated. Individual E1fl/WT cell lines showed normal allele-specific Gnas methylation, yet revealed considerable differences in the maternal contribution to total Gαs mRNA expression; namely 72±0.34% (n=5), 55±1.9% (n=12), or 25±4.8% (n=2). The relative contribution of each parental allele to *Gnas* mRNA expression was maintained for more than 30 passages. These novel cell lines can now be used to investigate the epigenetic and non-epigenetic mechanisms that contribute to the differential regulation of the Gnas allele inherited from mother versus father.

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High Prevalence (64%) of Mutations in the LRP5 and/or COLIA1 Genes in Male & Juvenile Female Patients with Osteoporosis. Christian Wüster*[1], Susanne Thomczyk[2], Wolfgang Höppner[3], Klaus Edgar Roth[2], Philipp Drees[2]. [1]Center for Hormones & Metabolism Prof. Wüster, Germany, [2]Orthopaedics, Orthopaedic & Rheumatoid Surgery, University Mainz, Germany, [3]Bioglobe GmbH Hamburg, Germany

Introduction: Several genetic causes of osteoporosis have been identified. Single mutations have been shown in some individual patients or several polymorphisms in population-based studies. The intention of the study was to investigate bone mineral density (BMD), fracture rates, markers of bone turnover (BTMs) and special point mutations (COLIA1 and LRP5) in a larger group of male and juvenile female patients with established osteoporosis in a clinical setting. Patient characteristics: 194 patients [39 males (20%) and 155 females (80%)] with a mean age of 48 years in man and 54 years in women were included into the study. BMD was measured by DXA (Medix DR, MediLink, France), fractures were recorded by history and BTM measured by ELISAs (ids, Frankfurt, Germany). Genetic mutations were analyzed by bioglobe, Hamburg, Germany. We divided patients into 4 groups: patients with no mutations (A), with mutations in COLIA1 (B) or LRP5 (C) and a mutation in both genes (COLIA1 & LRP5) (D). Results:124 patients (64%) [25 males (m) & 99 females (f)] had at least one mutation. Group A (no mutation) consisted of n=72 (37%) [15 m & 60 f], group B (COLIA1): n=38 (20%) [8 m & 30 f], group C (LRP5): n= 43 (22%) [12 m & 34 f] and group D (COLIA1 & LRP5): n=35 (18%) [6 m & 29 f]. There was no significant difference in fracture rates, mean BMD and BTMs between the 4 groups. Discussion: There is a high prevalence of mutations in the COLIA1 and/or LRP5 genes in selected patients with osteoporosis (males and young females). There was no difference in their clinical characteristics versus those patients without a mutation. Further studies have to show whether the presence of a mutation has a significant influence on treatment outcome. Conclusions: Genetic screening should be considered as a routine investigation in patients with osteoporosis.

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Jun N-terminal Kinases (JNKs) Act in Osteoblasts to Control Adolescent Bone Formation. Ren Xu*[1], Yeon Suk Yang[2], Sarfaraz Lalani[2], Na Li[3], Roger Davis[4], Jae-Hyuck Shim[3], Matthew Greenblatt[3]. [1]Dept of Medicine & Dept of Pathology, Weill Cornell Medical College, Cornell University, USA, [2]Department of Medicine, Weill Cornell Medical College, Cornell University, USA, [3]Department of Pathology & Laboratory Medicine, Weill Cornell Medical College, Cornell University, USA, [4]Program in Molecular Medicine, University of Massachusetts Medical School, USA

The c-Jun N-terminal kinases (JNKs) are members of the mitogen activated protein kinases (MAPKs) and are ancient and evolutionarily conserved regulators of proliferation, differentiation and cell death responses. Currently, *in vitro* studies using chemical inhibitors suggest that JNKs play an important role in osteoblast differentiation but offer conflicting data about whether the JNK pathway augments or represses osteoblast differentiation, and the contribution of JNK to regulation of bone mass *in vivo* remains unclear. Here we show that $Jnk1^{-/-}$ but not $Jnk2^{-/-}$ mice display osteopenia that is most severe during adolescent bone modeling at 3-4 weeks of age. Accompanying this, $Jnk1^{-/-}$ mice display an occipital-predominant impairment in calvarial mineralization. In order to both confirm that these effects were osteoblast intrinsic and assess whether redundancy with JNK1 masks a potential contribution of JNK2, mice with a conditional deletion of *Jnk1* and *Jnk2* floxed conditional alleles in osteoblasts ($Jnk1^{osx}Jnk2^{osx}$) were bred. These mice displayed a similar degree of osteopenia as $Jnk1^{-/-}$ mice, suggesting that JNK1 is the major mediator of JNK signaling in osteoblasts *in vivo*. Consistent with these *in vivo* findings, $Jnk1^{-/-}$ osteoblasts display a selective defect in the late stages of osteoblast differentiation *in vitro*, with impaired mineralization activity but intact upregulation of alkaline phosphatase. Downstream of JNK1, phosphorylation of JUN and *Fra-1* mRNA levels are impaired in $Jnk1^{-/-}$ osteoblasts. In order to identify the transcriptional targets of JNK1-regulation, RNA sequencing analysis of $Jnk1^{-/-}$ osteoblasts was performed, identifying that JNK1 is required for upregulation of several osteoblast-derived proangiogenic factors such as FGF2 and VEGF. Taken together, this study establishes that JNK1 plays a critical role in promoting osteoblast activity both *in vivo* and *in vitro,* displaying a robust contribution to postnatal modeling in both intramembranous craniofacial and endochondral bones.

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Mechanical Loading Induces CD31hiEmcnhi Vessel Formation by Preosteoclast Secretion of PDGF-BB. Weicheng Xu*[1], Hui Xie[1], Ryan Tomlinson[1], Zhuying Xia[1], Genevieve Brown[2], Maureen Pickarski[3], Le Duong[4], X. Edward Guo[2], Xu Cao[1]. [1]Department of Orthopaedic Surgery, Johns Hopkins University School of Medicine, USA, [2]Department of Biomedical Engineering, Columbia University, USA, [3]Merck Res. Lab, Bone Biology Group, USA, [4]Merck Res. Labs., Bone Biology Group, USA

Bone is remodeled to adapt to changes in mechanical loading, but the molecular and cellular mechanisms of this process are not well understood. Our previous research has shown that secretion of platelet-derived growth factor-BB (PDGF-BB) from tartrate-resistant acid phosphatase–positive (TRAP^{+}) preosteoclasts stimulates the production of a specific blood vessel subtype essential for new bone formation (CD31^{hi}Emcn^{hi} vessels). As a result, we hypothesized that preosteoclasts may also perform this important function at sites of load-induced bone formation. To test this hypothesis, we subjected adult mice to four weeks of *in vivo* mechanical loading by cyclic hind limb compression. This regimen stimulated the production of new cortical bone at the mid-diaphysis of the tibia and increased the number of TRAP^{+} preosteoclasts and CD31^{hi}Emcn^{hi} blood vessels in the tibial periosteum detected by μCT and immunofluorescence. Importantly, we observed that these periosteal TRAP+ preosteoclasts expressed PDGF-BB. Therefore, we examined whether secretion of PDGF-BB from TRAP^{+} preosteoclasts is required for the production of CD31^{hi}Emcn^{hi} blood vessels and bone formation following mechanical loading. In mice with the selective deletion of PDGF-BB from the osteoclast lineage (TRAP-Cre), mechanical loading did not stimulate new bone formation or periosteal CD31^{hi}Emcn^{hi} vessels relative to their WT littermates. These results suggested that secretion of PDGF-BB from TRAP+ preosteoclasts is required for load-induced bone formation. To further explore the role of preosteoclast-derived PDGF-BB in skeletal adaptation, mice were subjected to hind limb suspension (HLS) with or without administration of the Cathepsin K (CatK) inhibitor L-235 (Merck). As expected, HLS was associated with decreased bone mass and a diminished number of TRAP^{+} preosteoclasts and CD31^{hi}Emcn^{hi} blood vessels in the periosteum, but these effects were reversed by the administration of CatK inhibitor L-235. Taken together, the results from this study illustrate that osteogenic mechanical loading induces the accumulation of periosteal preosteoclasts that secrete PDGF-BB, leading to the formation of CD31^{hi}Emcn^{hi} vessels to support new bone.

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Genome-wide association study of knee bone marrow lesions and association with previously reported bone mineral density loci. Michelle S. Yau*[1], Braxton D. Mitchell[2], Rebecca D. Jackson[3], Marc C. Hochberg[2], Douglas P. Kiel[1], David T. Felson[4]. [1]Hebrew SeniorLife, BIDMC/Harvard, USA, [2]University of Maryland School of Medicine, USA, [3]The Ohio State University, USA, [4]Boston University School of Medicine, USA

Bone marrow lesions (BMLs) are areas of damage in the subchondral bone that may be an early sign of knee osteoarthritis (OA). BMLs have been associated with clinically relevant outcomes, such as pain and OA progression, and are associated with local BMD, suggesting that underlying bone pathology may be involved. We therefore conducted a genome-wide association study of BMLs in the Osteoarthritis Initiative (OAI) and the Framingham Osteoarthritis Study, and determined whether BMD genetic loci were also associated with BMLs. We included 1,027 Caucasians from OAI (57% women, age 65±9 yrs), a longitudinal study of individuals with or at high risk for knee OA and 304 Caucasians from Framingham (62% women, age 66±9 yrs), a community-based study of prevalent knee OA. Participants were genotyped with the Illumina Omni-Quad 2.5M array or Affymetrix 500K Dual GeneChip+50K gene-centered MIP set for OAI and Framingham studies, respectively. Both cohorts were imputed to 1000G. A BML summary index was derived using MRI-based semi-quantitative BML severity scores across 15 sub-regions of the knee (0-45). We conducted linear regression, adjusting for age, sex, and study-specific principal components, then combined results using an inverse variance fixed-effects meta-analysis. About 48% of OAI and 30% of Framingham participants had radiographic knee OA (KL≥2), and 77% of OAI and 79% of Framingham had a BML summary index>1. In both cohorts, mean BML summary index was higher in those with knee

OA (5 vs 2). We identified 22 loci that met suggestive significance ($p<1\times10^{-6}$), of which 3 met genome wide significance ($p<5\times10^{-8}$): 1) rs6070523 (maf=3%, beta=-2.0, se=0.4, $p=1.1\times10^{-8}$), 2) rs117994469 (maf=3%, beta=-3.0, se=0.5, $p=2.8\times10^{-8}$), 3) rs188681806 (maf=1%, beta=-4.5, se=0.8, $p=2.8\times10^{-8}$). Of 62 previously reported loci for BMD, we identified 3 nominally significant loci ($p<0.05$) associated with BMLs: 1) FOXL1 (rs10048146, maf=20%, beta=-0.5, se=0.2, $p=0.01$), 2) TXNDC3, rs10226308, maf=20%, beta=-0.4, se=0.2, $p=0.02$), 3) DCDC5 (rs163879, maf=31%, beta=0.29, se=0.13, $p=0.03$). For FOXL1 and TXNDC3, the high BMD allele was protective for BMLs. In conclusion, we identified 3 novel genome-wide significant loci and 3 previously reported BMD loci that were associated with BMLs, suggesting that there may be unique processes associated with BML development. Further work to understand the biology underlying these findings may reveal novel therapeutic targets for knee OA.

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Heritability of Thoracic Kyphosis and Genetic Correlations with Other Spine Traits: Framingham QCT Study.

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Hyperkyphosis is a common spinal disorder in older adults, characterized by excessive curvature of the thoracic spine (large kyphosis angle) and adverse health outcomes. The etiology of hyperkyphosis has not been firmly established, but may be related to changes in the vertebrae, discs, joints, and muscles, which function as a unit to support the spine. Determining the contribution of genetics to kyphosis and the degree of shared genetics with other spine traits may provide insight into the determinants of hyperkyphosis. The purpose of our study was to estimate heritability of kyphosis and genetic correlations between kyphosis and vertebral fracture (VF), intervertebral disc height narrowing (DHN), facet joint osteoarthritis (FJOA), lumbar spine volumetric bone mineral density (vBMD), and paraspinal muscle area and density. We included 2,063 individuals from the second and third generation cohorts of the Framingham Study. Thoracic kyphosis (T4-T12 Cobb) angle was measured from CT scout images and based on vertebral morphometry points identified by a semi-automated algorithm. A musculoskeletal radiologist used a semi-quantitative method (0=none, 1=mild, 2=moderate, 3=severe) to evaluate VF, DHN, and FJOA from T4 to L4 from CT images. Scores were summed across spinal levels to derive a summary index for each trait. We measured average paraspinal muscle size and density at T7 and T8 using spatially filtered CT scans (Analyze, Biomedical Imaging Resource). We used a variance components method to estimate heritability of each spine trait and bivariate models to estimate genetic correlation between kyphosis angle and each spine trait. All models were adjusted for age, sex, and weight. Mean age was 59±11 years; 49% were women. Mean kyphosis angle was 34.0±9.6 degrees. Heritability of kyphosis angle was h^2=54±6%. We found significant genetic correlations between kyphosis angle and muscle area (ρ_G=-0.46, $p<0.01$), VF (ρ_G=0.39, $p<0.01$), and vBMD (ρ_G=-0.23, $p=0.02$), but not between kyphosis angle and muscle density (ρ_G=-0.22, $p=0.09$), DHN (ρ_G=0.17, $p=0.14$), or FJOA (ρ_G=0.05, $p=0.64$). In conclusion, the severity of thoracic curvature is heritable and may share genetic contributions with bone loss, VF, and declines in muscle size that frequently occur with aging. Better understanding of the molecular processes underlying shared genetic variation among spine traits may provide biological insights into the etiology of hyperkyphosis and related spine conditions.

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Decreased JMJD3 Expression in Mesenchymal Stem Cells Contributes to Long-term Suppression of Osteoblast Differentiation in Multiple Myeloma.

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Multiple myeloma (MM) is the most frequent cancer to involve the skeleton, with over 80% of myeloma patients developing lytic bone disease (MMBD). Importantly, MM-associated bone lesions rarely heal even when patients are in complete remission. Mesenchymal stem cells (MSCs) isolated from MM patients have a distinct genetic profile and an impaired osteoblast (OB) differentiation capacity when compared to healthy donor MSCs. Utilizing an *in vivo* model of MMBD and patient samples, we showed that MSCs from tumor-bearing bones failed to differentiate into OBs weeks after removal of MM cells. Both Runx2 and Osterix, the master transcription factors for OB differentiation, remained suppressed in these MSCs. Currently molecular mechanisms for MM-induced long-term OB suppression are poorly understood. Therefore, we characterized both Runx2 and Osterix promoters in murine pre-osteoblast MC4 cells by ChIP to assess the transcriptional activity. The Runx2 and Osterix transcriptional start sites (TSSs) in untreated MC4 cells were co-occupied by transcriptionally active histone 3 lysine 4 tri-methylation (H3K4me3) and transcriptionally repressive histone 3 lysine 27 tri-methylation (H3K27me3), termed the "bivalent domain". These bivalent domains became transcriptionally silent with increasing H3K27me3 levels when MC4 cells were co-cultured with MM cells or treated with TNF-α, an inflammatory cytokine enriched in MM bone marrow. Furthermore, both MM cells and TNF-α downregulated the H3K27 demethylase JMJD3 and increased global H3K27me3 levels in MC4 cells and murine MSCs. Similarly, knockdown of JMJD3 in MC4 cells inhibited OB differentiation. Consistent with *in vitro* findings, MSCs from MM patients showed markedly decreased JMJD3 expression compared to healthy MSCs. Interestingly, the epigenetic signature at the Runx2 and Osterix TSSs in MC4 cells treated with TNF-α mimicked that of pluripotent MSCs. TNF-α enhanced the expression of MSC marker genes in MC4 cells. Our findings

link extracellular stimuli in the MM bone marrow microenvironment to changes of histone modifers in MSCs. We hypothesize that MM resolves the bivalent domains at the Runx2 and Osterix TSSs to transcriptionally silent domains by decreasing JMJD3 expression, which not only blocks OB differentiation but further reverses this apparent one-way process. Understanding the molecular mechanisms in this pathway should help identify targets to increase bone formation and possibly decrease tumor burden.

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Comprehensive genome characterization of alcohol-induced osteonecrosis of femoral head. Dewei Zhao*, Yan Ding. The Affiliated Zhongshan Hospital of Dalian University, China

Excessive alcohol consumption is widely recognized as a major risk factor for osteonecrosis of femoral head (ONFH). However, the pathogenesis of alcohol-induced ONHF still remains unclear. Here we performed deep sequencing of RNA isolated from paired necrosis (15) and normal (n=15) tissue samples collected from patients diagnosed as alcohol-induced ONFH. Genome-wide transcriptome analysis identified a total of 484 genes are differentially expressed in ONFH samples, with 46 down- and 428 -up regulated genes when compared to controls (FDR-adjusted p <0.05). Gene ontology and pathway analysis by GSEA reveal significant dysregulation of genes involved in alcohol metabolic process, extracellular matrix organization and collagen formation, hemoistasis, metabolism of lipids and lipoproteins, GPCR signaling, complement and coagulation cascade, and TGF-a signaling (PITX2,COMP,DCN, INHBA,THBS4), suggesting alcohol induces bone remodeling by osteoblasts. We identified polymorphism of Arg48His (rs1229984) for ADH1B and novel mutations at the 3'UTR region of ADH1B from the ONFH samples. Dysregulation of the differentially expressed genes were validated by real-time quantitative PCR and genotypes of the ADH1B were confirmed by DNA-PCR followed by Sanger sequencing.   We further found that ADH1B was over-expressed in osteocytes by Immunohistochemistry (IHC). Genetics linkage analysis between alcohol-induced ONFH patients and non-ONFH alcoholics (n=30) show likelihood of developing ONFH for individuals with concurrent SNP Arg48His and 3'UTR mutation in ADH1B. Take together, for the first time, we identified novel mutations in ADH1B gene and its overexpresion in osteocytes, and link ADH1B gene dysregulation in the osteocytes to the alcohol-induced osteonecrosis of femoral head.